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ASSESSING THE QUALITY OF BUFFALO MILK FROM A COAL MINING REGION: PHYSICOCHEMICAL ATTRIBUTES AND HEAVY METAL ANALYSIS BY ATOMIC ABSORPTION SPECTROSCOPY

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Abstract

Background: Buffalo milk is an important source of nutrition in Pakistan; however, contamination from environmental factors such as coal mining activities can severely affect its quality and safety.

Objectives: This study aimed to assess the physicochemical characteristics and heavy metal content of buffalo milk collected from Mach, a coal mining area in Balochistan, to evaluate potential public health risks.

Methods: Thirty buffalo milk samples were collected from various farms in Mach City. Physicochemical properties were analyzed using a Master Milk Quality Analyzer, while heavy metals were determined using Atomic Absorption Spectroscopy according to standard protocols.

Results: The milk samples generally met basic sensory standards, showing a uniform white color, normal flavor, and sweet taste. However, fat content ranged from 2.68% to 12.81%, protein content varied between 2.9% and 5.0%, and SNF levels fell below the PSQCA standard of 9.0% in a significant number of samples. Lactose and mineral salt deviations were found in 33% of the samples. Heavy metal analysis showed that copper and lead levels were within acceptable limits, while iron levels exceeded the Codex Alimentarius limit of 0.138 mg/L in 90% of samples. Cadmium levels ranged from 0.003 to 0.067 mg/L, and cobalt concentrations (0.010 mg/L) surpassed the IDF threshold in 57% of samples. Manganese levels (0.130–0.559 mg/L) also suggested potential health hazards.

Conclusion: These results highlight the urgent need for strict monitoring, improved feed and water management, and enforcement of regulatory standards to minimize health risks and ensure milk safety for consumers.

Keywords: Buffalo milk, Heavy metal contamination, Mach city, Physicochemical analysis

INTRODUCTION

Milk is a fundamental source of nutrition for mammals, providing essential nutrients and energy critical for early development and growth. It is considered a well-balanced and nutritious food, integral to a healthy diet (1). Livestock, particularly the dairy sector, plays a vital role in the economy of many countries, contributing significantly to GDP and exports. In Pakistan, for instance, it ranks as the fifth-largest milk-producing nation globally, with an annual production of approximately 46.44 billion liters of milk. Buffalo and cow are the primary sources of milk, accounting for 68% and 27% of the total production, respectively (2). The livestock sector directly supports over 55 million livelihoods in Pakistan.

Buffalo holds a special status in the dairy industry and is often referred to as "black gold" due to its substantial contribution to milk production and agriculture. Buffaloes are favored for their resistance to parasites, ability to thrive on low-quality high-roughage diets, rapid calf growth, and high-quality meat and milk production. They are widespread across Asia, Europe, the Middle East, and South America, with India and Pakistan being the top producers, accounting for 68% and 28% of global buffalo milk production, respectively (3).

Buffalo milk contains compounds like gangliosides, which offer antioxidant and neuronal protection, contributing to overall well-being (4-8). In Pakistan, there are approximately 28.4 million



buffaloes producing around 22 million tons of milk annually, and Punjab province is the primary hub for buffalo population, hosting about 70% of the total. Sindh province boasts the largest buffalo colony, housing around 2.5 million buffaloes (9).

The composition of milk varies among different species, with each offering unique physicochemical attributes and nutritional profiles. Studies have shown variations in parameters like pH, total solids, fat, lactose, specific gravity, titratable acidity, ash, and protein content in milk samples from buffalo, cows, sheep, and goats. These differences have implications for the suitability of milk from various species for specific dietary needs (10-12). However, the composition of milk is not static and can be influenced by various factors. Genetic factors, health status, stage of lactation, environmental conditions, feeding practices, and age can all contribute to the variability of milk properties (13). Furthermore, sodium chloride, formalin, hypochlorite, starch and sugar were detected in milk samples (14).

Recently, environmental contamination of milk, especially with heavy metals, has emerged as a serious public health concern. Heavy metals such as iron, manganese, lead, copper, cobalt, and cadmium can enter the food chain through various environmental pathways, including wastewater irrigation for cattle fodder, pesticide use, and coal mining activities (15).

These heavy metals pose severe health risks when present above permissible limits. Iron, though essential, can cause tissue damage if excessively consumed. Manganese is necessary for biological functions but can induce neurological disorders at high levels. Lead is highly toxic, leading to neurological and cognitive impairments. Copper is vital but harmful when in excess. Cobalt is required in trace amounts but becomes toxic at higher concentrations. Cadmium is a dangerous heavy metal linked to kidney damage and other chronic health issues (16, 17).

To ensure the safety of milk consumption, it is crucial to monitor heavy metal levels and comply with maximum permissible limits established by international bodies such as the Codex Alimentarius Commission and the World Health Organization (18).

However, there is limited data available on the contamination status of buffalo milk in coal mining regions like Mach, Balochistan. Therefore, this study aims to fill this research gap by evaluating the physicochemical characteristics and heavy metal content of buffalo milk from Mach. It specifically investigates potential public health risks and provides evidence to guide regulatory policies and improve environmental and food safety standards.

MATERIALS AND METHODS

In this research, a comprehensive study was conducted to assess the quality of buffalo milk from various dairy farms in Mach City.

SAMPLE COLLECTION

A total of 30 milk samples were collected from different farms in Mach City, with each farm contributing five samples, using a purposive sampling technique based on farm accessibility and animal health status. These samples were carefully collected in the morning right after milking to prevent post-milking contamination. They were then placed in clean plastic bottles on-site and transported to Quetta using ice packs to maintain their quality. No control group (such as milk from non-mining areas) was included in this study due to logistical limitations; however, standard reference limits were used for comparison.

PHYSICOCHEMICAL ANALYSIS

Upon arrival at the Balochistan Food Authority laboratory, the milk samples underwent a thorough evaluation of their physicochemical properties. This evaluation included an assessment of physical attributes such as color, taste, flavor, and pH. Additionally, measurements were taken to determine factors like adulteration, protein content, fat content, and lactose content. To ensure precise measurements, the samples were equilibrated in a water bath to reach a temperature range of 20 to 25°C and were stored in a refrigerator during this process.

To profile the milk samples, a Laktan 1-4M Model 600 Ultramax, known as a Master Milk Quality Analyzer, was employed. This analyzer utilized wet chemical calibrated ultrasonic technology, a globally recognized method. The process began by allowing the milk samples to stabilize, after which 50 ml portions were transferred to 100 ml beakers. The device was activated and calibrated, and the milk type was selected on the display. In about 30 seconds, the device provided essential data, including temperature, fat content, SNF (Solid-Not-Fat) content, density, freezing point, protein content, lactose content, salt content, adulteration, pH, and conductivity. These results were meticulously recorded for further analysis.

HEAVY METAL ANALYSIS

In addition to the physicochemical assessment, heavy metals in the 30 milk samples were analyzed. The analysis followed standard procedures described by the Association of Official Analytical Chemists (AOAC) and guidelines from the Atomic Absorption Spectroscopy (AAS) manual to ensure accuracy and repeatability.

This involved an acid digestion process before testing with an atomic absorption spectrophotometer, ensuring complete solubility of metals for accurate analysis. A 5 ml portion of each milk sample was added to a 100 ml conical flask, along with 10 ml of HNO₃. The mixture was heated to 80°C for 20 minutes, allowed to cool, and then 5 ml of hydrogen peroxide (H₂O₂) was added. It was heated again to 180°C until the milk sample volume was reduced to 2–3 ml. The resulting digested milk sample was diluted with deionized water to a 50 ml volume and filtered before analysis in the atomic absorption spectrometer (19).

The study involved daily preparation of calibration standards for copper, cobalt, cadmium, iron, manganese, and lead using a 1000 ppm stock solution from Merck, Pakistan. For each metal, three dilutions were prepared within specified concentration ranges, following guidelines from the Atomic Absorption Spectrometer cookbook. Copper dilutions ranged from 3 to 9 ppm, analyzed at 302.7 nm with a flow rate of 1.0–1.4 mL/min. Similarly, cadmium dilutions comprised 0.03 ppm, 0.06 ppm, and 0.09 ppm, analyzed at 201.5 nm. Iron dilutions ranged from 0.01 ppm to 0.03 ppm, with analysis at 279.5 nm. Manganese dilutions included 0.02 ppm, 0.05 ppm, and 0.07 ppm, analyzed at 255.7 nm. Lead dilutions comprised 0.007 ppm, 0.01 ppm, and 0.02 ppm, analyzed at 217 nm. Sample analysis employed an atomic absorption spectrometer to quantify metal concentrations.

STATISTICAL ANALYSIS

All quantitative data were statistically analyzed using ANOVA to compare mean values across different samples. Differences were considered statistically significant at $p < 0.05$. The inclusion of p-values or standard deviations strengthens the reliability of these findings and highlights real variations rather than random fluctuations.

RESULTS AND DISCUSSION

PHYSICAL PARAMETERS OF MILK

In this study, the primary objective was to evaluate the physical parameters of buffalo milk collected from Mach City in the Kachhi district. The initial assessment of color, flavor, taste, and texture showed no significant differences among the samples, indicating overall acceptable sensory quality.

All milk samples exhibited a consistent white color, attributed to the presence of fat, casein, and small amounts of coloring matter, which aligns with previous research (20). The milk had a typical milky flavor with a slight aroma and a sweet taste, and its texture was normal and free-flowing, confirming compliance with established quality and composition standards. The pH values of the milk samples ranged from 5.81 to 6.44 (Table I), slightly below the recommended pH range for buffalo milk (6.5–6.7) set by FAO, potentially indicating mild acidity caused by bacterial activity or storage conditions (22). These findings are comparable to those reported by Imran *et al.*, 2008 (21), who observed pH levels between 6.38 and 6.77. The variation in pH can be influenced by lactose content, solid-not-fat, breed, lactation stage, animal health, and microbial activity.

Out of 30 samples, 22 were found pure, while 8 samples showed signs of dilution with water, suggesting possible adulteration practices aimed at increasing volume and profit. This adulteration can lower nutritional value and pose public health risks. The freezing point ranged from -0.530 to -0.430°C , consistent with the typical range for buffalo milk (-0.53 to -0.54°C), suggesting that most samples were close to normal composition (23). However, deviations in freezing point may indicate adulteration or compositional changes.

Table I. Physical attributes measured in buffalo milk samples

Parameters	Minimum	Maximum	Mean	Standard Deviation
pH	5.807	6.437	6.119	0.159
Water	0%	20%	3%	0.061
Freezing Point	-0.530	-0.430	-0.517	0.031

CHEMICAL CONSTITUENTS OF MILK

Table II summarizes fat content, which ranged from 2.68% to 12.81%. Five samples fell below the PSQCA minimum standard of 5%, while ten samples exceeded the maximum standard of 9%, indicating substantial variability likely due to feeding practices and breed differences. This variability aligns with other studies reporting average fat content in buffalo milk from 6.57% to 7.79% (24). Excessive fat levels can impact caloric intake, contributing to dietary imbalances.

Protein content ranged from 2.05% to 3.10%, with five samples below the minimum threshold. While most samples met acceptable standards, deviations may result from breed differences, lactation stage, and nutritional status (25-27). Protein is vital for tissue growth and repair and supports essential metabolic functions.

The PSQCA sets a minimum SNF standard of 9% for buffalo milk; however, only four samples met this criterion. Low SNF values may suggest adulteration or poor-quality feed and can compromise nutritional value (28-30).

Lactose content ranged from 3.61% to 5.39%, with ten samples exceeding the typical buffalo milk range (3.28–4.8%). Lactose variations affect osmotic balance and milk properties and may reflect differences in animal health, particularly mastitis (25).

Mineral salt content ranged from 0.54% to 0.81%, with 33% of samples below the acceptable range of 0.7–0.9%. These deficiencies can reduce nutritional value and alter milk stability (31–33).

Table II. Chemical constituents of buffalo milk samples

Parameters	Min	Max	Mean	Standard Deviation
Fat	2.680	12.810	6.231	2.241
Protein	2.050	3.100	2.667	0.261
SNF	6.560	9.790	8.407	0.795
Lactose	3.610	5.390	4.624	0.437
Mineral Salts	0.540	0.810	0.697	0.066

HEAVY METALS ANALYSIS

COPPER

Copper levels ranged from 0.013 to 0.24 mg/L, with an average of 0.134 mg/L (Fig. 1). Although these values were below the local standard (0.04 mg/L), the mean was still higher than desirable, requiring periodic monitoring to avoid potential toxic effects (34, 35).

IRON

Iron levels exceeded the Codex Alimentarius Commission limit of 0.138 mg/L in 90% of samples (Fig. 1). Excessive iron intake can cause oxidative stress and liver damage, highlighting public health concerns and the need for strict control measures (36, 37).

CADMIUM

Cadmium levels ranged from 0.003 to 0.067 mg/L, exceeding the permissible limit of 0.0026 mg/L set by IDF in all samples (Fig. 1). Chronic exposure to cadmium may cause kidney dysfunction and bone damage, emphasizing the importance of reducing environmental contamination sources (38).



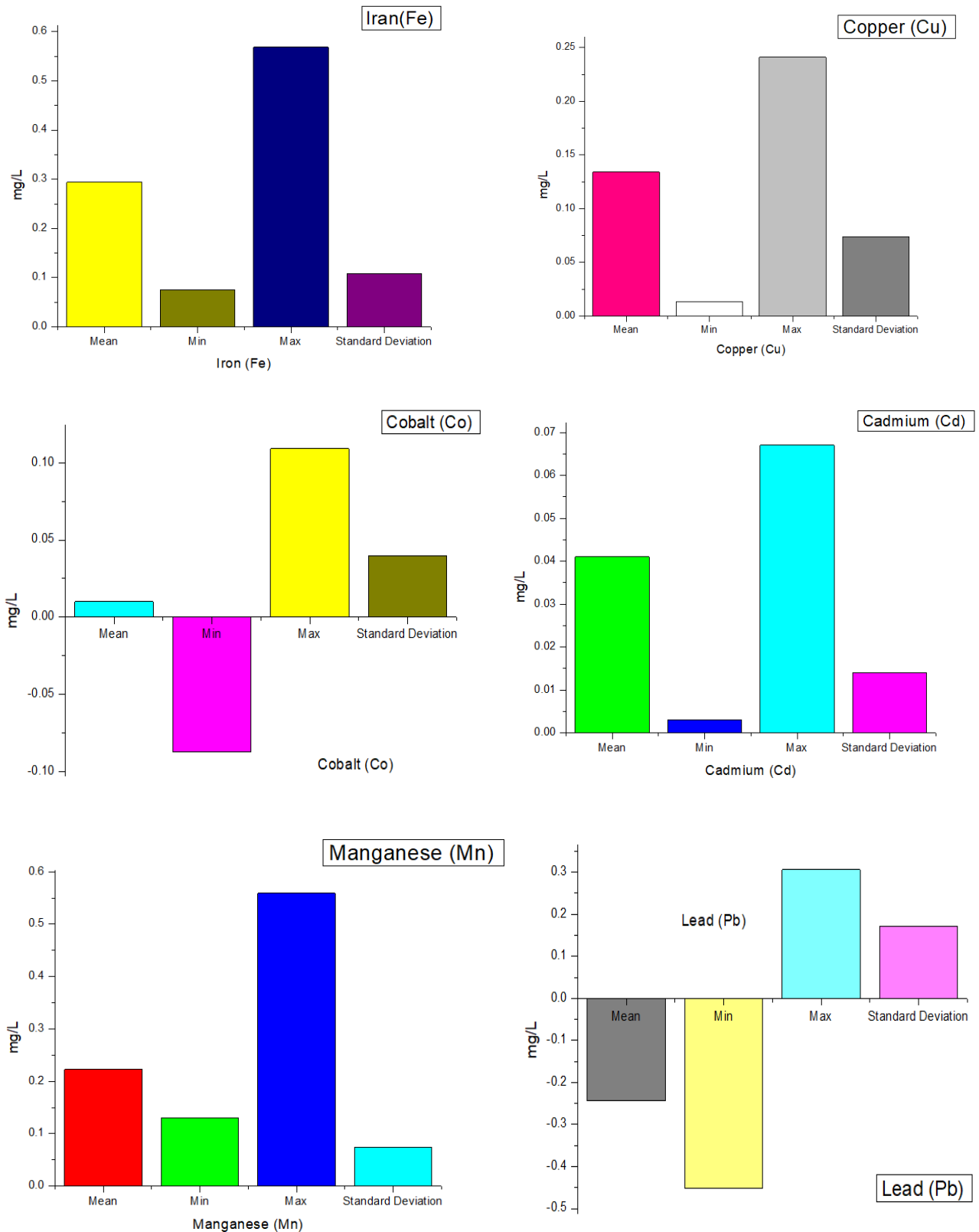


Fig. 1. Concentration of iron, copper, cobalt, cadmium, manganese and lead in the buffalo milk samples

LEAD

Lead levels ranged from -0.451 to 0.306 mg/L, with an average of -0.244 mg/L (Fig. 1). While most samples were within the IDF limit of 0.02 mg/L, a few exceeded it, underscoring the importance of preventing environmental lead exposure to safeguard public health (36, 39).

COBALT

Cobalt levels ranged from -0.087 to 0.109 mg/L, with an average of 0.010 mg/L. Approximately 57% of samples surpassed the limit of 0.006 mg/L (IDF), potentially causing thyroid and reproductive system disruptions if consumed continuously (40).



MANGANESE

Manganese levels ranged from 0.130 to 0.559 mg/L, exceeding the permissible range of 0.02 to 0.05 mg/L in all samples (Fig. 1). High manganese exposure is associated with neurotoxicity and reproductive health issues, requiring urgent attention (41, 42).

CONCLUSION

This study comprehensively evaluated the physicochemical characteristics, chemical composition, and heavy metal content of buffalo milk samples collected from various farms in Mach City, Balochistan, Pakistan. While the physical attributes—such as color, flavor, taste, and pH—were generally within acceptable thresholds, notable variability was detected in key chemical constituents including fat, protein, SNF, lactose, and mineral salts. These disparities point to inconsistencies in farm-level practices and emphasize the need for improved quality control and standardization.

Heavy metal analysis revealed concerning levels of contamination. Notably, 90% of the milk samples exceeded the permissible limits for iron, while all samples were found to contain cadmium above safe thresholds. In addition, a substantial number of samples surpassed acceptable limits for cobalt, manganese, and lead. These findings pose significant public health risks and call for immediate implementation of regular monitoring protocols, stricter regulatory enforcement, and effective intervention strategies to safeguard consumer health and ensure milk safety in the region.

Implications and recommendations:

The observed variations in physicochemical and mineral profiles indicate the influence of multiple factors including feed composition, seasonal and environmental conditions, farm hygiene practices, and potential mining-related contamination. The presence of heavy metals beyond permissible limits underscores the urgent need for stricter feed quality control, regular monitoring, and policy-level interventions. Educating farmers on best practices and introducing stricter regulatory checks could minimize contamination and improve milk safety for consumers.

Authors' contribution:

RR Conducted research work, writing initial manuscript and data acquisition; SA Conceptualization and supervision; UH & QR Data analysis and statistical analysis; FB, HT, AB, BS & AAB editing and revision of manuscript.

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