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Comparative determination of heavy metal concentrations in cattle and buffalo milk from north Gujarat using ICP-AES

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Abstract

The contamination of milk with heavy metals, particularly non-essential elements, poses significant risks to both livestock and human health. These pollutants primarily enter the dairy food chain via industrial effluents, vehicular emissions, construction dust, and intensive agrochemical use. The present study aimed to evaluate the residual concentrations of heavy metals like Lead (Pb), Chromium (Cr), and Nickel (Ni) in cattle and buffalo milk from three districts in northern Gujarat. 800 samples were collected across the summer and winter seasons. Following collection, samples were immediately stored at -20°C and later processed via acid digestion with 6% nitric acid (HNO_3) prior to analysis. Heavy metal analysis was performed using Inductively Coupled Plasma–Atomic Emission Spectroscopy (ICP-AES). In cattle milk, the mean concentrations of Pb, Cr, and Ni were recorded as 0.188 ± 0.014 mg/L, 0.121 ± 0.014 mg/L, and 0.144 ± 0.011 mg/L, respectively. For buffalo milk, the respective values were 0.139 ± 0.015 mg/L, 0.272 ± 0.029 mg/L, and 0.245 ± 0.015 mg/L. While buffalo milk generally showed higher levels of these heavy metals compared to cattle milk, the differences between the two species were not statistically significant.

Keywords: Heavy metals, milk contamination, cattle milk, buffalo milk, ICP-AES, north Gujarat

1. Introduction

Ensuring the safety and traceability of dairy products has become increasingly important to meet consumer demands for foods that promote health and reduce the risk of disease. Milk, a key component of many daily diets, provides essential nutrients and is particularly important for the growth and development of children. However, despite its nutritional value, milk can also serve as a pathway for environmental contaminants, such as heavy metals. Elements like lead (Pb), chromium (Cr), and nickel (Ni) can enter the food chain through contaminated water, soil, animal feed, industrial emissions, and agricultural practices. Regular consumption of milk containing these metals can lead to serious health issues, including neurological disorders, kidney damage, and other chronic conditions. Consequently, monitoring the levels of heavy metals in milk is critical to safeguarding public health and ensuring food safety.

Milk is widely consumed across India, both in urban and rural areas. Given its extensive presence in daily diets, it is essential to monitor milk for any toxic substances. Among the various potential contaminants, heavy metals are particularly important because of their significant health risks to humans and animals.

Heavy metals are those elements which have density more than 5 g/cm^3 , atomic weight 63.456 to 200.590 (Kennish, 1992) ^[26] and a specific gravity more than 4.0 (Connell and Miller, 1984) ^[16]. Although heavy metals by and large remain in ground water and soil yet they tend to accumulate and are very toxic at certain levels. Living organisms normally require some of these heavy metals up to certain limits in microquantities, but when excess accumulation occurs, they lead to severe detrimental effects (Kennish, 1992) ^[26]. Historically, the accumulation of heavy metals is not a new experience. Many historians believe lead coated utensils as a major cause for the fall of Roman Empire (Andrada *et al.*, 2006) ^[5]. Heavy metals undergo biotransfer, bioaccumulation, and biomagnification in food webs. Ruminants

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consuming contaminated forage accumulate metals in tissues, potentially causing toxicity or inert storage, with implications for trophic transfer (Nazir *et al.*, 2015) [32].

Crops cultivated in agricultural soil or watered with contaminated water can accumulate metals, becoming a significant source of heavy metal exposure for both animals and humans. These metals are toxic in nature and at relatively low concentration can cause adverse effects (Aslam *et al.*, 2011) [7]. Accumulation of toxic metals in food chain, and their rising concentration in the environment are mainly due to increase in urban, agricultural and industrial emissions (Birghila *et al.*, 2008) [11].

Milk is a basic food product but unfortunately it is likely to contain numerous xenobiotics like pesticides, disinfectants, drugs, metals and various environmental pollutants associated with high risk for the health of the end user (Beretta, 1984; Casarett, 2000) [9, 15]. Levels of toxic metals in dairy milk not only serve as a direct indicator of food safety and hygiene for consumers but also reflect the extent of environmental contamination in the regions of milk production or sourcing (Licata *et al.*, 2004; Gonzalez-Montana *et al.*, 2012) [28, 22]. Heavy metals are found in milk at relatively lower concentration (Unnikrishnan *et al.*, 2005) [38]. The presence of toxic metals in milk represents a significant public health concern (Caggiano *et al.*, 2005) [14]. Heavy metal contamination in milk and related dairy products may arise from multiple sources, including the ingestion of contaminated feed and water by livestock, as well as exposure during industrial processing and packaging operations (Anastasio *et al.*, 2006; Ayer *et al.*, 2009) [4]. Industrial and agricultural processes have resulted in an increased concentration of heavy metals in air, water, soil and subsequently, these metals are taken by plants or animals and find their ways into food chain (Salah *et al.*, 2012) [36]. Additionally, physio-chemical property of milk favors retention of metals due to formation of bioactive (lipophilic) complexes (Leeuwen and Pinheiro, 2001, Buechler *et al.*, 2002) [27, 12].

Lead contamination is a global environmental issue. It is easily absorbed from milk (Morisson and Quarterman, 1987) and, as a non-essential toxic metal, can cause Alzheimer's disease, kidney, reproductive, endocrine, and nervous system disorders, as well as lower children's IQ (Ahmad *et al.*, 2011) [3].

Chromium which is very toxic metal may cause abdominal pain, hepatotoxicity, dizziness, cardiotoxicity, hyperemia, neurotoxicity ulceration and vomiting (Hussain *et al.*, 2010) [23]. Inspiring air containing high concentration of nickel may cause lung diseases and irritates mucosal layer of circulatory system.

The presence of toxic metals in milk being a major threat to consumer health needs an immediate attention of health regulatory authorities and the researchers as well. Thus, contamination of milk with heavy metals adversely affects health of consumers. Additionally, presence of toxic metals in milk also constitutes a trade barriers for dairy industries because of technological risk involved in processing and packaging. North Gujarat contributes over 50% of Gujarat's milk production, with Banaskantha, Mehsana, and Gandhinagar districts accounting for 13.19%, 7.03%, and 3.27%, respectively. Dairying and animal husbandry are vital for regional livelihoods and food security. Using Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES), this study quantified heavy metal concentrations in cattle and buffalo milk from multiple locations in North Gujarat and

assessed inter-species differences in metal accumulation. The findings provide critical insights into milk safety and potential public health implications.

Materials and Methods

Sample collection and storage

Milk samples from cattle and buffalo were collected from selected locations in North Gujarat, including the districts of Gandhinagar, Mehsana, and Banaskantha, representing areas with varying climatic, industrial, and geographic conditions. In each selected area, five villages were chosen for sampling. Samples were collected during two seasons, summer (April–June) and winter (October–December). A total of 400 samples were obtained in each season, resulting in 800 samples for the entire study. Fresh milk samples were collected directly from animals at the collection point during the morning milking hours. Milk (10–15 ml) was collected directly from animals during morning milking into clean, dry centrifuge tubes. The samples were immediately stored in an ice-packed container and transported to the laboratory on the same day. After arrival, they were preserved at -20°C until analysis using ICP-AES.

Chemical and reagents

Trichloroacetic acid and nitric acid (extra pure grade, SDFL, India) were procured and used for the determination of toxic metals. De-ionized water used throughout the study was obtained from an Ultra Water Purifier (SG, Germany). All glassware employed during the analysis was initially washed with a laboratory detergent solution and subsequently rinsed with metal-free water. The glassware was then soaked overnight, or for an extended duration, in a covered acid bath containing 10% nitric acid (HNO_3) solution to eliminate potential metal contaminants. After acid treatment, the glassware was thoroughly rinsed multiple times with de-ionized metal-free water and dried in a hot air oven prior to use. Porcelain crucibles were carefully cleaned before each use by washing with diluted nitric acid followed by rinsing with distilled water to prevent any possible contamination that might interfere with the analytical results.

Acid digestion of milk samples

Milk samples were prepared following a standard protein precipitation and ash digestion procedure. Briefly, 5 mL of milk was diluted with distilled water to reduce viscosity. Subsequently, 5 mL of 0.1 M trichloroacetic acid (TCA) was added to precipitate proteins. The mixture was centrifuged at 4000 rpm for 20 min, and the supernatant was separated by decantation and transferred to labeled porcelain crucibles. The samples were then ashed in a muffle furnace at 500°C for 3 h. The resulting ash was dissolved in 15 mL of 6% nitric acid and filtered through Whatman No. 41 filter paper. The filtrate was collected in centrifuge tubes previously rinsed with deionized water and stored until further analysis. The final volume of each sample was adjusted to 15 mL prior to assay.

Instrumental analysis

Standard preparation for ICP-AES analysis

A composite standard of multi elements (Cetripur®, Merck KGaA, Frankfurter, Germany) was used for preparation of standard curve for ICP-AES analysis. The standard provided by manufacturer was traceable to NIST SRM (National Institute of Standard Technology Standard Reference Material). A stock solution of 100 mg L^{-1} was prepared and further used to prepare working standard solutions of 0.01,

0.1, 1.0 and 10.00 ppm for ICP-AES analysis. Quantification of element concentrations were performed after establishing calibration curves with external standards prepared in 6% v/v ultrapure HNO₃ for analysis of all samples. A calibration curve was constructed using four standard concentrations and their corresponding counts, which was then used to quantify heavy metal concentrations in the unknown samples.

Analytical Procedure for ICP-AES

The levels of heavy metals (Pb, Cr, and Ni) in milk samples were quantified using Inductively Coupled Plasma–Atomic Emission Spectroscopy (ICP–AES) with a Thermo Fisher Scientific iCAP 6000 ICP–AES (Spectroflame-P) system. Prior to sample measurement, the instrument was calibrated using blank and standard solutions, which were scanned over the selected wavelength range to identify suitable analytical lines. Background signals were corrected by manually selecting positions close to each analyte emission peak in order to minimize spectral interference. Details of the instrumental configuration and instrumental parameters are provided in Table 1. Each milk sample was analyzed in triplicate under identical conditions, and the results were reported as mean values. Analytical Wavelengths for Determination of Heavy Metals (LOD)) for the studied elements (Table 2) were estimated using the standard addition approach and were defined as three times the standard deviation of the blank signal. The obtained detection limits indicated that the method provides sufficient sensitivity for determining trace levels of these metals in milk samples.

Table 1: ICP-AES instrumental parameters

Sr No.	Operating conditions	
1	RF Frequency	27.12 Hz
2	RF Power	2.5 Kw
3	Outer gas flow rate	Ar 17 L/min
4	Intermediate gas flow rate	Ar 1 L/min
5	Carrier gas flow rate	Ar 1 L/min
6	Observation height	18 mm above work coil
7	Plasma's temperature	8000-9000 K

Table 2: Analytical Wavelengths for Determination of Heavy Metals

Sr No.	Element	Analytical wavelength (nm)
1	Chromium	267.716 nm
2	Nickel	231.604 nm
3	Lead	220.353 nm

Statistical analysis

The statistical significance of variations between cattle and buffalo was determined by applying the Student's *t*-test. Moreover, the influence of species differences on heavy metal concentrations in milk samples was analysed using SPSS (version 16.0 for Windows) and GraphPad Prism (version 7.00).

Results and Discussion

Mean $\pm$ SE concentrations of Pb, Cr, and Ni (mg/L) in cattle and buffalo milk across all four regions are summarized in Table 4, showing no significant differences between species. Interspecies comparison of a range of Pb, Cr and Ni (mg/L) in cattle and buffalo milk collected from all four areas is presented in Table 5. Interspecies differences in milk were observed for all measured metals across the four regions. Table 6 summarizes cattle and buffalo milk in relation to heavy metal content and corresponding MRLs. All samples were below the MRLs for Pb, Cr, and Ni, indicating compliance with safety standards. Heavy metal presence remains a potential health concern in milk.

Therefore, the monitoring of toxic/heavy metals in milk of dairy animals is an important issue of today's time. Heavy metals have an easy access to the food chain and have been reported to exert many pathophysiological alterations within the human body. Therefore, attention of the researchers has been diverted to its bio-hazardous potential. Heavy metals can accumulate in the body over a lifetime (Bernard, 2008) [10]. Exposure of lactating animals to high levels of toxic metals such as Cr, Ni, and Pb can lead to their transfer into milk, posing health risks to consumers. Monitoring heavy metal concentrations in dairy products is essential for food safety and provides an indirect assessment of environmental contamination and animal health.

North Gujarat was selected as a study area because of having reputation of Milk Bowl of Gujarat State producing 50% of total state milk production as well as harboring many industries and manufacturing units. The region also has distinct geographical and anthropogenic activities ranging from mainly agriculture/animal husbandry in Banskantha / North West of Mehsana to highly industrial development in South West of Mehsana and Gandhinagar district.

The inter species variation of Buffalo milk exhibited higher concentrations of heavy metals than cattle milk, with Pb (0.139 ± 0.015 mg/L), Cr (0.272 ± 0.029 mg/L), and Ni (0.245 ± 0.015 mg/L) compared to Pb (0.121 ± 0.014 mg/L), Cr (0.188 ± 0.014 mg/L), and Ni (0.144 ± 0.011 mg/L) in cattle milk. These interspecies differences may reflect variations in feeding and grazing behaviours, species-specific metal metabolism, and the intensity and duration of environmental exposure.

In previous studies have reported higher levels of heavy metals in milk than observed in the present study. For example, Birghila *et al.* (2008) [11] found elevated Pb in cow milk in Romania, Qin *et al.* (2009) [35] reported higher Cr in cattle milk in China and Japan, and Aslam *et al.* (2011) [7] detected increased Cr, Ni, and Pb in Pakistan. Abdulkhaliq *et al.* (2012) [2] also recorded higher Pb in Palestine, whereas lower Cr and Pb levels were reported by Birghila *et al.* (2008) [11] and Tona *et al.* (2013) [37] in Romania and Nigeria, respectively. In the current study, Pb concentrations ranged from 0.001 to 2.019 mg/L in cattle milk (mean 0.121 mg/L) and 0.001 to 2.442 mg/L in buffalo milk (mean 0.139 mg/L) across both seasons. Exceptionally high levels were reported in Faisalabad (18.870 mg/L) and Peshawar (2.082–2.024 mg/L) in Pakistan (Javed *et al.*, 2009; Iftikhar *et al.*, 2014) [25, 24]. Variations among studies likely reflect differences in environmental exposure, feeding practices, and grazing patterns.

Table 3: Species and Area wise details of sample collection

Sl. No.	District	Number of Cattle Samples	Number of Buffalo Samples	Total Samples Collected
1	Mehsana	284	116	400
2	Gandhinagar	65	135	200
3	Banaskantha	141	59	200
Total	3	490	310	800

Table 4: Interspecies comparison of Pb, Cr, and Ni (mg/L) concentrations (mean $\pm$ SE) in milk from cattle and buffalo across four regions

Residue	Cattle	Buffalo
Pb	0.121 ± 0.014	0.139 ± 0.015
Cr	0.188 ± 0.014	0.272 ± 0.029
Ni	0.144 ± 0.011	0.245 ± 0.015

*Significantly ($p \leq 0.05$) different from their respective values

Table.5: Interspecies comparison of range of Pb, Cr and Ni (mg/L) in cattle and buffalo milk collected from all four areas

Residue	Cattle (Range)	Buffalo(Range)
Pb	0.001-2.019	0.001-2.442
Cr	0.009-1.481	0.001-2.455
Ni	0.013-0.875	0.003-0.875

*=Significantly ($p \leq 0.05$) different from their respective values

Table 6: Comparative summary of between cattle and buffalo milk

Residue	Species	Detectable Samples	Non-Detectable Samples	Samples Above MRL
Cr	Cattle	251	59	-
	Buffalo	316	84	-
Ni	Cattle	241	69	-
	Buffalo	376	24	-
Pb	Cattle	193	117	0
	Buffalo	205	195	0

MRLs of Pb is taken as 2.5 and as per FSSAI (2011) [20].

Table 7: Permissible Limits of Heavy Metals in Milk According to International Standards

Heavy metal	Permissible limit (mg/kg) or ppm	Standard Authority
Lead	0.02 ppm	WHO/FAO/Codex Alimentarius
Chromium	0.005 ppm	WHO
Nickel	0.2 ppm	WHO

In this study, Pb concentrations in cattle and buffalo milk ranged from 0.001–2.019 mg/L and 0.001–2.442 mg/L, respectively, across both seasons. These values were lower than those reported for cattle and buffalo milk in Pakistan (Javed *et al.*, 2009; Iftikhar *et al.*, 2014) [25, 24] but exceeded maximum concentrations previously documented in buffalo milk in India (Nirgude and Bhagure, 2015) [33] and in cattle and buffalo milk in Egypt (Arafa *et al.*, 2014) [6]. Similarly, the highest Pb levels in cow milk observed here surpassed the 0.052 mg/L reported in Tamil Nadu, India (Dhanalakshmi and Gawdaman, 2013) [17], yet remained lower than levels reported in Iran (Nejatolahi *et al.*, 2014) and Nigeria (Farid and Baloch, 2012) [19]. Higher values of Pb in milk occur due to fodder contamination, climatic factors such as wind, use of agro-chemicals and very importantly drinking water higher in its level. Livestock often graze near roads and railways, exposing them to environmental lead from vehicle emissions. Dairy farms located close to high-traffic areas are therefore at risk of lead contamination. Elevated lead levels in milk are particularly concerning, as infants and children who are highly susceptible to lead's neurotoxic effects are the primary consumers of dairy products (Javed *et al.*, 2009) [25]. The concentration range of Pb concentration in present study does not implicate any toxicological or safety risk in consumption of cattle or buffalo milk, because no sample individually exceeded MRL value (2.5 mg/L) as recommended by FSSAI. This indicated that the milk collected from all areas (mahesana, banaskantha and Gandhinagar) were found to be safe with respect to concentration of lead in milk of both cattle and buffalo.

The present study demonstrated the concentration ranges of Cr in cattle and buffalo milk as 0.0009 – 1.481 and 0.001 – 2.455 mg/L, respectively. The average concentration of Cr was found to be 0.188 and 0.272 mg/L in cattle and buffalo milk, respectively. The values of Cr reported in cow milk by Cadar *et al.*, (2015) [13] as 0.00456 mg/L; Abdalla *et al.*, (2013) [1] as 0.0006 mg/L; Murra (2013) [30] as 0.136 and Gebrelibanos *et al.* (2015) [21] as 0.010 ± 0.064 mg/L were less than the findings of our study. In contrast, high concentration of Cr in cattle milk as 1.066 mg/L and 1.199 mg/L by Javed *et al.*,

(2009) [25] and Aslam *et al.*, (2011) [7], respectively, in Faisalabad of Pakistan were observed. The high concentration of Cr in cattle and buffalo milk could be attributed to discharges released from tanneries and fuel residuals of brick kilns. The acceptable daily intake of Cr is reported as 50-200 µg/day (Ogabiela *et al.*, 2011) [34].

In present investigation, the concentration ranges of Ni in cattle and buffalo milk were observed to be 0.013 to 0.875 and 0.003 to 0.875, respectively. The average values of Ni for cattle and buffalo milk were observed to be 0.144 and 0.245 mg/L, respectively. The very high concentration of Ni in cattle milk 22.394 mg/L by Javed *et al.*, (2009) [25] and 22.395 mg /L by Aslam *et al.*, (2011) [7] in Faisalabad, Pakistan were observed. Comparatively, higher levels of Ni in cattle (Etonihu and Alichio, 2010) [18] in Nigeria and in Buffalo milk (Nazir *et al.*, 2015) [32] in Khyber Pakhtunkhwa area of Pakistan were also observed. Zodape *et al.* (2013) [39] reported slightly lower range of concentration of Ni (0.010-0.131 mg/L) in cattle milk collected from different location in and around Mumbai city as compared to range of Ni concentration observed in present investigation.

The overall summary of the study revealed that, across all samples collected from various regions and from different species, the levels of metals such as lead (Pb), chromium (Cr), and nickel (Ni) did not exceed the maximum residue limits (MRLs) established by the Food Safety and Standards Authority of India (FSSAI) [20], indicating compliance with the recommended safety standards. Moreover, the study revealed that the concentrations of the analysed metals did not differ significantly between cattle and buffalo milk, indicating a uniform distribution of these metals across the two species.

Conflict of Interest

Not available

Financial Support

Not available

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