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Original Research Article

Analysis of Heavy Metals Levels in Milk and Dairy Products and Assessing the Associated Health Risks

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Abstract: Milk has long been recognized globally as a vital and comprehensive source of nutrition. However, heavy metals and metalloids in milk have become a significant global concern. These metalloids, originating from the soil due to natural geological processes and various human activities, are transferred into animal feed and accumulate in milk through bio transfer processes. These contaminants enter the food chain through the bio-transfer process from soil to feed and milk. The primary metalloids of concern include arsenic (As), mercury (Hg), lead (Pb), and cadmium (Cd). This study examines the levels of heavy metals, specifically lead (Pb) and cadmium (Cd), and fat and protein content in milk and dairy products, alongside assessing their potential health risks. It compares the findings to international safety and quality standards. Furthermore, it evaluates the possible health effects of consuming these products, particularly for vulnerable groups. The study aims to ensure milk and dairy products' safety, quality, and nutritional value while minimizing health risks.

Keywords: Milk, Heavy Metals, Dairy Products, Cadmium, Lead, Fat and Protein.

INTRODUCTION

Milk is considered an essential and complete food in the human diet and is widely consumed worldwide [1, 2]. Milk's rich array of essential nutrients, vital for our bodies, solidifies its status as a superfood. For instance, milk contains casein, which is crucial for developing bones and teeth and repairing tissues. It also provides milk fat, which aids in absorbing fat-soluble vitamins (A, D, E, and K) and vitamin B2, promoting radiant skin and sharp vision. Additionally, milk is a significant source of minerals like calcium and phosphorus, essential for maintaining strong bones and teeth [3].

Due to their high biological value and easy accessibility, believed to involve low health risks, consumers highly prefer milk and dairy products, considering them an essential part of the human diet [4]. Milk's primary nutrients include proteins, fatty acids, lactose, vitamins, and minerals, which are influenced by various factors such as feed intake, livestock health, environment, and genetics. Numerous studies highlight the importance of determining heavy metal and nutrient elements in milk and its products. Additionally, ensuring the quality and concentration of raw milk in terms of protein, fat, and heavy metal content is of utmost importance to the dairy industry [5]. On average, cow's milk comprises about 80% casein and 20% whey proteins. Milk is one of the most complex natural fats, containing over 400 fatty acids. Milk fat is predominantly (98%) composed of triglyceride molecules. Approximately 65% of the fatty acids in milk are saturated, 32% are monounsaturated, and 3% are polyunsaturated, depending on the levels of milk. The fat composition can vary significantly due to feeding strategies and genetic differences [6].

In addition to protein and fat, milk contains numerous mineral elements, some essential for human health, as the body cannot synthesize them. These elements play crucial roles in biological functions and are cofactors in enzymes [7]. Essential minerals are divided into primary and trace elements. Major components such as calcium (Ca), magnesium (Mg), and phosphorus (P) are present in the body in much higher quantities than trace elements like iron (Fe), zinc (Zn), and manganese (Mn).

Moreover, milk contains trace elements like lead and cadmium through various pathways. We can categorize milk into essential elements like iron, copper, and zinc, which are required in small amounts, and harmful elements like lead and cadmium. Lead (Pb) and cadmium (Cd) are significant trace elements that can enter milk through manufacturing processes, livestock diets, contaminated water, and industrial activities near milk production areas. These elements can easily transfer through food chains and are highly toxic; dairy products contain essential elements such as calcium, zinc, iron, selenium, cobalt, and heavy metals like lead, mercury, and cadmium. For instance, calcium reduces the impact of lead and arsenic on children's hair [8]. Conversely, a sufficient intake of selenium inversely correlates with mercury toxicity [9].

Consumers have identified many trace elements as toxic due to their adverse health effects on biological systems, even at low concentrations. Therefore, exposure to these metals is concerning, especially over long periods, due to their tendency to accumulate in the body [10]. Milk and dairy products constitute an essential source of macronutrients and microminerals, such as calcium, magnesium, zinc, and selenium, which are crucial in metabolic and growth processes, especially for children [11]. The global burden of metal contamination is significant. In 2015, the ingestion of arsenic (As), mercury (Hg) (methylmercury), lead (Pb), and cadmium (Cd) resulted in more than one million illnesses, over 56,000 deaths, and well over 9 million disability-adjusted life years (DALYs) worldwide [12]. These elements are present as inorganic salts or organic mineral compounds, such as porphyrins [13]. There is often an imbalance of essential trace elements in people with chronic kidney disease (CKD), which is linked to a higher risk of heart disease and death. Monitoring these vital trace element levels in CKD patients is crucial for effective treatment, as it can slow down the disease's progression and enhance outcomes [14]. Various sources contribute to the presence of heavy metals in the environment, including both natural and industrial origins. Natural sources, such as weathering and volcanic eruptions, play a significant role in heavy metal pollution [15].

MATERIALS AND METHODS

Collection of samples

A total of 100 samples of milk and dairy products (20 samples of cow milk, 20 samples of sheep milk, 20 samples of goat milk, and 20 samples of buffalo milk) were collected from November 2023 to July 2024 from various dairy farms, dairy shops/vendors, and retail dairy centers in Kirkuk (Laylan, Altun Kupri) and Sulaymaniyah, Iraq. All the samples collected from the dairy shops, vendors, and dairy centers originated from intensive farming. Each 50-ml sample was individually packed in a polyethylene bag. Then, all samples were immediately conveyed to the laboratory in an insulated icebox containing crushed ice and stored in a deep freezer at 20°C until analysis for their heavy metal content.

Sample Preparation

All the laboratory wares and working surfaces were washed thoroughly with deionized water and diluted HNO_3 (10%), then rinsed with deionized water and dried to ensure they were free from metal contamination. The digestion vessels were soaked in water and detergent for at least 3 hours, followed by several rinses: first with distilled water, then with a mixture of 80 mL H_2O_2, 200 mL HCl (37%), and 250 mL deionized water, and finally with 10% diluted HNO_3. Lastly, we washed all the equipment thrice with deionized water and thoroughly air-dried it in an incubator [16].

Samples Digestion

The samples were processed using the wet digestion method. Two milliliters or two grams of dairy samples were mixed well and then put into a 20-milliliter screw-capped tube with 10 milliliters of HNO_3 97% and 2 milliliters of $HClO_3$ 72%. The tube was heated in a water bath at 53°C until all the proteins were broken down, and then it was left to cool at room temperature. After the mixture had cooled, it was put into a 50 ml volumetric flask with distilled water to dilute it. Whatman filter paper (No. 42 Merck-Darmstadt-Germany) was then used to filter it into a clean Pyrex glass tube, and it was kept at room temperature (25°C) until the levels of lead and cadmium were measured. We also prepared standard or blank solutions in the same manner as the wet digestion technique but without adding any samples. We analyzed the blank solutions to identify any heavy metal contamination of the chemicals and subtracted them from the final results.

Heavy Metals Analysis

The collected filtrates were analyzed for lead and cadmium at the Central Laboratory. The analysis used an atomic absorption spectrophotometer (AAS; Buck Scientific 210 VGP Inc.), which features an autosampler and digital readout for absorbance and concentration. The spectrophotometer was adjusted to specific lamp wavelengths of 217.0, 228.8, 324.7, 357.9, 253.7, and 193.7 nm and lamp currents of 5, 4, 4, 7, 4, and 10 mA for measuring lead and cadmium, using air-acetylene flame AAS.

RESULTS AND DISCUSSION

The concentration of heavy metals and trace elements in milk and its products depends on various conditions related to geographical locations and the intensity of industrial activity in those areas. The level of contamination of milk and its products with heavy metals was determined in different locations in the governorates of Sulaymaniyah and Kirkuk, specifically in Laylan and Altun Kupri. Tables 1 and 2 present the results, showing the concentration of cadmium (Cd) and lead (Pb) in milk from these locations. There is a significant variation in the concentration of heavy metals in milk and its products across these sites.

Table 1: Concentration range of metal in milk of different locations

Metal mg/kg	Laylan	Altun Kupri	Sulaymaniyah	P-Value
	Min – Max (Mean)	Min – Max (Mean)	Min – Max (Mean)	
Cadmium (Cd)	0.03 – 0.06 (0.045)	– 0.01 (0.005)	– 0.05 (0.03)	0.002**
Lead (Pb)	0.41 – 0.53 (0.470)	0. 05 – 0.13 (0.090)	0.12 – 0.25 (0.185)	0.0004**
Total	**0.44 – 0.59 (0.515)**	**0.05 – 0.14 (0.095)**	**0.13 -0.3 (0.215)**	**0.0003 ****

Table 1 shows the concentration of cadmium (Cd) and lead (Pb) in milk from three regions (Laylan, Altun Kupri, and Sulaymaniyah), with the statistical values (P-value) displayed to ensure that there are significant differences between different areas. The concentration range is between 0.03 and 0.03–0.06, with an average of 0.045. This data indicates that Laylan suffers from higher cadmium pollution than other regions. The pollution could be due to agricultural fertilizers containing cadmium or industrial activities. Altun Kupri concentration is the lowest (0.01–0.01) with an average of 0.005, indicating a cleaner environment regarding cadmium pollution.

In Sulaymaniyah, the concentration ranges between 0.02 and 0.05, with an average of 0.03, making it intermediate between Laylan and Altun Kupri. The P-value (0.002) shows statistically significant differences between the cadmium concentrations in milk from different regions. The concentration range of lead in Laylan was between 0.41 and 0.53, with a mean of 0.47. This result shows that milk from Laylan has the highest concentration of lead. The concentration ranged in Altun Kupri between 0.05 and 0.13, with a mean of 0.09, the region's lowest concentration. The concentrations in Sulaymaniyah ranged between 0.12 and 0.25, with a mean of 0.185, which places it second after Laylan. The P-value (0.0004) indicates statistically significant differences among the three regions.

Total concentration ranges in Laylan between 0.44 and 0.59, with an average of 0.515, the highest among the three regions. Altun Kupri is between 0.05 and 0.14, with an average of 0.095, indicating the lowest level of total contamination, and Sulaymaniyah concentration ranges between 0.13 and 0.30, with an average of 0.215, which makes it intermediate between Laylan and Altun Kupri. The P-value (0.0003) confirms the existence of statistically significant differences in the total concentration of metals between the regions. The statistical results are then interpreted. The statistical values (P-value) are less than 0.05 for all minerals and total concentration, meaning there are significant statistical differences between the three regions. The horizontal lowercase letters (a, b, c) indicate a clear difference between the regions based on the average. Conduct broader studies to identify sources of pollution in the Laylan area. Assess the quality of water, air, and soil used in agriculture. Numerous studies have focused on the variation in heavy metal contamination rates across different regions. In these areas, several studies have highlighted the disparities in heavy metal contamination levels among various cities [17, 18].

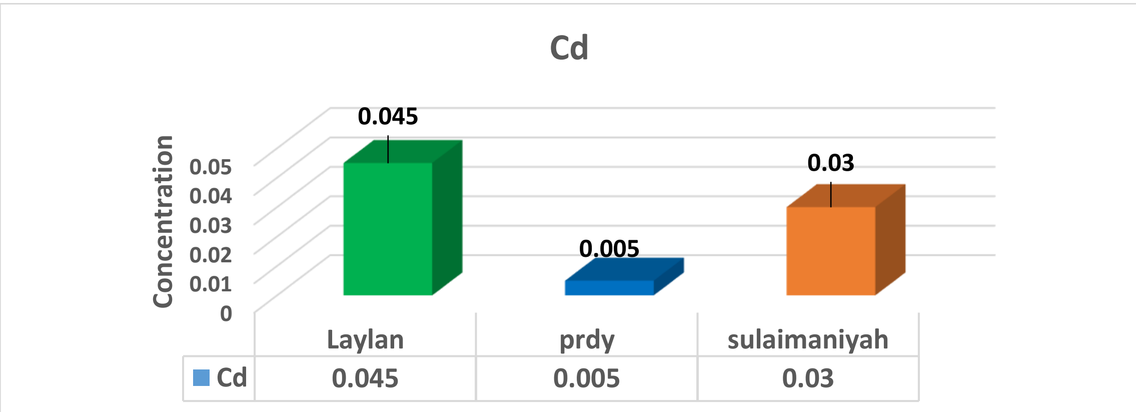

Figure 1: Shows the concentrations of cadmium (Cd) in three different areas: Laylan, Altun Kupri and Sulaymaniyah

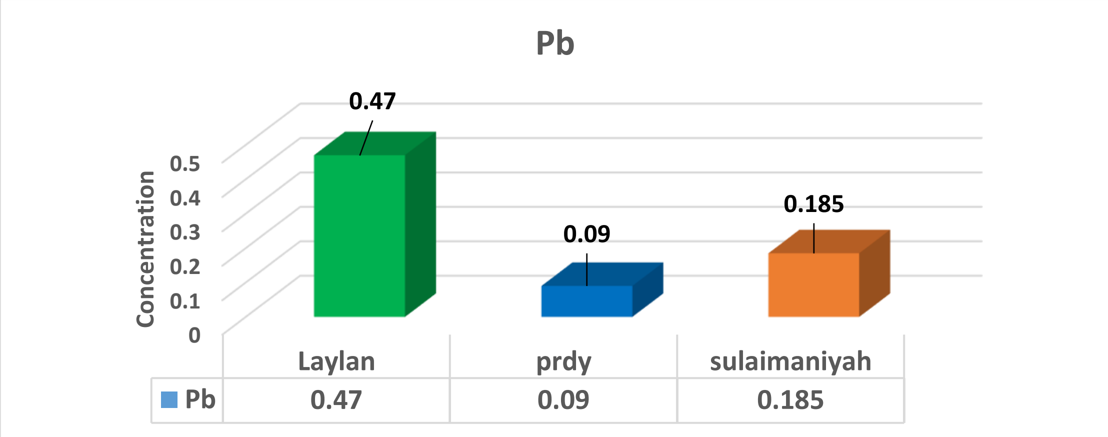

Figure 2: Shows the concentrations of cadmium (Pd) in three different areas: Laylan, Altun Kupri and Sulaymaniyah

Table 2: Heavy metals content of different types of milk

Metal mg/kg	**Cow**	**Sheep**	**Goat**	**Buffalo**	**P-Value**
	Min – Max (Mean)	**Min – Max (Mean)**	**Min – Max (Mean)**	**Min –Max (Mean)**	
Cadmium (Cd)	0.01 – 0.05 (0.03)	0.02– 0.06 (0.04)	0.02 - 0.06 (0.04)	0.01 - 0.04 (0.025)	0.341
Lead (Pb)	0.01– 0.03 (0.02)	0.05 – 0.1 (0.03)	0.07 – 0.1 (0.04)	0.06 – 0.09 (0.075)	0.022 *
Total	**- 0.08 (0.05)**	**0.07 – 0.16 (0.12)**	**0.10 -0.16 (0.13)**	**0.07 – 0.13 (0.10)**	**0.037 ***

Table 2 provides information about cadmium concentration in various milk types. Table 2 indicates that cadmium concentration in cow's milk ranges from 0.01 to 0.05 mg/kg, from 0.02 to 0.06 mg/kg in sheep and goat milk, and from 0.01 to 0.04 mg/kg in buffalo milk. There are no significant differences in the concentration of cadmium. The p-value (0.341) indicates no significant differences between different types of milk regarding cadmium content compared with permissible limits. According to the World Health Organization (WHO) and the Codex Alimentarius, the maximum permitted limit for cadmium in milk is 0.02–0.05 mg/kg, which means that some samples have exceeded the safe limit, which poses a risk to public health. Lead levels vary significantly between different types of milk. Cow milk: 0.01 - 0.03 mg/kg (mean 0.02 mg/kg); sheep milk: 0.05 - 0.1 mg/kg (mean 0.03 mg/kg); goat milk: 0.07 - 0.1 mg/kg (mean 0.04 mg/kg); buffalo milk: 0.06 - 0.09 mg/kg (mean 0.075 mg/kg). The p-value (0.022) indicates significant differences between different types of milk concerning lead content.

High lead concentration in goat and buffalo milk: this may be related to the diet of the animals or environmental exposure to sources of pollution, such as polluted water and pastures near industrial areas [19]. Some values exceed the permissible limits: The maximum permissible limit for lead in milk, according to the World Health Organization, is 0.02 mg/kg, which means that sheep, goat, and buffalo milk contain concentrations higher than the safe level, which poses a risk to human health, especially children, who are more affected by lead poisoning [20].

Total heavy metal concentration in milk the results show clear differences in total heavy metal concentration between milk types:

Cow milk: 1.03 - 0.08 mg/kg, average 0.05 mg/kg

Ep milk has an average concentration of 0.12 mg/kg.

Goat milk: 0.10 - 0.16 mg/kg (average 0.13 mg/kg).

Buffalo milk: 0.07-0.13 mg/kg (average: 0.10 mg/kg).

P value (0.037) indicates a significant difference between the different types.

There is a high accumulation of lead in sheep and goat milk. The historical use of lead in various products across the Americas may have contributed to elevated lead concentrations in the environment, which eventually found their way in to milk sample. Furthermore, the country's high level of industrialization, surpassing that of many other nations, could lead to increased environmental lead concentrations [21]. There is a lot of lead exposure in many urban areas across the United States. This is because of things like older homes, old industrial practices, and different types of lead pollution, such as water that has lead in it from paint, soil that has lead in it from paint, and industrial emissions [22].

Health effects of heavy metal content

Cadmium: Chronic exposure can lead to kidney damage, osteoporosis, and an increased risk of cancer [23]. Lead: Even at low doses, lead is very toxic and can affect the nervous system, liver function, and mental development of children [24]. Long-term exposure: Continuous consumption of milk containing heavy metals can lead to their accumulation in the body, causing chronic diseases and metabolic disorders [25].

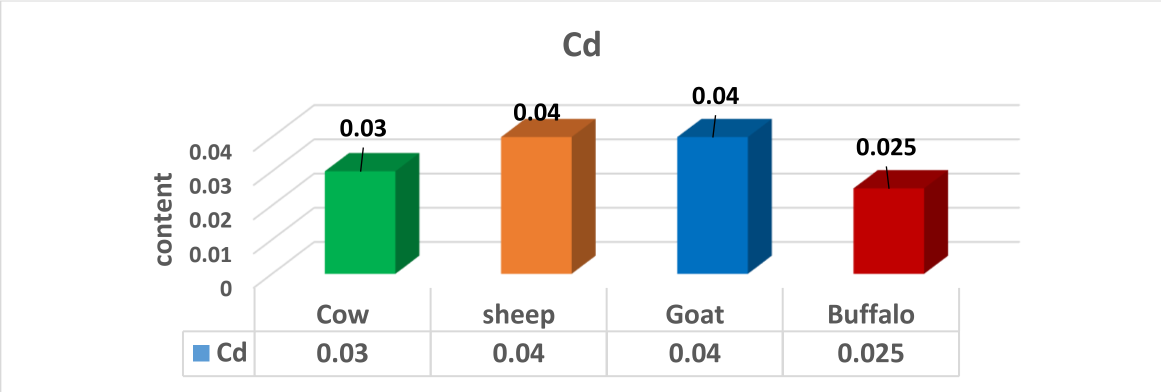

Figure 3: Shows the concentration of cadmium (Cd) in milk extracted from four different types of animals: cow, sheep, goat, and buffalo

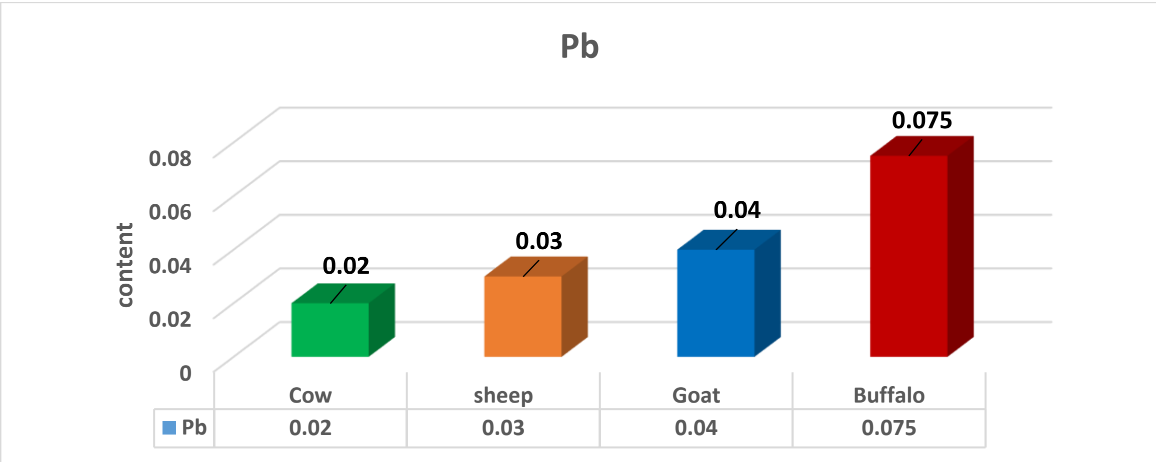

Figure 4: Shows the concentration of cadmium (Pb) in milk extracted from four different types of animals: cow, sheep, goat, and buffalo

Table3. Mean value of protein in raw milk samples

Months Station	Jan	Feb	Mar	Apr	M ay	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Mean of Stations
0	2.99	2.97	2.94	2.90	2.98	2.95	2.90	3.00	3.01	2.99	2.89	3.06	2.965
1	3.10	3.01	2.99	3. 01	2.99	2.99	2.99	3.01	3.01	2.97	2.87	3.00	2.994
2	2.91	2.92	2.96	2.98	2.97	3.04	2.94	2.99	3.01	3.00	2.99	3.01	2.9767
3	3.08	2.99	3.00	2.88	3.01	2.93	2.85	2.89	2.99	2.87	3.02	3.00	2.959
4	2.91	3.02	3.01	3.02	2.98	3.00	3. 00	2.93	2.97	2.96	3.00	2.96	2.978
5	2.92	3.00	3.00	2.97	3.00	2.98	3.01	3.03	2.95	2.98	3.04	2.99	2.989
6	2.95	2.98	2.99	2.95	2.89	2.99	2.99	3.02	2.96	2.95	2.99	2.89	2.963
7	3.03	2.90	2.89	3.05	2.90	3.00	2.95	3.01	2.92	3.01	2.98	2.98	2.968
8	3.02	3.02	2.79	2.99	2.95	3.01	2.88	2.89	2.94	3.00	2.85	2.95	2.941
9	2.94	2.98	2.94	2.78	3.03	2.97	2.93	2.85	2.98	2.94	3.00	3.00	2.945
10	2.88	2.79	2.95	2.95	3.01	2.78	2.91	2.86	3.00	2.97	3.06	3.01	2.931
11	2.65	2.99	2.99	3.00	2.98	2.76	2.90	2.95	3.04	2.96	2.09	3.02	2.861
12	2.72	2.93	3.00	3.01	2.94	2.84	2.89	2.98	2.96	3.00	3.00	2.99	2.938
13	2.65	3.03	3.02	2.95	3.04	2.85	2.78	2.97	2.96	3.01	2.98	2.87	2.926
14	2.96	3.01	3.01	2.79	2.99	2.90	2.76	2.95	2.98	2.99	2.99	2.96	2.941
Mean of Months	2.914	2.969	2.965	2.944	2.977	2.933	2.906	2.955	2.979	2.973	2.917	2.979	

Table (3) shows the monthly average values of protein content in raw milk from several measuring stations throughout the year. These data indicate a slight variation in protein content between different months, with values ranging between 2.79% and 3.10%. The quality and quantity of feed play a significant role in determining the protein content in milk, as feeds rich in protein (such as alfalfa and soybeans) increase the protein content of milk. Some cow breeds, such as Holstein, produce milk with a lower protein concentration than others, such as Jersey. [26] The health status of cows and the production level affect the protein content, as cows with high production tend to produce milk with a lower protein content due to the dilution of nutrients in the milk. The lowest protein content was observed in February and April at some stations, while the highest was recorded in January, May, and August. The monthly variations may reflect the cows' response to seasonal factors, especially nutrition and heat stress. The overall mean for the measurement stations ranged between 2.931% and 2.994%, indicating a relative stability in protein levels.

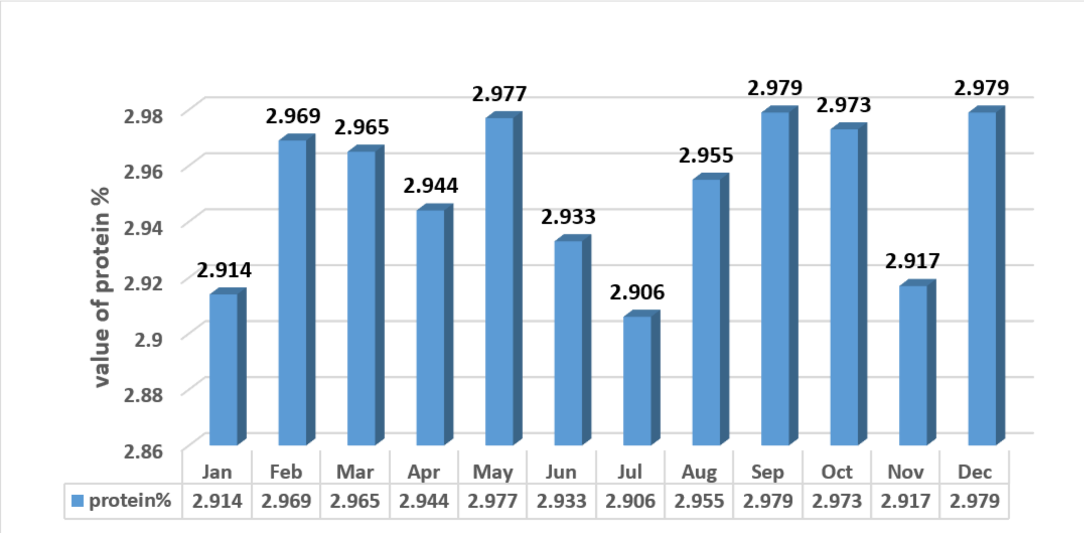

Figure 5: Shows the change in protein content in raw milk during the months of the year. Based on the data, the following trends can be observed

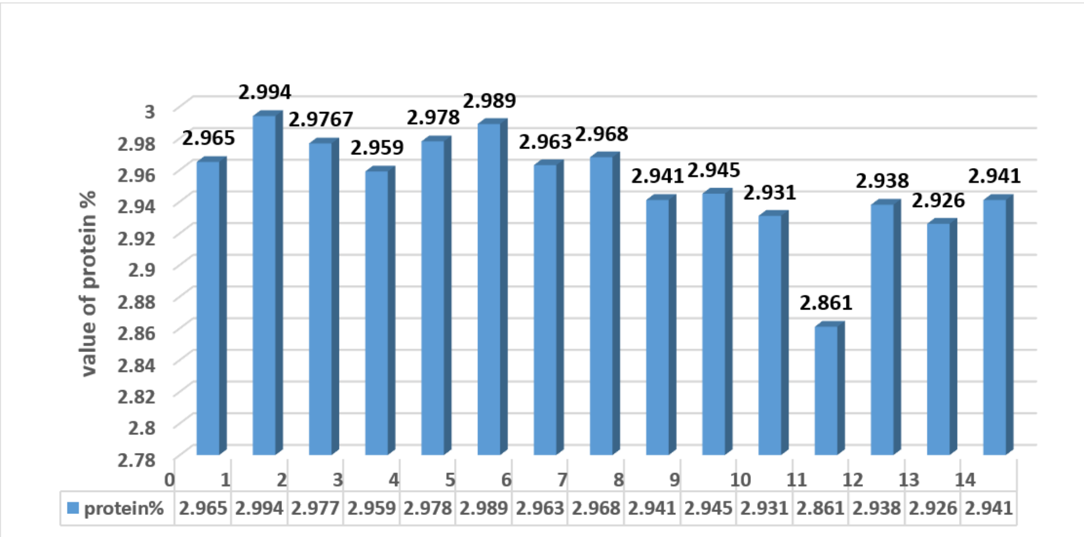

Figure 6: Shows the change in protein content in raw milk during the station of the year. Based on the data, the following trends can be observed

Table 4: Mean value of fat in raw milk samples

Months Station	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Mean of Stations
0	2.98	3.54	3.32	3.00	2.99	3.23	3.00	2.98	3.00	2.99	3.17	3.00	3.100
1.	3.00	3.34	3.04	2.98	2.47	3.20	2.98	2.93	3.24	2.86	2.95	3.02	3.001
2.	3.08	3.98	2.85	2.76	3.00	2.97	2.97	2.94	3.12	2.98	2.48	3.06	3.016
3.	3.01	3.02	3.00	2.95	3.10	2.94	2.95	2.49	2.94	2.95	2.69	2.99	2.919

Months Station	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Mean of Stations
4.	2.99	3.01	2.94	2.95	3.00	2.94	2.57	3.03	2.89	3.00	2.49	2.98	2.899
5.	2.85	2.99	4.00	3.02	2.98	2.49	2.97	3.49	2.49	3.40	2.59	2.97	3.020
6.	3.04	2.89	2.94	3.09	2.79	2.79	3.56	2.97	2.78	3.04	3.00	2.69	2.965
7.	3.04	4.01	2.97	3.11	2.96	2.88	3.96	2.48	2.98	2.54	3.28	3.00	3.101
8.	2.58	3.99	3.05	3.12	3.17	3.88	2.91	2.74	2.69	2.43	3.05	3.05	3.055
9.	3.2	3.87	2.90	3.78	3.28	3.96	2.89	3.00	3.86	2.85	3.06	2.49	3.262
10.	3.89	3.98	3.00	2.89	3.20	3.67	3.45	2.94	3.54	2.98	2.98	2.95	3.289
11.	3.99	2.99	3.01	2.95	2.94	3.56	3.65	2.04	4.00	2.94	2.59	2.99	3.137
12.	3.96	2.78	3.00	2.99	2.94	3.97	3.27	2.09	3.45	2.94	2.49	4.00	3.157
13.	4.0	3.00	2.98	2.78	2.99	3.96	2.95	2.95	3.23	2.49	3.09	2.39	3.068
14.	3.90	3.29	2.78	2.66	2.48	4.00	2.59	3.12	2.99	2.04	3.05	3.00	2.992
Mean of Months	3.301	3.379	3.052	3.002	2.953	3.363	3.111	2.813	3.147	2.829	2.864	2.972	

Table (4) presents the average fat percentages in raw milk samples measured across 14 monitoring stations throughout the year. It also displays the average values for each station and each month, allowing for an analysis of seasonal and spatial variations in fat content.

Fat percentages fluctuate throughout the months, ranging from 2.813% in August (the lowest value) to 3.379% in February (the highest value). Higher values were recorded during the colder months (January–March), while lower values appeared during the hotter months (June–August). The average fat content ranges from 2.899% at Station 5 to 3.157% at Station 13. Feed rich in fibre and fat can enhance the fat content of milk (Jenkin). The text discusses the affinity of heavy metals for milk fat globules due to their lipophilic nature. The text suggests that we should expect full-fat milk to contain more heavy metals, like lead (Pb). At the same time, cadmium (Cd) is lipophobic and has both lipophilic and hydrophilic properties. However, variations in detection methods and fat removal procedures may influence the measured concentrations of heavy metals in milk. Interestingly, despite the lipophilic nature of lead, it is also ionic and may dissolve more in the aqueous phase of milk rather than in fat. This phenomenon could explain why some studies, such as that of [27], found higher levels of lead in low-fat milk compared to full-fat milk. However, conflicting results exist, as some studies have reported different findings [28]. Some breeds, such as Holstein, produce milk with lower fat than breeds like Guernsey or Jersey [29]. Heat stress, health, and milking methods can influence milk fat content. Adjusting feeds or nutritional strategies during the summer may be necessary to compensate for the drop in fat content. Modifications in fat content may be required to maintain the final products. Some prefer high-fat milk, which affects pricing and marketing based on seasonal variations.

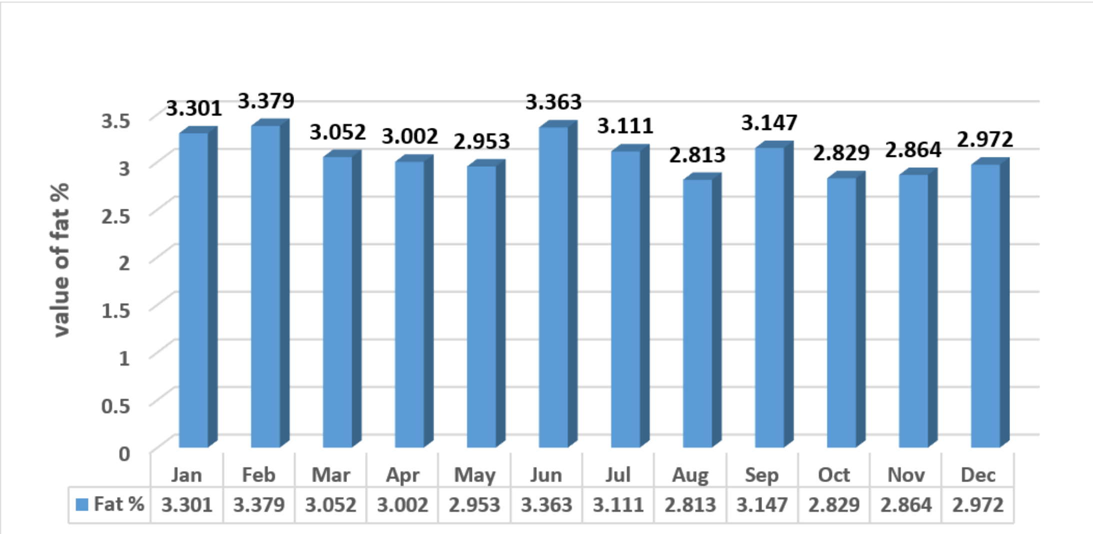

Figure 7: Shows the monthly changes in the percentage of fat in raw milk throughout the year. By analyzing the data, several important trends can be observed:

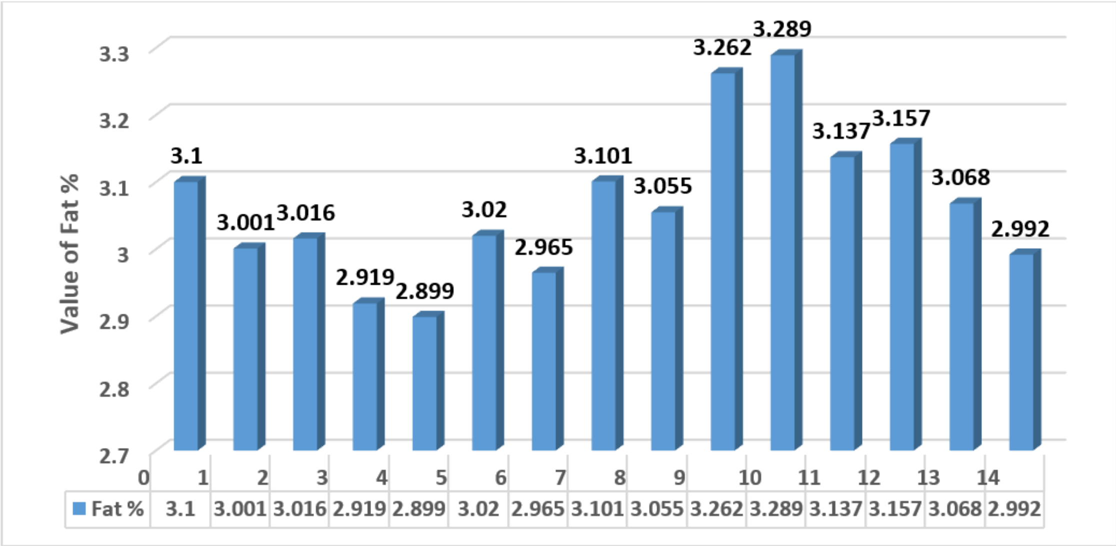

Figure 8: Shows the Stations changes in the percentage of fat in raw milk throughout the year. By analyzing the data, several important trends can be observed:

CONCLUSIONS

The study found that the concentrations of heavy metals, particularly lead (Pb) and cadmium (Cd), in milk and dairy products varied among the samples, with some exceeding the internationally established safety limits. These toxic metals present potential health hazards, especially to sensitive populations like children and pregnant women, due to their cumulative and harmful effects. The results highlight the importance of consistent monitoring, enforcing stricter regulations, and adopting better agricultural and production practices to reduce contamination and ensure the safety of milk and dairy products for consumers. The study reveals varying concentrations of heavy metals in different types of milk, with relatively high levels recorded in sheep and goat milk, especially for lead. These results emphasize the need for strict control of milk production to ensure consumer safety. It might be possible to attain better stability by enhancing feed quality during the months with low protein content. To ensure milk quality throughout the year, we should study the influence of environmental and management factors more deeply. The study reveals varying concentrations of heavy metals in different types of milk, with relatively high levels recorded in sheep and goat milk, especially for lead. These results emphasize the need for strict control of milk production to ensure consumer safety. Farmers must ensure that feed and water are free from metallic contaminants, conduct early detection, and take necessary measures. It is advisable to avoid raising livestock near industrial areas and highways and to adhere to the standards of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) to ensure food safety. These findings highlight the impact of environmental and managerial factors on raw milk quality. Farmers and producers should consider these variations to ensure product consistency and maximize economic returns.

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