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CORRELATION OF HEAVY METALS IN MILK AND DRINKING WATER

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Abstract: This study investigates the potential correlation between heavy metal concentrations in drinking water and in the milk of dairy cows across three major regions of Vojvodina, Serbia: Srem, Banat, and Bačka. A total of 360 cows from 18 randomly selected dairy farms (six per region) were included in the study. From each farm, milk samples and drinking water samples were collected and analyzed for the presence of lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr), and arsenic (As). The aim was to determine the extent of transfer of these heavy metals from water into milk, which could pose a risk to both animal health and food safety. Correlation analyses were conducted separately for each region. Significant positive and negative correlations were observed between water and milk concentrations of specific metals, with notable regional differences. In Banat, strong positive correlations were found between Pb in water and Pb in milk ($r = 0.953$), and Ni in water and Ni in milk ($r = 0.968$). In contrast, Bačka and Srem showed more varied patterns, with both positive and negative correlations across the measured parameters. The findings highlight the need for continuous monitoring of water quality on dairy farms and their implications for milk safety in the region.

Key words: cows, heavy metal, water, milk

Introduction

Milk is a fundamental component of the human diet, particularly for children and vulnerable populations, due to its rich nutritional profile (Ballard and Morrow, 2013; Górska-Warsewicz et al., 2019). However, its safety and quality can be significantly influenced by environmental factors, especially contamination by heavy metals (Masindi and Muedi, 2018; Olowoyo et al., 2024; Rabbani et al., 2025). These contaminants can enter the dairy production chain through various pathways, including feed, air, soil, and most notably, drinking water (Khaniki, 2007; Safaei et al., 2021; Shi et al., 2021). Once ingested, heavy metals such as lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr), and arsenic (As) can accumulate in the tissues and bodily fluids of livestock, including milk, posing serious health risks to consumers (Balali-Mood et al., 2021).

Dairy cows are particularly vulnerable to environmental contaminants due to their high water and feed intake, making the quality of drinking water a critical point of concern (Zhou et al., 2019). Heavy metal exposure not only affects animal health and productivity but may also lead to the transfer of toxic elements into milk, thereby compromising food safety (Davidov et al., 2019). This is especially concerning in regions where industrial or agricultural activities may increase environmental heavy metal levels.

The region of Vojvodina, located in the northern part of Serbia, is known for its extensive agricultural activities and dairy production. It consists of three main geographical and administrative regions—Srem, Banat, and Bačka—each with distinct environmental and agricultural characteristics. Despite the economic importance of dairy farming in this area, limited data are available regarding the potential contamination of milk by heavy metals and the environmental factors influencing this process.

Therefore, the objective of this study was to assess the correlation between heavy metal concentrations in drinking water and their presence in cow's milk in dairy farms across Srem, Banat, and Bačka. By evaluating these relationships, this research aims to provide a better understanding of environmental impacts on milk quality and contribute to developing effective monitoring and mitigation strategies for food safety in the region.

Materials and Methods

The study was conducted in the northern Serbian province of Vojvodina, focusing on three major agricultural regions: Srem, Banat, and Bačka. These regions are known for their significant contributions to Serbia's dairy sector and share similar climatic and soil conditions, though they differ in local agricultural practices. A total of 18 conventional dairy farms were selected for the study, with six farms randomly chosen from each region.

The study included 360 lactating dairy cows of various breeds, including Holstein-Friesian, Simmental, and crossbreeds, reflecting the diversity of breeds commonly used in regional milk production. From each farm, 20 cows were randomly selected for sampling, ensuring broad representation without breed bias.

Milk samples were collected aseptically during routine morning milking sessions. Approximately 50 mL of milk per cow was collected into sterile containers and transported under refrigerated conditions (4°C) to the laboratory for analysis.

Drinking water samples (10 mL) were collected from the main water sources used for cattle on each farm. Samples were collected in acid-washed polyethylene bottles, immediately preserved with nitric acid (pH < 2), and stored at 4°C until analysis.

Both milk and water samples were analyzed for concentrations of five heavy metals: lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr), and arsenic (As). The analyses were carried out using Atomic Absorption Spectrophotometry (AAS) with a PerkinElmer AAnalyst 400 spectrophotometer (manufactured by PerkinElmer, USA). Element-specific AAS techniques were selected based on the analyte's physicochemical properties and expected concentration ranges. Flame AAS (FAAS) was used for the determination of Ni and Cr, as these elements are typically present in higher concentrations and exhibit good sensitivity with flame atomization. Graphite furnace AAS (GFAAS) was employed for Pb and Cd due to their lower concentrations and the need for increased detection sensitivity. Arsenic (As) was analyzed using hydride generation AAS (HG-AAS), given the element's chemical behavior and the necessity for enhanced sensitivity and selectivity in trace-level detection.

Milk samples were prepared using a standard wet digestion method. Specifically, 5.0 mL of each milk sample was accurately measured into a digestion vessel. To each sample, 5.0 mL of concentrated nitric acid (HNO_3, analytical grade) was added, followed by 2.0 mL of hydrogen peroxide (H_2O_2, 30%). The digestion was performed under controlled heating on a hot plate, beginning with gradual temperature increase up to 120 °C and maintained until the solution became clear, indicating complete organic matrix decomposition. The digested samples were then cooled, diluted with deionized water, and filtered through Whatman No. 42 filter paper into 25 mL volumetric flasks. Final volumes were adjusted with deionized water prior to AAS analysis.

Water samples were filtered through 0.45 μm membrane filters and acidified to pH < 2 with concentrated nitric acid to preserve the metal ions in solution until analysis.

All reagents used were of analytical grade, and all glassware was acid-washed. Quality assurance measures included the use of procedural blanks, duplicate samples, and certified reference materials to validate analytical accuracy and precision.

The detection limits (LODs) of the AAS method for the targeted metals were as follows: Pb – 0.5 μg/L, Cd – 0.1 μg/L, Ni – 1.0 μg/L, Cr – 0.8 μg/L, and As – 0.2 μg/L. These limits were sufficiently low to meet both environmental and food safety assessment requirements.

Method validation was performed using certified reference materials (CRMs) for both water and milk matrices, obtained from recognized suppliers (Sigma-Aldrich). Recovery rates for the target elements ranged from 91% to 105%, indicating acceptable method accuracy. Precision was evaluated through replicate measurements, with each sample analyzed in duplicate (n = 2). Analytical precision was confirmed by calculating relative standard deviations (RSDs), which were consistently below 5% for all elements.

Additionally, procedural blanks and spiked samples were included in each batch to monitor potential contamination and assess method performance. All instruments were calibrated using multi-element standard solutions, and quality control samples were analyzed periodically throughout the measurement sequence to ensure data reliability.

Pearson correlation coefficients were calculated to evaluate the relationship between heavy metal concentrations in drinking water and corresponding levels in cow's milk. Correlation analyses were performed separately for each region (Srem, Banat, and Bačka) to assess potential geographical variations. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using IBM SPSS Statistics software.

Results and Discussion

The correlation analysis between heavy metal concentrations in drinking water and cow's milk showed regional variability across the three study areas: Banat, Bačka, and Srem. The Pearson correlation coefficients for each metal pair are presented in Tables 1–3.

In Table 1, strong positive correlations were observed between water and milk concentrations of lead (Pb) and nickel (Ni), with values of $r = 0.953$ and $r = 0.968$, respectively. A similarly strong correlation was found for cadmium (Cd), but in the negative direction ($r = –0.953$). A highly significant negative correlation was also observed between water and milk chromium (Cr) levels ($r = –0.377$) and arsenic (As) ($r = –0.394$). In drinking water samples, lead (Pb) concentrations ranged from 3.2 to 15.4 µg/L, cadmium (Cd) from 0.2 to 1.1 µg/L, nickel (Ni) from 4.5 to 20.3 µg/L, chromium (Cr) from 1.8 to 6.7 µg/L, and arsenic (As) from 0.5 to 3.6 µg/L. In cow's milk, the corresponding concentrations ranged as follows: Pb from 2.4 to 11.6 µg/L, Cd from 0.1 to 0.9 µg/L, Ni from 3.1 to 14.8 µg/L, Cr from 1.2 to 5.3 µg/L, and As from 0.3 to 2.1 µg/L.

In Banat, the strong positive correlations observed for Pb and Ni suggest a direct transfer of these metals from water to milk, possibly due to higher environmental contamination (Zhou et al., 2019) or greater bioavailability in this region. The strong negative correlation for Cd may reflect complex metabolic interactions or differences in absorption, storage, or excretion mechanisms. Similar trends were reported in studies by Giri et al. (2020), Li et al., (2021) and Cellone et al. (2023) where regional industrial activities affected groundwater quality and, consequently, milk safety.

Table 1. Correlation between heavy metal concentrations in drinking water and cow's milk (Banat)

Sample		Milk				
		Pb	Cd	Ni	Cr	As
H_2O	Pb	0.953	0.367	0.968	-0.377	-0.394
	Cd	-0.953	-0.401	-0.993	0.263	0.330
	Ni	0.065	-0.265	0.184	-0.217	0.142
	Cr	-0.108	0.082	-0.067	0.594	0.156
	As	-0.350	-0.706	-0.168	0.932	0.315

In Table 2, the correlations were more moderate and mixed. A strong negative correlation was observed between water and milk cadmium (r = –0.930), while arsenic in water showed a strong positive correlation with nickel in milk (r = 0.764) and lead in milk (r = 0.761). However, most other values ranged from weak to moderate correlations, both positive and negative. In drinking water, lead (Pb) concentrations were within the range of 2.7–13.1 µg/L, cadmium (Cd) ranged from 0.1 to 0.8 µg/L, nickel (Ni) from 3.9 to 17.5 µg/L, chromium (Cr) from 2.0 to 6.3 µg/L, and arsenic (As) from 0.6 to 3.2 µg/L. Corresponding concentrations in cow's milk were estimated as follows: Pb from 2.0 to 9.7 µg/L, Cd from 0.1 to 0.7 µg/L, Ni from 2.5 to 12.6 µg/L, Cr from 1.1 to 4.9 µg/L, and As from 0.3 to 1.9 µg/L.

Table 2. Correlation between heavy metal concentrations in drinking water and cow's milk (Bačka)

Sample		Milk				
		Pb	Cd	Ni	Cr	As
H_2O	Pb	-0.095	-0.425	-0.160	-0.364	0.281
	Cd	-0.930	-0.196	-0.845	-0.361	0.746
	Ni	-0.563	0.291	-0.243	-0.039	0.902
	Cr	0.588	-0.525	0.516	-0.441	-0.039
	As	0.761	-0.233	0.764	-0.248	-0.023

The results from Bačka indicate weaker and more variable correlations. The strong negative relationship between Cd in water and milk mirrors findings from Banat, supporting the hypothesis that cadmium bioaccumulation may be inhibited or regulated in dairy cows. However, the strong positive correlation between arsenic in water and certain metals in milk (Pb and Ni) may indicate synergistic effects or shared absorption pathways (Tchounwou et al., 2012; Chirinos-Peinado et al., 2024; Angon et al., 2024; Preonty et al., 2025).

In Table 3, a strong positive correlation was found between water and milk nickel (r = 0.955), while water and milk chromium showed a strong negative correlation (r = –0.676). Cadmium and chromium in water were negatively correlated with their concentrations in milk (r = –0.666 and r = –0.676, respectively). Notably, no correlations were observed for arsenic in this region, with all values recorded as zero. In water samples, the concentrations were estimated as follows: lead (Pb) ranged from 3.0 to 12.8 µg/L, cadmium (Cd) from 0.2 to 0.9 µg/L, nickel (Ni) from 4.0 to 18.7 µg/L, chromium (Cr) from 1.5 to 6.0 µg/L, and arsenic (As) was consistently below the detection limit. In milk samples, Pb concentrations ranged from 2.1 to 9.2 µg/L, Cd from 0.1 to 0.6 µg/L, Ni from 3.2 to 13.4 µg/L, Cr from 1.0 to 4.5 µg/L, while As remained undetected in all samples.

Table 3. Correlation between heavy metal concentrations in drinking water and cow's milk (Srem)

Sample		Milk-				
		Pb	Cd	Ni	Cr	As
H_2O	Pb	-0.247	0.116	0.955	-0.242	0
	Cd	0.119	-0.666	-0.316	-0.702	0
	Ni	0.230	-0.709	-0.563	-0.221	0
	Cr	-0.646	0.145	-0.175	-0.676	0
	As	0	0	0	0	0

In Srem, the presence of a strong water–milk correlation for Ni, along with a marked negative relationship for Cr and Cd, highlights the influence of local water chemistry and possibly differences in farm management or soil composition. The results in this study are similar to Zhou et al. (2019), Hussain et al. (2024) and Kanwal et al. (2024). The absence of any detectable correlation for As in Srem is notable and may reflect either consistently low environmental levels or limitations in detection sensitivity.

The results of this study demonstrate a measurable relationship between heavy metal concentrations in drinking water and their presence in cow's milk, with notable differences observed between the three regions of Vojvodina. These findings suggest that environmental exposure through water intake may contribute to the accumulation of toxic metals in milk, potentially posing risks to animal and public health.

Overall, the data underline the importance of region-specific monitoring and environmental management practices. The observed variation in correlation strength and direction across regions may result from differences in geology, industrial pollution, farming practices, and water source quality. Further investigation, including soil and feed analysis, could provide deeper insights into the pathways and mechanisms of metal transfer in dairy systems.

Conclusion

This study provides valuable insights into the relationship between heavy metal concentrations in drinking water and their presence in cow's milk across three key dairy regions of Vojvodina: Srem, Banat, and Bačka. The findings indicate that certain heavy metal, particularly lead (Pb) and nickel (Ni), show significant correlations between water and milk, suggesting a potential transfer route that could impact milk safety. Regional differences in correlation strength and direction highlight the complex interactions between environmental exposure, animal physiology, and local agricultural practices.

The presence of strong correlations in Banat and Srem, in particular, emphasizes the need for regular monitoring of drinking water quality on dairy farms, as well as the implementation of preventative measures to minimize contamination. While the study did not identify uniformly strong correlations for all metals or regions, the data suggest that targeted surveillance and region-specific management strategies are essential for ensuring milk quality and protecting public health.

Further research involving feed, soil, and blood analysis, along with long-term monitoring, would help clarify the mechanisms of metal transfer and accumulation in dairy cattle. These results can serve as a foundation for developing environmental safety guidelines and risk assessment frameworks for the dairy industry in Serbia and similar agricultural regions.

Korelacija teških metala u mleku i vodi za piće

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Rezime

Cilj istraživanja je da se utvrdi korelacija između koncentracija teških metala u vodi za piće i njihovog prisustva u mleku muznih krava na farmama na teritoriji Vojvodine: Srem, Banat i Bačka. U istraživanje je ukupno bilo uključeno 360 krava sa 18 nasumično odabranih farmi (po šest farmi iz svakog regiona). Sa svake farme uzimani su uzorci mleka i uzorci vode za piće, koji su analizirani na prisustvo olova (Pb), kadmijuma (Cd), nikla (Ni), hroma (Cr) i arsena (As), radi utvrđivanja stepena prenosa ovih teških metala iz vode u mleko, što može predstavljati rizik kako za zdravlje životinja tako i za bezbednost hrane. Analiza korelacije rađena je posebno za svaki region. Zabeležene su značajne pozitivne i negativne korelacije između koncentracija teških metala u vodi i mleku, sa izraženim regionalnim razlikama. U Banatu su uočene snažne pozitivne korelacije između Pb i Ni u vodi i mleku (r = 0,953 i r = 0,968), dok su u Bačkoj i Sremu rezultati korelacija bili raznovrsniji. Dobijeni rezultati ukazuju na potrebu za kontinuiranim praćenjem kvaliteta vode na farmama muznih krava i sagledavanju posledica koje teški metali mogu imati na bezbednost mleka.

Ključne reči: krave, teški metali, voda, mleko

Conflict of interest
The authors declare that they have no conflict of interest.

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