

DOI 10.7251/VETJSR074D
Original Scientific Paper

UDK 636.2.085:616-008:636.2

IMPACT OF HEAVY METALS IN WATER ON METAL CONCENTRATIONS IN THE BLOOD AND MILK OF HIGH-YIELDING DAIRY COWS

Ivana DAVIDOV*, Dragica STOJANOVIĆ, Miodrag RADINOVIĆ, Jovan STANOJEVIĆ, Mihajlo ERDELJAN, Annamaria GALFI VUKOMANOVIĆ, Nikola DAVIDOV, Milica VRANEŠEVIĆ

University of Novi Sad, Faculty of Agriculture, Novi Sad, Republic of Serbia

*Corresponding author: Ivana Davidov, ivana.davidov@polj.edu.rs

Abstract: The aim of this study was to investigate the presence of heavy metals in water and their impact on metal concentrations in the blood and milk of high-yielding dairy cows. The study was conducted on six farms in Vojvodina, where 20 cows were randomly selected from each farm. Blood and milk samples were collected from the cows, as well as water samples from the farms, originating from the public water supply system, which is subject to regular sanitary monitoring in accordance with current regulations. In all samples, the levels of five heavy metals were analyzed: lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr), and arsenic (As).

The results of descriptive statistics showed that metal concentrations in water were relatively low, while concentrations in cow milk and blood varied, with the highest levels recorded for lead and nickel. Simple linear regression was used to examine correlations between metal concentrations in water and their levels in milk. Although lead concentrations showed a high correlation ($r = 0.955$), the statistical significance of this relationship was marginal ($p = 0.088$). Other metals, such as cadmium, nickel, chromium, and arsenic, did not show a significant correlation between concentrations in water and milk.

These results suggest that lead may influence its concentration in milk, whereas other heavy metals do not show a significant association. Further research is needed to clarify the potential long-term effects of heavy metal exposure on cow health and milk safety.

Keywords: heavy metals, water, blood, milk, cow

INTRODUCTION

Environmental pollution with heavy metals represents a serious global problem that directly affects ecosystems and the health of animals and humans. Heavy metals such as lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr), and arsenic (As) are present in various segments of the environment, including water resources, air, soil, and food. Due to their specific characteristics, such as long persistence, bioaccumulation, and toxicity, these metals can have serious effects on biological systems, including

domestic animals (Mitra et al., 2022; Pujari and Kapoor, 2021; Tchounwou et al., 2012).

On high-yielding dairy cow farms, which represent an important source of milk production, contamination with heavy metals may occur through various pathways, most commonly via contaminated feed, water, or direct contact with a polluted environment. Given that cows regularly consume water and that milk is a widely consumed product, investigating the presence of heavy metals in this context becomes crucial for both animal health and human safety. Water contamination can lead to increased concentrations of heavy metals in the bodies of cows (Tahir and Alkheraije, 2023), which in turn may negatively affect milk quality and the safety of milk and dairy products.

Although numerous studies (Mitra et al., 2022; Awasthi et al., 2022; Katare et al., 2021; Pujari and Kapoor, 2021; Davidov et al., 2019; Adeleye et al., 2016; Sankhla et al., 2016; Tchounwou et al., 2012) have demonstrated the presence of heavy metals in different components of the ecosystem, very few studies have focused on the specific impact of heavy metals in water on their concentrations in the blood and milk of high-yielding dairy cows. Moreover, it is still not fully understood which metals have the greatest impact on cow health and milk quality, nor how contamination of water resources may directly affect the metabolism and bioaccumulation of these metals in cows.

Due to these gaps in the literature, it is necessary to conduct studies that will provide clearer insight into the relationship between heavy metal contamination of water and metal concentrations in the milk and blood of animals.

The presence of heavy metals in water can affect animal organs through various biological processes (Sankhla et al., 2016), including absorption through the digestive system, accumulation in tissues, and excretion via milk (Mitra et al., 2022; Jaishankar et al., 2014; Licata et al., 2004). Because of their bioaccumulative nature, heavy metals can be stored in vital organs such as the liver, kidneys, and lungs (Hossini et al., 2022; Witkowska et al., 2021), which may impair the overall health of cows (Tahir and Alkheraije, 2023). In farm conditions, the impact of heavy metals in water can be particularly concerning, as many farms use water sources with potential heavy metal contamination. This water becomes a key factor for heavy metal intake in cows, leading to their accumulation in milk.

Despite the well-established effects of heavy metals on cow health, there is very limited information on how the presence of metals in water specifically influences metal levels in milk. Understanding this dynamic is important for minimizing risks to human health, since milk as a food product may be directly contaminated, potentially leading to health problems in consumers. Contamination of milk with heavy metals can also have negative effects on animal fertility and health (Hossini et al., 2022; Wrzecińska et al., 2021), reducing milk production and resulting in economic consequences for farmers. Therefore, the aim of this study is to examine the impact of heavy metals in water on metal concentrations in the blood and milk of high-yielding dairy cows. Although previous studies indicate the importance of presence of heavy metals in the

environment, research linking metal levels in water with their concentrations in the milk and blood of high-yielding dairy cows represents a novel contribution. Thus, this study may provide insight into the current situation on farms and enable the formulation of recommendations for reducing contamination and improving food safety.

MATERIALS AND METHODS

The study was conducted during the summer period on six farms located in the territory of the Autonomous Province of Vojvodina, Republic of Serbia. Twenty cows were randomly selected from each farm, resulting in a total of 120 cows included in the study. The cows were in good health condition, aged between two and five years, in good body condition, and in different stages of lactation, while being maintained under a standard feeding regime for high-yielding dairy cows.

Blood, milk, and water samples were collected from all six farms. From each cow, 5 mL of blood was collected from the coccygeal vein, and 10 mL of milk was obtained during the first morning milking. Prior to blood sampling, the venipuncture site was disinfected with alcohol, and blood was collected using a sterile needle. Immediately before milk sampling, the udders were washed with warm water and dried with a clean, dry cloth. In addition, 10 mL of water was collected from each source used for livestock watering to assess the quality of the water consumed by the cows. The cows were supplied with water from the local water supply system, which is used for both livestock and household purposes. According to farm managers, the water originated from the public water supply system and was subject to regular sanitary monitoring in accordance with current regulations. Detailed data on water quality control results were not available; however, it was assumed that the water met standards for agricultural use and did not contain heavy metals at concentrations exceeding permissible limits. All blood, milk, and water samples were placed in sterile containers, labeled, and stored under refrigeration until transported to the laboratory for analysis.

In the laboratory, all samples were analyzed for the presence of heavy metals, including arsenic, lead, cadmium, nickel, and chromium. Metal concentrations were determined using atomic absorption spectrometry (AAS), a method that provides high precision for the detection and quantitative determination of heavy metal concentrations. Water samples were analyzed directly, while milk and blood samples were subjected to a digestion process prior to analysis in order to release metal particles from their matrices. Instrument calibration was performed using standard solutions with known metal concentrations, ensuring accurate and precise analytical results. For quality control purposes, blank samples and samples with known metal concentrations were also analyzed, and results were verified using internal standards. All analyses were conducted according to standard procedures to ensure data reliability.

Statistical analysis was performed using standard methods of descriptive and analytical statistics. Mean values, minimum and maximum values, and standard deviations were used to describe the results. Pearson correlation analysis was applied to assess statistical significance, with results considered statistically significant at p-values less

than 0.05. A statistical software package, such as SPSS 26.0, was used for data processing and analysis. Additionally, simple linear regression analysis was applied to examine the relationship between heavy metal concentrations in water and their concentrations in milk and blood.

All procedures involving blood and milk sampling were conducted in accordance with ethical guidelines for animal research, with prior consent obtained from the animal owners. The study did not cause any adverse effects on animal health and was carried out in compliance with the animal welfare regulations of the Republic of Serbia

RESULTS

By analyzing samples of drinking water, cow blood, and cow milk collected from six farms in Vojvodina, the obtained descriptive statistical values are presented in Table 1. This table contains the results of the analysis of five heavy metals in three different sample types: drinking water, cow blood, and cow milk. For each metal, mean concentrations (mg/L) and standard deviations (SD) were analyzed, providing an overview of metal concentrations and their variability in the samples.

Table 1. Descriptive statistical results for heavy metals in drinking water, cow blood, and cow milk

Metals in milk	mg/L ± Sd	Metals in water	mg/L ± Sd	Metals in blood	µg/L ± Sd
Pb	0.206 ± 0.183	Pb	0.012 ± 0.008	Pb	0.018 ± 0.021
Cd	0.006 ± 0.046	Cd	0.000 ± 0.000	Cd	0.011 ± 0.017
Ni	0.398 ± 0.578	Ni	0.053 ± 0.010	Ni	0.022 ± 0.017
Cr	0.021 ± 0.022	Cr	0.165 ± 0.004	Cr	0.016 ± 0.012
As	0.067 ± 0.054	As	0.028 ± 0.026	As	0.042 ± 0.030

For lead (Pb), the mean concentration in milk was 0.206 mg/L with a standard deviation of 0.183, indicating a relatively low concentration with some variability. In drinking water, the mean lead concentration was 0.012 mg/L with a standard deviation of 0.008, suggesting a very low and stable concentration. The mean lead concentration in blood was 0.018 µg/L with a standard deviation of 0.021, indicating a low concentration with greater variability.

For cadmium (Cd), the mean concentration in milk was 0.006 mg/L, with a high standard deviation of 0.046, indicating a very low concentration accompanied by high variability. Cadmium was not detected in drinking water (0.000 ± 0.000), while the

mean concentration in blood was 0.011 $\mu\text{g/L}$ with a standard deviation of 0.017, indicating a low concentration with low variability.

The mean nickel (Ni) concentration in milk was 0.398 mg/L, with a high standard deviation of 0.578, indicating a relatively high concentration and substantial variability. In drinking water, nickel concentration was very low (0.053 mg/L with a standard deviation of 0.010), and in blood, nickel concentration was also low, with a mean value of 0.022 $\mu\text{g/L}$ and a standard deviation of 0.017.

Chromium (Cr) showed a very low concentration in milk (0.021 mg/L with a standard deviation of 0.022), while a slightly higher concentration was observed in drinking water (0.165 mg/L with a standard deviation of 0.004). In blood, chromium was also present at low levels (0.016 $\mu\text{g/L}$ with a standard deviation of 0.012).

Arsenic (As) had a mean concentration in milk of 0.067 mg/L with a standard deviation of 0.054, indicating a low concentration with some variability. In drinking water, arsenic was present at low levels (0.028 mg/L with a standard deviation of 0.026), while the mean concentration in blood was 0.042 $\mu\text{g/L}$ with a standard deviation of 0.030

Table 2 presents the results of the regression analysis examining the effect of heavy metal concentrations in drinking water on the presence of these metals in cow milk. The results are shown in three columns: correlation coefficient (r), F-statistic (F), and p-value (p). The correlation coefficient (r) indicates the strength and direction of the relationship between metal concentrations in water and their concentrations in milk. Values close to 1 or -1 indicate a strong correlation, whereas values close to 0 indicate a weak or no correlation. The F-statistic tests the overall statistical significance of the regression model, while the p-value indicates whether the observed relationship is statistically significant.

Table 2. Results of the regression analysis of the effect of heavy metals in drinking water on the presence of heavy metals in cow milk

Metal	r	F	p
Pb	0.955	10.333	0.088
Cd	0.411	0.203	0.831
Ni	0.506	0.344	0.744
Cr	0.596	0.552	0.644
As	0.908	4.685	0.176

The results for lead (Pb) indicate a strong positive correlation ($r = 0.955$) between its concentration in drinking water and its presence in milk. However, the F-statistic was 10.333, and the p-value was 0.088, indicating that the correlation was not statistically significant at the 5% level, although it was close to significance.

For cadmium (Cd), the correlation coefficient was 0.411, indicating a weak positive correlation. The F-statistic was very low (0.203), and the p-value was high (0.831), demonstrating that this relationship was not statistically significant.

Nickel (Ni) showed a correlation coefficient of 0.506, suggesting a weak to moderate positive correlation; however, the F-statistic (0.344) and p-value (0.744) indicated that this correlation was not statistically significant.

For chromium (Cr), the correlation coefficient was 0.596, indicating a moderate positive correlation. Nevertheless, the low F-statistic (0.552) and the p-value (0.644) showed that this relationship was not statistically significant.

Arsenic (As) exhibited a strong positive correlation ($r = 0.908$) between concentrations in water and milk; however, the F-statistic (4.685) and p-value (0.176) indicated that the correlation was not statistically significant at the 5% level, although it may be considered significant at the 10% level.

In Abstract, lead (Pb) and arsenic (As) showed relatively strong correlations with their concentrations in milk, but none of the observed correlations were statistically significant at the 5% level. Cadmium (Cd), nickel (Ni), and chromium (Cr) did not exhibit a significant influence on their concentrations in milk, as reflected by low correlation coefficients and high p-values.

DISCUSSION

This study aimed to investigate the concentrations of heavy metals in water, milk, and blood of high-yielding cows on six farms. The results showed that Pb concentrations were highest in milk, whereas other metals, such as Cd, Ni, Cr, and As, were present at lower levels. Regression analysis indicated a strong correlation between Pb concentrations in water and milk, while other metals did not show significant associations (Cowan and Blakley, 2016).

Many researchers (Boudebouz et al., 2021; Davidov et al., 2019; Licata et al., 2004) have examined the presence of heavy metals in milk and their potential impact on human and animal health. According to Giri et al. (2020), Pb is often one of the most prevalent metals in milk, which aligns with the findings of this study. They also reported that Pb concentrations in milk may result from contamination of feed and water, which corresponds to the potential sources of contamination in the present study. Oraby et al. (2021) and Bilandžić et al. (2021) investigated Cd and Ni in milk and animal blood and observed similarly low concentrations of these metals in milk. In this study, Cd was detected at very low levels in milk (0.006 ± 0.046 mg/L), consistent with these previous findings. These metals are often not significantly accumulated in milk because their bioavailability in the cow's body is lower compared to metals such as Pb. Regarding As, the results of this study are consistent with Rana et al. (2010), who also reported low As concentrations in milk. Although As can be present in feed and water, its levels in milk often remain below detectable limits, suggesting that cows efficiently eliminate this metal. In contrast, Davidov et al. (2021) concluded that As in cow drinking water had a statistically strong positive correlation with As concentrations in blood and milk, indicating that increased As in water leads to higher As concentrations in blood and milk.

One key factor explaining the results of this study is the way heavy metals enter the cow's body. Pb, for example, tends to accumulate in milk because it can pass through the digestive system, be absorbed into the bloodstream, bind to proteins, and be transported to the mammary gland (Swarup et al., 2005). This could explain why Pb concentrations in milk were significantly higher than in water or blood in this study.

On the other hand, Cd and Ni are absorbed more slowly and tend to accumulate in bones, which may result in much lower concentrations in milk. This phenomenon is well documented in the literature (Roczniak et al., 2017).

Additionally, the relatively low metal concentrations in farm water suggest that environmental contamination levels were insufficient to cause high metal concentrations in blood or milk. Similar findings were reported by Ullah et al. (2024) on farms using water with low heavy metal content.

The results of this study highlight the potential risk of Pb accumulation in cow milk, which may pose a health hazard for humans, especially if milk is not adequately monitored. Although the concentrations of other metals were relatively low, the significance of Pb in milk, directly linked to water contamination, represents an important finding. This aligns with Mukherjee et al. (2023), who suggest that monitoring heavy metal levels in food and water is essential to ensure the safety of milk and other dairy products.

These findings are important for the dairy industry, emphasizing the need for improved quality control systems for water and feed, particularly on farms using water from natural sources that may be contaminated. They can also inform the development of guidelines for maximum allowable heavy metal concentrations in milk.

One limitation of this study is the small number of farms (six), which may limit the representativeness of the results for the entire region. Additionally, samples were collected only during the summer, which could influence metal concentration variability, as seasonal changes may affect contamination levels.

Future research should expand the number of farms and include sampling across different seasons. It should also focus on specific pollution sources, such as pesticides and industrial waste, and investigate the effects of these metals on cow health, providing further insights into potential risks for both animals and humans

CONCLUSION

The results of this study showed that Pb concentrations were highest in milk, while other metals, such as Cd, Ni, Cr, and As, were present at considerably lower levels. Furthermore, regression analysis indicated a significant correlation between Pb concentrations in water and milk, whereas the other metals did not show a similar association. These findings suggest that water is the primary source of Pb contamination in milk, while the other metals appear to have a limited impact.

These results have important implications for the dairy industry, highlighting the need for improved quality control systems for water and feed, particularly to reduce heavy metal contamination in livestock inputs. Considering the potential risks to human

health, especially through milk consumption, stricter standards for permissible heavy metal levels in milk are recommended.

Moreover, the study emphasizes the need for further research including a larger number of farms and samples across different seasons, while also considering the analysis of animal feed, to obtain more precise data on environmental variations in metal concentrations and their impact on cow health. Future studies should also investigate the effects of heavy metals on animal health to better understand the long-term consequences of exposure.

Conflict of Interest: The authors declare that they have no conflicts of interest related to this study

REFERENCES

- Adeleye A.S., Conway J.R., Garner K., Huang Y., Su Y., Keller A.A. (2016): Engineered nanomaterials for water treatment and remediation: Costs, benefits, and applicability. *Chemical Engineering Journal*, 286:640-662. DOI: 10.1016/j.cej.2015.10.105
- Awasthi G., Nagar V., Mandzhieva S., Minkina T., Sankhla M.S., Pandit P.P., Aseri V., Awasthi K.K., Rajput V.D., Bauer T. (2022): Sustainable amelioration of heavy metals in soil ecosystem: Existing developments to emerging trends. *Minerals*, 12(1):85. DOI: (MDPI): 10.3390/min12010085
- Bilandžić N., Calopek B., Sedak M., Dokić M., Gajger I.T., Murati T. (2021): Essential and potentially toxic elements in raw milk from different geographical regions of Croatia and their health risk assessment in the adult population. *Journal of Food Composition and Analysis*, 104:104152. DOI: 10.1016/j.jfca.2021.104152
- Boudebouz A., Boudalia S., Bousbia A., Habila S., Boussadia M.I., Gueroui Y. (2021): Heavy metals levels in raw cow milk and health risk assessment across the globe: A systematic review. *Science of the Total Environment*, 751:141830. DOI: 10.1016/j.scitotenv.2020.141830
- Cowan V., Blakley B. (2016): Acute lead poisoning in western Canadian cattle - A 16-year retrospective study of diagnostic case records. *Canadian Veterinary Journal*, 57(4):421-426.
- Davidov I., Stojanović D., Galfi Vukomanović A., Vranešević M., Radinović M., Erdeljan M., Lakić B. (2021): Uticaj arsena na parenhim vimena krava. *Veterinarski žurnal Republike Srpske*, 21(1-2):294-301. DOI: 10.7251/VETJEN2101286D
- Davidov I., Kovačević Z., Stojanović D., Pucarević M., Radinović M., Stojić N., Erdeljan M. (2019): Contamination of cow milk by heavy metals in Serbia. *Acta Scientiae Veterinariae*, 47(1):1682-1685. DOI: 10.22456/1679-9216.96366
- Giri A., Bharti V.K., Kalia S., Arora A., Balaje S.S., Chaurasia O.P. (2020): A review on water quality and dairy cattle health: A special emphasis on high-altitude region. *Applied Water Science*, 10:79. DOI: 10.1007/s13201-020-1160-0
-

- Hossini H., Shafie B., Niri A.D., Nazari M., Esfahlan A.J., Ahmadpour M. (2022): Comprehensive review on human health effects of chromium: insights on induced toxicity. *Environmental Science and Pollution Research*, 2022(47): 70686-70705. DOI: 10.1007/s11356-022-22705-6
- Jaishankar M., Tseten T., Anbalagan N., Mathew B.B., Beeregowda K.N. (2014): Toxicity, mechanism and health effects of some heavy metals. *Interdiscip Toxicol*, 7(2):60-72. DOI: 10.2478/intox-2014-0009
- Katara P.Y., Sankhla M.S., Singhal M., Ekta B., Jadhav K.P., Bhagyashri T.N., Bhardwaj L. (2021): Microplastics in aquatic environments: Sources, ecotoxicity, detection & remediation. *Biointerface Research in Applied Chemistry*, 12:3407-3428. DOI: 10.33263/BRIAC123.34073428
- Licata P., Trombetta D., Cristani M., Giofre F., Martino D., Calo M., Naccari F. (2004): Levels of “toxic” and “essential” metals in samples of bovine milk from various dairy farms in Calabria, Italy. *Environment International*, 30(1):1-6. DOI: 10.1016/S0160-4120(03)00139-9
- Mitra S., Chakraborty A.J., Tareq A.M., Emran T.B., Nainu F., Khusro A., Idris A.M., Khandaker M.U., Osman H., Alhumaydhi F.A., Simal-Gandara J. (2022): Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. *Journal of King Saud University-Science*, 34(3):101865. DOI: 10.1016/j.jksus.2022.101865
- Mukherjee A.G., Renu K., Gopalakrishnan A.V., Veeraraghavan V.P., Vinayagam S., Paz-Montelongo S., Dey A., Vellingiri B., George A., Madhyastha H., Ganesan R. (2023): Heavy metal and metalloid contamination in food and emerging technologies for its detection. *Sustainability*, 15(2):1195. DOI: 10.3390/su15021195
- Oraby M.I., Baraka T.A., Rakha G.H. (2021): Impact of cadmium intoxication on health status, rumen and blood constituents in Egyptian Ossimi sheep. *International Journal of Veterinary Science*, 10:102-106. DOI: 10.47278/journal.ijvs/2021.040
- Pujari M., Kapoor D. (2021): 1- Heavy metals in the ecosystem: Sources and their effects. *Heavy Metals in the Environment*, 2021:1-7. DOI: 10.1016/B978-0-12-821656-9.00001-8
- Rana T., Bera A.K., Das S., Bhattacharya D., Bandyopadhyay S., Pan D. (2010): Effect of chronic intake of arsenic-contaminated water on blood oxidative stress indices in cattle in an arsenic-affected zone. *Ecotoxicology and Environmental Safety*, 73:1327-1332. DOI: 10.1016/j.ecoenv.2010.07.019
- Roczniak W., Brodziak-Dopierała B., Cipora E., Jakóbi-Kolon A., Kluczka J., Babuška-Roczniak M. (2017): Factors that affect the content of cadmium, nickel, copper and zinc in tissues of the knee joint. *Biological Trace Element Research*, 178(2):201-209. DOI: 10.1007/s12011-017-0934-5
- Sankhla M.S., Kumari M., Nandan M., Kumar R., Agrawal P. (2016): Heavy metals contamination in water and their hazardous effect on human health- A review.
-

- International Journal of Current Microbiology and Applied Sciences*, 5:759-766. DOI: 10.20546/ijcmas.2016.510.082
- Swarup D., Patra R.C., Naresh R., Kumar P., Shekhar P. (2005): Blood lead levels in lactating cows reared around polluted localities; transfer of lead into milk. *Science of the Total Environment*, 347:106-110. DOI: 10.1016/j.scitotenv.2004.12.055
- Tahir I., Alkheraije K.A. (2023): A review of important heavy metals toxicity with special emphasis on nephrotoxicity and its management in cattle. *Frontiers in Veterinary Science*, DOI: 10.3389/fvets.2023.1149720
- Tchounwou P.B., Yedjou C.G., Patlolla A.K., Sutton D.J. (2012): Heavy metal toxicity and the environment. *Experientia Supplementum*, 101:133-164. DOI: 10.1007/978-3-7643-8340-4_6
- Ullah W., Ahmad K., Rehman G., Ullah K., Kebaili I., Majeed A., Subhanullah M., Rawan B., Hussain S. (2024): Heavy metal impacts on antioxidants in cow blood from wastewater-irrigated areas. *Scientific Reports*, 2024:16918. DOI: 10.1038/s41598-024-67792-2
- Witkowska D., Słowik J., Chilicka K. (2021): Heavy metals and human health: Possible exposure pathways and the competition for protein binding sites. *Molecules*, 26(19):6060. DOI: 10.3390/molecules26196060
- Wrzecińska M., Kowalczyk A., Cwynar P., Czerniawska-Piątkowska E. (2021): Disorders of the reproductive health of cattle as a response to exposure to toxic metals. *Biology (Basel)*, 10(9):882. DOI: 10.3390/biology10090882
-