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Meat Technology — Special Issue 66/3

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 664.9

ID: 179596553

https://doi.org/10.18485/meattech.2025.66.3.87

Original Scientific Paper

Cadmium bioaccumulation in the liver and muscle tissue of mallards (*Anas platyrhynchos*) as bioindicators of environmental pollution in Serbia

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A R T I C L E I N F O

Keywords:
Cadmium
Mallard
Liver
Muscle
Biomonitoring

A B S T R A C T

The aim of this study was to assess the level of cadmium (Cd) contamination in the liver and muscle tissue of mallards (*Anas platyrhynchos*) from three different locations in Serbia: Belegiš, Pločica, and the Vršački ritovi. A total of 55 samples were analyzed, collected during 2024. Cadmium concentrations were determined using the ICP-MS method following microwave digestion of the samples. The highest mean concentration in the liver was recorded at the Belegiš site (0.643 mg kg^{-1}), while the highest average value in muscle tissue was measured in Pločica (0.021 mg kg^{-1}). Although no statistically significant differences were found between locations, 40.7% of liver samples exceeded the reference value of 0.5 mg kg^{-1} established for poultry, indicating a potential risk to human health. This study highlights the importance of wild birds as bioindicators of heavy metal pollution and the need for continued monitoring of cadmium presence in wildlife.

1. Introduction

Toxic metals such as cadmium (Cd) are naturally present in the Earth's crust; however, elevated levels in the environment are largely a consequence of intensive industrial activities over the past centuries, which have led to significant pollution of air, water, and soil (*Durkalec et al.,* 2015*; Aloupi et al.,* 2017). Cadmium belongs to group XII of the periodic table. It is a soft, silvery-white metal with physicochemical properties similar to those of zinc and mercury. It is corrosion-resistant, insoluble in water, and nonflammable, which makes it widely used as a protective coating in industrial applications (*Genchi et al.,* 2020). Naturally, cadmium enters the environment primarily through volcanic eruptions; however, natural sources account for only about 10% of the total cadmium emissions. The majority of cadmium pollution originates from anthropogenic activities, particularly mining, metallurgy, and the combustion of coal in the energy sector. It is also widely used in the production of nickel-cadmium batteries, pigments, fluorescent coatings, solders, alloys, cadmium rods, plastic stabilizers, and fireworks (*Bombik et al.,* 2023).

Cadmium released into the environment can persist for decades. It enters the food chain through

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Paper received August 18th 2025. Paper accepted August 27th 2025.
The paper was presented at the 63rd International Meat Industry Conference "Food for Thought: Innovations in Food and Nutrition" – Zlatibor, October 05th-08th 2025.
Published by Institute of Meat Hygiene and Technology – Belgrade, Serbia.

plants that absorb it from the soil (*Govind & Madhuri,* 2014). The main routes of human and animal exposure are contaminated food, water, and tobacco smoke (*Genchi et al.,* 2020). Waterfowl are particularly vulnerable to metal exposure due to the incidental ingestion of soil and sediment during feeding, especially in heavily polluted areas (*Aloupi et al.,* 2017). Studies have shown that cadmium exposure in birds negatively affects their physiological condition, reduces reproductive capacity, impairs egg quality, and causes developmental disorders during embryogenesis (*Bombik et al.,* 2023).

Cadmium is a toxic heavy metal with no known physiological role in the body. It induces oxidative stress by increasing the production of reactive oxygen species (ROS), which leads to damage of DNA, proteins, and membrane phospholipids, thereby impairing mitochondrial function and reducing ATP synthesis. The key mechanisms of its toxicity include interference with antioxidant enzymes, inhibition of DNA repair, epigenetic modifications, and the activation of apoptosis via the mitochondrial pathway. Chronic exposure to cadmium has been associated with the development of lung, breast, prostate, pancreatic, kidney, and other cancers, as well as with renal, cardiovascular, reproductive, and neurological disorders (*Genchi et al.,* 2020).

Game and wild birds, as free-living species, independently select their diet and are an integral part of the soil-plant-animal food chain (*Nikolić et al.,* 2017). The level of cadmium contamination in wild animals is often used as an indicator in ecological monitoring of cadmium poisoning (*Gasparik et al.,* 2010). Based on these facts, the mallard was used in this study to assess cadmium contamination at three sites in Serbia: Belegiš (Srem District), Pločica (South Banat District), and the Vršački ritovi (South Banat District). These locations were selected to investigate the effects of contamination on wild birds as part of an ecological cadmium pollution monitoring program.

2. Materials and methods

Cadmium levels were measured in liver and muscle tissues samples of mallards at three different locations: Belegiš (Srem District) and Pločica (South Banat District), along the course of the Danube River, and in a fishpond near the Vršački ritovi during the 2024 calendar year. The total number of analyzed samples was 55, including both liver and muscle tissues of mallards. A total of 23 samples were collected from the Belegiš site (12 muscle tissue samples and 11 liver samples), 12 samples from the Pločica site (6 liver and 6 muscle samples), and 20 samples from the Vršački ritovi site (10 liver and 10 muscle tissue samples).

The samples were stored at -18 °C until the time of analysis. One day prior to laboratory processing, the frozen samples were gradually thawed at 4 °C and subsequently homogenized. Approximately 0.3 g of tissue (± 0.001 g) was weighed for each sample and transferred into a Teflon vessel of a microwave digestion system. Nitric acid (67% Trace Metal Grade, Fisher Scientific, Bishop, UK) and deionized water (0.063 µS) obtained from a water purification system (Purelab DV35, ELGA, Buckinghamshire, UK), were added to the sample in quantities of 5 mL each. The microwave digestion system (MARS 6, CEM Corporation, Matthews, NC, USA) was programmed as follows: 5 min from initial temperature to 180°C, hold at 180°C for another 10 min, cooling and venting for 20 min. Digested samples were quantitatively transferred into 100 mL polypropylene volumetric flasks and diluted with deionized water (0.063 µS/cm).

The determination of the ^{111}Cd isotope was performed using inductively coupled plasma mass spectrometry (ICP-MS) on an iCap Q instrument (Thermo Scientific, Bremen, Germany), equipped with a collision cell and operated in kinetic energy discrimination (KED) mode. Quantitative analysis was based on a five-point calibration curve, including a zero point. A multielement internal standard (^{6}Li and ^{45}Sc at a concentration of 10 ng ml^{-1}; ^{71}Ga, ^{89}Y, and ^{209}Bi at a concentration of 2 ng mL^{-1}) was introduced via an additional peristaltic pump line to ensure measurement accuracy. Each sample was measured in duplicate, and the mean value was used, corrected for internal standard response factors. The quality of the analytical procedure was verified by the analysis of certified reference material (NIST 1577c – bovine liver, Gaithersburg, MD, USA), which was prepared in the same manner as the samples, using microwave digestion. Repeated measurements of the reference material yielded results within the range of certified values.

2.1 Statistical analysis

Statistical analysis was performed using the Minitab 17 software package. Descriptive statistics were conducted for each location to determine basic distribution parameters, including mean, standard deviation and range. To determine statistically significant differences between locations for muscle tissue and liver samples, a one-way analysis of variance (ANOVA) was applied.

3. Results and discussion

Tables 1 and 2 present the results of descriptive statistics for cadmium concentrations in liver and muscle tissue samples of mallards from different locations. The tables include arithmetic means, ranges (min–max), standard deviations, and the number of analyzed samples (n) for each sampling site.

As can be seen from Tables 1 and 2, the concentration of cadmium is significantly higher in the liver. This can be explained by the fact that the liver intensively produces the protein metallothionein to which cadmium binds (*Binkowski & Sawicka-Kapusta, 2015)*. Mean cadmium concentrations in the liver of mallards varied depending on the sampling site. The highest mean value was recorded at the Belegiš location (0.643 ± 0.566 mg kg^{-1}), while slightly lower values were observed at Pločica (0.583 ± 0.423 mg kg^{-1}) and the Vršački ritovi (0.496 ± 0.337 mg kg^{-1}), and the range of liver cadmium concentrations spanned from 0.098 to 2.134 mg kg^{-1}. In contrast, cadmium concentrations in muscle tissue were significantly lower compared to those in the liver. The highest mean value was recorded in samples from the Pločica site (0.021 ± 0.007 mg kg^{-1}), while slightly lower concentrations were observed in Belegiš (0.017 ± 0.016 mg kg^{-1}) and Vršački ritovi (0.008 ± 0.006 mg kg^{-1}). The cadmium concentration ranged in muscle tissue was from 0.002 to 0.071 mg kg^{-1}.

The results of the one-way ANOVA test indicated no statistically significant difference in cadmium concentrations in the liver of mallards among the studied locations ($p = 0.772$). In the case of muscle tissue, although the p-value of 0.077 suggested a possible trend, the difference between sites did not reach statistical significance. Nevertheless, descriptive statistics showed that the mean cadmium concentration was lowest in samples from the Vršački ritovi (0.008 mg kg^{-1}), while higher values were recorded at the Belegiš (0.017 mg kg^{-1}) and Pločica (0.021 mg kg^{-1}) sites. This difference in mean values, although not confirmed by statistical analysis, may indicate the influence of local pollution sources and justifies the need for an increased sample size in future studies.

A comparative analysis with previous studies conducted in Serbia and Croatia provides additional context for interpreting the obtained cadmium concentrations. *Nikolić et al*. (2017), analyzed cadmium levels in the liver and leg muscle tissue of mallards and reported average values of 0.186 mg kg^{-1} in the liver and 0.005 mg kg^{-1} in muscle tissue. *Borjan et al.* (2022), examined cadmium presence in the liver and leg muscles tissue of pheasants over four consecutive years (2018–2021), with mean liver concentrations of 0.354, 0.247, 0.306, and 0.296 mg kg^{-1}, and corresponding muscle tissue concentrations of 0.009, 0.006, 0.011, and 0.011 mg kg^{-1}, respectively. Additionally, *Florijančić et al.* (2009) from Croatia reported an average cadmium concentration of 0.332 mg kg^{-1} in the liver of mallards. The results of the present study are comparable with the aforementioned studies in terms of muscle tissue, where cadmium concentrations are generally low and consistent. On the other hand, as expected, cadmium concentrations were significantly higher in the liver, indicating potential bioaccumulation of this metal. This finding is significant as it highlights the need for more frequent monitoring, as well

Table 1. Cadmium Concentration in Liver Tissue of Mallards

Location	Number of Samples (n)	Range (mg kg^{-1})	Mean ± standard deviation (mg kg^{-1})
Belegiš	11	0.098-2.134	0.643±0.566
Pločica	6	0.195-1.212	0.583±0.423
Vršački ritovi	10	0.125-1.156	0.496±0.337

Table 2. Cadmium Concentration in Muscle Tissue of Mallards

Location	Number of Samples (n)	Range (mg kg^{-1})	Mean ± standard deviation (mg kg^{-1})
Belegiš	12	0.005-0.065	0.017±0.016
Pločica	6	0.010-0.026	0.021±0.007
Vršački ritovi	10	0.002-0.017	0.008±0.006

as the potential influence of local pollution sources on cadmium levels in wild species. Furthermore, the results underscore the necessity of conducting additional ecological investigations, including studies of sediments, water, soil, and air, in order to better understand the sources and pathways of cadmium entry into the environment and food chain.

According to the current Regulation on Maximum Levels of Certain Contaminants in Food (Official Gazette of the Republic of Serbia, Nos. 73/2024, 90/2024, 47/2025 and 61/2025), maximum permissible levels (MPLs) for cadmium are not specified for the meat and organs of wild game, but are defined for domestic animals. In this study, the MPLs established for poultry were used as reference values, given the biological similarity to mallards, with limits of 0.5 mg kg^{-1} for liver and 0.05 mg kg^{-1} for muscle tissue. Out of the 28 analyzed muscle tissues samples, only one sample (3.6%) from the Belegiš site exceeded the reference value, with a concentration of 0.065 mg kg^{-1}. In the case of liver tissue, 11 out of 27 samples (40.7%) had cadmium concentrations above the MPL. By location, at the Vršački ritovi, 4 out of 10 samples (40%) exceeded the threshold. At Belegiš, exceedances were recorded in 5 out of 11 samples (45.5%), while at Pločica, 2 out of 6 samples (33.3%) showed concentrations above the permitted limit, with values of 0.818 and 1.212 mg kg^{-1}. These findings indicate a potential health risk associated with the consumption of wild bird liver and highlight the need for continued monitoring of cadmium levels, as well as consideration of establishing regulatory limits for wild game.

4. Conclusion

The obtained results indicate the presence of cadmium in the liver and muscle tissue of mallards (*Anas platyrhynchos*) at all three investigated sites in Serbia, with notably higher concentrations in the liver compared to muscle tissue. Although differences in cadmium concentrations between the locations were not statistically significant, certain variations suggest a potential influence of local pollution sources. The highest mean cadmium level in the liver was recorded at the Belegiš site, while the lowest values in muscle tissue were found in the Vršački ritovi. A significant proportion of liver samples (40.7%) exceeded the reference value established for poultry, potentially indicating a health risk associated with the consumption of these organs. The results of this study highlight the need for continued monitoring and consideration of the introduction of specific regulatory limits for cadmium in the meat and organs of game, in order to protect human health and maintain ecological balance.

Disclosure Statement: No potential conflict of interest was reported by the authors.

Funding: The research results presented in this paper were funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, under the Agreement on the Implementation and Financing of Scientific Research Work of NIO for the year 2025, No. 451-03-136/2025-03/200050 dated 4.2.2025.

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