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Assessment of Mercury contamination in liver and muscle tissue of mallards (Anas platyrhynchos) as bioindicators at three locations in Serbia

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ABSTRACT

The aim of this study was to investigate the presence and distribution of mercury (Hg) in the liver and muscle tissue of Mallards (Anas platyrhynchos) collected from three locations in Serbia. A total of 55 samples were analyzed, including 23 from the Belegiš site, 12 from Pločica, and 20 from Vršački Ritovi. Mercury concentrations were determined using the ICP-MS method. Mercury levels in the liver ranged from 0.009 to 0.239 mg kg^{-1}, while concentrations in muscle tissue ranged from 0.005 to 0.069 mg kg^{-1}. The mean values for liver tissue were 0.097 mg/kg (Belegiš), 0.108 mg/kg (Pločica), and 0.025 mg kg^{-1} (Vršački Ritovi), whereas for muscle tissue they were 0.030 mg kg^{-1} (Belegiš and Pločica) and 0.008 mg kg^{-1} (Vršački Ritovi). Statistical analysis included descriptive statistics, Pearson correlation test, analysis of variance (ANOVA) with Tukey post hoc test, Levene's test for homogeneity of variances, Kruskal–Wallis test, and Mann–Whitney test. The findings provide insight into potential differences in mercury contamination levels between different ecosystems, as well as the relationship between different tissues in wild birds.

1. Introduction

Heavy metals have always been present as natural components of the environment, typically occurring at very low concentrations under normal conditions. However, both historically and in the present day, human activities have significantly contributed to their release into the environment. Particular concern is raised by the effects of certain metals, such as mercury, which can seriously threaten ecosystem stability. With the rise of public awareness regarding environmental issues, there is an increasing need for systematic monitoring, assessment, management, and remediation of pollution-related damage. Given the complexity of ecosystems, it is impossible to monitor all of their components, functions, and properties; therefore, selected biological indicators are used for this purpose. Birds, as one of the organism groups for which exposure to and toxicity of heavy metals have been extensively studied, serve as useful bioindicators of overall ecological status (*Zolfaghari et al.* 2007).

Mercury is released into the environment through both natural processes (such as volcanic activity, oceans, and vegetation) and anthropogenic activities (*Ribeiro & Germano,* 2015). Anthropogenic sources of mercury emissions are divided into primary sources, in which geologically derived mercury is mobilized into the environment (e.g., mining and the combustion of fossil fuels), and secondary sources, which involve the intentional use of mercury in industry, consumer products, dentistry, and artisanal and small scale

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gold mining (ASGM). Among the primary sources, the largest contribution to pollution comes from sectors where mercury is released as an unintentional byproduct, particularly coal combustion, metal production, and cement manufacturing. Secondary sources also significantly contribute to mercury emissions, with ASGM predominantly occurring in impoverished regions of Africa, Asia, and South America remaining the largest global consumer of mercury (*Pacyna et al.,* 2010). In the environment, mercury can exist in three forms: elemental (Hg^0), inorganic (Hg^{2+}), and organic (methylmercury, MeHg). Through complex biogeochemical cycles, it enters the food chain and accumulates in the tissues of animals and fish. According to EFSA (European Food Safety Authority), the majority of mercury found in fish is in the form of methylmercury (80–100%), whereas in other food sources, it is predominantly present in its inorganic form (*Ribeiro & Germano,* 2015).

Over the past decades, the potential harmful effects of mercury (Hg) have been studied from both ecological and public health perspectives. Mercury is particularly dangerous because it can cause adverse effects in humans and other organisms, with these effects depending on various factors such as the chemical form of mercury, the amount, the route of exposure, and individual susceptibility. Humans can be exposed to mercury through different pathways, including food consumption (especially fish). The effects of mercury encompass a wide range of syndromes, including neurological, immunological, renal, cardiovascular, endocrine, and reproductive disorders. Exposure to high levels of mercury can cause tremors, emotional changes, insomnia, weakness, and sensory disturbances. Although toxic effects of mercury are present in many ecosystems, research on mercury contamination has primarily focused on freshwater fish and birds, as these

Table 1. Mercury Levels in Different Organs of Birds

Animal	Sample	Range (mg kg^{-1})	Mean Value (mg kg^{-1})	Technique	Source
Pheasant (Phasianidae)	Tail feathers	0.150–0.220	0.180	AAS	Zolfaghari et al. (2007)
Hawk	Tail feathers	0.950–1.500	1.250	AAS	Zolfaghari et al. (2007)
Mallard	Liver	0.010–0.689	0.154	CV-AAS	Żarski et al. (2017)
Mallard	Kidney	0.013–0.423	0.122	CV-AAS	Żarski et al. (2017)
Mallard	Muscle	0.009–0.925	0.110	CV-AAS	Żarski et al. (2017)
Mallard	Breast muscle	0.008–0.938	0.133	AAS	Kalisinska et al. (2013)
Mallard	Liver	0.016–0.966	0.248	AAS	Kalisinska et al. (2013)
Mallard	Kidney	0.010–1.499	0.270	AAS	Kalisinska et al. (2013)
Mallard	Breast feathers	0.037–3.475	0.634	AAS	Kalisinska et al. (2013)
Mallard	Liver	0.080–0.510	0.300	CV-AAS	Aazami et al. (2011)
Mallard	Kidney	0.070–0.450	0.260	CV-AAS	Aazami et al. (2011)
Mallard	Feathers	0.500–1.600	1.040	CV-AAS	Aazami et al. (2011)
Mallard	Muscle	0.030–0.160	0.110	CV-AAS	Aazami et al. (2011)
Pheasant	Leg skeletal muscle	0.003–0.019	0.009	AAS	Gasparik et al. (2010)
Mallard	Leg skeletal muscle	0.001–0.023	0.009	AAS	Gasparik et al. (2010)
Eurasian coot	Leg skeletal muscle	0.005–0.015	0.010	AAS	Gasparik et al. (2010)
Common pochard	Liver female Liver male	0.001–0.277 0.001–0.016	0.093 0.006	AAS	Florijancic et al. (2009)
Mallard	Liver female Liver male	0.037–0.197 0.072–0.262	0.111 0.122	AAS	*Florijancic et al. (2009)*

species represent a major protein source for many human populations (*Lemaire et al.,* 2018*; Kim et al.,* 2016).

The mallard (Anas platyrhynchos) is frequently used as a bioindicator of habitat pollution due to its wide distribution. This species is suitable for ecological studies because of its large population size, pronounced sexual dimorphism, long lifespan, and the ability to study long-term exposure to pollutants. Additionally, the mallard is a game species, and its meat is consumed by humans. Therefore, understanding the levels of toxic metals in its body is important for food safety (*Żarski et al.,* 2017).

This study investigated mercury concentrations in liver and muscle tissue samples of wild mallards collected from three locations: near Belegiš on the Danube (Srem District), near Pločica on the Danube (South Banat), and from a fishpond near Vršački Ritovi during 2024. To better understand the extent of contamination, Table 1 presents results from scientific studies addressing similar topics, providing an overview of mercury concentrations in various tissues of wild birds.

The aim of this study was to examine and compare whether there is a statistically significant difference in mercury concentrations in the liver and muscle tissues of mallards collected from three different locations, each representing a distinct type of ecosystem. Two of the sites, Belegiš (Srem District) and Pločica (South Banat District), are located along the Danube River and are exposed to pollution due to intensive industrial activities in this section of the river. In contrast, the third site a fishpond near the Vršački Ritovi represents a relatively isolated and preserved ecosystem, without direct sources of anthropogenic contamination.

2. Materials and Methods

Mercury levels were measured in liver and muscle 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 wild 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/cm) 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).

The determination of the ^{202}Hg isotope was performed using inductively coupled plasma mass spectrometry (ICP-MS) on an iCap Qc 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.

2.1.1 Statistical analysis

Statistical analysis was performed using Minitab® 17.1.0. Descriptive statistics and Pearson correlation were applied. ANOVA with Tukey post hoc test was used to assess significant differences in liver mercury concentrations between locations. For muscle tissue, the Kruskal–Wallis and Mann–Whitney U tests were employed to determine differences among sites.

3. Results

Tables 2 and 3 present the results of descriptive statistics for mercury concentrations in liver and muscle tissue samples of mallards from different locations. The tables include median values, ranges (min–max), arithmetic means, standard deviations, and the number of analyzed samples (n) for each sampling site.

Based on the presented results, mercury (Hg) concentrations in the liver of mallards from all three locations ranged from 0.009 to 0.239 mg kg^{-1}, with the highest recorded concentration found in an individual from the Belegiš site, measuring 0.239 mg kg^{-1} (Table 2). According to the boxplot diagram (Figure 1), the highest

Table 2. Mercury Concentration in Liver Tissue of Mallards

Location	Number of Samples (n)	Median (mg kg^{-1})	Range (mg kg^{-1})	Mean ± standard deviation (mg kg^{-1})
Belegiš	11	0.100	0.023-0.239	0.097±0.056
Pločica	6	0.069	0.039-0.205	0.108±0.073
Vršački Ritovi	10	0.020	0.009-0.070	0.025±0.018

Table 3. Mercury Concentration in Muscle Tissue of Mallards

Location	Number of Samples (n)	Median (mg kg^{-1})	Range (mg kg^{-1})	Mean ± standard deviation (mg kg^{-1})
Belegiš	12	0.022	0.007-0.069	0.030±**0.021**
Pločica	6	0.027	0.013-0.054	0.030±**0.018**
Vršački Ritovi	10	0.007	0.005-0.022	0.008±0.005

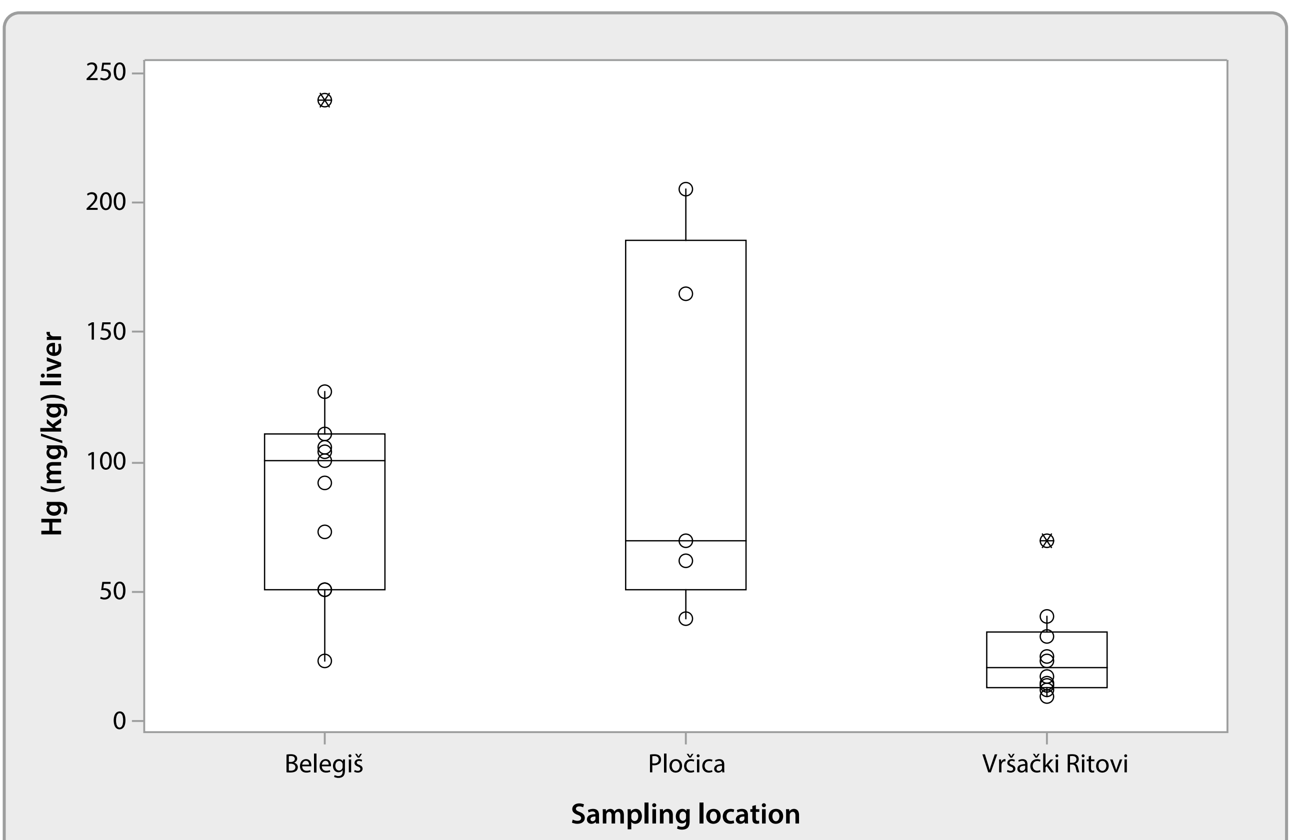

Figure 1. Mercury Levels (mg kg^{-1}) in Liver Samples of Mallards

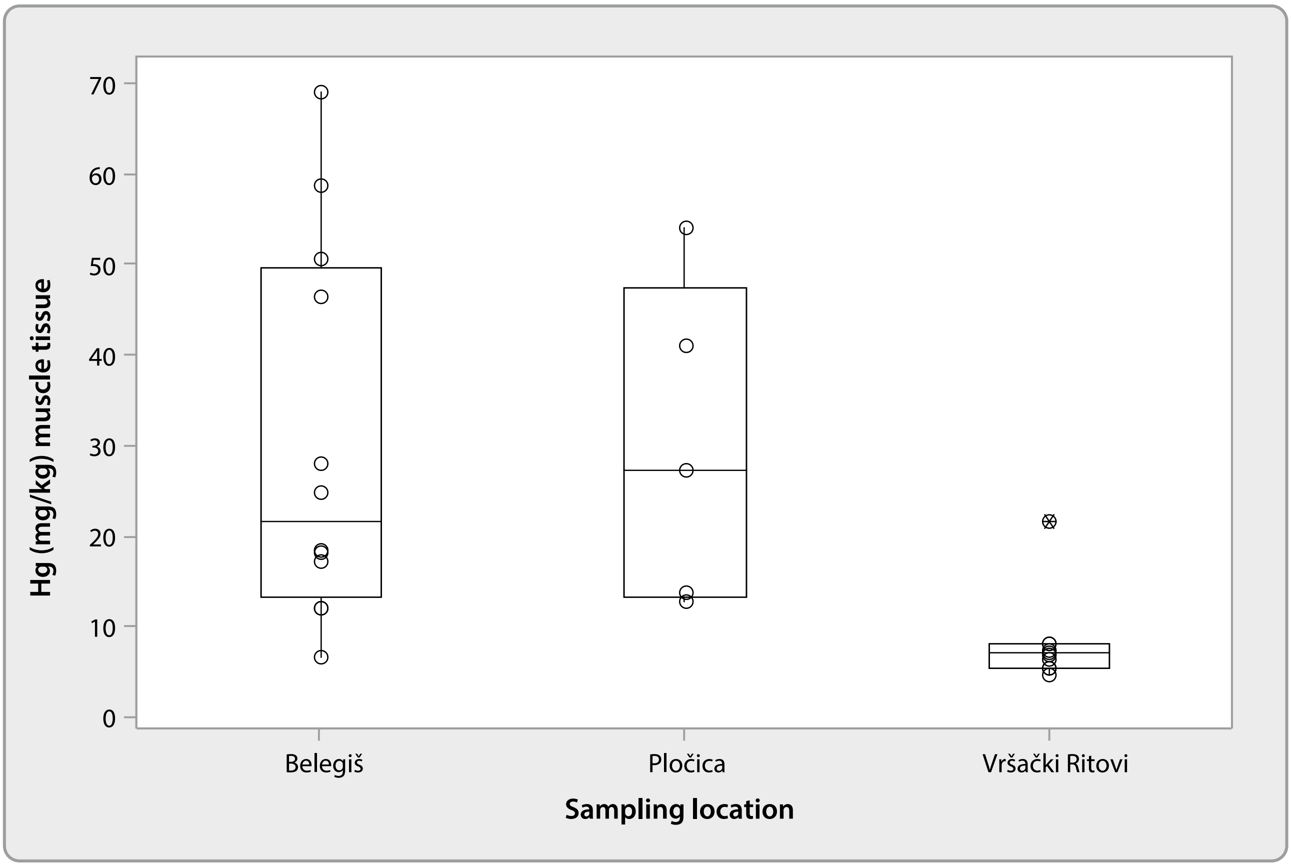

Figure 2. Mercury Levels (mg kg^{-1}) in Muscle Tissue Samples of Mallards

mean mercury concentration in liver samples was observed at the Pločica site, while the highest median value was recorded at Belegiš. Regarding mercury (Hg) concentrations in the muscle tissue of mallards, values at all three locations ranged from 0.005 to 0.069 mg kg^{-1}. The highest concentration was again recorded at the Belegiš site, measuring 0.069 mg kg^{-1} (Table 3 and Figure 2). The mean values for Belegiš and Pločica were identical at 0.030 mg kg^{-1}, while the median was slightly higher at Pločica.

Pearson correlation tests were performed at all three locations to examine the relationship between mercury concentrations in the liver and muscle tissues of mallards. At the Vršački Ritovi site, a very strong positive correlation coefficient ($r = 0.880$) was obtained, with a statistically significant p-value ($p = 0.001$), indicating a clear association between the two tissues. At Belegiš, a moderate positive correlation was found ($r = 0.539$), but the p-value ($p = 0.087$) suggests the relationship is not statistically significant. Similarly, at Pločica, a comparable correlation coefficient ($r = 0.508$) was observed, but with a high p-value ($p = 0.382$), indicating no statistically significant correlation.

Based on one-way analysis of variance (One-Way ANOVA), a statistically significant difference was found in mercury concentrations in the liver of mallards among the three studied locations ($P = 0.004$), indicating that location significantly affects mercury accumulation in liver tissue. A post hoc Tukey HSD test revealed no significant difference between Pločica and Belegiš, grouping them both into statistical group A, while Vršački Ritovi was significantly different from both (group B) with a notably lower average mercury concentration. Before analyzing differences in mercury concentrations in muscle tissue of mallards across the three studied locations, Levene's test was performed to check for homogeneity of variances. Since the test indicated unequal variances, the nonparametric Kruskal–Wallis test was applied. The results showed a statistically significant difference in mercury concentration in muscle tissue among the locations ($P = 0.001$). Following the Kruskal–Wallis test, a post hoc Mann–Whitney test revealed significant differences between Vršački Ritovi and Belegiš ($p = 0.0014$), as well as between Vršački Ritovi and Pločica ($p = 0.0059$). In contrast, no significant difference was found between Belegiš and Pločica ($p = 0.9580$).

4. Discussion

Differences in mercury concentrations between the examined locations were statistically confirmed using one-way analysis of variance (ANOVA) and Tukey post hoc test, with the most pronounced differences observed in liver tissue. The results indicate that habitat characteristics, as well as the presence of potential pollution sources along the course of the Danube River, significantly influence mercury accumulation in organisms. The Vršacki Ritovi site clearly stands out as the least contaminated, which may be attributed to its isolation from direct sources of pollution and limited bird migration. In contrast, the Belegiš and Počica sites, located along the open flow of the river, exhibited higher levels of contamination. Although it was expected, based on geographic position, that mercury concentrations would be higher at Pločica located downstream from Belegiš and directly following the urban and industrial zones of Belgrade and Pančevo the statistical analysis did not confirm this assumption, which is most likely explained by the limited number of samples collected at that site. A similar pattern of differences was observed in muscle tissue, where the Kruskal–Wallis and Mann–Whitney U tests revealed statistically significant differences between locations. The lowest mercury concentrations in this tissue were also recorded at the Vršački Ritovi site, clearly demonstrating the difference in mercury accumulation between distinct ecosystems, compared to the higher and more variable contamination levels observed at sites along the Danube River.

The results of this study were compared with data from two studies conducted in Poland. In the study by *Żarski et al.* (2017), carried out in the area of the Włocławek reservoir, the average mercury concentrations in the liver and muscle tissue of mallards were 0.154 mg kg^{-1} and 0.110 mg kg^{-1}, respectively. Similarly, in the study by *Kalisinska et al.* (2013), conducted near the city of Szczecin, even higher concentrations were reported, with mean values of 0.248 mg kg^{-1} in the liver and 0.133 mg kg^{-1} in the pectoral muscles. In comparison, mercury concentrations at all study sites in Serbia were significantly lower. These differences are most likely the result of varying degrees of industrialization and exposure to anthropogenic pollution sources. The Polish sites, particularly near Szczecin, are heavily influenced by coal-fired power plants, chemical industries, and shipyards, whereas the Serbian locations especially Vršački Ritovi are situated in considerably less contaminated ecosystems. The lower mercury burden observed in mallards from Serbia supports the conclusion that local ecological conditions and anthropogenic pressures play a crucial role in determining the level of heavy metal accumulation in bird tissues.

Additionally, the results obtained in this study were compared with those reported by *Gasparik et al.* (2010), in which the average mercury concentration in the skeletal muscles of mallards in Slovakia was 0.0086 mg kg^{-1}. This value is nearly identical to the concentration measured at the Vršački Ritovi site (0.008 mg kg^{-1}), suggesting that both locations share similar ecological characteristics, with limited industrial influence. In contrast, slightly higher mercury concentrations were recorded at the Belegiš and Pločica sites, which can be associated with greater anthropogenic impact, although these values remain substantially lower than those reported in studies from Poland. Furthermore, a comparison was made with the results of the study by *Florijančić et al.* (2009), conducted in eastern Croatia, where the average mercury concentration in mallard liver tissue was 0.116 mg kg^{-1}. In comparison, the values recorded in this study were slightly lower at Belegiš (0.097 mg kg^{-1}) and Pločica (0.108 mg kg^{-1}), and substantially lower at Vršački Ritovi (0.025 mg kg^{-1}). Given that the Croatian study site is geographically and ecologically closer to the Serbian localities than the Polish and Slovak sites, these findings further support the conclusion that the mercury concentrations observed in this study are consistent with regional environmental conditions. The differences in mercury levels between sites are likely attributable to varying degrees of exposure to local pollution sources, as well as differences in the feeding behavior of the birds.

5. Conclusion

The results of this study indicate that mallards can be considered reliable bioindicators of mercury contamination in aquatic ecosystems, due to their pronounced ability to accumulate this metal in both liver and muscle tissues. Statistical analyses revealed significant differences in mercury concentrations between the Vršački Ritovi ecosystem, which is isolated and subject to limited anthropogenic influence, and the Belegiš and Pločica sites, located along the open-flow section of the Danube River. However, no statistically significant difference was observed between Belegiš and Pločica, which is likely attributable to the limited number of samples collected from the Pločica site. Based on the mean values of mercury concentrations in liver and muscle tissues, a higher degree of contamination can be inferred in Pločica, consistent with its downstream position and greater exposure to potential urban and industrial pollution sources originating from Belgrade and Pančevo.

Procena kontaminacije živom u jetri i mišićnom tkivu divljih pataka (Anas platyrhynchos) sa tri lokaliteta u Srbiji

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INFORMACIJE O RADU

Ključne reči:
Živa
Divlja patka
Jetra
Mišić
Biomonitoring

APSTRAKT

Cilj ovog rada bio je da se ispita prisustvo i distribucija žive (Hg) u jetri i mišićnom tkivu divljih pataka (Anas platyrhynchos), prikupljenih na tri lokaliteta u Srbiji: Belegiš, Pločica i Vršački Ritovi. Ukupno je analizirano 55 uzorka, od čega 23 sa lokaliteta Belegiš, 12 sa lokaliteta Pločica i 20 sa lokaliteta Vršački Ritovi. Određivanje koncentracije žive vršeno je primenom ICP-MS metode. Koncentracije žive u jetri kretale su se u opsegu od 0,009 do 0,239 mg kg^{-1}, dok su u mišićnom tkivu bile u rasponu od 0,005 do 0,069 mg kg^{-1}. Srednje vrednosti za jetru iznosile su 0,097 mg kg^{-1} (Belegiš), 0,108 mg kg^{-1} (Pločica) i 0,025 mg kg^{-1} (Vršački Ritovi), dok su za mišićno tkivo iznosile 0,030 mg kg^{-1} (Belegiš i Pločica) i 0,008 mg kg^{-1} (Vršački Ritovi). Statistička analiza obuhvatila je deskriptivnu statistiku, Pirsonov test korelacije, analizu varijanse (ANOVA) sa Tukey post hoc testom, test homogenosti varijansi (Levene), Kruskal–Wallis test i Mann–Whitney test. Dobijeni nalazi pružaju uvid u potencijalne razlike u nivou kontaminacije živom između dva različita ekosistema, kao i povezanost između različitih tkiva divljih pataka.

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