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Research article

MONITORING OF HEAVY METALS CONTENT IN FISH MUSCLE TISSUE ALONG THE SAVA RIVER IN SERBIA

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The aim of this study was to examine the presence of heavy metals (Pb, Cd and Hg) in the muscle tissue of fish from the Sava River, from two locations: upstream of Belgrade near Obrenovac and in Belgrade. In order to determine the content of heavy metals in fish muscle tissue, 196 samples of different types of fish were collected: Prussian carp, carp, perch and catfish, during the fall and spring of 2023 – 2025. The content of heavy metals was determined by means of atomic absorption spetrometry. The highest average content of Pb (0.282±0.068 mg/kg) and Hg (0.303±0.116 mg/kg) was found in the muscle tissue of catfish, while the lowest average content of Pb and Hg was determined in the muscle tissue of perch (0.088±0.025 mg/kg), (0.090±0.038 mg/kg), respectively. The highest average content of Cd was recorded in the muscle tissue of carp (0.103±0.030 mg/kg), while the lowest was found in the muscle tissue of catfish (0.032±0.015 mg/kg). In all tested samples, the determined concentrations of Pb and Hg were below the maximum allowed concentrations regulated by the Rulebook of the Republic of Serbia and European Union regulations. The determined concentrations of Cd in the muscle tissue of carp, catfish and Prussian carp were on several locations above the maximum allowed concentration of 0.05 mg/kg of fresh fish. Fish from this part of the Sava river cannot be considered completely safe for human consumption, especially when it comes to species that show a tendency to accumulate Cd. Overall, seasonal variations were more evident than spatial differences, with several species showing moderately higher metal concentrations in spring, particularly in 2025, indicating subtle but recurring seasonal tendencies. Differences between the territories upstream of Belgrade and within Belgrade were generally small, although mercury levels were consistently higher at the downstream location, suggesting a mild but stable spatial influence.

Keywords: heavy metals, fish, environmental pollution, meat quality, nutrition

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INTRODUCTION

Environmental pollution today is increasingly pronounced due to various biological and anthropological influences such as volcanic activity, soil and rock erosion, industrial development, chemicalization of agriculture, burning of fossil fuels, urbanization, as well as due to the application of inadequate environmental protection measures [1,2]. These are some of the main factors of pollution and presence of various contaminants in the ecosystem. The main environmental contaminants are various organic pollutants (Persistent Organic Pollutants – POP's), which include polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), organochlorine pesticides (OCP), dioxins [3], and heavy metals such as lead (Pb), cadmium (Cd) and mercury (Hg) are among the most important industrial pollutants [4,5]. Contamination with these metals is present in air, soil, sediment, plants and water. In the water environment, metals are found in low quantities, but due to the mentioned sources of contamination, their concentrations in the waters have increased [6]. When heavy metals infiltrate into water systems, they are dissolved, stored in sediments and cannot be degraded [7]. Therefore, their occurrence in sediments, water courses and living organisms indicates the existence of natural or anthropogenic sources of pollution [8,9]. As the majority of industrial wastewater in Serbia is discharged without adequate treatment [10], heavy metals released in this way will accumulate in aquatic organisms, including fish [11].

Bioaccumulation of heavy metals in fish tissues has been described in many studies, because they are at the top of the food chain in aquatic systems [5,12]. Heavy metals can accumulate in fish tissues through feed, gills, skin, and water intake [13]. The type of feed also has an important influence on the accumulation of heavy metals in fish tissues [14,15]. Thus, carnivorous fish, due to biomagnification, accumulate larger amounts of metals compared to species that are at a lower trophic level [16]. On the other hand, for bottom-feeding fish, sediment is the primary source of heavy metals [17]. The degree of bioaccumulation of metals in fish tissues is influenced by other factors such as body weight, age, sex, species and physiological state of the fish, calcium and organic ligand concentrations [18], as well as water temperature and pH value of the medium [19,20]. Also, the accumulation of heavy metals in all fish tissues does not have the same efficiency, so the muscle tissue of fish contains smaller amounts of heavy metals compared to the internal organs, liver, kidneys and gills [21].

As fish is an important food in the human diet, its consumption is one of the main sources of human exposure to heavy metals [12,22]. The assessment of the presence of heavy metals in fish muscle tissue is very important, so that they do not pose a danger to human health and that the concentrations are below the permitted levels [23]. The heavy metals, that is: lead (Pb), cadmium (Cd) and mercury (Hg) have no physiological role, persist in the ecosystem for a long time, and are toxic when present in traces [6,24]. They are known as non-essential elements and if ingested over a long period of time can show harmful effects for all organisms [25]. Many studies have described that high concentrations of mercury in fish negatively affect its cardioprotective effect, and its presence can lead to neurological diseases in humans [26,27]. Cadmium and lead have an extremely negative effect on the health of children and adults. Lead accumulates in soft tissues and can cause liver, kidney and brain damage. Cadmium can lead to anemia, hypertension and prostate cancer [28]. Although the benefits of consuming fish are significant (quality proteins, the presence of polyunsaturated fatty acids, vitamins and minerals) [29,30], the presence of heavy metals in fish meat can indicate the harmfulness of consuming this food for human health. As the Sava River is important for fishing [16,31], caught fish can pose a significant risk to human health [32].

The aim of this research was to examine the content of heavy metals (Pb, Cd and Hg) in the muscle tissue of four fish species from the Sava River. The determined content of heavy metals in fish meat, indicates the pollution of the environment with harmful metals, which are found in the sediment and in the water, and consequently also in various fish tissues. An even bigger problem is that the Sava River flows through several European countries, where it can be polluted by various pollutants, and the level of contamination can even further increase in Serbia.

The content of the before mentioned toxic metals is regulated by National and European Regulations, that are harmonized, and is defined as the maximum allowed concentration in fish meat.

MATERIALS AND METHODS

Sampling and study area

For this study, fish were caught from the Sava River from two different locations. The first location is located 25 km upstream from Belgrade, Serbia, near the town of Obrenovac (44°44’49.3"N;19°47'24.5"E) (sampling point A), and the other is downstream, in Belgrade, before the confluence of the Sava River and the Danube River (44°47’48.1"N;20°24'43.4"E) (sampling point B), in order to observe the levels of heavy metals from the perspective of big city pollutants. The determination of the content of heavy metals was carried out on the following types of fish: Prussian Carp (*Carassius auratius gibelio*), carp (*Cyprinus carpio*), catfish (*Silurus glanis*) and perch (*Sander lucioperca*). All types of fish were collected from professional fishermen in four periods, during the fall and spring of 2023 – 2025. A total of 192 muscle samples of four fish species were analyzed, six samples of each species from the Sava River from two locations in four periods. After harvesting, each fish was identified to species level and then euthanized with 3-aminobenzoic acid ethyl ester (MS-222). The fish were then transported to the laboratory in a cooling box (hand-held refrigerator) at + 4 ºC. The fish were dissected in the laboratory, and muscle samples (about 100 g) were taken from under the dorsal fin and transferred to polyethylene containers that were previously cleaned (10%) with nitric acid and washed three times with deionized water. The packed samples were then frozen and stored at – 20 °C until heavy metal analysis was performed. Fish muscle was chosen as the target tissue in order to determine the risk that metal pollution poses to human health, and at the same time it is an indicator of the state of the ecosystem.

Reagents

For the determination of the content of the tested metals (Pb, Cd and Hg), the chemicals used to digest the samples, nitric acid, HNO_3 (65%) and hydrogen peroxide, H_2O_2 (30%), were of high purity grade (J.T. Baker). Standard solutions of elements, which were used for calibration, were prepared by diluting basic solutions (J.T. Baker) with a concentration of 1000 mg L^{-1}. Deionized water (resistivity 18.2 $M\Omega cm^{-1}$) obtained from a Millipore Elix UV 10 system was used for all dilutions.

Apparatus

Samples preparation was performed using a MW 3000 microwave closed system (Anton Paar GmbH, Graz, Austria) with a MF 100 cuvette. The content of all elements was determined on an Analyst 700 atomic absorption spectrometer with an MHS system (Perkin Elmer, Shelton, USA). Concentrations of elements (Pb and Cd) were detected by the graphite technique (GFAAS-800) using an auto sampler on the Analyst 700 apparatus (Perkin Elmer), while the mercury content was determined by the cold vapor technique (CVAAS) with a discontinuous system (MHS-15) on the same apparatus. The operating parameters of the atomic absorption spectrometer (Analyst 700) for the determination of heavy metals were adjusted according to the manufacturer's recommendations (Perkin Elmer) and are shown in Tables 1 and 2.

Table 1. Instrumental parameters for graphite furnance (GFAAS) using Analist 700

Metals	Wavelength (nm)	Parameters	Step 1 (Drying)	Step 2 (Pyrolysis)	Step 3 (Atomization)	Step 4 (Cleaning)
Pb	283.3	Temperature (ºC)	130	450	1900	2500
		Ramp time (s)	10	15	0	0
		Hold time (s)	30	10	4	5
Cd	228.8	Temperature (ºC)	130	350	1200	2500
		Ramp time (s)	10	15	0	0
		Hold time (s)	30	10	4	5

Table 2. Instrumental parameters for cold vapor (CVAAS) using Analyst 700

Metals	Wavelength (nm)	Acetylene flow (L min^{-1})	Air flow (L min^{-1})	Slit (nm)	N_2 pressure (bar)
Hg	253.7	/	12.0	0.2	4.0-5.0

Heavy metals analysis

Fish muscle tissue samples were thawed at room temperature, then mechanically ground and homogenized. To determine the content of the tested metals, about 1±0.001 g of the sample was weighed on an analytical balance in cuvettes for sample preparation. The samples were treated with a mixture of HNO_3-H_2O_2 (7:1, v/v) and performed to microwave digestion for 30 minutes. The digestion process was carried out with a program for decomposing fish meat samples, with a range of 250 to 630 W, in four steps, for 30 minutes. After cooling to room temperature, the prepared samples were quantitatively transferred into a measuring vessel, which was filled up to the marked level with deionized water. After the preparation, the content of toxic elements (Pb, Cd and Hg) was determined on the Analyst 700 apparatus. According to the manufacturer's instructions for the graphite technique (GFAAS-800), prescribed modifiers were used. To determine the content of Pb, the modifier primary ammonium phosphate was used, while for Cd, a mixture of magnesium nitrate and palladium chloride was used. The Hg content was determined using a hydride discontinuous system (MHS-15) after reduction with sodium boron hydride ($NaBH_4$). All prepared samples were analyzed twice for each heavy metal. The content of each element (Pb, Cd and Hg) was measured in the peak area mode against a calibration curve, taking into account the masses of the samples and possibly applied dilutions. Metal concentrations are expressed in milligrams per kilogram of fresh fish (w/w). The detection limits of heavy metals in fresh fish meat were 0.02, 0.005 and 0.0125 mg kg^{-1} for Pb, Cd and Hg, respectively. The quality of the analyzes was controlled using a certified reference material (ERM®-BB422, JRC Institute for Reference Materials and Measurements, Geel, Belgium) – fish muscle and BCR-186 (IRMM, Geel, Belgium), lyophilised pig kidney. The concentrations determined in the reference material were within the permissible deviations specified in the obtained certificate and are shown in Table 3.

Table 3. Certified concentration and measured values for the Certified Reference Material ERM®-BB422 (fish muscle) and BCR-186 (lyophilized pig kidney) (mg/kg)

Element	Assigned values (mg/kg)	Measured value ± Uncertainty (mg/kg)
Pb	0.306±0.011	0.298±0.089
Cd	0.0075±0.0018	0.0070±0.0019
Hg	0.601±0.030	0.615±0.150

The concentrations in the tested samples were compared with the maximum allowed concentrations (MAC) in fish meat established by the Regulations. According to the legislation of the European Union [33] and the national legislation [34], the maximum allowed concentrations for Pb, Cd and Hg in fresh fish meat are 0.30, 0.05 and 0.50 mg/kg, respectively.

Statistical Analyses

Statistical analysis of the results was elaborated using Microsoft Office Excel 2010 and GraphPad Prism software, version 9.00 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). All parameters were described by mean and standard deviation (SD). One-way ANOVA with Tukey's post hoc test was performed to assess the significance of differences between heavy metal concentrations during a two-year period in spring and autumn. To determine the significance of the differences between heavy metal concentrations at two locations, t-test was used. Differences were considered significant if $p<0.01$ or $p<0.05$ were observed.

RESULTS

The total concentration of heavy metals (mg/kg) in the muscle tissue of certain fish species in the territories before Belgrade (sampling point A) and in Belgrade (sampling point B) during the two-year period of investigation is shown in Table 4.

Table 4. Total concentrations of heavy metals (mg/kg) in the muscle tissue of selected fish species from the Sava River, aggregated across sampling points A and B (n=12) during the two-year study period

Type of fish	Para-meter	$\bar{X} \pm Sd$			
		Autumn '23	**Spring '24**	**Autumn '24**	**Spring '25**
Catfish	**Pb**	$0.215^{A,B}\pm0.042$	$0.282^{ACD}\pm0.068$	$0.113^{BCE}\pm0.0163$	$0.187^{DE}\pm0.038$
	Cd	$0.069^{A}\pm0.020$	$0.087^{B}\pm0,025$	$0.082^{C}\pm0,012$	$0.032^{ABC}\pm0,015$
	Hg	0.278±0.106	0.303±0.116	0.261±0.088	0.289±0.088
Carp	**Pb**	$0.212^{A}\pm0.045$	$0.248^{B}\pm0.056$	$0.115^{ABC}\pm0.025$	$0.245^{C}\pm0.062$
	Cd	$0.086^{A}\pm0.020$	$0.103^{BC}\pm0.030$	$0.077^{BD}\pm0.015$	$0.021^{ACD}\pm0.008$
	Hg	0.212±0.106	$0.243^{a}\pm0.126$	$0.268^{A}\pm0.098$	$0.133^{Aa}\pm0.038$
Perch	**Pb**	$0.111^{A}\pm0.026$	$0.088^{B}\pm0.025$	$0.090^{C}\pm0.038$	$0.225^{ABC}\pm0.083$
	Cd	0.033±0.010	0.038±0.012	0.041±0.015	0.041±0.014
	Hg	0.154±0.061	$0.201^{A}\pm0.079$	$0.201^{B}\pm0.075$	$0.090^{AB}\pm0.038$
Prussian carp	**Pb**	0.134±0.035	0.148±0.051	0.164±0.036	0.177±0.050
	Cd	0.047±0.007	0.041±0.013	0.036±0.008	0.045±0.022
	Hg	$0.112^{AB}\pm0.026$	$0.125^{a}\pm0.034$	$0.161^{A}\pm0.037$	$0.163^{Ba}\pm0.037$

Legend: Same letter in row ^{a} – $p<0.05$; ^{ABCDE} – $p<0.01$.

A significantly higher ($p<0.01$) concentration of lead in the muscle tissue of catfish was found in the spring of 2024 (0.282±0.068 g/kg) compared to the fall of 2023 (0.215±0.042 mg/kg), as well as in the spring of 2025 (0.187±0.038 mg/kg) and the fall of 2024. (0.113±0.0163 mg/kg). Also, the concentration of lead in the muscle tissue of catfish in the spring of 2025 was significantly higher ($p<0.01$) than in the fall of 2024. In the fall of 2023, the concentration of lead in the examined catfish tissue was significantly higher ($p<0.01$) compared to the fall of the following year. In the spring of 2025, a significantly lower ($p<0.01$) cadmium concentration was found in the muscle tissue of catfish (0.032±0.015 mg/kg) compared to other comparison periods. Between the other periods of comparison, no significant differences were found in the concentration of cadmium in the examined catfish tissue. The concentration of mercury in the muscle tissue of catfish was generally uniform during the two-year study and no statistically significant differences were found between the compared periods.

The concentration of lead in the muscle tissue of carp in the fall of 2024 (0.115±0.025 mg/kg) was significantly lower ($p<0.01$) compared to the concentration of this heavy metal in all other comparison periods, while no significant differences were found between the compared concentrations in the other periods during the two-year study. In the spring of 2025, a significantly lower ($p<0.01$) concentration of cadmium (0.021±0.008 mg/kg) was found compared to the previous period (autumn 2023-autumn 2024), while in the spring of the previous year, the concentration of cadmium in the muscle tissue of carp (0.103±0.030 mg/kg) was significantly higher ($p<0.01$) compared to the concentration of cadmium in the examined carp tissue in the fall of 2024 (0.077±0.015 mg/kg). Similar results were obtained for mercury concentration in carp muscle tissue. In the spring of 2025, a significantly lower concentration of this heavy metal was found in the muscle tissue of carp (0.133±0.038 mg/kg) compared to the spring of 2024 (0.243±0.126 mg/kg) ($p<0.05$) and the fall of 2024 (0.268±0.098 mg/kg) ($p<0.01$).

In the muscle tissue of perch, the lead concentration was significantly higher ($p<0.01$) in the spring of 2025 (0.225±0.083 mg/kg) compared to the other three test periods. During the two-year study period, no significant differences were found between the concentration of cadmium in the muscle tissue of perch in all four comparison periods. The concentration of mercury in the muscle tissue of perch (0.090±0.038 mg/kg) was significantly lower ($p<0.01$) in the spring of 2025 compared to the spring and autumn of 2024 (0.201±0.079 mg/kg; 0.201±0.075 mg/kg, respectively).

The concentration of lead and cadmium in the muscle tissue of Prussian carp did not change significantly during the two-year test period, while the concentration of mercury in the examined tissue of Prussian carp was significantly lower ($p<0.01$) in the fall of 2023 (0.112±0.026 mg/kg) compared to the mercury concentration in the fall of the following year (0.161±0.037 mg/kg) and the spring of 2025. (0.163±0.037 mg/kg). Also, significant differences ($p<0.05$) were found in the concentration of this heavy metal in the muscle tissue of Prussian carp in the spring of 2024 (0.125±0.034

mg/kg) and the spring of 2025 (0.163±0.037 mg/kg), where the concentration increased significantly during one year.

Table 5 shows the concentrations of lead, cadmium and mercury in the muscle tissue of catfish (mg/kg) at the sampling point A and sampling point B during a two-year period. In the muscle tissue of catfish, no significant differences were found between the concentration of lead in the territory of the catch upstream of Belgrade and in Belgrade during the entire period of investigation. In the spring of 2025 at the sampling point B, a significantly higher ($p<0.05$) concentration of cadmium (0.041±0.015 mg/kg) was found in the muscle tissue of catfish than in muscle tissue of catfish caught at the sampling point A (0.024±0.009 mg/kg). In the other periods, no significant differences were found between the concentrations of cadmium in the muscle tissue of catfish in the two compared catch territories. The mercury concentration in the muscle tissue of catfish caught in sampling point B was significantly higher ($p<0.01$) in all four comparison periods (0.355±0.091 mg/kg; 0.385±0.092 mg/kg; 0.331±0.065 mg/kg; 0.364±0.053 mg/kg, respectively) compared to the mercury concentration in the same catfish tissue caught at the sampling point A (0.201±0.049 mg/kg; 0.222±0.073 mg/kg; 0.192±0.035 mg/kg; 0.213±0.023 mg/kg, respectively).

Table 5. Concentration of examined heavy metals (Pb, Cd, Hg) in muscle tissue of catfish (mg/kg) from the Sava River at sampling point A and sampling point B (n=6) during the two-year study period

Parameter	Location	$\bar{X} \pm Sd$			
		Autumn '23	**Spring '24**	**Autumn '24**	**Spring '25**
Pb	**Sampling point A**	0.204±0.039	0.270±0.076	0.110±0.014	0.178±0.037
	Sampling point B	0.227±0.047	0.294±0.064	0.118±0.019	0.196±0.040
Cd	**Sampling point A**	0.070±0.024	0.096±0.029	0.086±0.014	0.024^{a}±0.009
	Sampling point B	0.069±0.017	0.077±0.019	0.079±0.009	0.041^{a}±0.015
Hg	**Sampling point A**	0.201^{A}±0.049	0.222^{A}±0.073	0.192^{A}±0.035	0.213^{A}±0.023
	Sampling point B	0.355^{A}±0.091	0.385^{A}±0.092	0.331^{A}±0.065	0.364^{A}±0.053

Legend: Same letter in row ^{a} – $p<0.05$; ^{A} – $p<0.01$.

During the two-year study period, the concentrations of lead, cadmium and mercury in the muscle tissue of carp (mg/kg) caught in the sampling point A and sampling point B were compared (Table 6). The concentration of lead in the examined tissue of carp caught in the sampling point A was not significantly different from the concentration of lead in the same tissue of this species of fish caught in the sampling point B during the four comparison periods. A similar situation was found when comparing the concentration of cadmium in the muscle tissue of carp, where during the two-year period of testing, no significant differences were found between the concentrations of the examined heavy metal in carp caught in the sampling point A and sampling point B, except for the spring of 2025 when a significantly higher $p<0.01$ value of cadmium was found at the sampling point B. The concentration of mercury was significantly higher ($p<0.01$) in the muscle tissue of the examined species of fish at sampling point B in the fall of 2023 (0.303±0.068 mg/kg), spring of 2024 (0.343±0.094 mg/kg) and autumn of the same year (0.355±0.049 mg/kg) in relation to the concentration of this heavy metal in the muscle tissue of carp caught at sampling point A during the compared test periods (0.122^{A}±0.025 mg/kg; 0.143^{A}±0.046 mg/kg; 0.180^{A}±0.019 mg/kg, respectively). In the spring of 2025, no significant differences were found between the concentration of mercury in the muscle tissue of the investigated species of fish spawned at the sampling points A and B.

Table 6. Concentration of examined heavy metals (Pb, Cd, Hg) in muscle tissue of carp (mg/kg) from the Sava River at sampling point A and sampling point B (n=6) during the two-year study period

Parameter	Location	$\bar{X} \pm Sd$			
		Autumn '23	**Spring '24**	**Autumn '24**	**Spring '25**
Pb	**Sampling point A**	0.200±0.047	0.232±0.056	0.104±0.022	0.212±0.049
	Sampling point B	0.224±0.043	0.263±0.057	0.127±0.024	0.278±0.060
Cd	**Sampling point A**	0.081±0.020	0.097±0.029	0.072±0.015	0.016^{A}±0.005
	Sampling point B	0.092±0.019	0.110±0.032	0.081±0.015	0.027^{A}±0.006
Hg	**Sampling point A**	0.122^{A}±0.025	0.143^{A}±0.046	0.180^{A}±0.019	0.123±0.037
	Sampling point B	0.303^{A}±0.068	0.343^{A}±0.094	0.355^{A}±0.049	0.144±0.040

Legend: Same letter in row ^{A} – $p<0.01$.

The concentrations of lead, cadmium and mercury in the muscle tissue of perch (mg/kg) caught at the sampling points A and B during a two-year period are shown in Table 7. No significant differences were found in the concentration of lead and cadmium in the muscle tissue of this type of fish caught in the observed locations, except for the last two examined periods when a significant difference was determined for lead. However, the concentration of mercury in the muscle tissue of perch caught at the sampling point A was significantly higher ($p<0.01$) in the fall of 2023 (0.201±0.044 mg/kg), spring (0.262±0.057 mg/kg) and fall of 2024 (0.259±0.063 mg/kg) in relation to the concentration of mercury in the muscle tissue of the examined species of fish caught at the sampling point B in the compared periods of the study (0.107±0.031 mg/kg; 0.140±0.040 mg/kg; 0.144±0.022 mg/kg. respectively). Completely opposite results were obtained in the spring of 2025, where the mercury concentration in the examined tissue of perch caught in the sampling point A (0.056±0.007 mg/kg) was significantly lower ($p<0.01$) compared to the mercury concentration of perch caught at sampling point B (0.124±0.018 mg/kg).

Table 7. Concentration of examined heavy metals (Pb, Cd, Hg) in muscle tissue of perch (mg/kg) from the Sava River at sampling point A and sampling point B (n=6) during the two-year study period

Parameter	Location	$\bar{X} \pm Sd$			
		Autumn '23	**Spring '24**	**Autumn '24**	**Spring '25**
Pb	**Sampling point A**	0.099±0.022	0.078±0.022	$0.056^{A}\pm0.007$	$0.292^{A}\pm0.053$
	Sampling point B	0.123±0.026	0.099±0.024	$0.124^{A}\pm0.018$	$0.157^{A}\pm0.036$
Cd	**Sampling point A**	0.028±0.009	0.032±0.008	0.037±0.016	0.037±0.016
	Sampling point B	0.037±0.008	0.044±0.014	0.046±0.013	0.046±0.013
Hg	**Sampling point A**	$0.201^{A}\pm0.044$	$0.262^{A}\pm0.057$	$0.259^{A}\pm0.063$	$0.056^{A}\pm0.007$
	Sampling point B	$0.107^{A}\pm0.031$	$0.140^{A}\pm0.040$	$0.144^{A}\pm0.022$	$0.124^{A}\pm0.018$

Legend: Same letter in row ^{A} – $p<0.01$.

In the spring of 2024, the concentration of lead in the muscle tissue of Prussian carp caught at the sampling point A (0.177±0.053 mg/kg) was statistically significantly higher ($p<0.05$) compared to the concentration of lead in the same tissue of Prussian carp caught in the sampling point B (0.120±0.031 mg/kg). The concentration of this heavy metal in the examined tissue of Prussian carp caught at the observed locations was not significantly different in the other three periods of comparison (autumn 2023, autumn 2024 and spring 2025) (Table 8). No significant differences were found between the concentration of cadmium in the muscle tissue of this species of fish caught in the sampling points A and B. In the muscle tissue of Prussian carp caught at the sampling point B, the concentration of mercury (0.131±0.019 mg/kg; 0.144±0.031 mg/kg; 0.191±0.019 mg/kg; 0.194±0.020 mg/kg. respectively) was significantly higher ($p<0.05$; $p<0.01$) compared to the muscle tissue of Prussian carp caught at sampling point A (0.093±0.016 mg/kg; 0.107±0.027 mg/kg; 0.131±0.021 mg/kg; 0.132±0.018 mg/kg. respectively) during all four comparison periods.

Table 8. Concentration of examined heavy metals (Pb, Cd, Hg) in muscle tissue of Prussian carp (mg/kg) from the Sava River at sampling point A and sampling point B (n=6) during the two-year study period

Para-meter	Location	$\bar{X} \pm Sd$			
		Autumn '23	Spring '24	Autumn '24	Spring '25
Pb	Sampling point A	0.149±0.041	0.177^{a}±0.053	0.181±0.041	0.200±0.061
	Sampling point B	0.119±0.021	0.120^{a}±0.031	0.147±0.021	0.154±0.020
Cd	Sampling point A	0.043±0.004	0.038±0.012	0.034±0.008	0.037±0.015
	Sampling point B	0.051±0.008	0.045±0.014	0.039±0.008	0.053±0.026
Hg	Sampling point A	0.093^{A}±0.016	0.107^{a}±0.027	0.131^{A}±0.021	0.132^{A}±0.018
	Sampling point B	0.131^{A}±0.019	0.144^{a}±0.031	0.191^{A}±0.019	0.194^{A}±0.020

Legend: Same letter in row ^{a} – $p<0.05$; ^{A} – $p<0.01$.

DISCUSSION

Investigation of the presence of heavy metals in the muscle tissue of four species of freshwater fish (catfish – *Silurus glanis*, carp – *Cyprinus carpio*, perch – *Sander lucioperca*, Prussian carp – *Carassius auratius gibelio*) from the territory of the Sava River at sampling points A and B, indicated certain spatial and seasonal differences, especially in relation to the concentration of mercury and cadmium. The overall results indicate a moderate, but clearly detected influence of the urban area on the quality of the river ichthyofauna, considering the different response of the observed fish species regarding the accumulation of lead, cadmium and mercury.

Lead was present in most samples in concentrations that did not exceed legally permitted values and did not show significant differences between locations, except for Prussian carp in spring 2024, where the lead level was higher at sampling point A, as well as perch in autumn 2024 and spring 2025, where higher and lower values were observed at the sampling point B, respectively. This result may indicate localized sources of contamination, including soil erosion and agricultural sources, which is consistent with research in the region [35]. Also, seasonal fluctuations of lead concentrations were observed in catfish and perch, which may indicate changes in the biological availability of the metal due to hydrological and temperature factors. Perch, especially in the spring of 2025, showed a significantly higher concentration of lead, which may be related to the increased mobilization of the metal from the sediments due to the rise in the water level in the spring months.

Cadmium was detected in the majority of samples in low concentrations, but unlike lead, exceeding the maximum allowed concentration (0.05 mg/kg) was recorded in catfish, carp and Prussian carp in several seasons. The values of cadmium in carp were above the MAC in autumn 2023 (0.086 mg/kg), spring 2024 (0.103 mg/kg) and autumn 2024 (0.077 mg/kg), while in catfish, exceedances were recorded in autumn 2023 (0.069 mg/kg), spring 2024 (0.087 mg/kg) and autumn in 2024 (0.082 mg/kg). In the case of Prussian carp, exceedances were determined in the fall of 2023 (0.047 mg/kg close to the limit value), but they were especially pronounced in sampling point B in the fall of 2023 (0.051 mg/kg) and spring of 2025 (0.053 mg/kg). This makes cadmium stand out as the riskiest metal in this research, which indicates a potential health risk for consumers and the need for strict monitoring. Although in the spring of 2025 the concentration of cadmium was significantly lower in catfish and carp (0.032 and 0.021 mg/kg), the previously determined exceedances cannot be ignored. Similar findings of cadmium accumulation in fish downstream of urban and industrial zones have been described in other papers [36,37]. It is worth noting that cadmium is deposited to a greater extent in the liver and kidneys, and the analysis of those tissues could give an even more precise insight into the actual degree of exposure.

Unlike the concentration of lead and cadmium, the most pronounced spatial differences were found in the concentration of mercury. In catfish, carp and Prussian carp, the concentration of mercury was significantly higher in samples from sampling point B during several seasons. This indicates a clear anthropogenic influence of urban and industrial sources. Given that methylmercury is particularly toxic and has a high potential for bioaccumulation and biomagnification in the trophic chain, these results are particularly significant from the point of view of consumer protection and ecosystem preservation. Similar findings were reported in large rivers in Europe and Asia, where Hg concentrations were significantly higher in benthic and omnivorous species [38,39]. The increased concentration of mercury in samples downstream from urban areas was also confirmed in works indicating that urban wastewater and atmospheric deposition are key sources of this metal in aquatic ecosystems [37,40]. It is interesting that in the case of perch, as a higher predator, the opposite trend was recorded – higher concentrations of mercury were present on the sampling point A during three of the four analyzed periods. This phenomenon may be the result of localized sources of pollution or changes in prey availability, as perch show high sensitivity to disturbances in the food chain. It is also possible that perch in this part of the river feed on species that migrate from tributaries with greater contamination, which further complicates the interpretation of spatial patterns. In addition, a significant seasonal oscillation of mercury concentration was observed in perch, which may be related to its migration patterns, changes in diet or metabolism depending on temperature and reproductive cycle [41].

Based on the obtained results, it can be concluded that the concentrations of lead and mercury in the largest number of samples were in accordance with the prescribed limit values, while cadmium repeatedly exceeded the maximum allowed concentration in the muscle tissue of carp, catfish and Prussian carp. This indicates that the fish from this part of the river Sava cannot be considered completely safe for human consumption, especially when it comes to species that show a tendency to accumulate cadmium. Therefore, it is necessary to introduce continuous monitoring, expand and confirm toxicological analyzes on a larger number of fish samples, including the monitoring of other tissues and biomarkers of exposure. The obtained results supplement and confirm findings from earlier research in Serbia and the region [4,5] and indicate the importance of a multispecies approach in assessing the state of aquatic ecosystems, because different fish species react differently to the same stressors in the environment. These findings further reinforce the need for an integrated analysis that includes physicochemical, biological, and toxicological parameters for a complete assessment of the ecosystem health of river courses in urban areas.

CONCLUSION

The research results show that the presence of heavy metals in freshwater fish from the Sava River reflects the influence of various anthropogenic and natural factors. While lead and mercury concentrations were generally within legal limits, cadmium exceeded permissible values in catfish, carp, and Prussian carp, indicating a potential health risk. Variations between species and seasons suggest complex bioaccumulation patterns, highlighting the need for systematic monitoring of heavy metals in fish to ensure food safety and protect aquatic ecosystems.

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Authors' contributions

DJ and JJ participated in conducting the experiment and drafting the manuscript. RM participated in the design of the experiment. DP, SR and VD participated in conducting the experiment. JJ performed the statistical analysis of the experiment results. SG participated as coordinator in all activities. DŠ designed the study, participated in drafting the manuscript and was coordinator of all activities. All authors read and approved the final manuscript.

Declaration of competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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REFERENCES

1. Gupta N, Yadav K K, Kumar V, Kumar S, Chadd R P, Kumar A: Trace elements in soil-vegetables interface: Translocation, bioaccumulation, toxicity and amelioration-A review. Sci Total Environ 2019, 651: 2927 – 2942.
2. Kumar V, Thakur R K, Kumar P: Assessment of heavy metals uptake by cauliflower (*Brassica oleracea* var. *botrytis*) grown in integrated industrial effluent irrigated soils: A prediction modeling study. Sci Hortic 2019, 257: 108684.
3. Trbović D, Janković S, Ćirković M, Nikolić D, Matekalo-Sverak V, Đorđević V, Spirić A: Bezbednost i kvalitet mesa nekih slatkovodnih riba u Srbiji. Tehnologija mesa 2011, 52(2): 276–282.
4. Aleksić J, Glamočlija N, Laudanović M, Ivanović S, Milijašević M, Baltić B, Starčević M: The content and associated health risk assessment of toxic elements, micro – and macrominerals in common carp, Wels catfish, and silver carp from the Danube River in Serbia. Environ Geochem Health 2025, 47: 60.
5. Jovanović D, Marković R, Teodorović V, Sefer D, Krstić M, Radulović S, Ivanović Ćirić J, Janjić J, Baltić M Z: Determination of heavy metals in muscle tissue of six fish species with different feeding habits from the Danube River, Belgrade—public health and environmental risk assessment. Environ Sci Pollut Res 2017, 24(12): 11383–11391.
6. Azar H, Vajargah M F: Investigating the effects of accumulation of lead and cadmium metals in fish and its impact on human health. J Aquacult Mar Biol 2023, 12(2): 209–213.

7. Linnik P M, Zubenko I B: Role of bottom sediments in the secondary pollution of aquatic environments by heavy-metal compounds. Lakes Reserv Res Manag 2000, 5(1): 11–21.
8. Nabi Bidhendi G R, Karbassi A R, Nasrabadi T, Hoveidi H: Influence of copper mine on surface water quality. Int J Environ Sci Technol 2007, 4(1): 85–91.
9. Mehrdadi N, Nabi-Bidhendi G R, Nasrabadi T, Hoveidi H, Amjadi M, Shojaee M A: Monitoring the arsenic concentration in groundwater resources, case study: Ghezel Ozan water basin, Kurdistan, Iran. Asian J Chem 2009, 21(1): 446–450.
10. Milanović A, Kovačević-Majkić J, Milivojević M: Water quality analysis of Danube River in Serbia—pollution and protection problems. Glas Srp Geogr Društ 2010, 90: 47–68.
11. Ginsberg G L, Toal B F: Quantitative approach for incorporating methylmercury risks and omega-3 fatty acid benefits in developing species-specific fish consumption advice. Environ Health Perspect 2009, 117: 267–275.
12. Milanov R, Krstić M, Marković R, Jovanović D, Baltić B, Ivanović J, Jovetić M, Baltić M: Analysis of heavy metals concentration in tissues of three different fish species included in human diet from Danube River, in the Belgrade region, Serbia. Acta Vet (Beograd) 2016, 66: 89–102.
13. Nussey G, van Vuren J H J, van Preez H H: Bioaccumulation of chromium, manganese, nickel and lead in the tissues of the moggel, Labeo umbratus (Cyprinidae), from Witbank Dam, Mpumalanga. Water SA 2000, 26: 269–284.
14. Cheng Z, Chen K, Li K, Nie X, Wu S C, Kong C, Wong C, Wong M: Arsenic contamination in the freshwater fish ponds of Pearl River Delta: bioaccumulation and health risk assessment. Environ Sci Pollut Res 2013, 20: 4484–4495.
15. Merciai R, Guasch H, Kumar A, Sabater S, García-Berthou E: Trace metal concentration and fish size: Variation among fish species in a Mediterranean river. Ecotox Environ Safe 2014, 107: 154–161.
16. Subotić S, Spasić S, Višnjić-Jeftić Ž, Hegediš A, Krpo-Ćetković J, Mićković B, Skorić S, Lenhardt M: Heavy metal and trace element bioaccumulation in target tissues of four edible fish species from the Danube River (Serbia). Ecotox Environ Safe 2013, 98: 196–202.
17. Rajeshkumar S, Li X: Bioaccumulation of heavy metals in fish species from the Meiliang Bay, Taihu Lake, China. Toxicol Rep 2018, 5: 288–295.
18. Moiseenko T I, Gashkina N A: Distribution and bioaccumulation of heavy metals (Hg, Cd and Pb) in fish: influence of the aquatic environment and climate. Environ Res Lett 2020, 15: 115013.
19. Kehrig H A, Seixas T G, Malm O, Di Beneditto A P, Rezende C E: Mercury and selenium biomagnification in a Brazilian coastal food web using nitrogen stable isotope analysis: a case study in an area under the influence of the Paraiba do Sul River plume. Mar Pollut Bull 2013, 75: 283–290.
20. Has-Schön E, Bogut I, Strelec I: Heavy metal profile in five fish species included in human diet, domiciled in the end flow of River Neretva (Croatia). Arch Environ Contam Toxicol 2006, 50: 545–551.
21. Akan J C, Mohmoud S, Yikala B S, Ogugbuaja V O: Bioaccumulation of some heavy metals in fish samples from River Benue in Vinikilang, Adamawa State, Nigeria. Am J Anal Chem 2012, 3: 727–736.
22. Squadrone S, Prearo M, Brizio P, Gavinelli S, Pellegrino M, Scanzio T, Guarise S, Benedetto A, Abete M: Heavy metals distribution in muscle, liver, kidney and gill of European catfish (Silurus glanis) from Italian rivers. Chemosphere 2013, 90: 358–365.

23. Uysal K, Emre Y, Köse E: The determination of heavy metal accumulation ratios in muscle, skin and gills of some migratory fish species by inductively coupled plasma-optical emission spectrometry (ICP-OES) in Beymelek Lagoon (Antalya/Turkey). Microchem J 2008, 90(1): 67–70.
24. Vardhan K H, Kumar P S, Panda R C: A review on heavy metal pollution, toxicity and remedial measures: Current trends and future perspectives. J Mol Liq 2019, 290: 111197.
25. Zheng G, Tian L, Liang Y, Broberg K, Lei L, Guo W, Nilsson J, Bergdahl I A, Skerfving S, Jin T: δ-Aminolevulinic acid dehydratase genotype predicts toxic effects of lead on workers' peripheral nervous system. Neurotoxicology 2011, 32: 374–382.
26. Bardach A E, Ciapponi A, Soto N, Chaparro M R, Calderon M, Briatore A, Cadoppi N, Tassara R, Litter M I: Epidemiology of chronic disease related to arsenic in Argentina: A systematic review. Sci Total Environ 2015, 538: 802–816.
27. Griboff J, Wunderlin D A, Monferran M V: Metals, As and Se determination by inductively coupled plasma-mass spectrometry (ICP-MS) in edible fish collected from three eutrophic reservoirs. Their consumption represents a risk for human health? Microchem J 2017, 130: 236–244. doi:10.1016/j.microc.2016.09.013.
28. Pelić M, Mihaljev Ž, Živkov Baloš M, Popov N, Gavrilović A, Jug-Dujaković J, Ljubojević Pelić D: Health risks associated with the concentration of heavy metals in sediment, water, and carp reared in treated wastewater from a slaughterhouse. Water 2024, 16: 94. doi:10.3390/w16010094.
29. Ackman R G: Nutritional composition of fats in seafood. Prog Food Nutr Sci 2000, 13: 161–241.
30. Kminkova M, Winterová R, Kučera J: Fatty acids in lipids of carp (Cyprinus carpio) tissues. Czech J Food Sci 2001, 19: 177–181.
31. Jaćimović M, Lenhardt M, Višnjić-Jeftić Ž, Jarić I, Gačić Z, Hegediš A, Krpo-Ćetković J: Elemental concentrations in different tissues of European perch and black bullhead from Sava lake (Serbia). Slov Vet Res 2015, 52(2): 57–65.
32. Cordeli A N, Oprea L, Cretu M, Dediu L, Coada M T, Mînzala D N: Bioaccumulation of metals in some fish species from the Romanian Danube River: A review. Fishes 2023, 8: 387. doi:10.3390/fishes8080387.
33. European Commission Regulation: Setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, Commission Regulation No. 1881/2006/EC 2006.
34. Official Gazette of the Republic of Serbia: Pravilnik o maksimalnim koncentracijama određenih kontaminenata u hrani ("Sl, Glasnik RS", br. 73/2024, 90/2024, 47/2025 i 61/2025) Prilog 1 – Maksimalne koncentracije određenih kontaminenata u hrani [In Serbian]. 2024.
35. Sakan S M, Dević JG, Relić JD, Anđelković BI, Sakan MN, Đorđević SD: Environmental assessment of heavy metal pollution in freshwater sediment, Serbia. Clean–Soil, Air, Water, 2015 43 (6): 838-845.
36. Ali M, et al.: Distribution of heavy metals in water and sediment of an urban river in a developing country: A probabilistic risk assessment. Int J Sediment Res 2022, 37(2): 173–187.
37. Driscoll C T, Mason R P, Chan H M, Jacob D J, Pirrone N: Mercury as a global pollutant: Sources, pathways, and effects. Environ Sci Technol 2013, 47(10): 4967–4983.
38. Zhou H Y, Ming H W: Mercury accumulation in freshwater fish with emphasis on the dietary influence. Water Res 2000 34 (17): 4234-4242.

39. Milošković A, Simić V: Bioaccumulation of potentially toxic elements in fish species of Serbia: A review. Environ Sci Pollut Res, 2023, 30(12): 32255–32277.
40. Pelić M, Mihaljev Ž, Živkov Baloš M, Popov N, Gavrilović A, Jug-Dujaković J, Ljubojević Pelić D: Health risks associated with the concentration of heavy metals in sediment, water, and carp reared in treated wastewater from a slaughterhouse. Water, 2024, (16): 94.
41. Suhareva N, Aigars J, Poikāne R, Tunens J: The influence of feeding ecology and location on total mercury concentrations in Eurasian perch (*Perca fluviatilis*). Environ Sci Eur 2021, 33(1): 82.

MONITORING SADRŽAJA TEŠKIH METALA U MIŠIĆNOM TKIVU RIBA DUŽ REKE SAVE U SRBIJI

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Cilj ove studije bio je ispitivanje prisustva teških metala (Pb, Cd i Hg) u mišićnom tkivu ribe iz reke Save, sa dve lokacije: pre Beograda u blizini Obrenovca i u Beogradu. Radi utvrđivanja sadržaja teških metala u mesu riba, sakupljeno je 196 uzoraka različitih vrsta riba: babuške, šarana, smuđa i soma, tokom jeseni i proleća 2023. – 2025. god. Za određivanje sadržaja teških metala korišćena je mikrotalasna peć i atomski apsorpcioni spektrometar. Najveći prosečan sadržaj Pb (0.282±0.068 mg/kg) i Hg (0.303±0.116 mg/kg) pronađen je u mišićnom tkivu soma, dok je najmanji prosečan sadžaj Pb i Hg utvrđen u mišićnom tkivu smuđa (0.088±0.025 mg/kg), (0.090±0.038 mg/kg), redom. Najveći prosečan sadržaj Cd zabeležen je u mišićnom tkivu šarana (0.103±0.030 mg/kg), dok je najmanji utvrđen u mišićnom tkivu soma (0.032±0.015 mg/kg). U svim ispitivanim uzorcima utvrđene koncentracije Pb i Hg su bile ispod maksimalno dozvoljenih koncentracija koje su regulisane Pravilnikom Republike Srbije i propisima Evropske Unije. Utvrđene koncentracije kadmijuma u mišićnom tkivu šarana, soma i babuške su u više navrata bile iznad maksimalno dozvoljenih koncentracija od 0.05 mg/kg sveže ribe. Riba iz ovog dela Save ne može se u potpunosti smatrati bezbednom za ljudsku ishranu, posebno kada je reč o vrstama koje pokazuju sklonost ka akumulaciji Cd. Uopšteno, sezonske varijacije su bile očiglednije od prostornih razlika, pri čemu je nekoliko vrsta pokazalo umereno veće koncentracije metala u proleće, posebno 2025. godine, što ukazuje na suptilne, ali ponavljajuće sezonske tendencije. Razlike između teritorija pre Beograda i unutar Beograda bile su generalno male, iako su koncentracije žive bile konstantno više na nizvodnoj lokaciji, što ukazuje na blag, ali stabilan prostorni uticaj.