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HEAVY METAL CONCENTRATIONS IN THE MUSCLE TISSUES AND LIVER OF THREE MOST COMMERCIAL FISH SPECIES FROM THE RIVER VJOSA – AOOS (ALBANIA – GREECE)

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ABSTRACT

The concentrations of Fe, Mn, Zn, Cu, Pb and Cd in the muscle tissue and liver of chub *Squalius platyceps*, barbel *Barbus prespensis* and nase *Chondrostoma vardarense* were determined. These three species are among the most widely consumed by local people in the wide Vjosa Basin. Prior to this study, no research had been conducted on heavy metal bioaccumulation in the Vjosa River ecosystem. This research also intends to address the potential health risks for the local population consuming fish resources. The fish species were sampled between the Petran and Kelcyra sites during spring and autumn 2024, with analyses conducted on the concentrations of six heavy metals in muscle tissue and liver. Two instrumental techniques were used for these analyses: atomic absorption spectrophotometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS). The results were analyzed using SPSS software version 30.

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INTRODUCTION

The Vjosa River, the last free-flowing river in Europe and an environmentally significant region, is a vital aquatic habitat for Albanian social, cultural, and ecological elements (Schiemer et al., 2019; Shumka et al., 2018a; Meulenbroek et al., 2018). Among the diverse life in this area, fish and other aquatic animals are noteworthy (Shumka et al., 2018b; Meulenbroek et al., 2020; Shumka et al., 2023). The Pindos Mountains in northern Greece, near the city of Vouvousa, are the source of the 270-kilometer-long River Vjosa, also known as the Aoos in Greek. This system is free-flowing and unrestricted by longitudinal barriers, with the exception of the first 10 kilometers. The source of the river is 2159 meters above sea level, where the Aoos flows from southeast to northwest. The river's route primarily passes within the Vikos-Aoos National Park in Greece for the first 80 kilometers. It is distinguished by a steep slope and a restricted riverbed (Hammerschmied, 2019). The river is known as the Vjosa from the Albanian border downstream, where the Sarandoporos, a major tributary, enters. In the Permet region, the river exhibits a narrow channel pattern, as sediment erosion surpasses sediment deposition, and it immerses itself in alluvial sediments. Upstream of the mouth, the river's slope decreases and its appearance changes to a meandering form, before it enters the Adriatic Sea north of the city of Vlore and the Narta Lagoon (Hauer, 2019; Schiemer et al., 2018; Hammerschmied, 2019).

The Vjosa River is home to at least 34 species of fish inhabiting the river and delta system, of which 29 are native, including eight species endemic to the Balkans (Šanda et al., 2008; Snoj et al., 2009; Shumka et al., 208b; Markova et al., 2010; Stierandová et al., 2016; Shumka et al., 2023 Hammerschmied, 2019). With 12 species, *Cyprinidae* are by far the most specious family, followed by *Mugilidae* (five). *Salmonidae* and *Acipenseridae* are represented by 2 species each. The remaining ten families are represented by a single species. At least four species (*Pseudorasbora parva*, *Oncorhynchus mykiss*, *Carassius* sp., *Gambusia holbrooki*) were introduced into the Vjosa Basin. The lower river reach is populated by other species such as European eel *Anguilla anguilla*, species of the family *Mugilidae* (*Mugil cephalus, Liza ramada, Liza salienes* and *Chelon labrosus*), seabream *Sparus aurata*, seabass *Dincentrarchus labrax*, flatfish *Platichthys flesus*, common sole *Solea* spp., etc., while the resident species associated with saline water are numerically prevalent with the two species *Atherina boyeri* and *Aphanius fasciatus*, showing presence and significance.

As a result of population growth and the development of agriculture and industry, water pollution from heavy metals has increased, leading to growing concerns about the contamination of aquatic organisms by these pollutants, mainly fish, which are an important protein source in our diet. The accumulation of heavy metals, depending on bioaccumulation levels, is considered lethal for organisms in these environments, as heavy metals enter the food chain directly through food or indirectly through muscle membranes and gills (Ribeiro et al., 2005; Jordanova et al., 2018).

Metals can enter aquatic ecosystems through a variety of channels, including mining, industry, agriculture, and atmospheric deposition and erosion. The existence of these ecosystems should be seen as threatened by the presence of heavy metals in the environment. Because heavy metals have a low capacity for biodegradation and are essentially indestructible (Macfarlane and Burchett, 2000), their contamination of the environment is particularly detrimental (Sary and Mohammadi, 2012).

MATERIALS AND METHODS

Originating in the Pindos highlands, east of Ioannina at the base of Mavrovouni (2159 m above sea level) in Greece, the Aoos-Vjosa River flows for more than 272 km across southern Albania and terminates at the Adriatic Sea. It travels in a SE-NW direction. The Aoos River flows through Greece for the first 80 kilometers. The entire catchment area is 6,704 km^2, of which 4,365 km^2 are located within Albania. An outline of the Vjosa watershed, including its principal tributaries and major cities, is shown in Figure 1. Samples of barbel (n = 24), chub (n = 24) and nase were collected in the area between Petran and Kelcyra sites between April 2024 and August 2024 at five sampling locations in two seasons (spring – summer and autumn – winter). The material was collected from the following sampling sites: Kelcyra (40° 18' 14.79" N; 20° 12' 30.18" E), Piskova (40° 16' 36.56" N; 20° 16' 13.10 "E), Permet (40° 12' 55.84" N; 20° 23' 0.96" E), Lengarica (40° 13' 28.67" N; 20° 24' 59.73" E) and Petran (40° 11' 56.94" N; 20° 25' 55.79" E). These species were selected because they are regularly consumed by local people living along the upper reaches of the Vjosa River. Electrofishing was used to catch the fish in accordance with CEN EN 14011, 2003. From each sampling location and season, we gathered and examined fish that were roughly the same size (15 – 20 cm in length) and weight (30 – 50 g). Fish were brought into the lab in containers with aerated river water after being captured. The capture and handling of fish complies with Albania's current legal requirements. Each fish was killed in the lab by cutting its spinal cord directly behind its operculum. The liver and muscle (just behind the dorsal fin) were removed and preserved in liquid nitrogen at -70 °C.

After being measured on an analytical balance (0.0001 g), a sample of 0.2 – 1.0 g of all tissues was put into digestion flasks. All tissue samples were placed into 25 mL plastic volumetric flasks after being wet-digested in a solution of concentrated HNO_3, H_2SO_4, and $HClO_4$ (10:2:1) (Allen, 1989). Air-acetylene flame atomic absorption spectrometry on an Agilent 55A was used to analyze the

Fig 1. Map of the Vjosa-Aoos catchment area. Indicated are major mountain ranges in the Albanian part (dotted line) and the position of major cities: Mif (Mifoli), Sel (Selenica), Poc (Poçem), Tep (Tepelena), Per (Permet), Car (Çarshova), Gji (Gjirokastro)

concentrations of Cu (λ = 324.7 nm; detection limits = 0.06 – 5 mg/L), Mn (λ = 279.5 nm; detection limits = 0.02 – 5 mg/L), Zn (λ = 213.9 nm; detection limits = 0.06–1.5 mg/L), and Fe (λ = 248.3 nm; detection limits = 0.06–15 mg/L) in the liver and kidney. Samples were diluted to 1:5 or 1:10 if needed to provide findings that fell within the calibration curves and detection limits. A graphite furnace Agilent 240Z AA was used to analyze the concentrations of Cu (λ = 324.7 nm; low detection limit = 1 μg/L) in the gills, bones, and muscles, Pb (λ = 217.0 nm; low detection limit = 1 μg/L), and Cd (λ = 228.8 nm; low detection limit = 0.1 μg/L) in an argon atmosphere. Cu, Pb, and Cd were measured using Zeeman background correction in both flame and graphite furnace methods. Solutions and instrument zero were prepared using redistilled water. The same process was used for the blanks, which were deducted from the sample results (three blanks were made for every batch of about 50 samples). Standard additions of the metals under analysis (5, 8, 10, and 15 μg/mL) were used to verify the method's validity, and a linear relationship with a recovery of 95.1%–103.8% was achieved.

All data were analyzed using SPSS 30.00. Data were subjected to descriptive statistical analysis to calculate the mean and standard deviation (SD) for each metal. To assess variations in metal concentrations across different tissues and seasons, a one-way ANOVA was conducted. Post-hoc comparisons were performed using the Tukey HSD test to identify significant differences between groups. The statistical significance was set at $P < 0.05$. To determine the proportion of variance explained by seasonal and tissue differences, Eta-Squared (η^2) values were calculated.

RESULTS AND DISCUSSION

The analysis of heavy metal accumulation in the muscle and liver tissues of 66 fish specimens, conducted during two seasons (April and September 2024), provided significant insights into the pollution levels of the aquatic ecosystem. After Dindi and Shehu (2023) during the monitoring period 2015–2021, a decrease in the concentration of heavy metals in the well water was observed in the Vjosa River Basin. It was thought that this decrease was caused by the improvement in Vjosa River water quality as a result of the closure of Memaliaj lignite mine, located ca. 52 km upstream of the Adriatic coast. Furthermore, according to Dindi and Shehu (2023), the improvement in the quality of Albanian river waters due to the partial cessation of mining activity is reflected in the quality of seawater. The Albanian standards of heavy metal content in drinking water (Albanian Government, 2016) are identical to the EU Directive 98/83/EC standards (European Council,1998), which were updated by the EU Directive 2020/2184 (European Council,2020) that entered into force in January 2023. The recorded data published by Dindi and Shehu (2023) showed values below thresholds of the abovementioned standards. It is worth noting that for the investigated period, there was a high variation of Zn content in water, oscillating from

0.001 to 0.035 mg/l. This may also be linked to subsequent analyses of Zn content in fish muscles and liver.

Metal accumulation patterns differed between muscle and liver tissues across both seasons. In muscle tissue, the concentration hierarchy followed Zn > Fe > Cd > Cu > Mn > Pb, while liver tissue exhibited Fe > Zn > Cu > Cd > Mn > Pb. In muscle tissue, the highest concentration was observed for Zn (27.3 µg/g) in *C. vardarense* during spring, while the lowest was Pb (0.1 µg/g) in *S. platyceps* during the same season. For liver tissue, Fe showed the highest concentration (77.1 µg/g) in *C. vardarense* during autumn, while the lowest was Pb (0.06 µg/g) in *S. platyceps* during spring. Jordanova et al. (2018) recorded similar zinc concentrations with a similar pattern: liver > muscle while working with chub and barber. For both species, the liver's zinc content is noticeably higher in the fall and winter than it is in the spring and summer. The same authors found that copper concentrations decreased in the following order: liver > kidney > bone > gills > muscle. For the liver in both seasons, barbel contained more Cu than chub, and this has also been revealed in our case.

A two-way ANOVA was performed to analyze differences in heavy metal concentrations across species, tissues, and seasons. Descriptive statistics were first calculated for metal concentrations in two tissues (liver and muscle) during two seasons (autumn and spring) across three species of the family *Cyprinidae*. The highest mean metal concentration was observed in liver tissue during autumn (23.578 µg/g), while the lowest was found in muscle tissue during spring (5.217 µg/g). Amongst the species studied, *Chondrostoma vardarensis* showed the highest mean concentration (15.328 µg/g), though differences between species were not statistically significant. Following Meulenbroek et al. (2018), *B. prespensis* and *Ch. vardarense* appear to be part of long migratory species/potamodromous, covering average annual migration distances of 20 kilometers or more, while *S. platyceps* is part of a short migratory species, covering average annual migration distances of less than 20 kilometers. Based on the rheophilic guild and spawning guild, both *B. prespensis* and *Ch. vardarense* are respectively rheophilic and lithophilic, while *S. platyceps* is indifferent and lithophilic. Furthermore, referring to the feeding guild, the first two species are respectively insectivorous, herbivorous and herbivorous, while *S. platyceps* is omnivorous (FishBase, 2025; Kottelat and Freyhof, 2007).

The descriptive analysis, as displayed in Table 1 and Figure 2, provides an overview of heavy metal concentrations in muscle and liver samples collected during the spring and autumn seasons. The concentration of Fe in muscle was found to be relatively high compared to other metals, with a mean of 7.40 ± 0.70 in spring and 8.10 ± 1.25 in autumn. Zn exhibited substantial variability, with a mean of 19.53 ± 9.05 in spring and 18.17 ± 3.69 in autumn, indicating an inconsistent distribution across samples. Pb showed the lowest concentration levels in both seasons, with mean values of 0.17 ± 0.06 in spring and 0.20 ± 0.00 in autumn. Regarding the liver samples, the Fe concentration was significantly higher than in muscle samples, with mean values of 60.93 ± 4.84 in spring and 70.63 ± 5.71 in autumn. Zn concentration was notably higher in liver samples compared to muscle, with a mean of 30.23 ± 13.48 in spring and 54.00 ± 10.10 in autumn. Pb levels remained consistently low in both spring (0.07 ± 0.01) and autumn (0.10 ± 0.02). The metal with the highest average concentration is iron (Fe), and the species in which this metal appears at the highest concentration is *Chondrostoma vardarensis*, with a concentration of 66.4 µg/g in the liver during spring. Heavy metal concentrations in muscle and liver samples from the three fish species caught in both seasons follow the sequence: Zn > Fe > Cd > Cu > Mn >Pb (muscle samples) and Fe > Zn > Cu > Cd > Mn > Pb (liver samples).

A one-way ANOVA was conducted to assess the statistical significance of differences in heavy metal concentrations among the different groups, as displayed in Table 2. Our data ($P < 0.001$) indicate significant differences in heavy metal concentrations across the analyzed groups. Eta-Squared Values range from 0.83 to 0.98, indicating that the majority of variance in metal concentrations is explained by seasonal and tissue differences. The post-hoc Tukey HSD test was performed to identify specific significant differences between heavy metal concentrations in different sample groups. Our data show that the Zn concentrations in muscle samples during the spring season differed significantly from Fe, Mn, Cu, Cd, and Pb ($P < 0.05$). Furthermore, no significant differences were observed among other metals. Also, Fe concentration levels in muscle during autumn seasons were significantly different from Mn, Zn, Cu, Cd, and Pb ($P < 0.05$). Zn exhibited the most significant variations between groups. Fe, Zn, Cu, and Cd concentrations in the liver during spring and autumn exhibited significant differences when compared to Mn and Pb ($P < 0.001$). The highest variability was observed in Fe and Zn concentrations.

Our findings showed that the species of fish and the time of year affected the metal content of fish. Our work is the first to examine the levels of heavy metals in fish species found in the River Vjosa-Aoos. We assume that greater concentrations of metals in water and/or sediment throughout the spring–autumn season are likely caused by seasonal variations in water flow. Therefore, it is most likely the cause of the seasonal variations in fish metal content, with higher levels in spring compared to autumn. All measured values of heavy metal content in fish's muscle and liver do not exceed the regulatory limits for fish consumption (EC, 2023).

Table 1. Heavy metal concentrations in muscle and liver samples collected during the spring and autumn of 2024

Species	HM	N spring	N autumn	Muscle		Liver	
				Spring	Autumn	Spring	Autumn
Barbus perspensis	Fe (µg/g)	12	12	6.9 ± 2.2	7.1 ± 2.7	59.2 ± 12.2	68.5 ± 22.5
Squalius platyceps		12	12	7.1 ± 2.3	7.7 ± 2.6	57.2 ± 32.2	66.3 ± 41.2
Chondrostoma vardarensis		10	8	8.2 ± 7.4	9.5 ± 12.6	66.4 ± 44.1	77.1 ± 51.1
Barbus perspensis	Mn (µg/g)	12	12	0.6 ± 0.1	0.5 ± 0.3	1.4 ± 0.2	1.3 ± 0.5
Squalius platyceps		12	12	0.7 ± 0.1	0.6 ± 0.2	1.6 ± 0.5	1.8 ± 0.6
Chondrostoma vardarensis		10	8	0.8 ± 0.3	0.7 ± 0.1	1.7 ± 0.4	1.9 ± 0.3
Barbus perspensis	Zn (µg/g)	12	12	9.6 ± 1.8	16.5 ± 8.9	16.3 ± 7.2	43.2 ± 25.5
Squalius platyceps		12	12	21.7 ± 11.1	15.6 ± 11.2	31.2 ± 6.5	55.6 ± 33.4
Chondrostoma vardarensis		10	8	27.3 ± 18.2	22.4 ± 10.3	43.2 ± 12.8	63.2 ± 26.3
Barbus perspensis	Cu (µg/g)	12	12	1.0 ± 0.1	1.1 ± 0.5	9.6 ± 3.9	9.7 ± 4.6
Squalius platyceps		12	12	0.9 ± 0.2	0.8 ± 0.5	11.3 ± 6.8	4.8 ± 2.6
Chondrostoma vardarensis		10	8	1.1 ± 0.6	1.2 ± 0.7	10.8 ± 3.1	8.9 ± 4.6
Barbus perspensis	Cd (µg/g)	12	12	4.5 ± 0.8	4.8 ± 1.4	9.6 ± 3.9	9.7 ± 4.6
Squalius platyceps		12	12	0.8 ± 0.2	0.9 ± 0.4	2.6 ± 0.8	2.9 ± 0.5
Chondrostoma vardarensis		10	8	2.2 ± 0.5	2.6 ± 0.6	8.9 ± 4.2	9.2 ± 4.3
Barbus perspensis	Pb (µg/g)	12	12	0.2 ± 0.1	0.2 ± 0.1	0.07 ± 0.1	0.09 ± 0.1
Squalius platyceps		12	12	0.1 ± 0.2	0.2 ± 0.4	0.06 ± 0.1	0.12 ± 0.1
Chondrostoma vardarensis		10	8	0.2 ± 0.1	0.2 ± 0.1	0.08 ± 0.1	0.09 ± 0.1

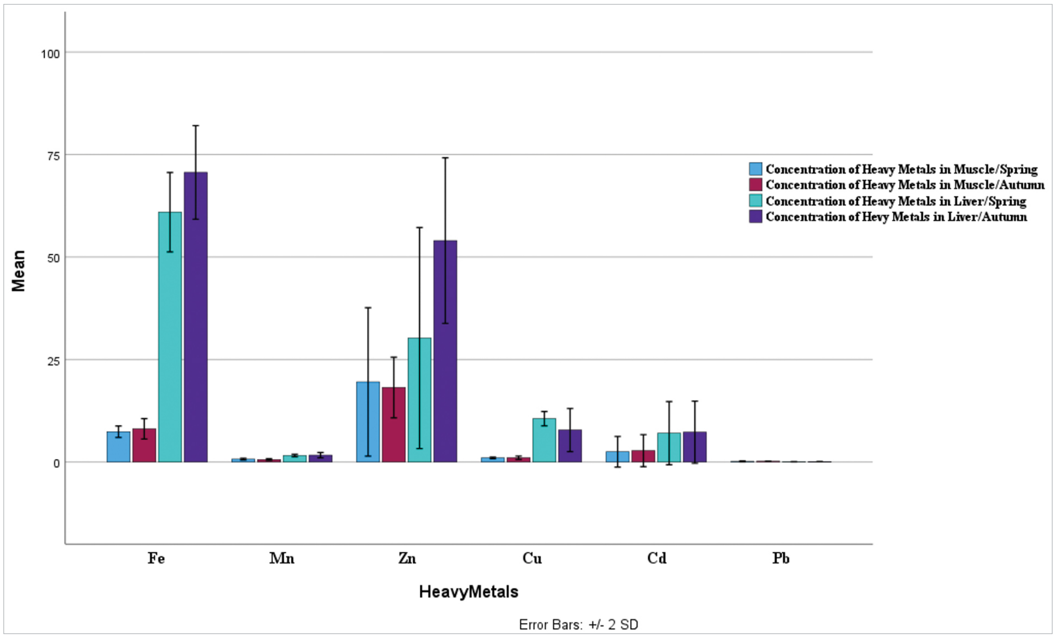

Fig 2. Mean concentration of heavy metals in muscle and liver tissues during spring and autumn

Table 2. Concentration of heavy metals in the muscle and liver of barbell, chub and nase collected from River Vjosa-Aoos spring–autumn 2024

Variables	Heavy Metals	N	Mean ± SD	F	Sig.
Concentration of Heavy Metals in Muscle Samples/ Spring	Fe	3	7.40000 ± 0.700000	11.775	<0.001
	Mn	3	0.70000 ± 0.100000		
	Zn	3	19.53333 ± 9.046731		
	Cu	3	1.00000 ±.100000		
	Cd	3	2.50000 ±1.868154		
	Pb	3	0.16667 ± 0.057735		
Concentration of Heavy Metals in Muscle Samples/Autumn	Fe	3	8.10000 ± 1.249000	46.409	<0.001
	Mn	3	0.60000 ± 0.100000		
	Zn	3	18.16667 ± 3.693688		
	Cu	3	1.03333 ± 0.208167		
	Cd	3	2.76667 ± 1.955335		
	Pb	3	0.20000 ± 0.000000		
Concentration of Heavy Metals in Liver/Spring	Fe	3	60.9333 ± 4.83873	45.011	<0.001
	Mn	3	1.5667 ± 0.15275		
	Zn	3	30.2333 ± 13.47603		
	Cu	3	10.5667 ± 0.87369		
	Cd	3	7.0333 ± 3.85530		
	Pb	3	0.0700 ± 0.01000		
Concentration of Heavy Metals in Liver/Autumn	Fe	3	70.6333 ± 5.70731	108.229	<0.001
	Mn	3	1.6667 ± 0.32146		
	Zn	3	54.0000 ± 10.09554		
	Cu	3	7.8000 ± 2.62869		
	Cd	3	7.2667 ± 3.78990		
	Pb	3	0.1000 ± 0.01732		

CONCLUSION

The statistical analysis revealed significant seasonal and tissue-based variations in heavy metal concentrations. Fe and Zn were the most abundant metals across all samples, with liver tissues displaying substantially higher concentrations than muscle. Pb exhibited consistently low levels in both muscle and liver, irrespective of the season. ANOVA and post-hoc analyses confirmed that these differences were statistically significant, emphasizing the need for further research into the environmental and biological factors influencing these metal accumulations.

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KONCENTRACIJE TEŠKIH METALA U MIŠIĆNOM TKIVU I JETRI TRI NAJPOPULARNIJE VRSTE RIBA IZ RIJEKE VJOSA – AOOS (ALBANIJA-GRČKA)

SAŽETAK

Određene su koncentracije Fe, Mn, Zn, Cu, Pb i Cd u mišićnom tkivu i jetri klena (*Squalius platyceps*), mrene (*Barbus prespensis*) i vardarskog podusta (*Chondrostoma vardarense*). Ove tri vrste su među onima koje lokalno stanovništvo u širokom slivu rijeke Vjose često koristi za konzumaciju. Prije ove studije nisu provedena istraživanja o bioakumulaciji teških metala u ekosustavu rijeke Vjose. Ovo istraživanje također ima za cilj pozabaviti se potencijalnim zdravstvenim rizicima za lokalno stanovništvo koje konzumira riblje resurse. Ribe su uzorkovane na području između lokacija Petran i Kelcyra u proljeće i ljeto 2024. godine, a provedene su analize koncentracija šest teških metala u mišićnom tkivu i jetri. Za ove analize korištene su dvije instrumentalne tehnike: atomska apsorpcijska spektrofotometrija (AAS) i masena spektrometrija s induktivno spregnutom plazmom (ICP-MS). Rezultati su analizirani pomoću SPSS softvera verzije 30.

Ključne riječi: teški metali, ribe, riječni ekosustav, akumulacija, *Cyprinidae*

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