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ASSESSMENT OF POTENTIALLY TOXIC ELEMENTS IN FISH FROM ALEKSANDROVAC RESERVOIR AFTER A MASS MORTALITY

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ABSTRACT. Small water bodies are particularly vulnerable to environmental disturbances, with fish mass mortality increasingly associated with anthropogenic stressors. This study researches the potential role of potentially toxic elements (PTEs) in the mass fish die-off in December 2012 at the Aleksandrovac Reservoir in Serbia. Muscle, liver, and gills of twenty specimens of silver carp (*Hypophthalmichthys molitrix*) and common bream (*Abramis brama*) were analyzed for concentrations of 14 PTEs. Common breams had higher PTE levels due to their bottom-feeding habits and sediment exposure. The liver showed the highest concentrations of potentially toxic elements (PTEs), and elevated Metal Pollution Index (MPI) values in this organ indicate prolonged exposure to environmental contaminants. The concentrations of As, Cd, Pb, Hg, Fe, Cu, and Zn in muscles were below Serbian and EU legislation limits, though some common bream specimens showed slightly elevated Cd and Pb concentrations. While PTEs did not directly cause the die-off, their accumulation poses an ecological risk, emphasizing the need for permanent monitoring.

Keywords: microaccumulation, silver carp, common bream, fish tissue, human health risk.

INTRODUCTION

Small water bodies, such as lakes and ponds, are particularly vulnerable to environmental changes that often have no or minimal impact on larger water bodies (JEPPESEN *et al*., 2007). According to BIGGS *et al.* (2014), the risk of pollution is magnified in these ecosystems due to their smaller water bodies, which offer less capacity to buffer against contaminants compared to larger lakes or rivers. This sensitivity places small water bodies

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among the most threatened ecosystems, as their biological communities are often poorly able to adapt to sudden shifts in water quality, temperature, or chemical composition.

Mass fish mortality is a common appearance in marine, estuarine, or freshwater systems (MEYER and BARCLAY, 1990). These incidents are happening more often worldwide, driven by a combination of natural and human-induced factors. Human-related contributors include agricultural pollutants (such as pesticides, fertilizers, and animal waste), industrial contaminants (like metals and hydrocarbons), municipal wastewater, and transportation activities (HASLOUER, 1979).

Mass fish mortality in freshwater ecosystems has also been documented in Serbia. One such incident occurred at Aleksandrovac Reservoir, located 8 km southwest of Vranje, Serbia. In 2008, two major die-offs resulted in the loss of 3,000 kg of fish. Interestingly, the reservoir was drained at the end of 2009, and a restoration program began in the spring of 2010. The remaining fish were removed, the water was drained from the reservoir, and full rehabilitation was carried out based on the preliminary project by PROKIĆ *et al.* (2008). In the summer of 2010, the reservoir was re-filled and subsequently used for tourism and recreation. On December 20, 2012, another large-scale fish die-off took place. Almost the entire fish population, over 1,700 kg in total has perished, including species such as common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), common bream (*Abramis brama*), catfish (*Silurus glanis*), Prussian carp (*Carassius gibelio*), asp (*Aspius aspius*), and chub (*Squalius cephalus*). ĐORĐEVIĆ and SIMIĆ (2014), ĐORĐEVIĆ *et al.* (2015), and SVIRČEV *et al.* (2016) identified cyanobacterial blooms as the primary cause of fish mortality, along with specific environmental conditions in this small water body. Additionally, low oxygen levels, large silt deposits, macrophyte overgrowth, and various waste materials were suspected to be contributing factors; however, the exact cause was not conclusively determined.

Given the various potential causes of fish mortality in Aleksandrovac Reservoir, this study investigates whether the presence of potentially toxic elements in fish contributed to the mass die-off.

MATERIALS AND METHODS

Aleksandrovac Reservoir is located in the southernmost part of Serbia (N 42º29′22″, E 21º53′54″) at an elevation of 412 m (Fig. 1). Created in 1964 on the Aleksandrovac River for irrigation purposes, it has undergone significant ecological changes over time. Increasing anthropogenic activity and nutrient runoff from nearby agricultural fields, driven by wind and water erosion, have contributed to severe eutrophication. The reservoir's relatively shallow depth (maximum 4 m, average 2 m) has further worsened this issue. By the early 21st century, it had become highly eutrophic, with conditions approaching anoxia.

After the second large-scale fish die off on December 20, 2012, sampling was conducted on December 22. Two species were selected and sampled from the entire fish population that died off: silver carp (*H. molitrix*) and common bream (*A. brama*). A total of 20 individuals were sampled, with ten from each species. Before dissection, total length (TL; measured to the nearest mm) and body weight (BW; measured to the nearest g) were recorded. Fish health status was assessed using Fulton's condition factor (CF), calculated according to the formula proposed by RICKER (1975):

$$CF = 100BW/TL^3 \quad (1)$$

Fish dissection was performed using a decontaminated ceramic knife. Tissue samples (right dorsal muscle below the dorsal fin, right gills – second arch, and liver) were rinsed with distilled water, packed in polyethylene bags, and transported on ice in a portable hand-held refrigerator to the laboratory.

Figure 1. Map of the studied Aleksandrovac Reservoir.

In the laboratory, samples were weighed on an electronic scale (with an accuracy of ±0.01 g) and then stored at –20 °C until analysis. Prior to digestion with a microwave (Christ Alpha 2-4 LD, Harz, Germany), the samples were dried in a lyophilizer (Christ Alpha 2-4 LD, Harz, Germany) and weighed again. Portions of the dried samples, weighing between 0.3 and 0.5 g, were digested in a mixture of 65% nitric acid and 30% hydrogen peroxide (Suprapur®, Merck, Darmstadt, Germany) in a 10:2 volume ratio at 200 °C for 20 minutes. After cooling to room temperature, the solution was not filtered but diluted to a final volume of 25 ml with ultrapure water.

The concentrations of elements in fish tissues were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) with a Thermo Fisher Scientific iCAP 6500 Duo ICP (Cambridge, UK). The analysis was conducted at the following wavelengths (nm): Al 391.402, As 188.032, Cd 226.602, Co 221.618, Cr 204.542, Cu 322.764, Fe 257.921, Hg 183.940, Mn 260.353, Ni 234.606, Pb 222.354, Se 199.093, Sn 245.162, and Zn 207.194. Standard muscle reference material (DORM-4, National Research Council of Canada) was digested and analyzed in triplicate to ensure quality control. Recovery rates ranged from 95.6% to 107.14%. Mean values and standard deviations were calculated for each group, and element concentrations were expressed as mg/kg dry weight (dw). These concentrations were then converted to mg/kg wet weight and used to calculate the metal pollution index (MPI). Additionally, the concentrations of Cd, Hg, Pb, As, Cu, and Zn in fish muscles were compared with maximum permissible concentrations (MPC) in fish meat as determined by the national legislation of Republic of Serbia (ANONYMUS, 2018) and the European Union (ANONYMUS, 2006). According to these regulations, the MPCs for As, Cd, Cu, Hg, Pb, and Zn are 2.0, 0.05, 30.0, 0.50, 0.30, and 100.0 mg/kg ww, respectively. These analyses are essential for evaluating the safety of fish consumption and ensuring compliance with established health standards.

The Metal Pollution Index (MPI) was calculated to compare the total metal concentration in fish tissues using the following formula proposed by USERO *et al.* (1997):

$$MPI = (c1 \times c2 \times \ldots \times cn)^{1/n} \quad (2)$$

where c is the concentration of the metal n in the sample (mg/kg ww).

All values are presented as mean ± standard deviation (SD). The Shapiro-Wilk test was used to assess data normality to begin the statistical analysis. If the data followed a normal distribution, differences between the two fish species were analyzed using the Independent Samples T-Test. Otherwise, the non-parametric Mann-Whitney test was applied. The significance level (α) was set at 5%. All analyses were conducted using the SPSS 26.0 statistical software for Windows (IBM Corp., 2019).

RESULTS AND DISCUSSION

The analyzed silver carp specimens had an average weight of 902.33 ± 147.80 g and a total length of 42.20 ± 2.42 cm, with a CF of 0.97 ± 0.19. In contrast, the common bream specimens weighed 103.00 ± 11.30 g on average, with a total length of 22.50 ± 0.60 cm and a CF of 0.90 ± 0.34. The CF value recorded in this study highlights the potential environmental decline in the Aleksandrovac Reservoir, given that a CF below one suggests that fish species are in poor overall health. While previous research by LAFLAMME *et al.* (2000), RAJOTTE and COUTURE (2002), and ZHELEV and TSONEV (2018) has linked a decline in CF to highly polluted environments, KROON *et al.* (2017) emphasized the importance of critically evaluating the specificity and reliability of CF as a biomarker.

When both fish species are observed together, the highest concentrations of Hg, Cd, and Pb were found in muscle tissue, whereas As, Co, Cr, Cu, Fe, Se, Sn, and Zn were the most concentrated in the liver. Meanwhile, the gills contained the highest concentrations of Al, Mn, and Ni (Table 1). Conversely, the lowest concentrations of Al, As, Cu, Fe, Hg, Mn, Se, Sn, and Zn were observed in muscle tissue, Ni and Pb had the lowest levels in the liver, while Cd, Co, and Cr showed the lowest concentrations in the gills. The highest concentrations of As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, Sn, and Zn were detected in common bream, while silver carp exhibited the highest concentrations of Al, Fe, and Hg (Table 1). Statistical analysis revealed significant differences between the two fish species in Cr, Mn, and Zn concentrations, with notably higher levels found in the tissues of common bream (except Cr, which had a lower concentration in the gills of common bream). Given that the element concentrations in the water column of the Aleksandrovac Reservoir were relatively low (SVIRČEV *et al.*, 2016), the elevated PTE levels detected in common bream support the hypothesis that dietary intake is the main pathway for PTE accumulation (DALLINGER and KAUTZKY, 1985). Our results indicate that common bream feeding on invertebrates, which have absorbed elements from both the water and surrounding sediment, tend to accumulate higher concentrations of PTEs. Conversely, STORELLI *et al.* (2005) reported that planktivorous species tend to accumulate higher levels of As. Nevertheless, our findings did not align with this pattern, as common bream showed consistently higher As concentrations in all three tissues compared to silver carp.

In the study by SVIRČEV *et al.* (2016), where the fish species researched in Aleksandrovac Reservoir was not specified, higher concentrations of Co, Cr, Cu, Mn, and Ni were found compared to silver carp, and higher concentrations of Co, Cr, Cu, Hg, Mn, and Ni were detected compared to common bream in our study. In our earlier research (MILOŠKOVIĆ *et al.*, 2022) examining PTE levels in Prussian carp, we observed elevated concentrations of As, Cd, Cr, Hg, and Mn compared to silver carp. Additionally, the levels of Al, Fe, and Hg were higher in Prussian carp compared to common bream in the current study.

The concentrations of As, Cd, Pb, Hg, Fe, Cu, and Zn found in the muscle tissue of both fish species were below the MPCs established by the European Union (ANONYMUS, 2006) and the Republic of Serbia (ANONYMUS, 2018). These results indicate that contamination of fish by these elements in the Aleksandrovac Reservoir is highly unlikely and does not pose a significant health risk.

Table 1. Element concentrations (mg/kg dw) in the muscle, liver, and gill tissues of two fish species – silver carp and common bream from the Aleksandrovac Reservoir.
Values are presented as mean ± SD, with ND denoting concentrations below the detection limit.

		Silver carp	Common bream
Al	Muscle	3.95 ± 3.80[a*]	3.80 ± 2.80[a]
	Liver	ND	ND
	Gills	16.05 ± 13.00[a]	14.30 ± 3.15[a]
As	Muscle	0.45 ± 0.20[a]	0.55 ± 0.25[a]
	Liver	0.950 ± 0.015[a]	2.75 ± 0.25[a]
	Gills	0.50 ± 0.15[a]	1.00 ± 0.03[a]
Cd	Muscle	0.150 ± 0.005[a]	0.350 ± 0.015[a]
	Liver	0.20 ± 0.001[a]	0.300 ± 0.025[a]
	Gills	0.015 ± 0.005[a]	0.010 ± 0.005[a]
Co	Muscle	0.150 ± 0.025[a]	0.015 ± 0.015[a]
	Liver	0.30 ± 0.10[a]	2.70 ± 0.65[a]
	Gills	ND	ND
Cr	Muscle	0.8 ± 0.1[a]	1.35 ± 0.25[b]
	Liver	0.24 ± 0.10[a]	7.40 ± 2.05[b]
	Gills	1.35 ± 0.65[a]	0.015 ± 0.015[b]
Cu	Muscle	0.85 ± 0.05[a]	0.95 ± 0.40[a]
	Liver	11.40 ± 3.00[a]	16.10 ± 5.25[a]
	Gills	2.65 ± 0.30[a]	2.60 ± 0.80[a]
Fe	Muscle	12.85 ± 10.40[a]	24.15 ± 8.60[a]
	Liver	1,139.50 ± 34.50[a]	250.00 ± 28.00[a]
	Gills	181.50 ± 76.75[a]	223.60 ± 43.20[a]
Hg	Muscle	0.2 ± 0.05[a]	0.0015 ± 0.0025[a]
	Liver	ND	ND
	Gills	0.045 ± 0.040	ND
Mn	Muscle	0.65 ± 0.50[a]	2.40 ± 0.35[b]
	Liver	3.50 ± 0.10[a]	7.30 ± 0.45[b]
	Gills	39.75 ± 16.30[a]	64.55 ± 10.55[b]
Ni	Muscle	0.05 ± 0.10[a]	0.13 ± 0.04[a]
	Liver	0.10 ± 0.10	ND
	Gills	0.15 ± 0.05[a]	0.20 ± 0.03[a]
Pb	Muscle	1.10 ± 0.45[a]	1.65 ± 0.70[a]
	Liver	ND	ND
	Gills	1.55 ± 0.90[a]	1.000 ± 0.025[a]
Se	Muscle	1.00 ± 0.15[a]	1.30 ± 0.20[a]
	Liver	3.15 ± 0.50[a]	4.45 ± 0.25[a]
	Gills	1.10 ± 0.10[a]	1.75 ± 0.30[a]
Sn	Muscle	ND	0.015 ± 0.03
	Liver	0.750 ± 0.005[a]	0.80 ± 0.01[a]
	Gills	0.60 ± 0.05[a]	0.65 ± 0.04[a]
Zn	Muscle	15.60 ± 4.25[a]	32.40 ± 5.80[b]
	Liver	106.05 ± 27.50[a]	915.30 ± 167.5[b]
	Gills	71.25 ± 12.60[a]	613.00 ± 127.85[b]

[*]different letters within a row indicate statistically significant differences in element concentrations between the tissues of the two fish species, with a significance level of $p < 0.05$.

However, several common bream individuals (three of ten) exhibited higher concentrations of Cd (0.07 mg/kg) and Pb (0.33 mg/kg) than the maximum allowed limits. As these concentrations were slightly above the MPCs and knowing that both Cd and Pb are of anthropogenic origin and can cause serious health problems in humans, the need for continuous monitoring is emphasized. Previous research conducted in the Aleksandrovac Reservoir by SVIRČEV *et al.* (2016) and MILOŠKOVIĆ *et al.* (2022) similarly reported that the concentrations of potentially toxic elements in the analyzed species were within the prescribed MPC limits.

The Metal Pollution Index (MPI) provides a more reliable assessment of fish contamination status than individual accumulation patterns, as it accounts for all analyzed metals simultaneously. According to the MPI values (Fig. 2), muscle tissue exhibited lower levels of metal contamination compared to the gills and liver. MPI is typically higher in fish liver than in other tissues because it serves as a primary site for metal accumulation due to its ability to bind and transform metals through specialized proteins and metabolic activity (CANLI and ATLI, 2003; YILMAZ, 2009; AUTHMAN *et al*., 2015), highlighting the liver's importance as both a metal sink and a reliable indicator for assessing aquatic metal pollution.

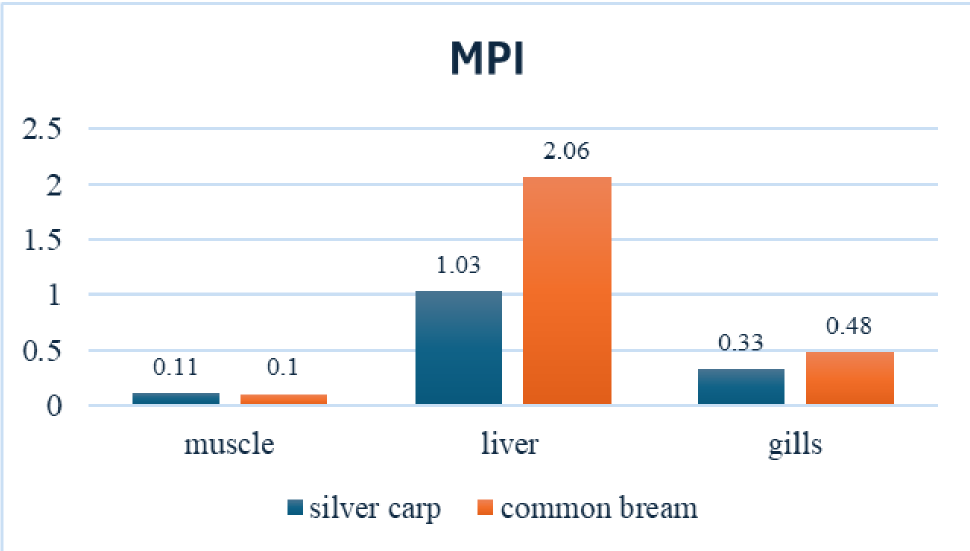

Figure 2. Metal Pollution Index (MPI) values indicating the overall accumulation of elements in the muscle, liver, and gill tissues of silver carp and common bream.

A statistically significant difference in MPI values for the liver was observed between the two examined species, with common bream exhibiting notably higher values. Common bream typically exhibits higher MPI values in the liver than other fish species, primarily due to its ecological characteristics and behavior. Its benthivorous feeding habits and a relatively sedentary lifestyle result in extended exposure to contaminated sediments and increased ingestion of organisms from polluted areas (BERVOETS *et al*., 2001; KLAVINS *et al*., 2009). These results are in agreement with the study by SVIRČEV *et al.* (2016), which reported increased concentrations of the analyzed elements in the sediments of the Aleksandrovac Reservoir.

CONCLUSIONS

This study does not confirm a direct link between PTE accumulation and the observed mass fish mortality in Aleksandrovac Reservoir. However, their presence and other stressors, such as cyanobacterial blooms, low oxygen levels, and poor water quality, indicate a combined impact on fish health.

The generally low concentrations of PTEs in muscle tissue suggest that these fish are safe for human consumption, with minimal associated health risks. Although PTE concentrations in fish tissues were largely within the MPC established by Serbian and European legislation, isolated cases in common bream showed slight exceedances for Cd and Pb, which warrants caution. These findings highlight the necessity for continuous ecological monitoring of small, eutrophic water bodies, particularly those exposed to anthropogenic pressure, in order to prevent future ecological disturbances and ensure both aquatic ecosystem and human health. The assessment protocol must encompass comprehensive chemical and biological analyses of the aquatic environment. In particular, measurements of dissolved oxygen should be combined with systematic evaluation of cyanobacterial populations, given that cyanobacterial blooms may precipitate the release of toxins harmful to aquatic fauna and potentially humans. Equally essential is the quantification of trace elements and other potentially toxic substances present both in the water column and within resident biota. To accurately characterize the ecosystem's health status, an initial monitoring phase should be conducted at monthly intervals; once baseline conditions are established and understood, subsequent surveillance may proceed on a seasonal basis.

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