Knowl. Manag. Aquat. Ecosyst. 2023, 424, 4
© K. Jovičić *et al.*, Published by EDP Sciences 2023
https://doi.org/10.1051/kmae/2022027

www.kmae-journal.org

Knowledge & Management of Aquatic Ecosystems

Journal fully supported by Office français de la biodiversité

RESEARCH PAPER OPEN ACCESS

Prospects of fish scale and fin samples usage for nonlethal monitoring of metal contamination: a study on five fish species from the Danube River

Katarina Jovičić[1,*], Saša Janković[2], Dragica M. Nikolić[2], Vesna Đikanović[1], Stefan Skorić[3], Jasmina Krpo-Ćetković[4] and Ivan Jarić[5,6,7]

[1] Institute for Biological Research "Siniša Stanković" – National Institute of the Republic of Serbia, University of Belgrade, Bulevar Despota Stefana 142, 11060 Belgrade, Serbia
[2] Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia
[3] Institute for Multidisciplinary Research, University of Belgrade, Kneza Višeslava 1, 11030 Belgrade, Serbia
[4] Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia
[5] Biology Centre of the Czech Academy of Sciences, Institute of Hydrobiology, České Budějovice, Czech Republic
[6] University of South Bohemia, Faculty of Science, Department of Ecosystem Biology, České Budějovice, Czech Republic
[7] Université Paris-Saclay, CNRS, AgroParisTech, Ecologie Systématique Evolution, Orsay, France

Received: 5 July 2022 / Accepted: 13 December 2022

Abstract – The development of nonlethal methods for the monitoring of environmental contamination is essential to minimize the negative effects on studied species and communities. Fish scales and fin clips can be used as nonlethal indicators of water quality given that they are in direct contact with the environment and can accumulate high concentrations of metals and trace elements. Fin clipping causes minimal harm to fish and it does not affect fish growth or survival. In this study, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn concentrations were measured by inductively-coupled plasma mass spectrometry (ICP-MS) in scales, fins, and muscle of common bream (*Abramis brama*), white bream (*Blicca bjoerkna*), wels catfish (*Silurus glanis*), northern pike (*Esox lucius*), and pikeperch (*Sander lucioperca*) from the Danube River. The analysis showed a positive correlation for Hg between scales and muscle in pikeperch. Anal fin and muscle were positively correlated in white bream for Hg, in wels catfish for Cu, and in northern pike and pikeperch for As. The results suggest that scales and fins have a potential to be used as indicators of muscle tissue contamination with As, Cu, and Hg, depending on species' ecological traits.

Keywords: Fish tissue / water quality indicators / toxic metals / trace elements / ICP-MS

1 Introduction

Numerous ecotoxicological studies and monitoring programs have been conducted worldwide on metal and trace element accumulation in various fish tissues (Uysal *et al.*, 2009; Begum *et al.*, 2013; Squadrone *et al.*, 2013). Most studies focused on gills, liver, and muscle tissue, while accumulation patterns in other tissues were rarely assessed. In Jovičić *et al.* (2014), the authors focused on the extent of elemental accumulation in such rarely studied tissues to identify which of them could be used as indicators in environmental monitoring programs. However, sampling of fish tissues for contamination analysis comes with an inherent problem – the necessity to sacrifice the fish, which can produce negative effects on studied species and communities. Moreover, lethal sampling may not be feasible in protected waters or in studies on threatened species.

For this reason, continuous efforts are made to minimize lethal sampling procedures and negative effects of monitoring programs by developing nonlethal methods for this purpose, and consequently reducing the number of fish killed. Nonlethal sampling has advantages over the whole tissue sampling procedures – it provides a possibility to collect larger samples, as well as to sample rare and endangered species without causing mortality (Baker *et al.*, 2004). Most studies on nonlethal sampling have focused on monitoring of mercury (Gremillion *et al.*, 2005; Schmitt and Brumbaugh, 2007; Rolfhus and Sandheinrich, 2008; Červenka *et al.*, 2011; Valová *et al.*, 2013; Cerveny *et al.*, 2016). Commonly considered

*Corresponding author: katarina.jovicic@ibiss.bg.ac.rs

nonlethal sampling techniques in fish include the analysis of scales and fins, as well as tissue biopsy of blood, liver, or muscle via needles or punches (Valová *et al.*, 2013). The use of a biopsy punch to obtain a small tissue sample, as an alternative to the homogenized fillet method, has been widely used in fish studies for more than 30 years, mainly for mercury and selenium (Uthe, 1971; Baker *et al.*, 2004; Knight *et al.*, 2019; Stahl *et al.*, 2021). Another nonlethal method used in studies of aquatic food webs is the stable isotope analysis, especially of carbon and nitrogen. In such studies, fish scales and fin clips are used as nonlethal alternatives to muscle tissue (Fincel *et al.*, 2012; Vašek *et al.*, 2017; McCloskey *et al.*, 2018).

Scales represent hard calciferous structures on fish skin and, due to their external position, they are more exposed to various contaminants from the environment. They increase in size during fish growth by incorporating nutrients and elements supplied in the blood, which are then permanently stored in the scale (Wells *et al.*, 2003). By knowing the age of fish when each growth ring on a scale was formed, it is possible to analyze its chemical composition and thus learn about the substances to which the fish was exposed at certain stages of its life (Farrell *et al.*, 2000; Beaudin *et al.*, 2010; Łuszczek-Trojnar and Nowacki, 2021). Elemental signature in scales remains the same even after maturation and migration (Yamada and Mulligan, 1982), and some studies suggest that it is possible to discriminate elemental signatures in scales of fish from rivers with different levels of pollution (Coillie and Rousseau, 1974). However, there is evidence that scales may stop growing or even be resorbed during periods of physiological stress (Bilton and Robins, 1971; Bilton, 1975). Scales can regenerate rapidly if lost or damaged, so entire time periods can be missed, and their elemental composition may change over time (Wells *et al.*, 2000b). Consequently, scales can be used as environmental tracers for analyses of both short and long-term metal and trace element pollution, provided that scales are randomly sampled from different body parts of each individual fish.

Trace element concentrations in fish scales show a linear correlation with concentrations in ambient water (Wells *et al.*, 2000a). Furthermore, metal accumulation in scales can be detected within days of exposure (Cooley and Klaverkamp, 2000). The main advantage of using scales as metal pollution indicators compared to other tissues is the possibility to use archived fish scales as predictors of past pollution (Cobelo-García *et al.*, 2017; Morán *et al.*, 2018). In addition, scales can be used as a sorption material for the extraction of various pollutants (heavy metal ions, dyes, antibiotics) from natural and wastewater (Shaikhiev *et al.*, 2020). For this reason, scales can act as potential nonlethal indicators of water quality (Khanna *et al.*, 2007). They can accumulate high concentrations of metals and trace elements due to the binding of metals to mucus and formation of strong metal-mucus complexes (Negi and Maurya, 2015). Exposure of scales to metals and trace elements can lead to ultrastructure damage in fish scales, which can be used as an effective noninvasive indicator of water pollution (Vaid and Hundal, 2019).

Overall, the use of scales as an indicator of water pollution is not fully validated because few studies have examined the relationship between the level of contamination in scales and other fish tissues. There is a need for a standardized protocol for cleaning scales to remove surface impurities. In addition, for further analyses of the interactions between metals and scales, it would be useful to obtain information on the structure of scales using methods such as scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM-EDS).

Removal of one or more fins from a fish has been used as a method for fish marking (Gjerde and Refstie, 1988). The advantage of using fin clips for metal and trace element analysis is that clipping can be conducted with a minimal harm to the organism and has no significant effect on its growth and survival (Gjerde and Refstie, 1988). Additionally, fins completely regenerate within 1–2 months after sampling (Sanderson *et al.*, 2009). Elemental signature in fins is highly correlated with ambient water chemistry and remains unchanged for at least two years, providing a permanent record of environmental conditions, which suggests that fins can be efficiently used to discriminate the origin of fish capture (Rosenthal, 1963; Veinott and Evans, 1999; Clarke *et al.*, 2007). Nevertheless, fins have rarely been studied as indicators of water pollution.

Metal and trace element accumulation and distribution patterns in fish tissues depend on the affinity of metals for the fish tissues and the rates of uptake, deposition, and excretion (Kalay and Canli, 2000). The amount of accumulation is affected by both biotic and abiotic factors, such as habitat type, chemical form of the metal in water, water temperature, pH, dissolved oxygen concentration, as well as age, sex, body weight, and physiological condition of the fish (Has-Schön *et al.*, 2006). Accumulation also varies among species, depending on their ecology and life history, and especially on their position in the food chain (Agah *et al.*, 2009). Carnivorous fish species tend to accumulate higher amounts of metals and trace elements than herbivorous, omnivorous, or planktivorous species (Phillips *et al.*, 1980). However, benthic species can sometimes accumulate even higher amounts of elements than carnivorous species through sediment absorption (Tayel and Shriadah, 1996).

In this study, we analyzed concentrations of eleven elements (As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Se, and Zn) in muscle, scales, and anal fin clips of five selected fish species. These elements are common constituents of agricultural, urban, and municipal wastewater (Wright and Welbourn, 1994; Nagajyoti *et al.*, 2010). We have chosen the Danube River as the study site because of the considerable industrial infrastructure that is located on its banks. The area of the city of Belgrade, where the sampling was conducted, represents the main industrial zone in Serbia (Petrović, 2015). Other important anthropogenic sources of metal pollution in this part of the Danube include municipal and agricultural wastewater. The major problem with water pollution in Belgrade is that wastewater is discharged into the river without any prior treatment (Milanović *et al.*, 2010).

Considering that studies of metal contamination in fish have traditionally focused on muscle tissue because of its relevance for human consumption, the main objective of this study was to evaluate whether metal and trace element concentrations in fish scales and fins could be measured as a substitute for muscle tissue samples, which should be indicated by the presence of positive correlations between element concentrations in these tissues. Most of the studies mainly focused on mercury, while we analyzed here additional ten elements, among which some have toxic effect (As, Cd, Cr, Co, Cu, Ni) on human health.

Table 1. Total body length (cm) and total body mass (g) of sampled fish.

Species	TL (cm) min–max (mean ± SE)	BM (g) min–max (mean ± SE)
Abramis brama (common bream)	26–45	225–1155
	31.6 ± 5.7	433.5 ± 277.1
Blicca bjoerkna (white bream)	21–29	140–440
	25.1 ± 2.4	259 ± 84.1
Silurus glanis (wels catfish)	55.5–69.0	1190–2390
	64.2 ± 4.5	1750.8 ± 330.6
Esox lucius (northern pike)	38.5–52	350–985
	45.7 ± 4.4	653.5 ± 205.1
Sander lucioperca (pikeperch)	44–54	810–1475
	48.5 ± 3.3	1115 ± 223.7

Species selected for this study belong to different trophic levels: three predatory fishes – wels catfish (*Silurus glanis*), northern pike (*Esox lucius*), and pikeperch (*Sander lucioperca*), and two benthivorous fishes – common bream (*Abramis brama*) and white bream (*Blicca bjoerkna*). Different element levels and bioaccumulation capacities of studied tissues in selected fish species were hypothesized, because of their different physiological characteristics (*i.e.*, metabolic rate, developed detoxification system, *etc.*).

2 Material and methods

2.1 Sample collection

Ten individuals of each species, common bream (*A. brama*), white bream (*B. bjoerkna*), northern pike (*E. lucius*), and pikeperch (*S. lucioperca*), were collected by professional fishers from November 2013 to March 2014 from the River Danube (1169 river km) in the vicinity of Belgrade, Serbia (44° 49' 54.48" N, 20° 27' 23.68" E). The sample was complemented with 13 individuals of wels catfish (*S. glanis*) that were used in a previous research (Jovičić *et al.*, 2014) to assess elemental accumulation in different tissues. Individuals were sacrificed with a quick blow to the head. Total body length (cm) and total body mass (g) were measured for each fish (Tab. 1). Samples of the muscle (right dorsal muscle), anal fin, and scales were collected. All samples were washed with distilled water and stored at −20 °C prior to analysis.

2.2 Sample preparation and analysis

The samples were freeze-dried using a rotary vacuum concentrator Christ, model GAMMA 1-16LSC (Osterode am Harz, Germany). Analytical portions of approximately 0.3 g (dry weight) were accurately weighted and subsequently processed in a microwave digestion system. Samples were mineralized by adding 6 mL of 65% HNO_3 and 4 mL of 30% H_2O_2 (Merck, Darmstadt, Germany). Microwave assisted digestion was performed in Speedwave™ MWS3+ oven (Berghof, GmbH, Eningen, Germany). The following temperature program was used (default food program): 5 min – 160 °C; 15 min – 190 °C; 20 min – 100 °C. After cooling, digested samples were quantitatively transferred into 100 mL polypropylene volumetric flasks and diluted to volume with ultrapure water. In order to assess the possible presence of trace elements in reagents or carry-over effects of digestion vessels, five reagent blank samples were prepared as well, one per each session, according to the described procedure. These samples were analyzed in each analytical batch.

Table 2. Operating conditions for ICP-MS.

RF power	1550 W
Cooling gas flow	14 L/min
Nebulizer flow	1 L/min
Collision gas flow	1 mL/min
Operating mode	KED
Dwell time	10 ms (Cr, Mn, Fe, Co, Ni, Cu, Zn, Se)
	100 ms (As, Cd, Pb)
Sampling cone	Platinum, 1 mm orifice diameter
Skimmer cone	Platinum, 0.75 mm orifice diameter

The analysis was performed by inductively-coupled plasma mass spectrometry (ICP-MS) using the instrument "iCap Q" (Thermo Scientific, Bremen, Germany), equipped with a collision cell and operating in kinetic energy discrimination (KED) mode. The following isotopes were measured: chromium (^{52}Cr), manganese (^{55}Mn), iron (^{57}Fe), cobalt (^{59}Co), nickel (^{60}Ni), copper (^{63}Cu), zinc (^{66}Zn), arsenic (^{75}As), selenium (^{77}Se), and cadmium (^{111}Cd). Basic operating conditions of the instrument are shown in Table 2.

Torch position, ion optics, and detector settings were adjusted daily using a tuning solution (Thermo Scientific Tune B), in order to optimize measurements and to minimize possible interferences. For the quantitative analysis of the samples, a five-point calibration curve (including zero) was constructed for each isotope in the concentration range of 0.1–2.0 µg/L for ^{75}As and ^{111}Cd, and 0.1–2.0 mg/L for ^{52}Cr, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, and ^{77}Se. An additional line of the peristaltic pump was used for an online introduction of a multi-element internal standard (^{6}Li, ^{45}Sc – 10 ng/mL; ^{71}Ga, ^{89}Y, ^{209}Bi – 2 ng/mL), covering a wide mass range. Concentrations of each measured isotope were corrected for response factors of both higher and lower mass internal standards using the interpolation method.

The quality of the analytical process with respect to the accuracy and precision was assessed by the analysis of the standard reference material SRM 1577c (NIST, Gaithersburg, MD, USA). Reference material was prepared in a random manner during microwave digestion of each sample batch and run at the beginning, in the middle, and at the end of each sample list. Measured concentrations were within the range of the certified values for all isotopes (Tab. 3).

Mercury (Hg) was measured using cold vapor technique by atomic absorption spectrometer SpectrAA 220 (Varian, Palo Alto, USA) with VGA 77 hydride system and $SnCl_2$ in HCl as a reductant. Calibration was performed in five points, standard concentration range was 0.5–15.0 ng/mL. Absorption was measured at 257.3 nm. The quality of the analytical process was controlled using BCR-186 certified reference material (IRMM, Geel, Belgium). Reference material preparation and

Table 3. Assigned and measured concentrations of the SRM 1577c and BCR-186 reference material used for quality control. Values are given with the standard uncertainties and with the 95% confidence interval.

Elements	Assigned values (NIST 1577c) ± U	Measured value ±U
^{75}As, μg/kg	19.6 ± 1.4	20.5 ± 1.1
^{111}Cd, μg/kg	97 ± 1.4	97.9 ± 2.6
^{208}Pb, μg/kg	62.8 ± 1.0 −1.6%	63.3 ± 2.6
^{63}Cu, mg/kg	275.2 ± 4.6	271.9 ± 5.7
^{57}Fe, mg/kg	197.94 ± 0.65	197.43 ± 5.21
^{66}Zn, mg/kg	181.1 ± 1.0	180.9 ± 1.8
^{55}Mn, mg/kg	10.46 ± 0.47	10.55 ± 0.25
^{52}Cr, μg/kg	53 ± 14	51 ± 2.8
^{59}Co, mg/kg	0.3 ± 0.018	0.31 ± 0.016
^{60}Ni, μg/kg	44.5 ± 9.2	52.7 ± 4.3
^{77}Se, mg/kg	2.031 ± 0.045	2.055 ± 0.066
Element	**Assigned value (BCR-186) ± U**	**Measured value ± U**
Hg (total), μg/g	1.97±0.04	2.02 ± 0.07

analysis was conducted in the same manner as described previously. Obtained Hg concentrations corresponded to the certified value. All concentrations were expressed as μg g^{-1} dry weight (dw).

2.3 Statistical analysis

The normality of distribution of analyzed samples was tested by the Kolmogorov–Smirnov test (STATISTICA software package, version 6.0, StatSoft Inc; Sokal and Rohlf, 1987). Since the variables lacked normality of distribution, nonparametric tests were applied. In order to assess the relationship between metal and trace element concentrations in muscle on one hand and anal fins and scales on the other, Spearman's nonparametric correlation test ($p < 0.01$) was used.

3 Results

Metal and trace element concentrations in muscles, scales, and fins of five analyzed fish species are presented in Table 4, and the ratio of contaminant levels in each organ of each species is given in Figures 1 and 2. The concentrations of nickel were below the threshold in all tissues analyzed.

The pattern of accumulation in all species studied was the same for iron, mercury, and zinc. Iron accumulation had the following trend: scale > fin > muscle, and the trend of zinc accumulation was: fin > scale > muscle. The trend of mercury was reversed in all five species: muscle > fin > scale. The trend of manganese was different between benthivorous (fin > scale > muscle) and carnivorous species (scale > fin > muscle). Arsenic and copper concentrations were lowest in the scales of all four studied species. The detected As, Cd, Cu, Fe, Hg, and Zn concentrations in fish muscle were below the maximum allowable concentration (MAC) set by both the EU (Official Journal of the EC, 2001) and the Republic of Serbia (Official Gazette of RS, 2010, 2011); there are no prescribed MAC for other analyzed elements. The results of Spearman's nonparametric correlation test ($p < 0.01$) showed that only one correlation between the muscle and scales was statistically significant, namely in pikeperch for Hg ($p < 0.01$). As for the correlation between the muscle and the anal fin, statistically significant correlations were found in white bream for Hg, in wels catfish for Cu, and in northern pike and pikeperch for As ($p < 0.01$).

4 Discussion

Numerous studies have shown that different metals preferentially accumulate in different tissues, depending on their route of uptake.

4.1 Mercury in muscle

In all five studied species, the highest accumulation of mercury was observed in muscle tissue. Mercury enters the fish body through the digestive tract, as well as through skin and gills. It is then transported within the organism, bound to erythrocytes, to fish organs, including blood, spleen, kidney, liver, muscle, and brain; muscle represents the primary storage site of redistributed methylmercury, where it binds to sulfhydryl groups in proteins (Wiener and Spry, 1996). Even though muscle represents the accumulation center for mercury, Cizdziel *et al.* (2003) noticed that at high concentrations in the fish body, its concentration tends to increase in other organs as well. According to Havelková *et al.* (2008), in less polluted waterbodies Hg concentrations in fish muscle are about twice as high as in the liver, while in heavily polluted waterbodies Hg is again redistributed to the liver. The highest mercury concentrations are most commonly found in the muscle of piscivorous fish at the top of food chains (Wiener and Spry 1996; Havelková *et al.*, 2008). Our results confirm this, as three piscivorous species had mercury levels 2–6 times higher than two benthivorous species.

4.2 Mercury in scales/fins compared to muscle

We observed a significantly positive correlation of mercury content between scales and muscle of pikeperch ($p < 0.01$), which is consistent with findings of Červenka *et al.* (2011),

Table 4. Metal and trace element concentrations in muscle, scales, and anal fin (means ± standard deviation). Concentrations are expressed as μg g^{-1}.

Element	Tissue	*Abramis brama* (common bream)	*Blicca bjoerkna* (white bream)	*Sander lucioperca* (pikeperch)	*Esox lucius* (northern pike)	*Silurus glanis* (wels catfish)*
As	Muscle	0.227 ± 0.095	0.416 ± 0.132	0.459 ± 0.280	0.685 ± 0.807	0.131 ± 0.097
	Scale	0.083 ± 0.020	0.058 ± 0.018	0.201 ± 0.144	0.381 ± 0.502	–
	Fin	0.336 ± 0.140	0.294 ± 0.084	0.491 ± 0.429	0.425 ± 0.665	0.767 ± 0.391
Cd	Muscle	0.003 ± 0.001	0.005 ± 0.003	0.004 ± 0.004	0.006 ± 0.003	0.004 ± 0.007
	Scale	0.004 ± 0.003	0.008 ± 0.009	0.007 ± 0.005	0.059 ± 0.096	–
	Fin	0.004 ± 0.003	0.206 ± 0.628	0.013 ± 0.013	0.020 ± 0.024	0.009 ± 0.008
Co	Muscle	0.219 ± 0.103	0.150 ± 0.024	0.221 ± 0.127	0.151 ± 0.049	0.018 ± 0.023
	Scale	0.208 ± 0.072	0.192 ± 0.028	0.179 ± 0.130	0.253 ± 0.130	–
	Fin	0.297 ± 0.076	0.193 ± 0.099	0.178 ± 0.042	0.258 ± 0.185	0.051 ± 0.024
Cr	Muscle	0.132 ± 0.109	1.138 ± 1.751	0.164 ± 0.128	0.074 ± 0.067	0.138 ± 0.325
	Scale	0.172 ± 0.136	0.113 ± 0.047	0.379 ± 0.317	0.752 ± 0.949	–
	Fin	0.239 ± 0.261	0.158 ± 0.087	0.620 ± 0.630	0.161 ± 0.137	0.083 ± 0.084
Cu	Muscle	1.523 ± 0.787	2.049 ± 1.616	0.914 ± 0.797	1.690 ± 1.136	0.950 ± 0.656
	Scale	0.691 ± 0.292	0.596 ± 0.271	0.855 ± 0.419	1.475 ± 1.369	–
	Fin	1.410 ± 0.488	1.728 ± 1.122	1.308 ± 0.483	1.604 ± 0.570	0.668 ± 0.589
Fe	Muscle	27.726 ± 14.143	40.373 ± 16.494	13.162 ± 5.976	46.444 ± 25.355	19.464 ± 15.075
	Scale	83.279 ± 33.045	131.145 ± 163.057	121.293 ± 94.258	324.051 ± 446.000	–
	Fin	75.696 ± 63.152	99.552 ± 61.922	53.117 ± 15.285	94.401 ± 64.179	34.014 ± 16.097
Hg	Muscle	0.286 ± 0.176	0.471 ± 0.203	1.427 ± 0.842	0.886 ± 0.341	1.599 ± 0.535
	Scale	0.002 ± oe-7	0.002 ± oe-7	0.015 ± 0.018	0.009 ± 0.008	–
	Fin	0.004 ± 0.002	0.006 ± 0.005	0.066 ± 0.078	0.016 ± 0.007	0.095 ± 0.041
Mn	Muscle	5.066 ± 4.155	2.345 ± 0.442	1.319 ± 0.754	3.446 ± 1.326	0.674 ± 0.401
	Scale	67.934 ± 23.250	29.219 ± 6.975	35.119 ± 6.155	80.293 ± 28.446	–
	Fin	87.438 ± 28.827	43.615 ± 10.330	31.199 ± 6.595	68.523 ± 21.533	14.475 ± 7.592
Se	Muscle	1.450 ± 0.316	0.921 ± 0.267	1.590 ± 0.233	1.136 ± 0.373	0.905 ± 0.333
	Scale	0.890 ± 0.321	0.674 ± 0.193	0.647 ± 0.177	1.168 ± 0.236	–
	Fin	1.086 ± 0.531	0.818 ± 0.344	1.192 ± 0.501	1.363 ± 0.503	0.824 ± 0.303
Zn	Muscle	17.056 ± 3.942	14.715 ± 3.409	16.173 ± 5.873	21.319 ± 6.956	19.621 ± 10.201
	Scale	79.554 ± 11.848	55.348 ± 2.683	82.130 ± 11.899	118.742 ± 26.260	–
	Fin	329.128 ± 59.971	222.900 ± 71.651	100.799 ± 27.967	175.889 ± 53.617	104.246 ± 39.346

*Scales in wels catfish have not been analyzed since the species does not have them.

who observed that predatory fish tend to accumulate significant amounts of mercury in scales. Lake *et al.* (2006) and Červenka *et al.* (2011), who analyzed the relationship in mercury concentrations between scales/fins and muscle, recommended the scale/fin analysis as a useful screening tool for assessing general trends in mercury concentration in fish tissue. Given that fish need more time to recover after fin clipping, the authors suggested that the analysis of scales seems more suitable for predicting metal concentrations in fish muscle. Gremillion *et al.* (2005) evaluated the use of fins to predict mercury concentration in the muscle of walleye (*Sander vitreus*) and northern pike, and observed positive correlations between these tissues. The authors suggested that the higher trophic state of a waterbody and better physiological condition of fish positively influence the accumulation of mercury in fins relative to its accumulation in the muscle, and recommended the use of fin clippings as a nonlethal indicator of mercury concentration in the muscle. In our study, in addition to the correlation between mercury content in scales and muscle of pikeperch, we also observed a significantly positive correlation between mercury content in anal fin and muscle of white bream.

Conversely, Valová *et al.*, (2013) found no significant correlation between Hg concentrations in scales and muscle of chub (*Squalius cephalus*), proposing that scales cannot be used as a reliable tool for metal analysis until further research has been done on methodology, particularly regarding the cleaning treatment of scales. Farrell *et al.* (2000) also found no relationship between Hg (as well as Se) concentrations in scales and muscle of Arctic grayling (*Thymallus arcticus*). The authors suggested that a narrow range of concentrations of the analyzed elements could explain the obtained results. They observed that the scale tissue accumulated certain metals to a greater extent when their concentrations in the muscle (and presumably in the environment) were low, which suggests that scale concentration could overestimate the metal concentration in muscle, but rarely more than fourfold. Nevertheless, they recommended the use of scale analysis as a promising nonlethal sampling tool, specifically in light of the fact that it could also generate a historical record of fish exposure, as metal content varies among the scale growth annuli.

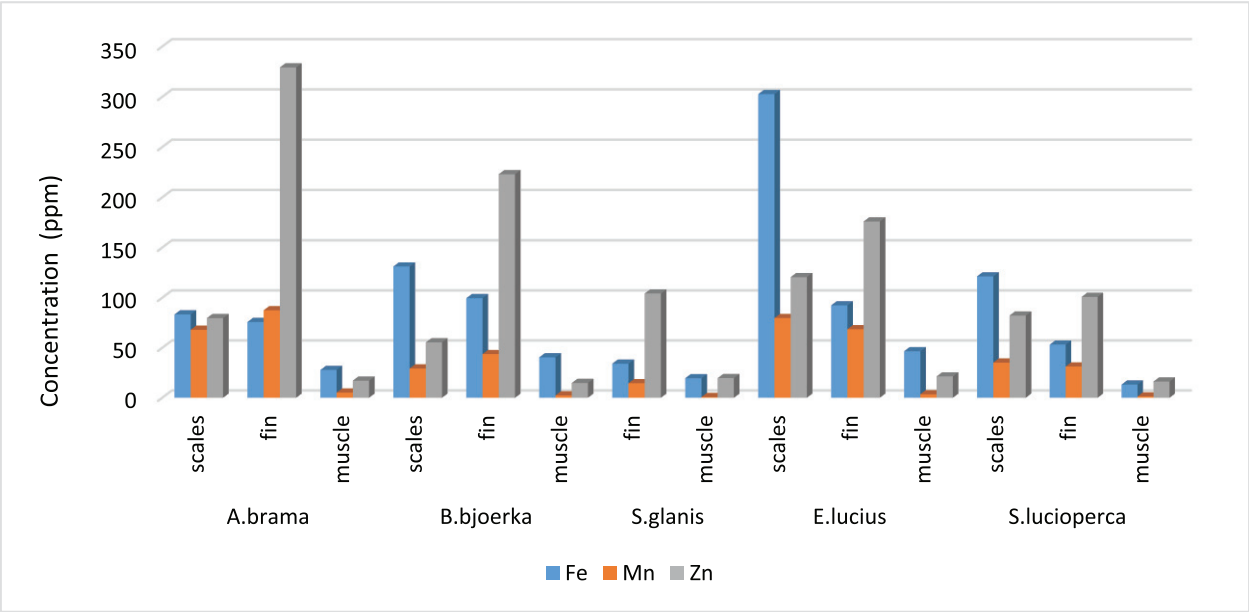

Fig. 1. Contaminant levels of Fe, Mn and Zn in muscle, fin and scales of analyzed species (*Abramis brama, Blicca bjoerkna*, *Silurus glanis, Esox Lucius* and *Sander lucioperca*).

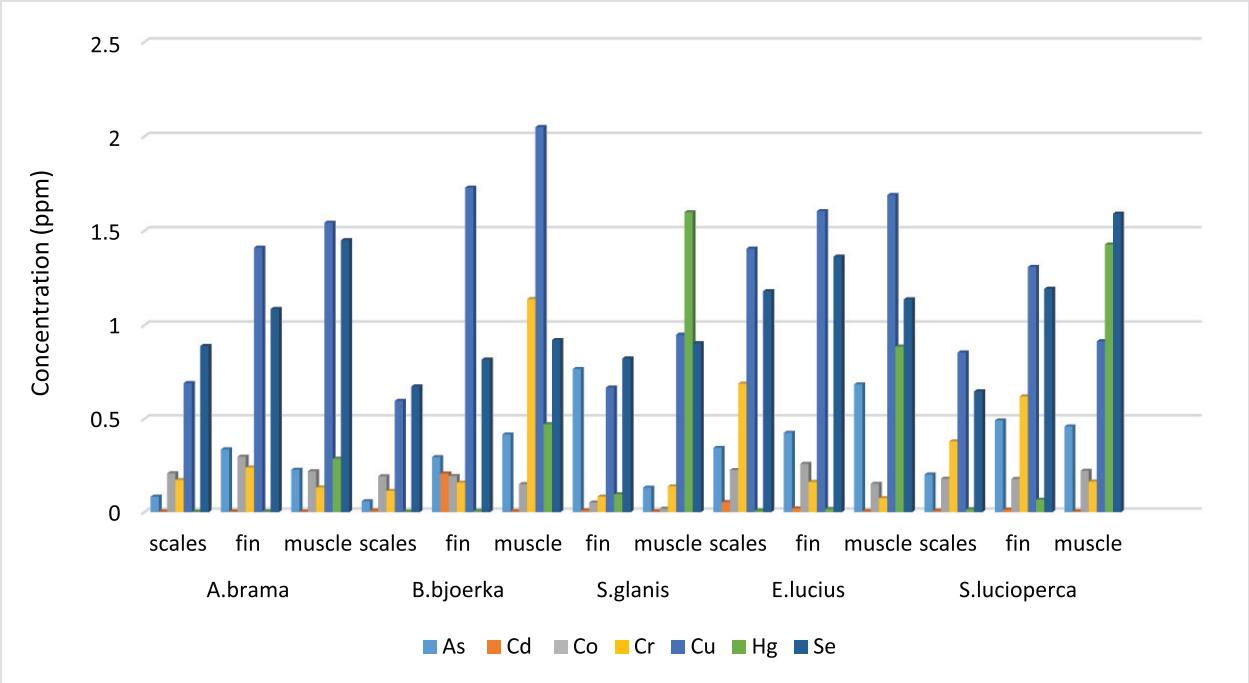

Fig. 2. Contaminant levels of As, Cd, Co, Cr, Cu, Hg and Se in muscle, fin and scales of analyzed species (*Abramis brama, Blicca bjoerkna*, *Silurus glanis, Esox Lucius* and *Sander lucioperca*).

4.3 Arsenic in fins and muscle

Another statistically significant correlation in our study was observed for arsenic between the anal fin and muscle of northern pike and pikeperch. Arsenic has a considerable tendency to accumulate in bottom sediments (Smedley and Kinniburgh, 2002). Before its sequestration in soils and sediments, arsenic is bioavailable to many organisms, but unlike mercury, its organic metabolites do not biomagnify across trophic levels (Dale and Freedman, 1982). Williams *et al.*, (2006) noticed that the highest tissue concentrations of As were measured in benthic species that feed on plant detritus, while the lowest levels were detected in species that feed on zoobenthos, which we also observed in our study, as two benthivorous species analyzed (common bream and white bream) had very low average arsenic levels. Total arsenic

concentrations in fishes are highest in the liver; however, a number of studies suggested that arsenic concentration in muscle is comparable to or slightly lower than in whole-body samples (Williams *et al.*, 2006).

4.4 Copper in fins

A significant correlation was also observed for copper between the anal fin and muscle of wels catfish. Copper is an essential element for fish that can become toxic when its cellular level is elevated (Pena *et al.*, 1999). The metabolism of copper is mainly controlled by the liver, which plays an important role in its homeostasis (Das and Gupta, 2013). The gills are the main route of waterborne Cu uptake in fish (Carvalho and Fernandes, 2008); however, copper has a distinct affinity for the liver, even at low concentrations in the environment (Jezierska and Witeska, 2006).

4.5 Other metals in scales and fins

Several studies have shown that fish scales are highly efficient at adsorbing trace metal ions (biosorption), including Fe and Zn, from wastewater (Zayadi and Othman, 2013; Othman *et al.*, 2016), suggesting that scales readily accumulate these metals. It is not known whether they then become bioavailable to other organs of the fish or whether they remain within the scale matrix. If the latter is the case, then the scales can be used as indicators of environmental pollution by Fe and Zn. In our study, the highest Fe concentrations were detected in scales of all species studied, while the highest Zn concentrations were detected in fins. The amount of Zn was particularly high in fins of two benthivorous species, and the amount of Fe was highest in scales of northern pike.

Furthermore, the presence of bone in fins, along with muscular fibers and connective tissue, allows for nonlethal monitoring of more divalent cations (such as Cu, Zn, Mn) that tend to accumulate in fish bony structures due to their ability to substitute for Ca during the assembly of bone matrix (Anderson *et al.*, 2017).

5 Conclusions

The obtained results of this study suggest that scales and anal fin clips may be used as indicators of metal and trace element contamination of muscle tissue, at least for three (As, Cu, and Hg) of the eleven elements analyzed. Because fish need more time to recover after fin clipping, analysis of scales seems more suitable for predicting metal concentrations in fish muscle.

The concentrations of As, Cu, and Hg were significantly correlated between scales/anal fin and muscle, even in fish with different diets and ecological characteristics. Taking into account that these elements are toxic in aquatic ecosystems and that both the European Union and the national legislation of Serbia prescribe the maximum allowable concentration in the environment for all three elements, we recommend the use of scales and fin clips as a useful noninvasive tool for an early warning of pollution in freshwater ecosystems and for biomonitoring of elemental contamination, without the need to kill a large number of individuals.

Acknowledgments. This study was supported by the Ministry of Education, Science, and Technological Development of the Republic of Serbia, contract numbers: 451-03-68/2022-14/200007, 451–03-68/2022–14/200053 and 451-03-68/2022-14/200178.

Supplementary material

Table S1. Correlation matrix showing Spearman correlation coeficients for muscle, fin and scales of sampled fish species ($p < 0.01$).

The Supplementary Material is available at https://www.kmae-journal.org/10.1051/kmae/2022027/olm.

References

Agah H, Leermakers M, Elskens M, Fatemi SMR, Baeyens W. 2009. Accumulation of trace metals in the muscle and liver tissues of five fish species from the Persian Gulf. *Environ Monit Assess* 157: 499–514.

Anderson J, Scrimgeour G, Palace V, Suitor M, Wilcockson J. 2017. Quantifying elements in Arctic grayling and bull trout in the South Nahanni River watershed, Northwest Territories, using nonlethal tissue samples. *N Am J Fish Manag* 37: 50–63.

Baker RF, Blanchfield PJ, Paterson MJ, Flett RJ, Wesson L. 2004. Evaluation of nonlethal methods for the analysis of mercury in fish tissue. *Trans Am Fish Soc* 133: 568–576.

Begum A, Mustafa AI, Amin MN, Chowdhury TR, Quraishi SB, Banu N. 2013. Levels of heavy metals in tissues of shingi fish (*Heteropneustes fossilis*) from Buriganga River, Bangladesh. *Environ Monit Assess* 185: 5461–5469.

Bilton HT. 1975. Factors influencing the formation of scale characters. *International North Pacific Fishery Commission Bulletin* 32: 102–108.

Bilton HT, Robins GL. 1971. Effects of starvation, feeding, and light period on circulus formation on scales of young sockeye salmon (*Onchorhyncus nerka*). *J Fish Res Board Can* 28: 1749–1755.

Carvalho CS, Fernandes MN. 2008. Effect of copper on liver key enzymes of anaerobic glucose metabolism from freshwater tropical fish *Prochilodus lineatus*. *Comp Biochem Physiol* 51A: 437–442.

Cerveny D, Roje S, Turek J, Randak T. 2016. Fish fin-clips as a non-lethal approach for biomonitoring of mercury contamination in aquatic environments and human health risk assessment. *Chemosphere* 163: 290–295.

Cizdziel J, Hinners T, Cross C, Pollard J. 2003. Distribution of mercury in the tissues of five species of freshwater fish from Lake Mead, USA. *J Environ Monit* 5: 802–807.

Clarke AD, Telmer KH, Shrimpton JM. 2007. Elemental analysis of otoliths, fin rays and scales: a comparison of bony structures to provide population and life-history information for the Arctic grayling (*Thymallus arcticus*). *Ecol Fresh Fish* 16: 354–361.

Cobelo-García A, Morán P, Almécija C, Caballero P. 2017. Historical record of trace elements (1983-2007) in scales from Atlantic salmon (*Salmo salar*): Study of past metal contamination from a copper mine (Ulla River, NW Iberian Peninsula). *Chemosphere* 188: 18–24.

Coillie VR, Rousseau A. 1974. Composition minerale des ecailles du Catostomus commersoni issu de deux milieux differents: etude par microscopie electronique and analytique. *J Fish Res Board Can* 31: 63–66.

Cooley HM, Klaverkamp JF. 2000. Accumulation and distribution of dietary 282 uranium in Lake Whitefish (*Coregonus clupeaformis*). *Aquat Toxicol* 48: 477–494.

Červenka R, Bednařík A, Komárek J, Ondračková M, Jurajda P, Vítek T, Spurný P. 2011. The relationship between the mercury concentration in fish muscles and scales/fins and its significance. *Cent Eur J Chem* 9: 1109–1116.

Dale JM, Freedman B. 1982. Arsenic pollution associated with tailings at an abandoned gold mine in Halifax County, Nova Scotia. *Proc N S Inst Sci* 32: 337–349.

Das S, Gupta A. 2013. Accumulation of copper in different tissues and changes in oxygen consumption rate in Indian flying barb, *Esomus danricus* (Hamilton-Buchanan) exposed to sub-lethal concentrations of copper. *Jordan J Biol Sci* 6: 21–24.

Farrell AP, Hodaly AH, Wang S. 2000. Metal analysis of scales taken from arctic grayling. *Arch Environ Contam Toxicol* 39: 515–522.

Fincel MJ, Vandehey JA, Chipps SR. 2012. Non-lethal sampling of walleye for stable isotope analysis: a comparison of three tissues. *Fish Manag Ecol* 19: 283–292.

Gjerde B, Refstie T. 1988. The effect of fin-clipping on growth rate, survival and sexual maturity of rainbow trout. *Aquaculture* 73: 383–389.

Gremillion PT, Cizdziel JV, Cody NR. 2005. Caudal fin mercury as a non-lethal predictor of fish-muscle mercury. *Environ Chem* 2: 96–99.

Has-Schön E, Bogut I, Strelec I. 2006. Heavy metal profile in five fish species included in human diet, domiciled in the end flow of River Neretva (Croatia). *Arch Environ Contam Toxicol* 50: 545–551.

Havelková M, Dušek L, Némethová D, Poleszczuk G, Svobodová Z. 2008. Comparison of mercury distribution between liver and muscle – a biomonitoring of fish from lightly and heavily contaminated localities. *Sens* 8: 4095–4109.

Jezierska B, Witeska M. 2006. The metal uptake and accumulation in fish living in polluted waters. In Soil and water pollution monitoring, protection and remediation Springer, Dordrecht. 107–114.

Jovičić K, Nikolić MD, Višnjić-Jeftić Ž, Đikanović V, Skorić S, Stefanović MS, Lenhardt M, Hegediš A, Krpo-Ćetković J, Jarić I. 2014. Mapping differential elemental accumulation in fish tissues: assessment of metal and trace element concentrations in wels catfish (*Silurus glanis*) from the Danube River by ICP-MS. *Environ Sci Pollut Res* 22: 3820–3827.

Kalay M, Canli M. 2000. Elimination of essential (Cu, Zn) and nonessential (Cd, Pb) metals from tissues of a freshwater fish *Tilapia zilli*. *Turk J Zool* 24: 429–436.

Khanna DR, Sarkar P, Gautam A, Bhutiani R. 2007. Fish scales as bioindicator of water quality of River Ganga. *Environ Monit Assess* 134: 153–160.

Knight A, Bhavsar SP, Branfireun BA, Drouin P, Prashad R, Petro S, Oke M. 2019. A comparison of fish tissue mercury concentrations from homogenized fillet and nonlethal biopsy plugs. *J Environ Sci* 80: 137–145.

Lake JL, Ryba SA, Serbst JR, Libby AD. 2006. Mercury in fish scales as an assessment method for predicting muscle tissue mercury concentrations in largemouth bass. *Arch Environ Contam Toxicol* 50: 539–544.

Łuszczek-Trojnar E, Nowacki P. 2021. Common carp (*Cyprinus carpio* L.) scales as a bioindicator reflecting its exposure to heavy metals throughout life. *J Appl Ichthyol* 37: 235–245.

McCloskey M, Yurkowski DJ, Semeniuk CA. 2018. Validating fin tissue as a non-lethal proxy to liver and muscle tissue for stable isotope analysis of yellow perch (*Perca flavescens*). *Isotopes Environ Health Stud* 54: 196–208.

Milanović A, Kovačević-Majkić J, Milivojević M. 2010. Water quality analysis of Danube river in Serbia: pollution and protection problems. *Bull Serb Geogr Soc* 90: 47–68.

Morán P, Cal L, Cobelo-García A, Almécija C, Caballero P, de Leaniz CG. 2018. Historical legacies of river pollution reconstructed from fish scales. *Environ Pollut* 234: 253–259.

Nagajyoti PC, Lee KD, Sreekanth TVM. 2010. Heavy metals, occurrence and toxicity for plants: a review. *Environ Chem lett* 8: 199–216.

Negi RK, Maurya A. 2015. Heavy metal concentrations in tissues of major carp and exotic carp from Bhagwanpur fish pond, India. *J Fish Aquat Sci* 10: 543–552.

Official Gazette of RS No. 25/ 2010 and No. 8/2011. Regulation on the quantities of pesticides, metals, metalloids, and other toxic substances, chemoterapeutics, anabolics, and other substances that could be found in food.

Official Journal of the European Communities. 2001. Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs.

Othman N, Abd-Kadir A, Zayadi N. 2016. Waste fish scale as cost effective adsorbent in removing zinc and ferum ion in wastewater. *ARPN J Eng Appl Sci* 11: 1584–1592.

Pena MMO, Lee J, Thiele D. 1999. A delicate balance: Homeostatic control of copper uptake and distribution. *J Nutr* 129: 1251–1260.

Petrović L. 2015. Spatial development analysis of the Danube region in Serbia in the function of sustainable development. *Bull Serbian Geogr Soc* 95: 141–158.

Phillips GR, Lenhart TE, Gregory RW. 1980. Relations between trophic position and mercury accumulation among fishes from the Tongue River Reservoir, Montana. *Environ Res* 22: 73–80.

Rolfhus KR, Sandheinrich MB. 2008. Analysis of fin clips as a nonlethal method for monitoring mercury in fish. *Environ Sci Technol* 42: 871–877.

Rosenthal HL. 1963. Uptake, turnover and transport of bone seeking elements in fishes. *Ann. N.Y. Acad. Sci.* No. 109. pp. 278–293.

Sanderson BL, Tran CD, Coe HJ, Pelekis V, Steel EA, Reichert WL. 2009. Nonlethal sampling of fish caudal fins yields valuable stable isotope data for threatened and endangered fishes. *Trans Am Fish Soc* 138: 1166–1177.

Schmitt CJ, Brumbaugh WG. 2007. Evaluation of potentially nonlethal sampling methods for monitoring mercury concentrations in smallmouth bass (*Micropterus dolomieu*). *Arch Environ Contam Toxicol* 53: 84–95.

Shaikhiev IG, Kraysman NV, Sverguzova SV, Spesivtseva SE, Yarothckina AN. 2020. Fish scales as a biosorbent of pollutants from wastewaters and natural waters (a literature review). *Biointerface Res Appl Chem* 10: 6893–6905.

Smedley PL, Kinniburgh DG. 2002. A review of the source, behaviour and distribution of arsenic in natural waters. *Appl Geochem* 17: 517–568.

Sokal RR, Rohlf FJ. 1987. Introduction to biostatistics. New York: Dover Publications.

Stahl LL, Snyder BD, McCarty HB, Cohen TR, Miller KM, Fernandez MB, Healey JC. 2021. An evaluation of fish tissue monitoring alternatives for mercury and selenium: fish muscle biopsy samples versus homogenized whole fillets. *Arch Environ Contam Toxicol* 81: 1–19.

Squadrone S, Prearo M, Brizio P, Gavinelli S, Pellegrino M, Scanzio T, Guarise S, Benedetto A, Abete MC. 2013. Heavy metals distribution in muscle, liver, kidney and gill of European catfish (*Silurus glanis*) from Italian Rivers. *Chemosphere* 90: 358–365.

Tayel FTR, Shriadah MMA. 1996. Fe, Cu, Mn, Pb and Cd in some fish species from Western Harbour of Alexandria, Eqypt. *Bull Natn Inst Oceanogr Fish* 22: 85–96.

Uthe JF. 1971. A simple field technique for obtaining small samples of muscle from living fish. *J Fish Res Board Can* 28: 1203–1204.

Uysal K, Köse E, Bülbül M, Dönmez M, Erdoğan Y, Koyun M, Ömeroğlu Ç, Özmal F. 2009. The comparison of heavy metal accumulation ratios of some fish species in Enne Dame Lake (Kütahya/Turkey). *Environ Monit Assess* 157: 355–362.

Valová Z, Hudcová H, Roche K, Svobodová J, Bernardová I, Jurajda P. 2013. No relationship found between mercury and lead concentrations in muscle and scales of chub *Squalius cephalus* L. *Environ Monit Assess* 185: 3359–3368.

Vaid V, Hundal SS. 2019. Light microscopic studies to evaluate fish scales as non-invasive indicators of heavy metal-contaminated waters. *Environ Monit Assess* 191: 1–11.

Vašek M, Vejřík L, Vejříková I, Šmejkal M, Baran R, Muška M, Kubečka J, Peterka J. 2017. Development of non-lethal monitoring of stable isotopes in asp (*Leuciscus aspius*): a comparison of muscle, fin and scale tissues. *Hydrobiologia* 785: 327–335.

Veinott GI, Evans RD. 1999. An examination of elemental stability in the fin ray of white sturgeon with laser ablation sampling-inductively coupled plasma – mass spectrometry (LAS-ICP-MS). *Trans Am Fish Soc* 128: 352–361.

Wells BK, Bath GE, Thorrold SR, Jones CM. 2000a. Incorporation of strontium, cadmium and barium in juvenile spot (*Leiostomus xanthurus*) scales reflects water chemistry. *Can J Fish Aquatic Sci* 57: 1–8.

Wells BK, Thorrold SR, Jones CM. 2000b. Geographic variation in trace element composition of juvenile weakfish scales. *Trans Am Fish Soc* 129: 889–900.

Wells BK, Thorrold SR, Jones CM. 2003. Stability of elemental signatures in the scales of spawning weakfish *Cynoscion regalis*. *Can J Fish Aquat Sci* 60: 361–369.

Wiener JG, Spry DJ. 1996. Toxicological significance of mercury in freshwater fish. In Beyer WN, Heinz GH, Redmon Norwood AW, eds, Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. Lewis, Boca Raton, FL, USA, 297–339.

Williams L, Schoof RA, Yager JW, Goodrich-Mahoney JW. 2006. Arsenic bioaccumulation in freshwater fishes. *Hum Ecol Risk Assess* 12: 904–923.

Wright DA, Welbourn PM. 1994. Cadmium in the aquatic environment: a review of ecological, physiological, and toxicological effects on biota. *Environ Rev* 2: 187–214.

Yamada SB, Mulligan TJ. 1982. Strontium marking of hatchery reared coho salmon, *Oncorhyncus kisutch* Walbaum, identification of adults. *J Fish Biol* 20: 5–9.

Zayadi N, Othman N. 2013. Characterization and optimization of heavy metals biosorption by fish scales. *Adv Mat Res* 795: 260–265.

Cite this article as: Jovičić K, Janković S, Nikolić DM, Đikanović V, Skorić S, Krpo-Ćetković J, Jarić I. 2023. Prospects of fish scale and fin samples usage for nonlethal monitoring of metal contamination: a study on five fish species from the Danube River. *Knowl. Manag. Aquat. Ecosyst.*, 424, 4.