

## Content of heavy metals in the muscle tissue of fish from two rivers in the Morava river basin (Czech Republic)

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### Abstract

Heavy metals are notorious environmental pollutants with serious health implications for both aquatic ecosystems and human consumers. In 2022, we assessed the cadmium, chromium, mercury, nickel, and lead contents in fish (in wet weight muscle tissue) from two rivers in the Czech Republic subject to pollution incidents, the rivers Bečva and Svatka, to identify potential risks for human consumption. Three fish species were examined: chub (*Squalius cephalus*) from the Bečva (four sites), and chub, perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) from the Svatka (one site). While concentrations of nickel, chromium, and lead were generally higher in water from the Bečva than the Svatka, all heavy metals (aside from cadmium) were higher in sediment from the Svatka. In chub, nickel concentrations varied greatly between individuals, with the highest concentration reaching 5029.9 µg/kg. In comparison, nickel concentrations in roach and perch were generally low and often below the detection limit (14.8 µg/kg). Mercury concentrations varied between 14.3 and 249.4 µg/kg, with the highest mean concentration found in perch. Cadmium concentrations were highest in the Svatka, though the difference was non-significant, with concentrations in the Bečva mostly under the detection limit (1.49 µg/kg). Lead and chromium concentrations only occasionally exceeded the detection limit, except for one chub in which chromium concentrations exceeded 159.94 µg/kg. In general, aside from nickel, heavy metal concentrations in fish muscle tissue were low and appear not to represent any threat to human health. However, the samples with the highest nickel content could represent a potential risk for nickel-sensitive individuals.

*Chub, bioaccumulation, aquatic pollution, health risk, nickel sensitivity*

Heavy metals are notorious environmental pollutants with serious health implications for both aquatic ecosystems and human consumers. Though the definition of heavy metals varies, they are usually defined as metallic elements of high density. While most heavy metals are considered toxic, with actual toxicity depending on type and dosage, some also represent essential microelements (Pourret et al. 2021). In aquatic ecosystems, the most common heavy metal pollutants are generally lead (Pb), chromium (Cr), nickel (Ni), arsenic (As), copper (Cu), mercury (Hg), cadmium (Cd) and zinc (Zn), all of which are toxic to fish in higher concentrations (Garai et al. 2021). Owing to their position in the upper levels of the aquatic food chain, predatory fish, which are the most common aquatic food source for human consumption, exhibit a higher tendency to bioaccumulate

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heavy metals than other fish, and consequently are most likely to impact human health (Has-Schön et al. 2015; FAO 2020).

Urban and agricultural runoff, combustion of fossil fuels, cement production, mining, extractive metallurgy, pulp and paper production and other anthropogenic activities can all result in heavy metal pollution of aquatic habitats, especially those close to or in urbanised or industrialised areas (Zuliani et al. 2019), while atmospheric deposition and erosion of the geological matrix may also serve as significant sources (Boudou and Ribeyre 1989). In such cases, metallic elements may become environmentally ubiquitous allowing them to be trophically bioaccumulated through direct ingestion of particles suspended in water, ingestion of lower trophic levels and membrane absorption. Though not all the heavy metals ingested or absorbed will be accumulated, with some being excreted through urination and via the respiratory membranes (Alam et al. 2002), enough will be accumulated in heavily polluted areas to potentially make human ingestion of fish harmful. Consequently, governments worldwide have set maximum limits for heavy metal concentrations in foodstuffs, including fish, to protect human health. In Europe, the maximum heavy metal content in foodstuffs is contained in the Commission Regulation (EC) No 2023/915 with the maximum content of Pb in fish meat set at 0.30 mg/kg wet weight, Cd at 0.05 mg/kg wet weight and Hg at 0.50 mg/kg wet weight.

In the Czech Republic, many lotic and lentic ecosystems have been studied for content of heavy metals and other pollutants. Svobodová and Hejtmánek (1976), for example, studied Hg content in the muscles of fish from the river Ohře and its tributaries, finding high concentrations in several species, including chub *Squalius cephalus*. While Kružíková et al. (2021) identified a significant relationship between fish weight and total Hg content in fish muscle for almost all species examined in drinking water reservoirs in the Morava river basin. During this study, some fish muscle samples exceeded the maximum Hg levels set by the Commission Regulation (EC) No 2023/915. Other studies of note in the Czech Republic include those of Špurný et al. (2009) on the contamination of the lower course of the river Jihlava, Sedláčková et al. (2014) on the mercury content in fish muscles from major Czech rivers, Vičarová et al. (2016) on heavy metal contents in common carp from Czech reservoirs and Šehonová et al. (2022), who studied mercury content in fish from Czech ponds with the conclusion that fish from ponds did not pose a risk for consumers.

In the present study, we evaluate the potential threat of heavy metal contamination in fish taken for human consumption from two frequently polluted rivers in the Morava river basin, the rivers Bečva and Svatka. Surface waters in the Czech Republic are classified into five quality classes based on numerous chemical and biological criteria. In 2019 and 2020, the lower course of the Svatka fell in class IV, i.e. heavily polluted water, due to the Svatka river basin's much higher human population and industry aggregation, while the Bečva fell in class III, i.e. polluted water (Ministry of Agriculture of the Czech Republic, Ministry of the Environment of the Czech Republic 2021). Consequently, we hypothesise that fish muscle tissue from the Svatka will contain higher concentrations of heavy metals than that from the Bečva.

## Materials and Methods

### Sampling localities

The two rivers examined in this study both differ in their characteristics. The 168.5 km long river Svatka mainly flows through the central Vysočina highlands and the southern Moravian regions of the Czech Republic, finally joining the river Dyje at the Nové Mlýny reservoirs (river basin area of 7,116 km<sup>2</sup>). A reservoir (Kníničky Reservoir) has been constructed on the river to serve the Brno city suburbs, after first flowing through industrial zones near the small towns of Veverská Bitýška and Tišnov upstream of the reservoir. The river channel downstream of the reservoir suffers from daily water-level fluctuations due to the running of hydroelectric power plants, and its section running through Brno is significantly influenced by several weirs and heavily modified banks.

The 62 km long Bečva (river basin area of 1,613 km<sup>2</sup>) begins at the confluence of the Vsetínská Bečva and Rožnovská Bečva streams in the town of Valašské Meziříčí. The river then flows through a series of industrial areas in the towns of Valašské Meziříčí, Hranice, Lipník nad Bečvou and Přerov. Compared to the Svatka with its more uniform channel, the Bečva has a gravel-bed and is more dynamic, forming a typical natural river landscape. Despite historical pollution, the river is now considered to be in a very good, mostly natural, ecological state, though disturbed in some parts by the presence of weirs (Ministry of Agriculture of the Czech Republic, Ministry of the Environment of the Czech Republic 2021).

#### Fish sampling

Fish sampling first took place following an accidental Ni leakage into the Bečva, and subsequent fish poisoning, in the autumn of 2020. Fish samples were acquired in October 2021 by electrofishing from four sites: Bečva 1 - 49.4855889N, 17.4996822E; Bečva 2 - 49.5234658N, 17.8492814E; Bečva 3 - 49.4867111N, 17.9494914E; Bečva 4 - 49.4710981N, 17.9543650E (Fig. 1). Bečva 4, a slightly regulated barbel zone stretch with a gravel riverbed, is situated upstream of most waste outlets, including the Valašské Meziříčí wastewater treatment plant, a large chemical plant outlet and the Rožnov pod Radhoštěm urban outlet. While Bečva 3 has similar characteristics to Bečva 4, it lies immediately downstream of most of the outlets mentioned above. Both Bečva 2 and Bečva 1 are situated increasingly downstream and are characterised as barbel zone stretches with gravel riverbeds below boulder weirs on a mostly natural, unregulated stretch of the river.

Fish were sampled from the Svatka in March 2022 from a trapezoidal regulated stretch of the river, with more natural parts both upstream and downstream, adjacent to Poříčí street (GPS: 49.1860886N, 16.5912917E; Fig. 1). The stretch is classified as barbel zone and has a mostly gravel riverbed with parts covered by sediment. Owing to its location close to the Brno city centre, several storm drain outlets are located here and upstream, and urban rubbish is frequently present.

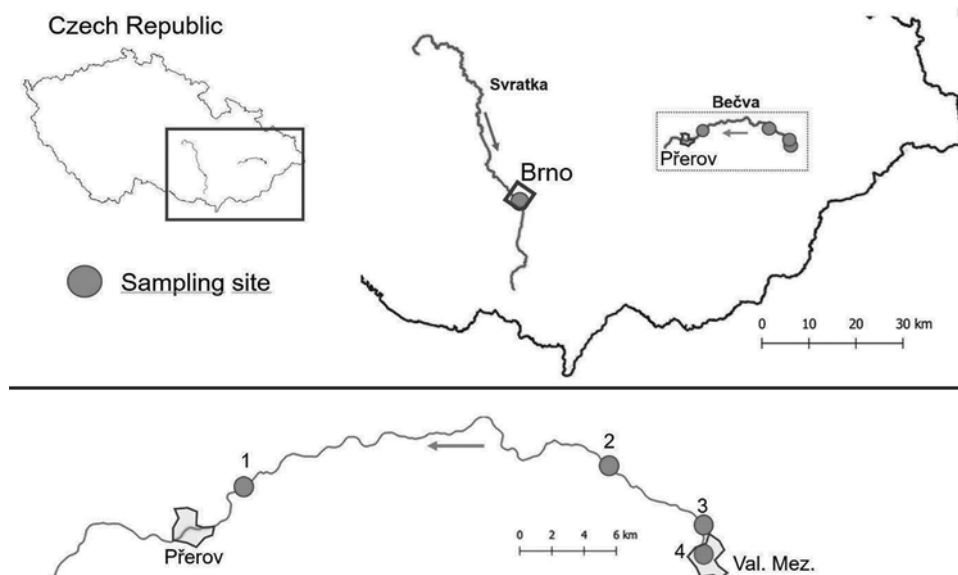


Fig. 1. Map of the Czech Republic with the rivers Bečva and Svatka indicated along with the approximate position of the five sampling sites.

Ten chub were taken for testing from each locality on the Bečva, and ten chub, perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) from the Svatka, each of these displaying different food preferences. Note that neither perch nor roach are found in the Bečva. After catching, the fish were individually weighed, and standard and total lengths were measured. At the same time, scales were taken from each fish for age determination. The fish were sacrificed humanely, filleted, skinned and a ca. 10 × 10 cm portion of muscle was excised from the entire width of the middle part of the fillet and stored in a freezer at -20 °C.

Data on heavy metal concentrations in the surface water of both rivers (2021–2022) and in the sediment from the Bečva (2020–2022) were obtained from the Povodí Moravy State Enterprise. Sediment samples from the Svatka were obtained by the authors themselves in 2022.

### Sediment sampling

Samples of sediments were taken from 2 places upstream of the locality on the Svratka (49.1852786N, 16.5707853E 49.1848836N, 16.5713719E), and one downstream of the locality (49.1826600N, 16.6087600E). The resulting value of the pollutant content in the sediments is the average of the values from these samples.

### Analytical methods

Heavy metals were analysed in river sediments and fish muscles. Sediment samples were first lyophilised using a Heto PowerDry LL 3000 Freeze Dryer (Thermo Fischer Scientific Inc. USA). Prior to determination of Cd, Cr, Pb and Ni,  $400 \pm 0.1$  mg of fresh fish muscle and  $100 \pm 0.1$  mg of dried homogenised sediment were decomposed/mineralised in a 10 ml aqueous solution of  $\text{HNO}_3$  (1:1) at  $210^\circ\text{C}$  for 30 min before being mineralised through microwave digestion (Ethos ONE, Milestone, Italy). Concentrations of Cd, Cr, Pb and Ni were then determined using a 280Z AA graphite furnace atomic absorption spectrometer (Agilent Technologies, USA) with Zeeman background correction. Determination of each element was carried out under conditions recommended by the manufacturer, with the wavelength for Cd set at 228.8 nm, Cr 357.9 nm, Pb 283.3 nm and Ni 32.0 nm. Ultrasensitive hollow cathode lamps (Agilent Technologies) were used as the radiation source, with the lamp current set at 5.0 mA Cd, 10.0 mA for Cr, 10.0 mA for Pb and 6.0 mA for Ni. In each case, 1% Pd/Mg( $\text{NO}_3$ )<sub>2</sub> was used as a modifier.

Total Hg analysis was performed on an AMA 254 advanced mercury analyser (Altec, Czech Republic) without pre-treatment of the sample (i.e. no decomposition or separation),  $100 \pm 0.1$  mg of fresh muscle or dry sediment sample being directly analysed under optimal measurement conditions, i.e. drying at  $120^\circ\text{C}$  for 90 s, followed by decomposition at  $550^\circ\text{C}$  for 180 s under a constant flow of  $\text{O}_2$ .

Calibration standards consisted of 1000 mg/l of stock Cd, Pb, Cr, Ni and Hg solutions (Merck & Co, Inc., USA), while ultrapure 69%  $\text{HNO}_3$ , m/m (Sigma Aldrich, USA) and Milli Q water with a maximum resistivity of  $18.2 \text{ M}\Omega/\text{cm}$  obtained from the Merck KGaA Millipore system (Merck & Co, Inc., USA), were used for sample (pre)treatment and sample dilution, respectively.

Fish protein DORM-4 (National Research Council of Canada) was used as a reference material for method validation of Hg, Cd, Pb, Cr and Ni analysis in fish muscles, while river sediment Metranal 1 (Analytika, Czech Republic) was used for validating Cd, Pb, Cr, Ni and Hg analysis of sediment samples. In all cases, our results agreed with the certified values. Limits of detection for the heavy metals were as follows:  $0.1 \mu\text{g/l}$  ( $0.1 \mu\text{g/kg}$ ) for Hg,  $0.052 \text{ mg/l}$  ( $1.49 \mu\text{g/kg}$ ) for Cd,  $0.65 \text{ mg/l}$  ( $18.57 \mu\text{g/kg}$ ) for Pb,  $0.12 \text{ mg/l}$  ( $3.43 \mu\text{g/kg}$ ) for Cr and  $0.52 \text{ mg/l}$  ( $14.8 \mu\text{g/kg}$ ) for Ni.

### Statistical analysis

Differences in heavy metal concentration between localities were tested using the non-parametric Kruskal-Wallis test, followed by non-parametric *post hoc* comparisons using the Statistica software package v. 14 (TIBCO Software Inc. 2020). Concentrations below the detection limit were treated as half the value of the concentration recalculated for fish biomass, i.e.  $0.05 \mu\text{g/kg}$  for Hg,  $0.745 \mu\text{g/kg}$  for Cd,  $9.285 \mu\text{g/kg}$  for Pb,  $1.715 \mu\text{g/kg}$  for Cr and  $7.4 \mu\text{g/kg}$  for Ni.

## Results

Chub total length differed significantly between both localities and rivers (Kruskal-Wallis test:  $H = 28.8$ ,  $df = 4$ ,  $P < 0.001$ ), with the largest chub ( $347.9 \text{ mm}$  on average) caught at Bečva 3, these differing significantly from all other localities except Bečva 4. Chub from Bečva 2 were the smallest caught ( $255 \text{ mm}$  on average) and differed significantly from those at Bečva 4 (Table 1). Body weight varied according to total length (Kruskal-Wallis test:  $H = 30.2$ ,  $df = 4$ ,  $P < 0.001$ ), with significant differences between localities same as in total length.

While heavy metal concentrations in river water were generally higher in the Bečva than the Svratka, concentrations in the sediment were higher in the Svratka than the Bečva (Table 2). Not only were mean concentrations of Ni in chub muscle tissue highest in the Svratka, the highest concentration recorded in the study ( $5029.87 \mu\text{g/kg}$ ) was also found there (Table 3). Significant differences were recorded between Ni concentrations at Bečva 1 and Svratka and between Bečva 2 and Svratka (Kruskal-Wallis test:  $H = 24.76$ ,  $df = 4$ ,  $P < 0.001$ ; Table 3). Concentrations of Hg in all chub muscle samples were above than the limit of detection ( $14.3\text{--}249.4 \mu\text{g/kg}$ ), with both mean and highest Hg concentration recorded at Bečva 1 and lowest values at Bečva 3, the difference being statistically significant (Kruskal-Wallis test:  $H = 22.81$ ,  $df = 4$ ,  $P < 0.001$ ). In comparison, Cr, Pb, and Cd concentrations were often below the limit of detection (Table 3). Mean

Cd concentrations in chub tissue were generally highest in the Svatka, which also had one sample with the highest Cd concentration recorded (13 µg/kg). While most samples from all localities had Cr concentrations below the limit of detection, one sample from Bečva 3 had a value 93.3 times above the limit of detection (159.94 µg/kg). Lead concentrations varied between 19 and 28.8 µg/kg in the Bečva (Table 3).

There were significant differences in both Ni concentrations (Kruskal-Wallis test:  $H = 21.76$ ,  $df = 2$ ,  $P = 0.001$ ) and Hg concentrations (Kruskal-Wallis test:  $H = 15.18$ ,  $df = 2$ ,  $P < 0.001$ ) in muscle tissue samples from chub and roach, and chub and perch (Table 4).

Table 1. Characteristics of fish caught in the rivers Bečva and Svatka for heavy metals analysis.

Locality	Species	Total length (mm)	Body weight (g)	Age range/mean age (years)
Bečva 1	chub	289.20 ± 27.19 <sup>AB</sup>	277.80 ± 67.58 <sup>AB</sup>	4–5 (4.6)
Bečva 2	chub	255.00 ± 20.60 <sup>A</sup>	216.00 ± 45.16 <sup>A</sup>	5–10 (6.6)
Bečva 3	chub	347.90 ± 28.23 <sup>C</sup>	581.00 ± 164.15 <sup>C</sup>	4–6 (5.2)
Bečva 4	chub	341.30 ± 29.16 <sup>CB</sup>	472.90 ± 130.97 <sup>CB</sup>	4–9 (5.9)
Svatka	chub	290.90 ± 44.48 <sup>AB</sup>	320.50 ± 166.49 <sup>AB</sup>	3–5 (3.6)
Svatka	perch	189.90 ± 20.93	87.00 ± 30.68	3–4 (3.6)
Svatka	roach	222.60 ± 34.16	137.70 ± 75.41	3–5 (3.6)

Length and weight: average ± SD; age: range (min–max) and mean;  $n = 10$  fish from each site. Different superscripts indicate significant differences between chub from different localities ( $P < 0.001$ ).

Table 2. Concentrations of heavy metal pollutants in water and sediment from the rivers Bečva and Svatka (mean ± SD).

Sample	River	Nickel	Mercury	Chromium	Lead	Cadmium
Water (µg/l)	Bečva	2.59 ± 1.5 (47)	N.A.	1.33 ± 1.43 (36)	1.35 ± 1.08 (14)	0.21 ± 1.82 (4)
	Svatka	2.21 ± 0.77 (48)	N.A.	0.76 ± 0.29 (16)	0.94 ± 0.61 (17)	≤ LOD
Sediment (mg/kg)	Bečva	32.77 ± 5.35 (6)	0.07 ± 0.03 (6)	42.12 ± 6.11 (6)	20.23 ± 4.77 (6)	1.14 ± 2.26 (6)
	Svatka	40.56 ± 11.22 (3)	0.37 ± 0.38 (3)	60.78 ± 14.08 (3)	78.33 ± 83.80 (3)	0.51 ± 0.25 (3)

Numbers in parentheses: number of samples above limit of detection (LOD) analysed; N.A.: non-applicable

Table 3. Concentrations of heavy metal pollutants in muscle of chub from the rivers Bečva and Svatka (mean ± SD).

	Nickel (µg/kg)	Mercury (µg/kg)	Chromium (µg/kg)	Lead (µg/kg)	Cadmium (µg/kg)
Bečva 1	393.96 ± 810.34 (5) <sup>A</sup>	125.88 ± 55.93 (10) <sup>B</sup>	≤ LOD	19.02 (1)	1.6 ± 0.13 (2)
Bečva 2	261.85 ± 556.08 (6) <sup>A</sup>	58.53 ± 13.98 (10) <sup>AB</sup>	3.91 (1)	≤ LOD	≤ LOD
Bečva 3	238.46 ± 297.79 (10) <sup>AB</sup>	32.27 ± 18.91 (10) <sup>A</sup>	100.68 ± 53.59 (3)	25.76 (1)	≤ LOD
Bečva 4	132.39 ± 142.17 (9) <sup>AB</sup>	66.78 ± 46.86 (10) <sup>AB</sup>	11.91 ± 1.57 (2)	28.79 (1)	≤ LOD
Svatka	1051.69 ± 1544.23 (10) <sup>B</sup>	56.05 ± 17.42 (10) <sup>AB</sup>	69.34 (1)	≤ LOD	4.66 ± 5.56 (4)

Numbers in parentheses: number of samples above limit of detection (LOD) analysed ( $n = 10$  in total). Different superscripts indicate significant differences between localities ( $P < 0.001$ ).

Table 4. Concentrations of heavy metal pollutants in muscle tissue of different fish species from the river Svatka (mean ± SD).

Species	Nickel (µg/kg)	Mercury (µg/kg)	Chromium (µg/kg)	Lead (µg/kg)	Cadmium (µg/kg)
Chub	1051.69 ± 1544.23 (10) <sup>B</sup>	56.05 ± 17.42 (10) <sup>A</sup>	69.34 ± 0 (1)	≤ LOD	4.66 ± 5.56 (4)
Perch	28.26 ± 15.33 (2) <sup>A</sup>	163.01 ± 66.52 (10) <sup>B</sup>	≤ LOD	32.34 ± 8.82 (5)	3.98 ± 2.46 (5)
Roach	92.72 ± 99.72 (5) <sup>A</sup>	114.45 ± 30.98 (10) <sup>B</sup>	≤ LOD	≤ LOD	1.71 ± 0.11 (4)

Numbers in parentheses: number of samples above limit of detection (LOD) analysed ( $n = 10$  in total). Different superscripts indicate significant differences between localities ( $P < 0.001$ ).

## Discussion

While all three fish species from the Svratka (chub, perch, roach) were of roughly the same age, chub from the Bečva were, on average, 1–2 years older than those from the Svratka. There were also slight differences between the Bečva localities, with the smallest fish from Bečva 2 being 1–2 years older than those from the other localities. This disparity in size/age may have been caused for instance by a lack of food or greater competition for food.

No correlation between the content of pollutants in muscle tissue with age and size was found. While some studies have confirmed the positive correlation of the pollutant content in fish muscle tissue with age and size, in other studies this trend was not confirmed, or the correlation was even negative. Due to its affinity to muscle tissue, the concentration of Hg is positively correlated with fish age and size, whereas the concentration of most other metals is negatively correlated with fish age and size (Jezierska and Witeska 2006). Dvořák et al. (2015) confirmed positive correlation between Hg, Pb, Cd, Cr and Zn load in chub muscle tissue with age and size, whereas Farkas et al. (2003) found positive correlation just for the Hg content of the bream (*Abramis brama*) muscle, no correlation for Cd, negative correlation for Pb and Zn. Klavins et al. (2008) found no correlation between age and content of heavy metals (Pb, Ni, Cd, e.g.) in perch. Has-Schöth et al. (2015) found positive correlation between age and content of Pb, Hg, and Cd in muscle tissue of carp. Negative correlation between the concentration of pollutants and age does not result from the fact that young fish are found in more polluted places, rather, it depends on differences in the food intake of young and older individuals (Farkas et al. 2003).

In this study, Cd concentrations tended to be either below the limit of detection or very low at all sites, which are typical results of previous studies on European freshwater fish (e.g. Dvořák et al. 2015; Vičarová et al. 2016; Cordeli et al. 2023). Similarly, Pb concentrations in muscle tissue were mostly below the limit of detection or very low, slightly higher in perch than in other species. Recently, Dvořák et al. (2015) reported Pb concentrations in chub muscle tissue from the lower course of the Bečva as around  $20 \pm 10 \mu\text{g}/\text{kg}$ , i.e. slightly lower than in the present study, and similar results have also been recorded in other rivers within the Morava river basin. In all cases, Cd and Pb concentrations were below the maximum allowable concentration in edible fish muscle, according to EC regulations, i.e.  $50 \mu\text{g}/\text{kg}$  for Cd and  $300 \mu\text{g}/\text{kg}$  for Pb (Commission Regulation (EC) No. 2023/915); thus, the results obtained in the present study are in accordance with those from previous studies.

In 2007 and 2008, Kružíková et al. (2011) sampled chub for Hg content at two localities on the Svratka, one upstream and the other downstream of our sampling site. Chub from the upstream locality had muscle Hg concentrations of  $110 \pm 40 \mu\text{g}/\text{kg}$  in 2007 and  $70 \pm 30 \mu\text{g}/\text{kg}$  in 2008, while the downstream locality had slightly higher concentrations at  $100 \pm 30 \mu\text{g}/\text{kg}$  in 2007 and  $120 \pm 143 \mu\text{g}/\text{kg}$  in 2008. Aside from the upstream locality sampled in 2008, these values are approximately twice as high as those recorded in this study ( $56.05 \pm 17.42 \mu\text{g}/\text{kg}$ ). Since the fish in both studies were of approximately the same age and size, increased Hg content in older fish (Zrnčić et al. 2012) is not applicable in this case. The highest muscle tissue Hg concentrations in this study were recorded in perch, suggesting that the species bioaccumulates Hg (and Pb) by consuming contaminated prey. While higher concentrations of Hg in perch compared to other fish species have also been observed in previous studies (e.g. Dušek et al. 2005; Havelková et al. 2008), our finding of higher Hg concentrations in roach muscle tissue than chub contradicts the findings of Havelková et al. (2008) and Falter and Schöler (1994), who both reported lower Hg in roach, which may be due to different food preferences in different locations. Dvořák et al. (2015) found relatively high Hg concentrations in chub muscle at sites close

to the Bečva's confluence with the river Morava ( $200 \pm 0.11 \mu\text{g/kg}$ ), however, they studied a site located more downstream, with another known potential source of pollution. It is also likely that heavy metal pollution levels in the Bečva have declined over the years, since the value of other pollution, presented by indicators used to assess the quality of surface waters ( $\text{COD}_{\text{Cr}}$ ,  $\text{BOD}_5$ ,  $\text{N-NH}_4$ ,  $\text{N-NO}_3$ ) decreased between years 1991–2021 (Ministry of Agriculture of the Czech Republic, Ministry of the Environment of the Czech Republic 2021).

Bečva 4, the site furthest upstream on this river, had high Hg concentrations in fish muscle tissue. However, Bečva 1, the site located furthest downstream and directly affected by its location within the city, exhibited even higher concentrations. Apart from this exception, the sites show a continuous downward trend in fish muscle Hg concentration further downstream. Similar, though slightly lower, muscle concentrations were also recorded in chub from the Svratka, though these fish were slightly younger than those from the Bečva. Nevertheless, all muscle Hg readings were below the maximum allowable concentrations in fish muscle according to EC regulations, i.e.  $500 \mu\text{g/kg}$  (Commission Regulation [EC] No 2023/915).

While Ni concentrations were low or below the limit of detection in perch and roach muscle tissue (Table 4), concentrations in chub muscle were high and varied greatly, both within individuals and across localities, when some samples were under the LOD ( $14.8 \mu\text{g/kg}$ ). In contrast, others had Ni concentration up to  $5.3 \text{ mg/kg}$ . Nickel leakage in the Bečva in 2020 was not reflected in the nickel content in the chub muscle tissue since it was generally lower compared to chubs from the Svratka, it was probably quickly diluted in the river current. Andreji et al. (2012) also recorded high Ni concentration variability in chub muscle tissue in fish from the River Nitra, Slovakia, and in other studies, high variability in Ni concentration in muscle tissue of one fish species from one locality occurs relatively often. Uysal et al. 2009 found high variability in Ni content ( $6.21\text{--}887.1 \text{ mg/kg}$ ) in muscle tissue of *Chondrostoma nasus* from Enne Dame Lake (Türkiye). Akbulut and Tuncer (2011) found high variability in Ni content in muscle tissue of *Capoeta capoeta* from Delice River ( $2.201\text{--}8.243 \text{ mg/kg}$ ) and in chub meat only in one of the samplings ( $0\text{--}0.441 \text{ mg/kg}$ ), while the variability was low on the later sampling ( $4.41\text{--}15.41 \text{ mg/kg}$ ). Svobodová et al. (2002), who studied Ni content in the muscle tissue of common carp in Czech fishponds also found high variability of Ni content in the muscle tissue of carp in some ponds. Regarding the dependence of the Ni content in the meat tissue and its content in the sediment, they recorded concentrations of just over  $0.5 \text{ mg/kg}$  in two muscle samples, in their study area, however, the highest sediment concentration was  $63.3 \text{ mg/kg}$  dry matter, while one site had a sediment concentration of just  $7.7 \text{ mg/kg}$  dry matter. Similarly, there were no clear correlations between fish muscle, water, or river sediment Ni concentrations, i.e. the relatively high Ni content in fish muscle could not be directly attributed to the relatively high Ni content in sediment in the Svratka (mean =  $40.56 \text{ mg/kg}$ ). The fact that the content of pollutants in sediments is not always positively correlated with the content of pollutants in muscle tissue is because bioaccumulation is rather influenced by the trophic chain, where bioaccumulation of heavy metals depends on what the food chain looks like in each locality/biotope (Yodzis 2001; Jurajda et al. 2020).

According to the European Food Safety Authority (EFSA), allergic manifestations may occur in individuals sensitive to Ni with a daily intake of as little as  $0.5 \text{ mg}$ , which corresponds to approximately  $6.6 \mu\text{g}$  per kg of body weight for an average individual of  $75 \text{ kg}$  (EFSA 2015). Consequently, some of the fish in this study represent a potential threat to Ni sensitive individuals and should be avoided for human consumption.

Chrome concentrations were mostly below the detection limit in all samples from all localities. Andreji et al. (2005) reported a mean muscle tissue Cr concentration of  $170 \pm 30 \mu\text{g/kg}$  in chub,  $190 \pm 30 \mu\text{g/kg}$  in perch and  $240 \pm 80 \mu\text{g/kg}$  in roach from

the river Nitra, suggesting that Cr bioaccumulates in these fish in the order roach > perch > chub; however, as all samples of perch and roach and almost all chub in this study had concentrations below the detection limit, we were unable to confirm this trend.

According to our study, concentrations of heavy metals do not pose a substantial risk to people wishing to consume fish; however, people who are sensitive to Ni should be cautious.

In our study we found that Hg bioaccumulates in muscle tissue of fish in the order perch > roach > chub. Compared to previous studies, we found evidence of a reduction in Hg concentrations in chub muscle tissue in the Svratka and the lower stretch of the Bečva.

Another positive finding is that the leak of Ni into the Bečva under the Valašské Meziříčí outlet in 2020 appears not to have been reflected in an increase in Ni concentrations in fish muscle. Note, however, that bioaccumulation may take time to become apparent, thus it may be appropriate to analyse the Ni content in fish muscle over a longer period on the Bečva.

Finally, we suggest that further research is needed on the potential factors causing such high variation in Ni concentrations in muscle between fish from the same locality.

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