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ORIGINAL PAPER

Anglers' Perceptions and Fish Consumption Risks in the Lower Tisza River Basin

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Abstract This study identifies risk perception and actual health risks from exposure to metals in fish from the Tisza River Basin of central Europe. Mining in the region has chronically introduced metals; however, two major mine-tailings spill in 2000 contributed an estimated 240,000 m^3 of wastewater and tailings contaminated with cyanide and metals to the system. In 2013 and 2014, water and fish ($N = 99$) collected from the lower Tisza River Basin were analyzed for cadmium, copper, lead, nickel, and zinc. Concurrently, surveys ($N = 45$) collected near sampling sites assessed fish-consumption patterns and risk perception. Metals in water exceeded regulatory criteria at multiple sites; however, metals are not bioaccumulating to a degree of undue concern in fish as bioaccumulation factors were below 1. Average concentrations of metals in fish fillets ($\mu g\ g^{-1}$ wet weight) in decreasing order were zinc $(8.8) >$ copper $(0.14) >$ nickel $(0.06) >$ lead $(0.02) >$ cadmium (0.004). Fillets were within European Food Safety Authority recommendations; however, the Target Hazard Quotient for lead was elevated at 1.5 for average consumers and 3.5 for people who consume fish twice weekly. The majority of survey participants were unconcerned with local fish consumption (87 %), citing the "clean" appearance of fishing locations. Participants also reported relatively low fish consumption, with most (76 %) eating basin fish once a week or less. While our study indicates fish are generally safe for human consumption, waters are polluted, suggesting that local fishing populations may be at risk from unseen pollutants and highlighting the need for monitoring and notification systems.

Keywords Metals · Exposure · Fish · Health risk · Risk perception · Risk assessment

Introduction

The pollution of freshwater fisheries is a global concern as over one-third of the available freshwater on the planet is subjected to human activities that often lead to contamination (Schwarzenbach et al. 2010). Humans consume approximately 80 % of the fish produced globally, and consumption rates are predicted to increase by at least 27 % by the year 2030 (Msangi et al. 2013). As such, fish are an important resource for feeding the growing human population (Msangi et al. 2013); however, fish consumption can expose humans to metal pollution in the environment (Medeiros et al. 2012). The evaluation of fisheries in regions with industrial activity is important for future food security as people must weigh the benefits of eating fish against potential risks of metal exposures, i.e., possible nerve, liver and kidney damages, and potential endocrine disrupters (Verbeke et al. 2005). Metals are persistent pollutants that do not break down, can be toxic at trace concentrations, and readily bioaccumulate into the food chain (Idriss and Ahmad 2015). One of the ways metals

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enter waterways is by direct or accidental discharge of mine tailings (Hudson-Edwards et al. 2011) such as the cyanide and metal spills in the year 2000 into the Tisza River in Central Europe (Koenig 2000).

The Tisza River is the largest tributary of the Danube River and the tenth largest river in Europe (Harka 2006). The basin receives on average 744 mm year^{-1} of precipitation. However, this varies across the basin, with less than 500 mm year^{-1} being received in the southwestern portion of the basin (ICPDR 2008). The central and lower portions of the Tisza River Basin encompass a region known as the Pannonian Plain, which has been intensively modified through channelization and drainage of wetlands to accommodate intensive agricultural activity. This extreme modification has led to soil erosion and increased flood vulnerability, with at least nine floods breaching Hungarian levees in the last 100 years (Schweitzer 2009). Floodplain reconnection has been suggested for the lower Tisza Basin (Guida et al. 2015), which may ameliorate flood risks while also offering ecological benefits. Crop production in Hungary declined significantly in the early 1990s, with some resurgence in 1994. However, the last 10–15 years have seen a general decline in both crop and livestock farming across the Tisza River Basin, with large portions of arable land left fallow. The major crop of the region is cereals. Aquaculture, and pig and cattle farms are locally important, especially for the Serbian economy (ICPDR 2008).

Tisza River fisheries have been an important resource for centuries, with accounts of rich stocks dating back to the 800s (Harka 2006). However, intensive river regulation and pollution have depleted and degraded fisheries (Harka 2006; ICPDR 2011), particularly for tributaries near mining activity (Harka 2006). In the last 15 years, the Tisza River Basin has experienced both man-made and natural disasters, in the form of record floods (Lóczy et al. 2009) and multiple failures of mine tailing ponds (Erős et al. 2015; Koenig 2000; László 2006). Two successive tailings-dam failures in the winter of 2000 contaminated the Tisza River with over 200,000 m^3 of mine tailings laced with cyanide and metals (Macklin et al. 2006). Fish were the most gravely affected group, with estimates of over 1240 tons of fish killed in Hungary alone (Koenig 2000). Assessments following the disasters reported elevated metals in river sediments, primarily cadmium, copper, lead, and zinc (Fleit and Lakatos 2003; Garvey et al. 2000; Kraft et al. 2006; Macklin et al. 2006; Schulz et al. 2005; UNEP 2000). Negative effects of the spills on the fishing industry were profound, including losses from both the 6-month ban on fishing and the delayed recovery of fish stocks to their former sizes and densities (Garvey et al. 2000). Concerns were also raised as to the long-term effects of residual metals in the river on fish and the humans who consume them (Csányi 2002; Garvey et al. 2000; UNEP 2000).

Understanding the patterns of fish consumption is important for a transboundary river like the Tisza, which encompasses five different countries and cultures and may reflect significant differences in exposure risk. Contamination of fish may disproportionately affect different demographic groups. Studies have shown significant correlations between race, ethnicity, and culture with the consumption of recreational fish catch (Boischio and Henshel 2000; Chess et al. 2005). Risks to minority populations may be increased by disparities between ethnic groups regarding the awareness of fish risks and benefits (Burger and Gochfeld 2008). Understanding differences in perception and opinion is important for sustainable fisheries management to successfully communicate risk across populations. To effectively weigh the consequences of food consumption, consumers need to be aware of both the true benefits and associated risks, which can differ across a population and are subject to some degrees of uncertainty (Frewer 2012). Risk communication should target the population of concern (Frewer 2012; Katner et al. 2011), and consider factors including the socioeconomic status, beliefs, and preferences of the target population (Frewer 2012). Subsistence fishers, those who rely on their catch as part of their daily food intake and perhaps an important source of protein, are less likely to heed warnings regarding fish consumption. Nonsubsistence fishers may be more open to adjusting their consumption habits in response to advisories, as their motivations may be driven more by the cultural and social values of fishing (Chess et al. 2005).

The objectives of this study were to first, quantify the bioaccumulation of metals in commercial fish species from the Tisza River Basin following more than a decade of recovery; and second, assess the health risks and public perception of these risks from eating contaminated fish from the Tisza. Specifically, we sought to compare metals in fish fillets from the river and adjacent oxbow lakes of the lower basin with maximum acceptable concentrations (MACs) set by the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO). We also sought to evaluate metals' exposure from consuming fish based on tolerance limits set by the European Food Safety Authority (EFSA). Given the volume of metals introduced into the basin, we expected to find high concentrations of metals in fish fillets that exceed recommendations for human consumption. We also expected fish from the Tisza River would have higher metal levels than in the oxbow lakes. In addition, we sought to gauge human risk perception in Tisza River Basin countries regarding adverse effects from eating locally caught fish. We expected to find low perception of risk from local anglers as they are actively engaged in fishing. Conversely, we expected to find a higher perception of risk in cities and urban centers where locals are less likely to engage in fishing as part of their diet. We then compared the dietary risks of eating local fish with the perception of those risks in order to identify disparities that may warrant further investigation (i.e., groups of fish or locations that present high risk where public perception of those risks are low).

Materials and Methods

Study Area

The study area encompassed the Tisza River Basin within portions of Hungary, Romania, and Serbia (Fig. 1). Sampling for metals analyses consisted of five locations distributed longitudinally along the Hungarian stretch of the Tisza River, sampled July 2013, as well as oxbow lakes along the lower Tisza, two lakes in southern Hungary and one lake in northern Serbia, sampled June 2014. Sampling sites 1–5 (Fig. 1) along the Tisza River consisted of five of the 27 sites from the ICPDR Joint Tisza Survey in 2001 (Csányi 2002), representing a longitudinal assessment of the Hungarian stretch of the river. Oxbows sampled included two in southern Hungary near the city of Szeged, Mártélyi Holt-Tisza and Körtvélyesi Holt-Tisza, and one in northern Serbia near the town of Čurug, the Mrtva Tisa. The oxbow lakes at the Mártélyi Holt-Tisza (46 ha) and Körtvélyesi Holt-Tisza (60 ha) locations (sites 6 and 7) are situated about 20 km north of Szeged, Hungary. These oxbows formed during the Vásárhelyi channelization and floodplain reconnection projects in the late nineteenth century (Pálfai 2003). This area is part of the Mártély Natural Conservation Area, and tends to flood annually at high flood stages (Kiss 1982). The region has been identified as a Ramsar protected site for its importance as an otter and bird breeding site, and is also a valuable site for recreation and commercial fishing (ICPDR 2008). A pumping station situated in the northern segment of the oxbow, with an outlet at the southern end, keeps the Mártélyi-Holt oxbow connected to the river system year-round. The Körtvélyesi Holt-Tisza, on the other hand, is reconnected to the main channel during high flood stages, but aside from a small channel on the oxbow's southern arm is otherwise disconnected (Pálfai 2003). This disconnect at Körtvélyesi Holt-Tisza has led to increases in both sedimentation and eutrophication in the oxbow (Kiss 1982; Pálfai 2003). In northern Serbia, samples were collected from an oxbow lake in the Stara Tisa Natural Area, known as the Mrtva Tisa (site 10), which was formed in 1858 from meander cutoffs (Gajin et al. 1989). The Mrtva Tisa lies outside the levee system and is not connected to the mainstem Tisza River; however, when the lake is at full capacity a pumping station in the northern section of the oxbow may be used to transfer water out of the lake into the river. Sites 8 and 9 represent survey collection sites in Szeged, Hungary; and Timişoara, Romania, respectively. Fish were not collected from Sites 8 or 9.

In order to gain the most relevant perspective for interdisciplinary fieldwork, surveys were collected in person from locations that coincided with metals sampling sites (with the exception of Timişoara, Romania, which

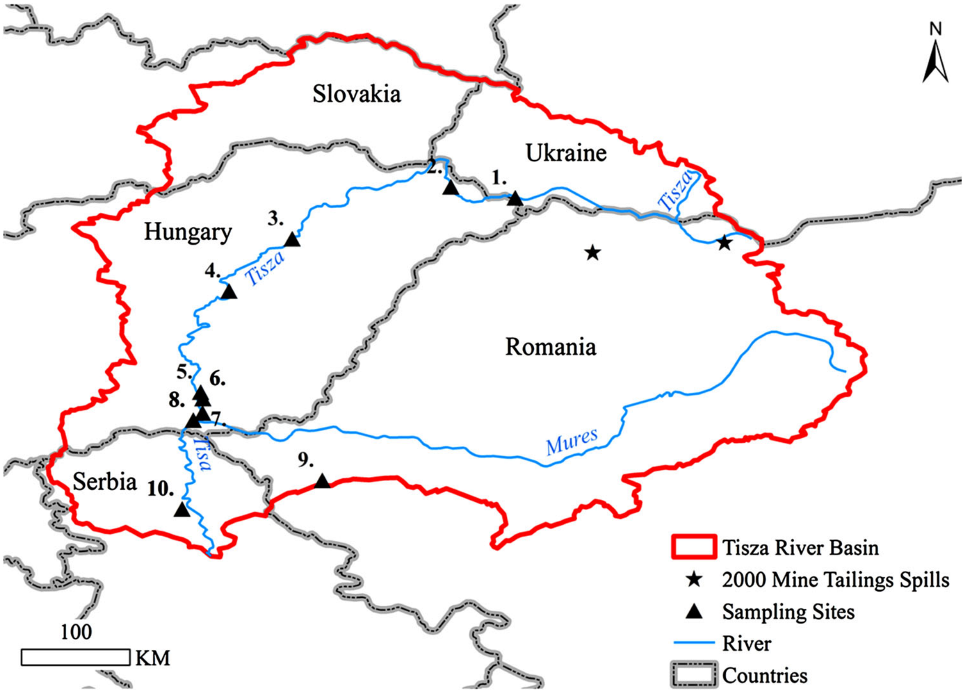

Fig. 1 Sampling and survey locations collected at sites in the lower Tisza River Basin, July 2015 and May–June 2014 in Romania, Hungary, and Serbia. *Triangles* correspond to sampling locations: *1–5*—Tisza River; *6*—Mártélyi Holt-Tisza oxbow lake; *7*—Körtvélyesi Holt-Tisza oxbow lake; *8*—Szeged, HU; *9*—Timişoara, RO; *10*—Mrtva Tisa oxbow

lacks metals data; and the upstream sampling locations on the Tisza River, which lack survey data). Samples from the furthest downstream location on the river, near the village of Tápé, Hungary, were compared with survey data from the village of Mindszent, Hungary. In addition, survey data were obtained from the city of Szeged, Hungary and the two oxbow lakes adjacent to Szeged, Mártélyi Holt-Tisza and Körtvélyesi Holt-Tisza. Surveys were also collected from the oxbow lake known as the Mrtva Tisa, located near Čurug, Serbia. These surveys were collected as near as possible to the sample collection sites, within a day of physical sampling if not simultaneously. Individuals were approached at random along the sampling site, with an accompanying translator at all sites except Timişoara, Romania.

Metals

Water quality, temperature, dissolved oxygen, and pH, was collected with a HACH HQ40d multiparameter meter. Water hardness and alkalinity were determined according to the U.S. standard methods (APHA 2005). Water was collected in acid-washed high-density polyethylene bottles, filtered through ashed glass fiber filters (0.45 µm) and frozen until analysis. Fish were collected using a combination of deep-water trawling by electrofishing, and backpack electrofishing. Triplicates within the same size class of each species were selected to minimize age-related variability. After length measurements were taken, fish were euthanized via manually applied blunt force trauma to the head followed by decapitation, according to American Veterinary Medical Association (AVMA) guidelines (Leary et al. 2013). Muscle fillets were removed, immediately placed on ice in the field, and then frozen at 4 °C until analysis.

Concentrations of copper, cadmium, nickel, lead, and zinc in water and fish were determined by graphite furnace atomic absorption spectrophotometry (GF-AAS), according to the U.S. standard methods (APHA 2005). Before analysis, fish fillets were freeze-dried and then microwave digested (MARS Xpress microwave digester, CEM, Corp.) in concentrated trace metal grade nitric acid (HNO_3). Digestate was diluted to a standard concentration of 5 % HNO_3. Following filtration to eliminate inorganic components, an aliquot was removed for analysis. Water samples were brought to 5 % HNO_3 before analysis. Quality-control procedures were performed by repeating any run that fell outside of the ±20 % of expected values. Preceding and following each run external quality checks (EQC, Fisher Brand reference standards) were analyzed, as well as a blank and middle calibration standard, for calibration verification approximately every 10 samples. In addition, at least one duplicate and sample spike (50 % of running standard and 50 % sample digestate) was chosen at random and analyzed during each run to verify consistent recovery. Detection limits of tissue digestate (µg L^{-1}) were: cadmium (0.26), copper (0.88), nickel (0.68), lead (0.73), and zinc (0.27).

Statistical analyses for metals included descriptive assessment and hypothesis testing. To obtain the most conservative estimates, observations below method detection limits were set to 0 in calculations of means and standard deviations for figures and tables, exposure risks, and bioaccumulation factors. In estimates of statistical differences, however, we set observations to the detection limit to avoid skewing data by including zeros. In practice, setting very low values to detection limits or to zero did not affect overall trends. Outliers were removed before data analysis, with criteria for outlier removal according to Grubb's test (Grubbs and Beck 1972). Statistical analyses included Kruskal–Wallis nonparametric analysis of variance followed by Welch's two-sample *t* test, which were performed with the open-source statistical package "R" (Team RC 2014). Fillet concentrations are reported in wet weight (ww).

Fish species were combined into the following groups by their common names and trophic levels (TL). Trophic Levels are averages from those reported on FishBase (Froese and Pauly 2016) (Table 1) followed by number of samples: Bream (TL $\approx$ 3.13, $N = 32$) (*Abramis brama, Ballerus ballerus, Ballerus sapa, Blicca bjoerkna*); Carp (TL $\approx$ 2.85, $N = 17$) (*Barbus, Carassius gibelio, Cyprinus carpio*); Catfish (TL $\approx$ 4.05, $N = 6$) (*Ameiurus nebulosus, Silurus glanis*); Chub (TL $\approx$ 2.7, $N = 6$) (*Squalius cephalus*); Sander (TL $\approx$ 4.05, $N = 14$) (*Sander lucioperca, Sander volgensis*); Whitefish (TL $\approx$ 3.5, $N = 3$) (*Coregonus nasus*); Sunfish (TL $\approx$ 3.25, $N = 10$) (*Lepomis gibbosus, Lopomis macrochirus*). The number of fish collected by location were: Mártélyi Holt-Tisza ($N = 12$), Körtvélyesi Holt-Tisza ($N = 14$), Mrtva Tisa ($N = 12$), Tisza1 ($N = 7$), Tisza2 ($N = 11$), Tisza3 ($N = 11$), Tisza4 ($N = 10$), Tisza5 ($N = 10$).

Data analysis for metals in fish tissues included hypothesis testing for differences and calculation of regulatory metrics. No significant differences in metals from water or tissue samples were observed between sampling sites on the Tisza River (Kruskal–Wallis nonparametric ANOVA, $\alpha = 0.05$). Thus, observations from the Tisza River were grouped together for statistical analysis. Bioaccumulation factors (BAFs), defined as the total concentration of contaminant in fish muscle tissue (µg g^{-1} ww) relative to total concentration of the contaminant in water (µg L^{-1}), were determined for each element at each sampling location. BAFs were calculated using the formula: $BAF = C_{tissue}/C_{water}$.

Table 1 Groups of fish identified by survey participants, followed by scientific name (*Genus species*) of individual species within each group

Fish	Trophic level	Reference
BREAM	**3.13**	
Abramis brama	*3.1*	Kottelat and Freyhof (2007)
Ballerus ballerus	*3.2*	Kottelat and Freyhof (2007)
Ballerus sapa	3.0	Kottelat and Freyhof (2007)
Blicca bjoerkna	3.2	Kottelat and Freyhof (2007)
CARP	**2.85**	
Barbus barbus	*3.1*	Bianco (1998)
Carassius gibelio	2.5	Kottelat and Freyhof (2007)
Cyprinus carpio	*3.1*	Kottelat and Freyhof (2007)
Squalius cephalus	*2.7*	Kottelat and Freyhof (2007)
CATFISH	**4.05**	
Ameiurus nebulosus	*3.7*	Page and Burr (1997)
Silurus glanis	*4.4*	Kottelat and Freyhof (2007)
CHUB	**2.7**	
Squalius cephalus	2.7	Kottelat and Freyhof (2007)
SANDER	**4.05**	
Sander lucioperca	*4.0*	Kottelat and Freyhof (2007)
Sander volgensis	*4.1*	Kottelat and Freyhof (2007)
SUNFISH	**3.25**	
Lepomis gibbosus	*3.3*	Page and Burr (1997)
Lepomis macrochirus	*3.2*	Page and Burr (1997)
WHITEFISH	**3.5**	
Coregonus nasus	*3.5*	Robins et al. (1991)

Group trophic levels (bold) were averaged from individual trophic levels obtaind from FishBase (Froese and Pauly 2016)

Mean concentrations of metals in fish fillets were compared against safety standards set by the joint Food and Agricultural Organization and World Health Organization Codex Alimentarius standards for food safety (FAO/WHO 2002). Estimated Daily Intake (EDI) values were calculated for an average adult (70 kg) and child (18 kg) with corresponding meal-sizes of 227 and 57 g, respectively, following U.S. Environmental Protection Agency (USEPA) guidelines (Brodberg and Klasing 2003):

$$\mathrm{EDI}_{\left(\mu\mathrm{g\ kg^{-1}\ body\ wt.\ per\ day}\right)} = \frac{\left(C_f \times M\right)}{W_{\mathrm{AB}}}$$

where C_f is the average fillet concentration (µg g^{-1} ww). M is the average meal size (g day^{-1}), and W_{AB} is the average body weight (kg). EDIs then were compared to Tolerable Daily Intake (TDI) and Tolerable Weekly Intake (TWI) recommendations set by the European Food Safety Authority (see EFSA 2009, 2010a, b, 2012, 2014, 2015a, b, c).

Human health risks were assessed by estimating Target Hazard Quotient (THQ):

$$\mathrm{THQ} = \frac{\left(E_{\mathrm{F}} \times E_{\mathrm{D}} \times F_{\mathrm{IR}} \times C_f \times 10^{-3}\right)}{\left(R_f D \times W_{\mathrm{AB}} \times T_{\mathrm{A}}\right)}$$

where E_{F} is the exposure frequency (365 days year^{-1}); E_{D} is the exposure duration or lifespan (70 years); and F_{IR} is the food ingestion rate (kg day^{-1}), which was estimated from our survey data. At an average consumption of 45 days out of the year and 227 g portions, the $\mathrm{F_{IR}}$ was estimated as 0.023 kg person^{-1} day^{-1} for the average adult from the Tisza Basin. $\mathbf{C_f}$ is the average metal concentration in fish fillets (mg kg^{-1} ww); $R_f D$ is the oral reference dose (mg kg^{-1}) (USEPA 2016); W_{AB} is average body weight (kg), corresponding to 77 and 18 kg for adult and child, respectively; and T_{A} is the average exposure time for non-carcinogens ($E_{\mathrm{D}} \times 365$ days year^{-1}). We calculated THQs for adults at the average reported fish consumption (estimated at 45 days year^{-1}) as well as more frequent consumers eating fish twice a week (104 days year^{-1}). The Hazard Index (HI) was estimated for each consumption frequency as the sum of the THQ for each metal:

$$\mathrm{HI} = \mathrm{THQ_{Cd}} + \mathrm{THQ_{Cu}} + \mathrm{THQ_{Ni}} + \mathrm{THQ_{Pb}} + \mathrm{THQ_{Zn}}$$

In addition, as an assessment of the greatest potential risk, the top three fish with the highest concentrations for each metal, which included outliers removed from previous analyses, were used to calculate the highest potentials of EDI, THQ, and HI from consuming Tisza River Basin fish.

Survey Design and Participants

In May–June 2014, 45 completed surveys were collected from a survey conducted among the general public at locations along the lower Tisza River Basin that coincided with metals sampling locations, as previously described (Fig. 1), which meant the surveys were conducted using a nonrandom, purposive sampling technique (Teddlie and Yu 2007). The survey design was expanded from a previous study conducted in 2013 through the addition of a set of questions regarding local fish collection and consumption habits, intended to gauge stakeholders' perceptions of fish health and their attitudes toward fishing guidelines. Following human subjects guidelines, all materials were translated into participants' native languages before the study by a certified translation service, participants were given a copy of the survey in their native language, and translators joined the researchers in the field to translate questions and to record answers in English onsite to ensure the most complete and accurate survey responses possible.

For each location, every person possible was approached regarding the survey to elicit as large a sample as possible. During the survey, participants were asked to describe their perception of the river basin and any changes affecting the system, personal fishing and local fish consumption habits, and general demographic information. Actual fish consumption species and numbers reported by participants may be biased by their ability to accurately recall their behavior over time (Tourangeau 1984).

Survey responses were analyzed with a mix of qualitative and quantitative analysis. Short answers were coded qualitatively using QSR International's NVivo 10 software to identify patterns of responses across participants and sampling sites (NVivo 2012). Rank-ordered and binomial (yes/no) responses were tabulated in Excel then imported into SPSS (IBM 2015), where descriptive statistics were calculated to enable comparison of responses across sampling sites. Responses were compared between groups of respondents who reported eating versus not eating locally caught fish to assess differences regarding knowledge and beliefs about consumption risks.

Results

Metals Results

Water quality data are given in Table 2. In general, values were similar between locations; with exceptions of dissolved oxygen in the Tisza River, which was slightly lower than the oxbow lakes, and alkalinity in the Mrtva Tisa, which was nearly double that of other locations. Metals were elevated across the basin with concentrations in water exceeding regulatory limits (Csányi 2002) for several elements at every location (Table 3). For example, cadmium concentrations were roughly 0.7 μg L^{-1} at nearly every site, well above the 0.1 μg L^{-1} criteria. Copper, nickel, and lead were also elevated across locations.

Fish fillet concentrations are given in Table 3. Average concentrations of metals in fish fillets (μg g^{-1} ww) were zinc (8.8) > copper (0.14) > nickel (0.06) > lead (0.02) > cadmium (0.004). Cadmium, copper, and lead were significantly higher in fish collected from the oxbows than those from the Tisza River (Kruskal–Wallis, $\chi^2 = 61.68$, $p < 0.001$ and $\chi^2 = 37.4$, $p < 0.001$, and $\chi^2 = 57.6$, $p < 0.001$, respectively, followed by post hoc t tests $\alpha = 0.05$). A similar pattern was observed for nickel; with the Mrtva Tisa also significantly higher than other locations (Kruskal–Wallis, $\chi^2 = 42.6$, $p < <0.001$, followed by post hoc t tests $\alpha = 0.05$). Consistently, the BAFs for the oxbows were higher than the Tisza River (Table 3). Körtvélyesi Holt-Tisza and Mrtva Tisa exhibited the highest BAFs for cadmium (0.01 L g^{-1}) and nickel (0.08 and 0.16 L g^{-1}), while Mártélyi Holt-Tisza had the highest BAF for lead at 0.05 L g^{-1}. BAFs for copper were 0.04 L g^{-1} in the oxbows and 0.02 L g^{-1} in the river mainstem.

Between groups of fish, chub fillets consistently contained lower metals than other groups of fish, particularly for highly toxic cadmium and lead (Table 3). Sunfish and whitefish fillets contained significantly more copper than most groups, whereas catfish and sander fillets were significantly lower than other groups of fish (Kruskal–Wallis, $\chi^2 = 17.5$, $p = 0.007$, followed by post hoc t tests $\alpha = 0.05$). Catfish fillets also contained significantly more nickel than bream, carp, or chub (Kruskal–Wallis, $\chi^2 = 17.5$, $p = 0.007$, followed by post hoc t tests $\alpha = 0.05$). Lead concentrations were the highest in sunfish, with chub fillets significantly lower than other groups (Kruskal–Wallis, $\chi^2 = 18.2$, $p = 0.006$, followed by post hoc t tests $\alpha = 0.05$). BAFs for cadmium were the highest in catfish at 0.015, followed by bream at 0.008 (Table 3). Nickel BAFs were also the highest in catfish at 0.15 L g^{-1}. BAFs for copper and lead were the highest in whitefish at 0.07 L g^{-1} and 0.04 L g^{-1}, respectively.

The Mrtva Tisa presented the highest human consumption risk for cadmium with one 227 g serving containing on average 2.7 μg and contributing to an EDI of 0.04 μg kg^{-1} body wt for a 70 kg person (Table 4). The Mrtva Tisa also presented the highest risks for nickel and lead, with one serving containing on average 54.5 and 17.4 μg, translating to a body burden of 0.78 and 0.25 μg kg^{-1} body wt for an average adult. Sunfish, followed by bream and carp species, contributed to higher EDIs for multiple metals. Given that meal sizes were scaled by body weight, the EDI for children did not differ substantially from that of adults. Although the TDI and TWI for children is less than adults, dietary recommendations were not exceeded for any of the metals assessed. To assess the non-carcinogenic health risks presented by consuming fish, THQs were calculated for an average adult in the Tisza River Basin. Averaged

Table 2 Water quality indicators for Tisza River Basin sample sites

	Temp (°C)	DO (mg L^{-1})	pH	Alkalinity (as mg $CaCO_3$ L^{-1})	Hardness (as mg $CaCO_3$ L^{-1})
Tisza River	23.9	8.1	8.1	141.1	174.8
Mártélyi Holt-Tisza	23.4	12.3	8.1	163.5	265.5
Körtvélyesi Holt-Tisza	19.7	15.7	8.5	150.1	165.5
Mrtva Tisa	23.6	13.3	8.6	276.4	271.8

Table 3 Metals in water and fish fillets (mean ± SD) and mean bioaccumulation factors (BAFs)

Water	Cd (μg L^{-1})	Cu (μg L^{-1})	Ni (μg L^{-1})	Pb (μg L^{-1})	Zn (μg L^{-1})
Tisza River	0.68 (±.43)	5.9 (±3.4)	0	2.1 (±2.8)	72.4 (±48.6)
Mártélyi Holt-Tisza	0.49 (±.33)	5.5 (±1.4)	1.5 (±0.0)	1.4 (±0.7)	28.2 (±13.0)
Körtvélyesi Holt-Tisza	0.68 (±.47)	5.3 (±3.0)	2.3 (±1.6)	2.0 (±1.2)	20.9 (±7.4)
Mrtva Tisa	0.67 (±.63)	7.6 (±4.0)	1.5 (±0.0)	1.7 (±1.3)	23.2 (±3.2)
Water Criteria	0.1	2	1	1	5
Fish fillets	Cd (μg g^{-1})	Cu (μg g^{-1})	Ni (μg g^{-1})	Pb (μg g^{-1})	Zn (μg g^{-1})
Tisza River	**0.002 (±0)**	**0.064 (±.01)**	**0.017 (±0.0)**	**0.004 (±0.0)**	5.6 (±0.9)
Mártélyi Holt-Tisza	**0.001 (±0)**	0.248 (±.03)	0.057 (±.02)	0.067 (±.01)	24.4 (±8.6)
Körtvélyesi Holt-Tisza	0.010 (±0)	0.220 (±.03)	0.176 (±.03)	0.021 (±.01)	13.2 (±2.0)
Mrtva Tisa	0.012 (±0)	0.296 (±.04)	**0.240 (±0.6)**	0.077 (±.03)	–
BREAM	0.006 (±0)	0.151 (±.03)	0.045 (±.02)	0.042 (±.01)	13.7 (±3.7)
CARP	0.003 (±0)	0.152 (±.03)	0.095 (±.04)	0.019 (±.01)	3.67 (±0.9)
CATFISH	0.006 (±0)	**0.058 (±.02)**	0.355 (±.01)	0.016 (±.01)	8.85 (±4.1)
CHUB	0	**0.084 (±.04)**	0.018 (±.01)	**0.004 (±0.0)**	3.42 (±0.7)
SANDER	0.001 (±0)	**0.057 (±.02)**	0.026 (±.01)	0	4.95 (±1.2)
SUNFISH	0.009 (±0)	0.310 (±.03)	0.120 (±.07)	0.041 (±.03)	13.0 (±6.3)
WHITEFISH	0.002 (±0)	0.264 (±.06)	0.056 (±.02)	0.024 (±.01)	3.75 (±1.0)
TOP FISH	0.062 (±0)	0.680 (±0.2)	1.1 (±0.5)	0.380 (±0.1)	104.1 (±22.6)
MAC	0.1	4.5*	0.8	0.5	–
BAF	Cd	Cu	Ni	Pb	Zn
Tisza River	0.003	0.017	0.028	0.007	0.387
Mártélyi Holt-Tisza	0.002	0.045	0.038	0.015	0.865
Körtvélyesi Holt-Tisza	0.008	0.039	0.017	0.010	0.633
Mrtva Tisa	0.014	0.033	0.159	0.029	–
BREAM	0.008	0.027	0.030	0.012	0.773
CARP	0.003	0.028	0.058	0.011	0.118
CATFISH	0.015	0.011	0.005	0.012	0.637
CHUB	0.000	0.021	0.033	0.006	0.039
SANDER	0.002	0.014	0.044	0.000	0.240
SUNFISH	0.002	0.042	0.082	0.018	0.495
WHITEFISH	0.005	0.068	0.104	0.041	0.113
TOP FISH	0.084	0.109	0.531	0.219	4.25

Fish results in μg/g wet wt Maximum Acceptable Concentrations (MAC) set by the World Health Organization (FAO/WHO 2002)

Bold values represent statistically significant (α=0.05) observations

* Maximum acceptable Concentration in mg

across species and locations, THQs were well below 1 indicating low non-carcinogenic health risks for consuming fish from the Tisza River Basin (Table 5). While the EFSA Scientific Committee for Food set a recommended minimum intake for zinc of 25 mg day^{-1} for adults (EFSA 2014), there is no general standard for upper limits of zinc and concentrations detected in this study were well below this intake level at every location.

However, the accumulation of metals by fish is a function of environmental factors that affect their bioavailability as well as ecological factors such as species and age, resulting in some fish with higher body burdens than others. To determine the maximum exposure risk, the top three fish collected with the highest concentrations for each metal were assessed, including outliers removed from previous analyses. These consisted of mostly sunfish and species of bream with the exception of one carp and one catfish. Average fillet concentrations for these top fish remained below MAC for all metals examined with the exception of nickel, although lead approached the MAC (Table 3). Based on the highest values, consuming fish from the Tisza River Basin does not exceed TDI or TWI recommendations. The recommended tolerable daily and weekly exposure for lead was recently revoked by the

Table 4 Estimated Daily Intake (EDI) for an average adult (70 kg, 227 g meal size) and child (18 kg, 57 g meal size)

	Cd (μg g^{-1})		Cu (μg g^{-1})		Ni (μg g^{-1})		Pb (μg g^{-1})		Zn (μg g^{-1})	
	Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child
Tisza River	0.01	0.01	0.21	0.20	0.05	0.05	0.01	0.01	18.07	17.65
Mártélyi Holt-Tisza	0.00	0.00	0.81	0.79	0.19	0.18	0.07	0.06	79.16	77.30
Körtvélyesi Holt-Tisza	0.02	0.02	0.71	0.70	0.13	0.12	0.07	0.07	42.84	41.84
Mrtva Tisa	0.04	0.04	0.75	0.73	0.78	0.76	0.25	0.24		
BREAM	0.02	0.02	0.49	0.48	0.15	0.14	0.08	0.07	44.40	43.35
CARP	0.01	0.01	0.49	0.48	0.31	0.30	0.06	0.06	11.89	11.61
CATFISH	0.02	0.02	0.19	0.18	0.02	0.02	0.05	0.05	28.70	28.03
CHUB	0.00	0.00	0.27	0.26	0.06	0.06	0.01	0.01	11.08	10.82
SANDER	0.00	0.00	0.19	0.18	0.09	0.08	0.00	0.00	16.05	15.67
SUNFISH	0.01	0.00	0.75	0.74	0.39	0.38	0.13	0.13	42.16	41.17
WHITEFISH	0.01	0.01	0.86	0.84	0.18	0.18	0.08	0.08	12.16	11.87
TOP FISH	0.20	0.20	2.20	2.15	3.39	3.31	1.25	1.22	337.67	329.74
TDI (μg day-1)	25.0	6.4	350*	90*	196.0	50.4	35.0	9.0	0.1**	8.5**
TWI (μg week-1)	175.0	45.0	2450*	630*	1372.0	352.8	245.0	63.0	0.8**	59.8**

Tolerable Daily Intake (TDI) and Tolerable Weekly Intake (TWI) recommendations set by the European Food Safety Authority (see EFSA 2009, 2010a, b, 2012, 2014, 2015a, b)

* Values in mg

** Values represent minimum nutritional requirements in mg

Table 5 Target Hazard Quotient (THQ) and the Hazard Index (HI) were calculated for adults at average fish consumption (45 days $year^{-1}$) and twice weekly consumption (104 days $year^{-1}$)

	Average Tisza basin fish THQ					
	Cd	Cu	Ni	Pb	Zn	HI
Average consumer	1.45E−06	1.38E−06	1.13E−06	0.08	1.17E−05	0.08
Twice weekly	3.36E−06	3.20E−06	2.61E−06	0.18	2.71E−05	0.18
	Top fish THQ					
	Cd	Cu	Ni	Pb	Zn	HI
Average consumer	2.48E−05	6.78E−06	2.09E−05	**1.54**	1.4E−04	**1.54**
Twice weekly	5.74E−05	1.57E−05	4.83E−05	**3.55**	3.2E−04	**3.55**

Bold values represent elevated non-cancerous health risks

Calculations were made for both average fish fillet concentrations and three fish with highest metals (top fish)

EFSA stating that there was no acceptable protective concentration (European Food Safety Authority 2010a). Based on the old recommended TDI of 0.5 μg kg^{-1} body weight for lead, fish from the Tisza River Basin present low risk for lead as the TDI was not met even when considering only the top fish fillets. However, the THQ for lead exceeded the risk threshold of 1 at 1.54 for the average adult consumer and 3.5 for adults who consume fish twice a week (Table 5).

Survey Results

The total survey sample size was 45. Of these 45, the average age was 45 years, with a minimum of 21 and a maximum of 73 years old. The majority of participants were male (67 %), and approximately 42 % of participants had some college education (Table 6). Of the 31 participants who reported that they consume locally caught fish (69 %), the average age was 52 and the participants were 77 % male; 35 % of this subset had attended college.

Regarding perception of water quality, on a scale of concern from 1 to 5, with 1 being least concern, participants who consumed local fish reported an average level of concern of 3.06. Concern for water quantity was below the median for fish consumers, at 2.26. Concern for health of the ecosystem was also slightly lower than the median for fish consumers, at 2.48. Comparing levels of concern for water quality, quantity, and ecosystem health among those who eat local fish with those who do not, levels of concern among those who reported not eating fish were about one

Table 6 Sample population characteristics for local fish survey

Participant demographics ($N = 45$)	Category	Count	Percentage
Gender	Male	30	67 %
	Female	15	33 %
Age	≤25	9	20 %
	26–35	6	13 %
	36–45	7	16 %
	46–55	7	16 %
	>55	15	33 %
	Mean	45.3	
	SD	16.6	
Education	College or higher	19	42.2 %
Fish consumption patterns	Eat local fish	31/45	68.9 %
	Catch fish their self	19/31	61.3 %
	Heard consumption warnings	7/31	22.6 %
	Concerned w. consumption	4/31	12.9 %
	Would follow warnings	28/31	90.3 %
	Cited health as reason to follow warnings	13/28	46.4 %

point higher in all three categories at 4.07, 3.5, and 3.58, respectively.

When asked to identify the most serious problem with the Tisza, pollution was reported most often (49 %). However, most respondents also said the reason they were unconcerned with fish consumption was that the site appeared clean; as a participant from the Mrtva site stated, "the water here is very clean," and another from the Tisza said "the river is not that polluted, it's not dangerous." In all, 17 of the 31 participants (55 %) made statements attributing their lack of concern to the apparent cleanliness of the water at their location. The term "clean" was used most frequently, followed by "not polluted." In addition, when asked to rank their concern on a scale of 1-5, with 5 being very concerned, participants noted median levels of concern for both water quality (3.06) and ecosystem health (2.48).

The majority of respondents consuming fish (87 %) reported that they were unconcerned with their consumption. The predominant reason given for this unconcern (63 %) was that the location was "clean," "not polluted," or "safe." Three respondents said they just do not think about the health of the fish. Two reported they are unconcerned because they eat fish that they received from their friends, implying they trust the source, or as one respondent said "[people] who don't want to kill me." Another two participants reported they are unconcerned because they can see on the fish whether there is something wrong, and "If we can see something wrong with the fish [we] just throw it back." Only one respondent said that they are unconcerned because they cook the fish very well, preparing the fish in a way to kill bacteria and parasites. Finally, two respondents claimed they are unconcerned because the testing after the cyanide pollution (2000) "proved there was no cyanide in the meat [of the fish]."

Of those who eat local fish, 61 % reported catching fish themselves. Only four of the 31 participants eating local fish reported being concerned with their consumption. Two of these four were located at the Mártély site in Hungary (site 6), one at the Szeged site (site 8), and the last in Serbia at the Mrtva Tisa site (site 10). One of the Mártély participants noted that he was concerned, but that "it's possible to see on the fish if it's healthy or not" and that "it [unhealthy fish] also smells different." The Szeged respondent noted that their concern was "because of the water pollution," while the Serbian respondent explained that even though he was concerned, he "thinks it [consumption] is safe." Only seven people (23 %) reported having ever heard any consumption warnings. However, the overwhelming majority (90 %) said that they would follow consumption guidelines if they heard them. The most commonly reported reason to follow warnings was concern for health (46 %).

When asked to identify what kind and how frequently they consumed local fish, 76 % reported eating fish once a week or less. The majority of participants reported consuming fish either once a week (23 %) or every few months (23 %). Only a small portion (3 %) reported eating fish every day. About 7 % of respondents stated their frequency and amount of consumption depended on the day's catch (Fig. 2). Two respondents alluded to the benefits of eating fish, saying they "know[s] we should eat fish two times a week," even though both reported eating fish less often. Participants identified eight fish species as ones they consumed (Table 7), with the most frequently consumed being the common carp (31 %) and common bream (22 %).

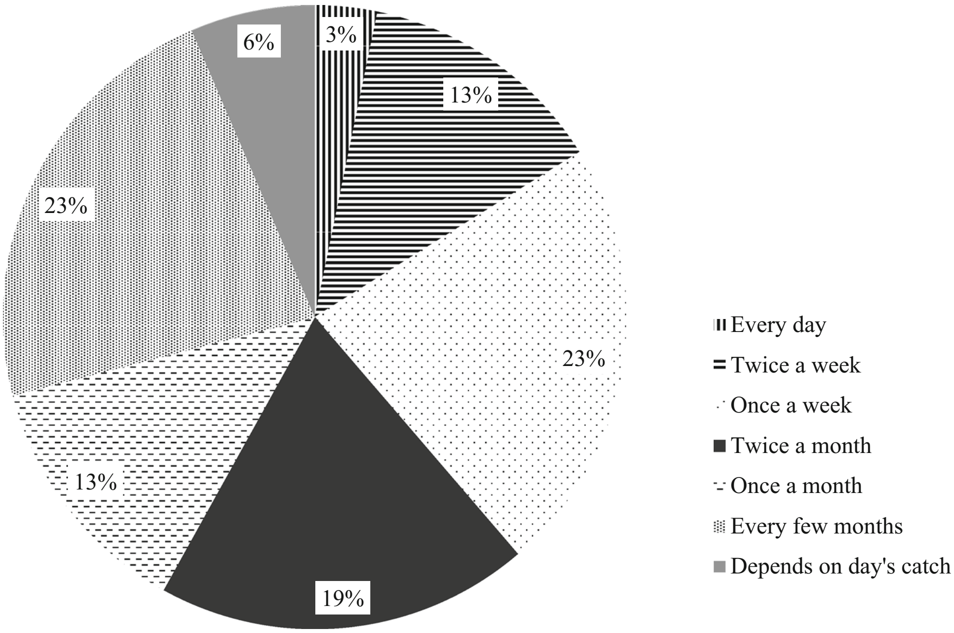

Fig. 2 Frequency of fish consumption by survey participants who reported eating local fish ($N = 31$)

These consumption reports may be subject to recall bias, i.e., the participants' ability to accurately recall their past behavior (Boischio and Henshel 2000).

As we hypothesized, participants in the urban sampling locations, Szeged and Timişoara, reported higher levels of concern for water quality (4.06 of 5) and ecosystem health (3.69 of 5) than the sample population (3.06 and 2.48, respectively), as well as lower levels of local fish consumption. Only 5 of 16 residents in these urban locations reported eating local fish: 31 % compared to the overall 69 % of the study (Fig. 3).

Discussion

The apparent cleanliness of the river basin and the medium-level concern for water quality and ecosystem health reported by participants is not wholly supported by water sampling test results, which showed metals levels exceeding regulatory criteria for cadmium, copper, and nickel at every location, and no significant difference between locations for water. However, the belief that a person could adequately detect risk based solely on appearance is not new. Previous studies have established that people tend to underestimate their own risk (Frewer 2012; Weinstein 1999) and that there is a tendency to believe pollution can be adequately perceived by the senses (Westphal et al. 2008). Cadmium, which can pose significant human health risk, was found in the basin at water concentrations which exceeded the 0.1 $\mu g\ L^{-1}$ regulatory limit by several orders of magnitude. If anglers are relying on sensory cues to determine safety, as suggested in their responses here and by the aforementioned literature, they may be vulnerable to unseen dangers such as heavy metals and chemical pollution. This disconnect between perception and reality in water quality highlights the importance of risk monitoring and communication, since pollutants, including the metals analyzed in this study, are not always apparent.

In spite of the elevated metals in water, concentrations in fish fillets from the Tisza River Basin are within the range of values reported for rivers across Central Europe, if not slightly lower (Table 8). Although water samples indicate elevated metals in the system, this pollution does not appear to be bioaccumulating in fish to a concerning degree as supported by low BAFs. However, we report the total dissolved concentrations for cadmium, copper, nickel, and zinc, of which generally less than half is biologically available for uptake by fish (Hallman and Brooks 2015a, b). Bioavailability of metals is dependent upon environmental factors including pH, temperature, water hardness (Moore and Ramamoorthy 2012), and dissolved organic carbon (Prusha and Clements 2004). The water quality indicators we measured were similar across the basin, with slightly alkaline waters and high water hardness, which limit the bioavailability of metals in the water and sediment. In addition, cadmium, copper, and lead do not typically biomagnify through freshwater foodwebs, although the mechanisms involved are not well understood (Cardwell et al. 2013). The application of bioaccumulation factors to assess risks from metals has been questioned (McGeer et al. 2003). Given the high concentrations observed in water samples collected in the Tisza Basin, further investigation into the bioavailable fraction and

Table 7 Fish species and reported consumption by survey participants (N = 73)

Fish species (common name/native/scientific)	Number of participants reported consuming	Percent of total fish reported (%)
BREAM	**16**	**22**
Common Bream/Dévér Keszeg/*Abramis brama*	16	22
CARP	**35**	**48**
Common Carp/Ponty/*Cyprinus carpio carpio*	23	31
Crucian Carp/Kárász/*Carassius carassius*	10	14
Silver Carp/Fehér busa/*Hypophthalmichthys molitrix*	2	3
CATFISH	**6**	**8**
Brown Bullhead/(Nonnative)/*Ameiurus nebulosus*	1	1
Wels Catfish/Harcsa/*Silurus glanis*	5	7
PIKE	**8**	**11**
Northern Pike/Csuka/*Esox lucius*	8	11
SANDER	**8**	**11**
Pike Perch (Zander)/Fogas Süllö/*Sander lucioperca*	8	11
SUNFISH	**0**	**0**
WHITEFISH	**0**	**0**
TOTAL	**73**	**100**

Bold values represent group designations followed by observations for individual species

bioaccumulation of metals in lower components of the foodweb may be warranted.

Overall, the type of fish consumed does not appear to affect metals exposure. Concentrations in fish fillets were below the maximum acceptable concentrations (MAC) set by the FAO/WHO for metals in fish fillets (Table 1), with the exception of lead in one fish (*Blicca bjoerkna*), which was above the 0.5 µg g^{-1} ww criteria at 0.62 µg g^{-1} ww. In addition, one fish (*Lepomis gibbosus*) approached the 0.8 µg g^{-1} ww criteria for nickel at 0.69 µg g^{-1} ww, while one catfish (*Ameiurus nebulosus*) well exceeded the criteria at 2 µg g^{-1} ww. Bream generally posed the greatest risk, having the highest levels of lead, nickel, and zinc, but these were outliers. Because participants reported eating carp (48 %) and bream (22 %) the most frequently, bream consumption may warrant further investigation with a larger sample size to rule out the risks posed by apparent outliers in this study. Even when considering the fish with the highest concentrations, dietary guidelines were not exceeded based on comparisons of EDI to TDI and TWI recommendations. The THQs calculated for an average adult indicate low dietary exposure risks for all metals except for lead, which exhibited higher THQs of 1.5 and 3.5 when consuming fish with elevated concentrations and also elevated risk for higher consumption frequency (Table 5). However, we found that most participants were not eating fish with great frequency (Fig. 2), with the majority of respondents reported eating fish once a week or less, and only 16 % eating fish twice a week or more, further lowering the risks of heavy metals exposure from fish consumption. The reduced consumption of local fish by urban residents is likely due in part to the higher perception of pollution in urban areas; four urban respondents specifically referenced pollution, with one citing discharge from factories as a concern. Another likely reason for reduced local fish consumption is convenience/ease of access, as one respondent stated they prefer to “buy [fish] in supermarkets.”

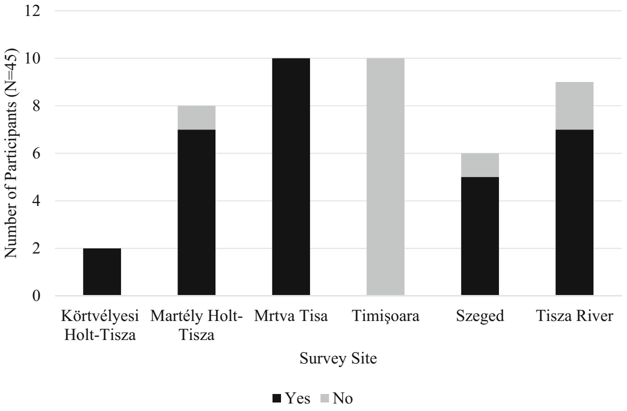

Fig. 3 Number of participants who reported either eating (*yes*) or not eating (*no*) local fish by survey site (*N* = 45)

Unexpectedly, even though some participants thought the oxbow lakes were safer than the river for fishing, fillets collected from the Tisza River contained significantly lower metals than the oxbow lakes. Similar to water quality, human health risk from fish consumption is not always apparent. Participant responses suggest this disconnect between perception and reality may warrant attention from policymakers, as some respondents reported

Table 8 Comparison of metals concentrations in fish fillets (μg g^{-1} wet wt)

Study location	Cd	Cu	Ni	Pb	Zn	Reference
Hungary/Serbia	0.05	0.88	0.36	0.20	8.8	This study
Moldova	0.03	2.93	3.83	1.97	8.65	Sapozhnikova et al. (2005)
Bosnia	0.03	3.02	–	0.17	–	Djedjibegovic et al. (2012)
Croatia	0.05	–	–	0.16	–	Has-Schön et al. (2006)
Malaysia	0.21	3.03	–	.72	64.86	Idriss and Ahmad (2015)*
Bangladesh	0.06	1.20	0.80	0.70	–	Islam et al. (2015)
Iran	0.26	1.6	0.21	0.67	7.2	Alipour et al. (2014)*
China	0.036	9.67	0.22	0.19	72.1	Wang et al. (2016)

* Reported values in dry weight

they were unconcerned because "it's possible to see on the fish if it's healthy or not." The majority of survey participants who eat locally caught fish were unconcerned with consumption (87 %), which was supported by physical data findings of moderate risk, and no exceedance of EFSA intake tolerances for an average adult. This agreement between perception and physical findings for the health of the system is similar to a study by Rochet et al. (2008), who found anglers' perceptions matched scientific data for a site in the English Channel, although this is not always the case (Boischio and Henshel 2000). Two of the four participants who reported concern fished at the Mártély site, where fish consumption poses only moderate risk of metals exposure. While fish tissue samples from Mártélyi Holt-Tisza presented the highest risk for copper, concentrations were within recommended tolerance limits for an average adult. The Mrtva Tisa presented the highest risks from cadmium, nickel, and lead in fish. A respondent from the Mrtva site reported concern, but thought the fish were still safe to eat. The fourth respondent who reported consumption concerns was from Szeged, along the Tisza River. His concern was "because of the water pollution."

In general, if the average adult consumes small quantities of recreationally caught fish (i.e., the majority of our survey population), then fish in the region do not present a significant risk of metals exposure. Although our risk estimates based on the recommended TWI did not indicate concern for children, they may in fact be at higher risk to metals from consuming Tisza River Basin fish. Children have a higher ratio of food consumption to body weight, and tend to have different food consumption patterns than adults (European Food Safety Authority 2009). Their lower body weight also means children tend to face different, often higher, exposure risks from food contaminants than adults. For example, the EFSA found that children under 12 had an average 60 % higher cadmium exposure than adults (European Food Safety Authority 2009). Lead is especially a problem for children because they absorb it more readily than do adults (European Food Safety Authority 2010a). Metals like lead can also be transferred through the placenta to the fetus, and to infants through nursing, therefore pregnant and nursing mothers should consider the added exposure risk to their children when making dietary choices (European Food Safety Authority 2010a). Although fish from the Tisza River Basin do not present a substantial health risk to adults if eaten occasionally, caution may be warranted for consumption of wild-caught fish by children or women who are pregnant or nursing. As of 2003, fish consumption guidelines for sportfish were not enforced in Hungary (Fleit and Lakatos 2003).

It is important to note these results only represent exposure through fish consumption, and do not include the multitude of other avenues of metals exposure including agricultural consumption, drinking water, inhalation from the atmosphere, workplace (especially industrial, manufacturing and mining), and smoking. For example, according to a recent assessment of cadmium exposure in 16 European Countries, cereals and vegetables present the highest exposure rate at roughly 4 μg/day, whereas fish contribute 1.7 μg/day (European Food Safety Authority 2009). Further studies of metals risk would benefit from a study of the total exposure pathway for local fishing populations.

Conclusion

The immediate effects of the cyanide and metals' spills in the winter of 2000 were extensive, with fish kills extending across nearly the entire Tisza River (Koenig 2000). Aside from a few outliers, however, our hypothesis that fish in the river and its oxbows contain metal levels that pose serious risk to human health was not supported.

Overall, locals' perceptions that the fish are safe to eat were supported by our findings. Although metals were elevated in water, metals in fish do not pose a significant hazard to human health in this system. This demonstrates that local perception can coincide with actual risk, as appears so in the case for the Tisza. On the other hand, it can also lead to greater risk if local beliefs regarding safe fish consumption are misinformed (Boischio and Henshel 2000). To avoid a possible disconnect between perceived and actual risk, it is important that regulatory agencies effectively communicate risk levels to the local community.

Risks need to be evaluated further, particularly among children and people with high exposure through other pathways. In the meantime, we recommend limiting consumption of species of bream and sunfish, as these groups contained fish samples with the highest levels of metals, including significantly higher levels of lead. In addition, we recommend children and women who are pregnant or nursing limit consumption of wild-caught fish from the basin. Causal factors for the lack of bioaccumulation found in Tisza Basin fish present an interesting area for further study.

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Compliance with Ethical Standards

Research Involving Human and Animal Rights The fish used in this study were collected following ICPDR methods (Csányi 2002), and euthanized according to American Veterinary Medical Association (AVMA) guidelines. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Ethical Approval The survey used in this study was approved by and conducted according to the guidelines for human subjects research of Southern Illinois University Carbondale's Human Subjects Committee. All procedures performed in studies involving human participants were in accordance with the ethical standards of Southern Illinois University and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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