Potentially Toxic Elements in Invasive Fish Species Prussian Carp (*Carassius Gibelio*) from Different Freshwater Ecosystems and Human Exposure Assessment

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Abstract

Concentrations of Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn, and Zn were detected in the muscle and gills of Prussian carp from three different freshwater ecosystems: isolated ponds and the South Morava River in Serbia, and Kopačko Lake in complex wetland ecosystem of the Kopački Rit Nature Reserve in Croatia. The main goals of the paper were to assess the concentrations of potentially toxic elements (PTEs) in the muscle and gills of Prussian carp (*Carassius gibelio*), to examine whether abiotic factors from three different freshwater ecosystems affect the accumulation of PTEs in fish tissues and to estimate the human health risk resulting from fish consumption. There were only six concentrations of PTEs in the gill tissue (Cr, Hg, Mn, Pb, Sn, and Zn) that were not significantly different among the different freshwater ecosystems. In the muscles, the differences were much less visible. Kopačko Lake distinguished with the highest values of metal pollution index (MPI) for muscles (0.24) and isolated ponds with the highest values of MPI for gills (0.8). The redundancy analysis (RDA) showed that concentrations of Al, Mn, Zn, Cu, and Fe in the gill tissue were significantly correlated with the environmental variables. In contrast, the RDA based on element concentrations in the fish muscles indicated no significant relationship with the environment. Isolated ponds, with no inflow of freshwater, stand out as the most polluted, followed by Kopačko Lake with occasional floods. Flowing freshwater ecosystem South Morava River can be single out as at least polluted with PTEs. The target hazard quotients (THQ) and hazard index (HI) suggested there were no significant noncarcinogenic health risks. The target carcinogenic risk factor (TR) for As and Pb confirmed there were no cancer risks related to human fish consumption. Since the elevated concentrations of toxic Cd and As in Prussian carp were estimated, an early warning should be assumed, especially for fishing activities in these areas.

1. Introduction

Potentially toxic elements (PTEs) are, among different xenobiotics from multiple anthropogenic stressors, particularly important environmental pollutants since they are not degraded or eliminated from the ecosystem (Klavins et al. 2000; Rajkowska and Protasowicki 2013). It is presumed that the world's large lakes and rivers cover the most area and therefore are the most important to study while small aquatic ecosystems studies have lagged over much of the past century (Downing et al. 2006). Regarding this, the studies of PTEs in Europe are mostly limited to large freshwater bodies, such as rivers and large lakes. Small water bodies, ponds and small lakes, also exposed to strong anthropogenic influences through various processes such as inputs of numerous pollutants (especially nutrients, pesticides, heavy metals, etc.) are the least researched freshwater ecosystems (Declerck et al. 2006). The assessment of PTEs in small water bodies is particularly important since they are vulnerable to pollutants that have little or no effect on larger water bodies (Kristensen and Globevnik 2014). Sediments play an important role in the accumulation and transport of contaminants so main rivers serve as conveyors for pollutants (Gómez-Gutiérrez et al. 2006; Davutluoglu et al. 2011). In addition, Has-Schön et al. (2015) found that water exchange intensity influences the bioaccumulation of metals in these ecosystems. Bioaccumulation of PTEs also depends on abiotic factors and intensities of pollution, so Dallinger and Kautzky (1985) stated that in low-level, chronic diffuse pollution, PTEs uptake in fish is predominantly from food rather than water.

To our knowledge, a significant knowledge gap was left regarding the influence of the type, size, depth, and abiotic variables of the water body (carbon content, oxygen, and nutrient concentrations, and water pH), ecosystem isolation, and the intensity of water exchange on the concentration of PTEs in fish. Guided by this, the field study is conducted at three different freshwater ecosystems – pond, lake, and river.

Fish respond to even low concentrations of PTEs (Radić et al. 2013) and therefore they are the most frequently used model systems in PTEs pollution assessment in water (Farkas et al. 2003). They are widespread and easy to sample, occupy the top of the food chain in water (Has-Schön et al. 2015), and the most importantly utilized in the human diet, so analysis can comprehend a potential risk to human health (Murtala et al. 2012).

Prussian carp (*Carassius gibelio*) is an invasive and recently highly dominant fish species in Europe and Asia, tolerant to unfavorable environmental conditions (low oxygen levels, variable temperatures), and high levels of anthropogenic pollution (De Boeck et al. 2004). Accordingly, it was an appropriate bioindicator in many PTEs assessment studies (Andreji et al. 2006; Has-Schön et al. 2008; Falfushynska et al. 2011; Milošković et al. 2013; Milošković and Simić 2015; Đikanović et al. 2016; Zhelyazkov et al. 2018; Mijošek et al. 2021). Common in rivers, ponds, eutrophic lakes, canals, and small water reservoirs in Europe (USFWS 2012), Prussian carp was selected as a bioindicator organism in this study.

Analysis of PTEs in various fish tissues is recommended by Has-Schon et al. (2015), Uysal et al. (2009), etc. The primary site of uptake from water are gills, so PTEs analysis in this tissue is desirable since they reflect the PTEs levels in the water (Raškovic et al. 2018). Muscles are of particular interest for routine environmental monitoring, fish meat is utilized in the human diet (Uysal et al. 2009), and represent the main uptake route of Hg into the human organism (Storelli 2008). It is recommended that these two tissues should be used in the assessment of PTEs to see, simultaneously, the current water pollution (based on PTEs in gills) and to assess the risk to human health (based on PTEs in muscle).

The study is conducted at isolated ponds near South Morava River and the South Morava River in Serbia, and Kopačko Lake in the complex wetland ecosystems of the Kopački Rit Nature Reserve in Croatia. The main differences between researched ecosystems are that isolated ponds are with standing water and without the inflow of freshwater, the South Morava River is a lowland river, and Kopačko Lake is an occasionally flooded lake (mostly in spring). We hypothesize that different freshwater ecosystems (the physical and chemical parameters of water) have an impact on PTEs accumulation in fish tissues. The main goals of the paper are to assess the concentrations of PTEs – Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn, and Zn in the muscle and gills of Prussian carp, to examine whether abiotic factors from three different freshwater ecosystems effect on the accumulation of PTEs in fish tissues and to estimate the human health risk resulting from fish consumption.

2. Material And Methods

2.1. Study area and sampling

The field study is conducted at three ponds near the South Morava River and the South Morava River in Serbia, and Kopačko Lake in the complex wetland ecosystem of the Kopački Rit Nature Reserve in Croatia in the autumn of 2016 (Fig. 1).

The South Morava River is a lowland river situated in south-eastern Serbia with a catchment area of 15 469 km^2. The study site at the South Morava River is located near a village Batušinac (43°26′19″N, 21°83′35″E) in the vicinity of highway and under the high agricultural influence. Ponds are located near the South Morava River, in village called Čokot (43°17'48"N, 21°47'57"E). Studied ponds are situated on the right side of the river, and according to Stamenković et al. (2019), they are created by sand and gravel excavation during the construction of a highway. This system of ponds is isolated and not flooded at any time of the year, but under anthropogenic pressure, since it is close to the highway, surrounded by agricultural land used for growing crops, and near a gravel factory. All study ponds are under groundwater influence. Both, South Morava River and isolated ponds are near the City of Niš, the third-largest city in Serbia.

Kopačko Lake is the largest lake (220 ha) in the wetland complex Kopački Rit Nature Park located in Northeast Croatia. This complex is situated close to the confluence of the Drava and the Danube rivers, subjected to occasional flooding. During periods of high-water levels, usually in spring and early summer, the inflow of water from the Danube into the Kopačko lake happens. The lake is very shallow, with an average lake depth of 1.5 to 2 m. With inflow, all pollution from the Danube River inflows in Kopačko Lake. Additionally, Kopačko Lake is in the vicinity of an urban center (Osijek, ~ 100,000 inhabitants), downstream from a nuclear power plant (Paks, Hungary) on the Danube, and close to regions of intense agriculture in Croatia, Hungary, and Serbia (Petrinec et al. 2018). Sampling site coordinates at Kopačko Lake are 45°36'32"N, 18°48'03"E.

Prior to Prussian carp sampling, the abiotic variables of the study sites were measured. Directly in the field, the temperature T (C°), pH, conductivity EC (µS), dissolved oxygen DO (mg l^{-1}), and oxygen saturation DO% were measured with a WTW multi 340i probe. The biochemical oxygen demand BOD (mg l^{-1}) was estimated using the standard methodology recommended by APHA (1999). The concentrations of ammonia-nitrogen NO_3-N (mg l^{-1}), ammonium-nitrogen NH_4-N (mg l^{-1}), total nitrogen N-TOT (mg l^{-1}), and phosphate ion PO_4 (mg l^{-1}) were measured using a Shimadzu UV–Vis's spectrophotometer. Total phosphates P-TOT (mg l^{-1}) was measured using a PC Multi Direct Lovibond® Photometer-System.

The Prussian carps were sampled from a boat using a DC Aquatech IG 1300 electrofisher (2.6 kW, 80–470 V). Fifteen specimens from the isolated ponds (five specimens from each pond), ten specimens from the South Morava River, and ten specimens from Kopačko Lake were caught, sacrificed with a quick blow to the head, and transferred on ice in a hand-held refrigerator to the laboratory.

2.2. Sample and element analysis

In the laboratory, fish were measured for their total body length (to the nearest cm), weighed (to the nearest g), and subsequently dissected with a decontaminated ceramic knife. The right dorsal muscle below the dorsal fin along with the gills of each specimen were dissected. Following dissection, the muscle and gill samples were weighed using an electronic scale (0.1 g) and stored at -20°C before analysis. For each specimen, the condition factor was calculated using the formula according to Bervoets and Blust (2003): $\mathrm{CF} = WTL^{-3} \times 100,$ where W represents the weight (g); TL is the total length of the fish (cm).

Before digestion in microwave Christ Alpha 2–4 LD, Harz, Germany, samples are dried in a lyophilizer Christ Alpha 2–4 LD, Harz, Germany and measured one more time. Digestion was conducted with a mixture of 65% nitric acid and 30% hydrogen peroxide (Suprapur®, Merck, Darmstadt, Germany, 10:2, v/v) at 200°C for 20 min. After cooling to room temperature and without filtration, the solution was diluted to a fixed volume of 25 ml with ultrapure water Fish-free samples were analyzed with each batch of samples to observe contamination by the reagents used. The concentrations of Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn, and Zn were analyzed with inductively coupled plasma optical emission spectrometry (ICP-OES), using a Thermo Fisher Scientific iCAP 6500 Duo ICP (Cambridge, United Kingdom). The following wavelengths were used for the ICP-OES analysis (nm): Al 391.402, As 188.032, Cd 226.602, Co 221.618, Cr 204.542, Cu 322.764, Fe 257.921, Hg 183.940, Mn 260.353, Ni 234.606, Pb 222.354, Se 199.093, Sn 245.162, and Zn 207.194. The limits of detection (LOD) were Al 0.077, As 0.011, Cd 0.026, Co 0.001, Cr 0.080, Cu 0.180, Fe 0.150, Hg 0.001, Mn 0.037, Ni 0.001, Pb 0.056, Se 0.121, Sn 0.001, and Zn 0.57 µg/L. The standard muscle reference material (DORM-4; National Research Council of Canada) was digested and analyzed in triplicate to support quality assurance and control. Recovery ranged from 95.6–108.1%. The mean values and standard deviations were calculated for each group and element concentrations were expressed as mg kg^{-1} wet weight (ww).

The metal pollution index (MPI) refers to the element concentration in fish tissue to assess the overall toxicity status of the sample. The MPI was calculated to compare the total PTEs content of fish muscles and gills to each other and from the various sampling sites using the following equation (Usero et al. 1997):

$$\mathrm{MPI} = (\mathrm{cf}_1 \times \mathrm{cf}_2 \times \mathrm{cf}_3 \times \ldots \times \mathrm{cf}_n)^{1/n}$$

where cf_n = concentration of the element n in the sample (mg kg^{-1} ww).

2.3. Statistical analysis

The Shapiro-Wilk test was employed to test the normality of data distribution. Kruskal-Wallis ANOVA test and Mann-Whitney post hoc test were employed to explore whether there were differences between the concentrations of PTEs in the fish tissues (gills and muscles) from the three groups of sampling sites. The first group comprised three ponds located in Southeastern Serbia, the second group was the South Morava River, and the third group was Kopačko Lake. The level of significance was set at $p \leq 0.05$ for the Kruskal-Wallis test, while for the post-hoc Mann-Whitney test the level of significance was $p \leq 0.016$. All ANOVA-based analyses were carried out using SPSS 19.0 statistical package programs for Windows (SPSS Inc., Chicago, IL, USA).

A redundancy analysis (RDA) was used to determine how much variation in the concentration of PTEs in the fish tissue could be explained by abiotic environmental variables. The fish tissue PTEs concentrations (gills and muscles) were selected as the response variables, while environmental parameters were treated as explanatory variables. The original data were transformed to log(x + 1) and standardized to avoid the influence of different data dimensions. The RDA analysis was run in R version 3.5.3 (R Core Team, Vienna, Austria).

2.4. Human health risk assessment

Prussian carp is used in human nutrition, so concentrations of the PTEs in muscle tissue (meat) were compared with the maximum permitted concentrations (MPCs) in fish meat for utilization in the human diet, according to legislations of the European Union (EU) and FAO. EU legislation (European Commission Regulation 2006) established the MPCs 0.05, 0.50, and 0.30 mg kg^{-1} ww for Cd, Hg, and Pb, respectively. MPCs for Cd, Hg, Pb, As, Cu, and Zn are 0.05, 0.50, 0.50, 0.10-4.00, 30, and 40–100 mg kg^{-1} ww, respectively according to FAO (FAO 1983).

We evaluated the target hazard quotients (THQ) and hazard index (HI), as well as the target carcinogenic risk factor (TR) for arsenic and lead to evaluate the need for an alert regarding possible harmful effects. The methodology for estimating the target hazard quotient (THQ) does not provide a quantitative estimate regarding the probability of an exposed population experiencing negative health effects. Still THQ indices the risk level due to PTEs exposure. This method was taken from the US EPA Region III Risk-based Concentration table (US EPA 2000). It is described by the following equation:

$$\mathrm{THQ} = \frac{(\mathrm{EF} \times \mathrm{ED} \times \mathrm{FIR} \times \mathrm{C})}{(\mathrm{RFD} \times \mathrm{WAB} \times \mathrm{TA})} \times 10^{-3}$$

where EF is the exposure frequency (365 days/year); ED is the exposure duration (70 years), equivalent to the average lifetime; FIR is the food ingestion rate (for freshwater fish for Serbia 7.03 g/person/day and for Croatia 3.82 g/person/day) (FAO 2005); C is the element concentration in Prussian carp (mg kg^{-1} ww); RFD is the oral reference dose (Hg = 0.0005 mg kg^{-1}/day, Cd = 0.001 mg kg^{-1}/day, Pb = 0.004 mg kg^{-1}/day, Cu = 0.04 mg kg^{-1}/day, Zn = 0.3 mg kg^{-1}/day, Cr = 1.5 mg kg^{-1}/day, Mn = 0.14 mg kg^{-1}/day, Al = 0.0004 mg kg^{-1}/day, As = 0.0003 mg kg^{-1}/day, Fe = 0.04 mg kg^{-1}/day, Co = 0.0003 mg kg^{-1}/day, Ni = 0.02 mg kg^{-1}/day) (US EPA 1997; FAO/WHO 1989; US EPA 2000); WAB is the average body weight of an adult (70 kg); and TA is the average exposure time (365 days/year x ED).

Humans are exposed to more than one pollutant (PTEs) and suffer their combined or interactive effects so the Hazard index, HI (also called The Total THQ) was also assessed. Each THQ value in the HI individually corresponds to the PTEs Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn, and Zn. The HI of PTEs for Prussian carp is the sum of the following composition:

$$\mathrm{HI} = \sum \mathrm{THQ}$$

Target carcinogenic risk factor (TR) for arsenic and lead was estimated using the equation:

$$\mathrm{TR} = \frac{\mathrm{EF} \times \mathrm{ED} \times \mathrm{FIR} \times \mathrm{C} \times \mathrm{CSFo}}{\mathrm{WAB} \times \mathrm{TA}} \times 10^{-3}$$

where CSFo is the oral carcinogenic slope factor (mg/kg-day) which is 1.5 for As and 0.0085 for Pb (USEPA 2009).

3. Results And Discussion

Since it has been introduced in European freshwaters in the 1970s, Prussian carp has been economically important for fisheries in Serbia (Smederevac-Lalić et al. 2011) as well as in Croatia, so estimating the human health risk resulting from consumption of this fish species is very important. Syasina et al. (2012) distinguished Prussian carp as one of the most resistant species to the effects of pollution, while Djikanović et al. (2016) comparing metal accumulation in liver, muscle, and gills of nine fish species from the Međuvršje Reservoir (Serbia) pointed out Prussian carp as the most effective accumulator of the most metals. Hence, we detected PTEs in the muscle and gills of this species caught from three different freshwater ecosystems – isolated and non-flooded ponds near the South Morava River, the South Morava River in Serbia, and Kopačko Lake (periodically flooded by the Danube) in Croatia, each with different physicochemical parameters, to determine if they affect the concentration of the elements in fish tissues. Data on physical-chemical parameters T (C°), pH, EC (µS), DO (mg l^{-1}), DO%, BOD (mg l^{-1}), NO3-N (mg l^{-1}), NH4-N (mg l^{-1}), PO4 (mg l^{-1}), N-TOT (mg l^{-1}), P-TOT (mg l^{-1}) measured once in parallel with fish sampling are presented in Table S1.

The weight of the specimens examined ranged from 45.20 ± 6.33 to 565.62 ± 176.44 g while the length ranged from 13.97 ± 0.65 to 30.34 ± 0.73 cm (Table 1). The highest CF was recorded for specimens from the South Morava River (1.94 ± 0.17) and the lowest CF was calculated for specimens from the isolated ponds (1.51 ± 0.05). There were significant differences between the isolated ponds and Kopačko Lake, as well as between the isolated ponds and South Morava River based on the CF values. As Froese (2006) stated, the condition factor is a measure of the general health of fish and it reflects the recent feeding conditions and environmental quality. Since the values of CF in our study were greater than one, it can be concluded that the general health of fish was good. In studies Laflamme et al. (2000), Rajotte and Couture (2002), and Zhelev et al. (2018) CF decline was observed at highly contaminated locations. Concerning the location of the isolated ponds (near a highway) and their origin (created by sand and gravel excavation during the construction of the highway) (Stamenković et al. 2019), as well as the high anthropogenic pressure and lack of connection with the river and freshwater, the lowest CF value in this ecosystem was logical. However, all the mean CF values for the fish analyzed in this study were lower than the findings for the same fish species in Lake Eğirdir (CF 2.401 for males and 2.594 for females) in study Balik et al. (2004), but higher than for the specimens from Ilova River in Croatia (CF 1.59 in autumn and 1.31 in spring) in study Mijošek et al. (2021).

Table 1
Weight, length, and condition factor with the standard deviation for the freshwater Prussian carp (*Carassius gibelio*) per sampling site

Site	Number of specimens	Weight (g)	Length (cm)	Condition factor
Isolated ponds	15	45.20 ± 6.33	13.97 ± 0.65	1.51 ± 0.05
South Morava River	10	543.80 ± 55.47	30.34 ± 0.73	1.94 ± 0.17
Kopačko Lake	10	565.62 ± 176.44	27.07 ± 3.55	1.83 ± 0.19

The highest PTEs values in muscle were recorded for IP – As, Co, Hg, Ni, Pb, Se, and Sn; for KL – Al, Cu, and Fe and for SMR – Cd, Cr, Mn, and Zn (Table 2). In both the muscle tissue and gills, the highest concentrations of Zn (muscle 7.978 ± 1.537 to 8.878 ± 1.799 mg kg^{-1} ww; gills 31.168 ± 3.100 to 72.23 ± 118.19 mg kg^{-1} ww) and Fe (muscle 3.813 ± 2.288 to 7.067 ± 3.834 mg kg^{-1}ww; gills 70.66 ± 60.98 to 178.30 ± 105.02 mg kg^{-1}ww) were recorded for all specimens. The lowest concentrations of Co (in muscle not detected (ND) at two sampling sites; in gills 0.019 ± 0.041 to 0.300 ± 0.390 mg kg^{-1} ww) and Pb (in muscle 0.094 ± 0.099 to 0.20 ± 0.022 mg kg^{-1} ww and in gills ND at two sampling sites) were recorded. The highest concentrations of essential elements Zn and Fe in this study are in accordance with our previous research (Milošković et al. 2018) and studies Syasina et al. (2012), Skorić et al., (2012), etc. Djikanović et al. (2016) pointed out, among nine fish species, Prussian carp from Međuvršje Reservoir with the highest concentrations of Fe and Zn were observed in all tissues. Goyer (1997) stated that the involvement of Zn and Fe in the regulation of key enzymatic detoxification processes may be the reason for the high concentrations of these elements in fish tissues. It is generally believed that fish actively regulate the essential element concentrations in their tissue. Bervoets and Blust (2003) emphasized that the levels of Zn and Fe in the tissue were influenced by several chemical and physiological processes in addition to the environmental concentrations. The concentration of Pb in both the muscle tissue and the gills was low, in fact, it was lower than in Prussian carp from Hutovo Blato nature reserve in Bosnia and Herzegovina (Has-Schön et al. 2008) and the Amur River basin (Syasina et al. 2012).

Table 2
The average element concentrations and standard deviation in the muscle tissue and gills of Prussian carp expressed in mg kg^{-1}ww (different letters in superscript denote significant differences in element concentrations among the study sites, $p < 0.05$) and the maximum permitted concentrations (MPCs) in fish meat for utilization in the human diet, established by the European Union (EU) and FAO

Elements	Tissue	Serbia		Croatia	MPC	
		Isolated ponds	**South Morava River**	**Kopačko Lake**	**MPC EU (2006)**	**MPC** FAO (1983**)**
Al	muscle	0.147 ± 0.373^{a}	0.174 ± 0.298^{a}	0.720 ± 1.828^{a}		
	gills	41.84 ± 80.93^{a}	5.269 ± 1.670^{b}	4.064 ± 2.940^{b}		
As	muscle	0.221 ± 0.255^{a}	0.077 ± 0.081^{a}	0.177 ± 0.176^{a}		0.1-4.0
	gills	0.32 ± 0.29^{a}	0.999 ± 0.267^{b}	0.747 ± 0.432$^{a, b}$		
Cd	muscle	0.076 ± 0.015^{a}	0.087 ± 0.023^{a}	0.074 ± 0.013^{a}	0.05	0.05
	gills	0.085 ± 0.015^{a}	0.243 ± 0.054^{b}	0.189 ± 0.078^{b}		
Co	muscle	0.0014 ± 0.0030	ND	ND		
	gills	0.019 ± 0.041^{a}	0.184 ± 0.084^{b}	0.300 ± 0.390^{b}		
Cr	muscle	0.5880 ± 0.1803^{a}	0.842 ± 0.124^{b}	0.680 ± 0.168$^{a, b}$		
	gills	0.880 ± 0.389^{a}	0.858 ± 0.182^{a}	1.075 ± 0.979^{a}		
Cu	muscle	0.297 ± 0.152^{a}	0.551 ± 0.145^{b}	0.587 ± 0.358$^{a, b}$		30
	gills	0.729 ± 0.136^{a}	3.001 ± 0.290^{b}	3.071 ± 1.210^{b}		
Fe	muscle	3.813 ± 2.288^{a}	5.892 ± 1.515^{a}	7.067 ± 3.834^{a}		
	gills	70.66 ± 60.98^{a}	153.61 ± 79.03^{b}	178.30 ± 105.02^{b}		
Hg	muscle	0.178 ± 0.175^{a}	0.059 ± 0.067^{a}	0.063 ± 0.070^{a}	0.5	0.5
	gills	ND	0.001 ± 0.002	0.0004 ± 0.0009		
Mn	muscle	0.350 ± 0.236^{a}	0.727 ± 0.126^{b}	0.724 ± 0.471$^{a, b}$		
	gills	5.08 ± 5.90^{a}	1.377 ± 0.290^{a}	1.334 ± 0.59^{a}		
Ni	muscle	0.098 ± 0.042^{a}	0.080 ± 0.016^{a}	0.085 ± 0.025^{a}		
	gills	0.128 ± 0.160^{a}	0.001 ± 0.002^{b}	0.008 ± 0.024^{b}		
Pb	muscle	0.094 ± 0.099^{b}	0.020 ± 0.022$^{a, b}$	0.020 ± 0.043^{a}	0.3	0.5
	gills	0.038 ± 0.056	ND	ND		
Se	muscle	0.205 ± 0.059^{a}	0.162 ± 0.041^{b}	0.162 ± 0.063^{b}		
	gills	0.241 ± 0.065^{a}	0.712 ± 0.135^{b}	0.642 ± 0.277^{b}		
Sn	muscle	0.029 ± 0.024^{b}	0.001 ± 0.001^{a}	0.003 ± 0.004^{a}		
	gills	0.219 ± 0.268^{a}	0.155 ± 0.008^{a}	0.154 ± 0.009^{a}		
Zn	muscle	7.978 ± 1.537^{a}	11.030 ± 1.155^{b}	8.878 ± 1.799$^{a, b}$		100
	gills	40.59 ± 60.60^{a}	31.168 ± 3.100^{a}	72.23 ± 118.19^{a}		

ND – not detected

The Kruskal-Wallis ANOVA test (p □ 0.05) confirmed significant differences in the concentrations of PTEs in the fish gills and muscle tissues among the three groups of sites. Besides, the Mann-Whitney post hoc test revealed that there were no significant differences for six concentrations of PTEs in the gill tissue (Cr, Hg, Mn, Pb, Sn, and Zn) in the groups. The most significant differences in the element concentrations in the gills were observed between the first (isolated ponds) and the second group (South Morava River) as well as between the first (isolated ponds) and third group (Kopačko Lake) (see Table 2). The specimens from isolated ponds can be singled out as having the highest values of Al and Ni and the lowest values of As, Cd, Co, Cu, Fe and Se in the gills in Prussian carp. On the other hand, any differences were much less visible in the muscles. The Mann-Whitney post hoc test revealed that only the concentrations of elements Cr, Cu, Mn, Sn, and Zn were significantly different between specimens from the isolated ponds and South Morava River, and Pb and Sn between the isolated ponds and Kopačko Lake (see Table 2). Bury et al. (2003) stated that Cu, Zn, and Cd mainly enter the fish through the food, but the higher concentrations of these elements in the gills of the fish in this study from all three sampling sites could be explained with high concentrations of those elements in the water. The highest concentrations of Pb, Se, and Sn and the lowest concentrations of Cr, Cu, Mn, Zn, Pb, Se, and Sn were recorded in the muscle tissue of the fish from the isolated ponds. Since fish muscle is not the primary tissue for the accumulation of PTEs, only concentrations of Hg, Pb, and Ni were accumulated in high amounts in the muscle tissue at all three sites. Likewise, in a study by Has-Schön et al. (2008), the highest concentration of Hg was detected in the muscle tissue of Prussian carp (concentrations in the same range at the values in our study; 0.142–0.151 mg kg^{-1} ww), but high concentrations of Pb were not detected in the muscle than in the gills. In a study by Syasina et al. (2012), higher levels of contamination by PTEs were detected in Prussian carp than in other species tested and the highest concentrations of Hg were detected in the muscle tissue. Concentrations of Pb, Cd, and Hg (0.052 ± 0.023, 0.017 ± 0.004 and 0.87 ± 0.006 µg/g dry weight, respectively) in Prussian carp from the Danube in Croatia in study Zrnčić et al. (2013) were higher, while the concentration of As (0.031 ± 0.011µg/g dry weight) was much lower than in Prussian carp from Kopačko Lake in this study. Prosi (1981) stated that the type of chemical, metabolic properties of the tissues, and the degree of environmental pollution affect the bioaccumulation levels in fish. Fish gills are responsive to the water quality since they are coming into immediate contact with the surrounding water (Syasina et al. 2012). When fish are exposed to high levels of elements in water, they typically exhibit elevated concentrations in the gills (Reynders et al. 2008), thus reflecting the concentration of elements in the water (Rao and Padmaja 2000). Particularly when there is a deficiency of trace elements in the food but a high concentration in the water, uptake from the water plays a considerable role (Kamunde et al. 2002). On the other hand, in low-level, chronic diffuse pollution, metal uptake in fish is predominantly from the food (Dallinger and Kautzky 1985).

The concentrations of PTEs affected the MPI values (Fig. 2). In the study Đikanović et al. (2016) Prussian carp was distinguished from others by higher MPI values. Our results pointed out the muscle exposed to the lower pressure of PTEs pollution than the gills. Kopačko Lake distinguished with the highest values of MPI for muscles (0.24) and isolated ponds with the highest values of MPI for gills (0.8). The reason for higher values of MPI for gills may be that the gills are in direct contact with pollutants in the water and the primary site of element uptake (Klavins et al., 2009). Based on above statement, we can conclude that the overall concentrations of PTEs are the highest in the water of isolated ponds. On the other hand, many authors reported muscle as less loaded with elements (lower MPI) (Subotić et al. 2013a; Djikanović et al. 2016, etc.). If we consider the statement by Subotić et al. (2013a, b) that the weight of the fish affects the MPI value for muscle tissue, the reason for the lower value for muscle tissue in the isolated ponds is comprehensible.

The RDA showed that concentrations of the elements in the gill tissues were significantly correlated with the environment (Monte Carlo F = 4.301, p < 0.001) (Fig. 3). The model obtained showed that 57.3% (R2adj = 0.573, p < 0.001) of the element concentrations in the fish tissue were driven by environmental attributes (listed in Table S1). The lengths of the environmental variable arrows reflect how much variance was explained by that factor, and the direction of the arrows for a particular environmental factor indicates an increasing concentration of that factor. The element arrows pointing in approximately the same direction as the environmental factor arrows indicate a high positive correlation (the longer the element line, the stronger the relationship). According to the angles between these arrows, the concentration of Al was positively correlated with T, pH, DO, and DO%, and negatively correlated with PO4-P, NH4-N, and NO3-N. In comparison, the concentration of Mn and Zn increased when the values of BOD, PTOT, and NTOT increased. Finally, Cu and Fe were slightly influenced by the environmental gradient. The other element concentrations did not show visible dependence on the physicochemical parameters of the water, as shown in Fig. 3.

In contrast, the RDA based on element concentrations in the fish muscles indicated no significant relationship with the environment (Monte Carlo F = 1.409, p = 0.178), providing a model that revealed that only 14.28% (R2adj = 0.1428, p = 0.178) of the element concentrations in the muscle tissue can be explained by environmental parameters (Fig. 3).

According to Chai et al. (2019), sources of pollution and different hydrological characteristics may be the main factors affecting the pathways of PTEs. The inorganic quality of natural water is affected by physicochemical parameters, such as pH, conductivity, and dissolved oxygen (Yılmaz and Doğan 2008; Chintala et al. 2013). The exposure period, the concentration of the chemical, environmental temperature, or the pH potentially affects the amounts of contaminants in aquatic organisms (Terra et al. 2008; Copa et al. 2012). Based on the above claims, we found that only the concentrations of Al, Mn, Zn, and to a lesser extent Cu and Fe in the gills were significantly correlated with the physicochemical parameters of the environment. Ghosh and Adhikari (2006) notified that increasing water hardness and acidified freshwaters reduce metal toxicity. In our study only the concentration of Al in gills was positively correlated to the pH in water. There were no significant relationships between the PTEs in the muscle tissue and environmental variables, which is in accordance with Zrnčić et al. (2013) who stated that it is not possible to correlate the measured element concentrations (Pb, Cd, Hg, As) in muscles in different fish species with water properties in Danube River in Croatia. Those results are in accordance with Syasina et al. (2012), who stated that, since Prussian carp is a bottom feeder, the dietary habits of this species subject it to the intensive influence of PTEs that accompanies food resources.

Fish samples from all the sites examined had concentrations of Cd above the MPC prescribed by the EU (2006) and FAO (1983) in both the muscle and gills (Table 2). Since EU legislation does not prescribe any MPC for As, and FAO prescribes MPC at a range of 0.1-4 mg kg^{-1}, we singled out Prussian carp from the isolated ponds and Kopačko Lake as having concentrations of As slightly above the MPC (Table 2). The negative impact of PTEs on human health was analyzed only by comparing the concentrations of these elements with the appropriate MPCs in several earlier studies dealing with pollution in aquatic environments (Milošković et al. 2016; Djikanović et al. 2016; Jovanović et al. 2018, etc.). We estimated elevated concentrations of Cd at all sampling sites, and of As in fish from the isolated ponds and Kopačko Lake. This information is useful but often very restricted because it does not consider the effect of intraspecific variations in the human population (exposure rate, human weight, meal size, etc.) or long-term exposure to pollutants (Nikolić et al. 2019). Because As and Cd are very toxic to humans, this information is doubtless useful as an early warning, especially for fishing activities in these areas.

As shown in Table 3, there were no THQ values over 1 for the elements in the freshwater Prussian carp from the isolated ponds, South Morava River, or Kopačko Lake, indicating that there was no health risk. The lowest potential health risk was that of Cr, which may be ascribed to a higher oral reference dose of this element. Concerning all the THQ values, the highest HI (see Table 3) values were assessed for the human population in Serbia, mainly ascribed to the consumption of Prussian carp from the isolated ponds, with a value of 0.16, and the South Morava River (0.10). The lowest HI value was assessed for the human population in Croatia (0.07).

Table 3
THQs and HI for potentially toxic elements in Prussian carp from isolated ponds and the South Morava River in Serbia, and Kopačko Lake in Croatia and TR population due to consumption of Prussian Carp

Study area/ Country of consumption		Al THQ_S	As THQ_S	Cd THQ_S	Co THQ_S	Cr THQ_S	Cu THQ_S	Fe THQ_S	Hg THQ_S	Mn THQ_S	Ni THQ_S	Pb THQ_S	Se THQ_S	Sn THQ_S
Isolated ponds	**Serbia**	0.033	0.066	0.007	0.0004	0.00004	0.0007	0.009	0.032	0.00022	0.0004	0.002	0.004	0.0009
South Morava River		0.039	0.023	0.0078	/	0.00005	0.0012	0.013	0.011	0.0005	0.0004	0.0004	0.003	0.00002
Kopačko Lake	**Croatia**	0.01	0.032	0.004	/	0.00002	0.0008	0.01	0.007	0.0003	0.0002	0.0003	0.0018	0.00005

*It was assumed that inorganic As was 3% of the total As

In this study, the highest TR values for inorganic As (9.9×10^{-7}) and Pb (1.1×10^{-8}) were recorded for Prussian carp from the isolated ponds in Serbia (Table 3). According to the result for TR obtained in this study, lower TR values were recorded for Pb than for As, as seen in study Nikolić et al. (2021). It is assumed that THQ value greater than one, and a TR above 1×10^{-6} is of concern because there is a high risk of developing chronic systemic effects (assessed by THQ) or cancer (assessed by TR) due to the intake of the contaminants (USEPA 2000). The THQ value for each element in each sampling site was lower than the hazard quotient threshold of 1, which indicates that there is no significant non-carcinogenic health risk resulting from the intake of these elements, as in studies by Chien et al. (2002) and Zheng et al. (2007). Consequently, the HI for all the study sites was lower than 1, suggesting there was also no potential health risk from consumption of the Prussian carp meat. We presented the levels of inorganic toxic As from the measured levels of the total As since organic As compounds (such as arsenobetaine) are considered to be nontoxic and therefore not a threat to human health (ATSDR 1998). The inorganic form of As causes a number of diseases, such as skin, lung, bladder, and kidney cancers (Castro-González and Méndez-Armenta 2008; Sirot et al. 2009) so the calculated TR is very important for human health assessment. All of the TR values for As ($3.5–9.9 \times10^{-7}$) and Pb ($2.42 \times 10^{-9} – 9.9 \times 10^{-8}$) were below 1×10^{-6}, as proposed Islam et al. (2014), suggesting there was no cancer risk for humans consuming fish from the sampling sites.

Based on the results of this research, it can be concluded that type of aquatic ecosystem in certain level affect the pollution status of the fish. This applies primarily to fish gills, which are an indicator of water pollution. Isolated ponds with no inflow of freshwater stand out as the most polluted, followed by Kopačko Lake with occasional floods. Flowing freshwater ecosystem South Morava River can be single out as at least polluted with PTEs.

4. Conclusions

The high concentrations of the essential elements Zn and Fe, and low concentrations of Pb were detected both in the muscle and gills of the Prussian carp from all three different types of freshwater ecosystems. Only the concentrations of Al, Mn, Zn, and to a lesser extent Cu and Fe in the gills were significantly correlated with the physicochemical parameters of the environment, and there were no significant relationships between the PTEs in the muscle tissue and environmental variables. Isolated ponds with no inflow of freshwater stand out as the most polluted, followed by Kopačko Lake with occasional floods. Flowing freshwater ecosystem South Morava River can be single out as at least polluted with PTEs. Kopačko Lake distinguishes with the highest values of MPI for muscles (0.24) and isolated ponds with the highest values of MPI for gills (0.8). Since the elevated concentrations of toxic Cd at all sampling sites and As in the fish from isolated ponds and Kopačko Lake were estimated, our results furthermore showed an early warning should be assumed, especially for fishing activities in these areas. The THQ and HI suggested that there was no significant non-carcinogenic health risk. Also, the TRs for As and Pb confirmed there was no cancer risk related to human fish consumption.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests

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Figures

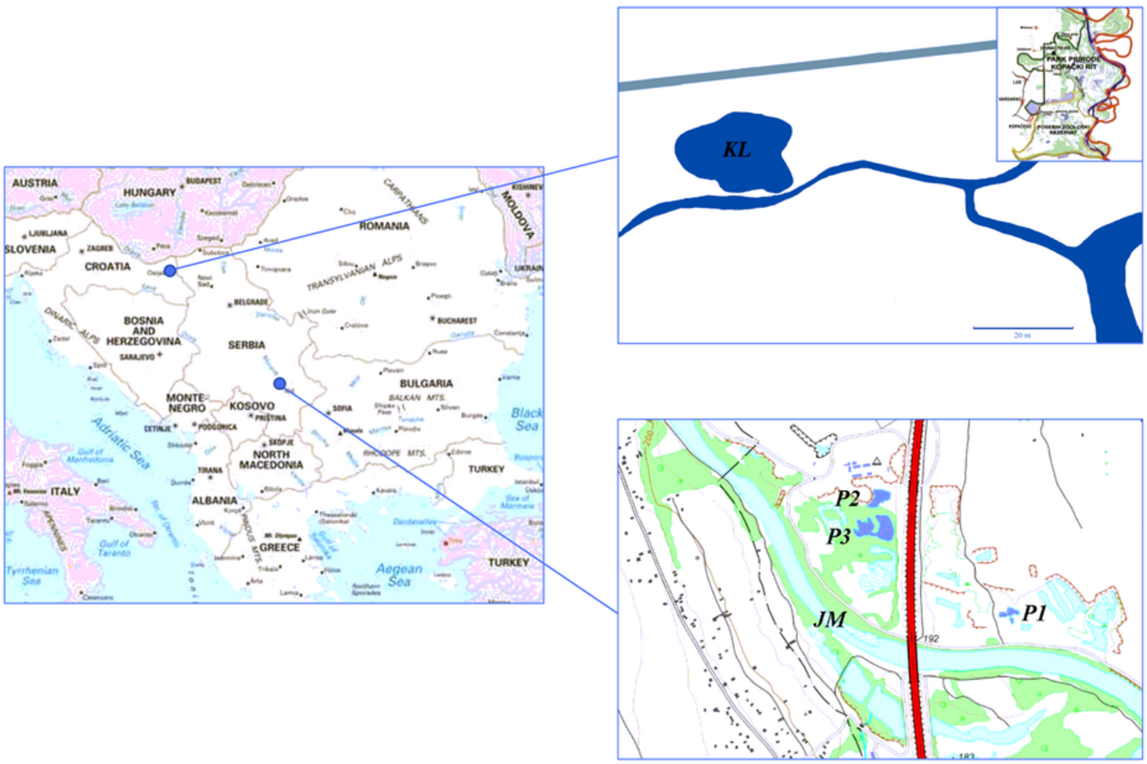

Figure 1

Map of the sampling sites: South Morava River - SMR and isolated ponds - IP1, IP2, IP3 in Serbia, and Kopačko Lake – KL in Croatia

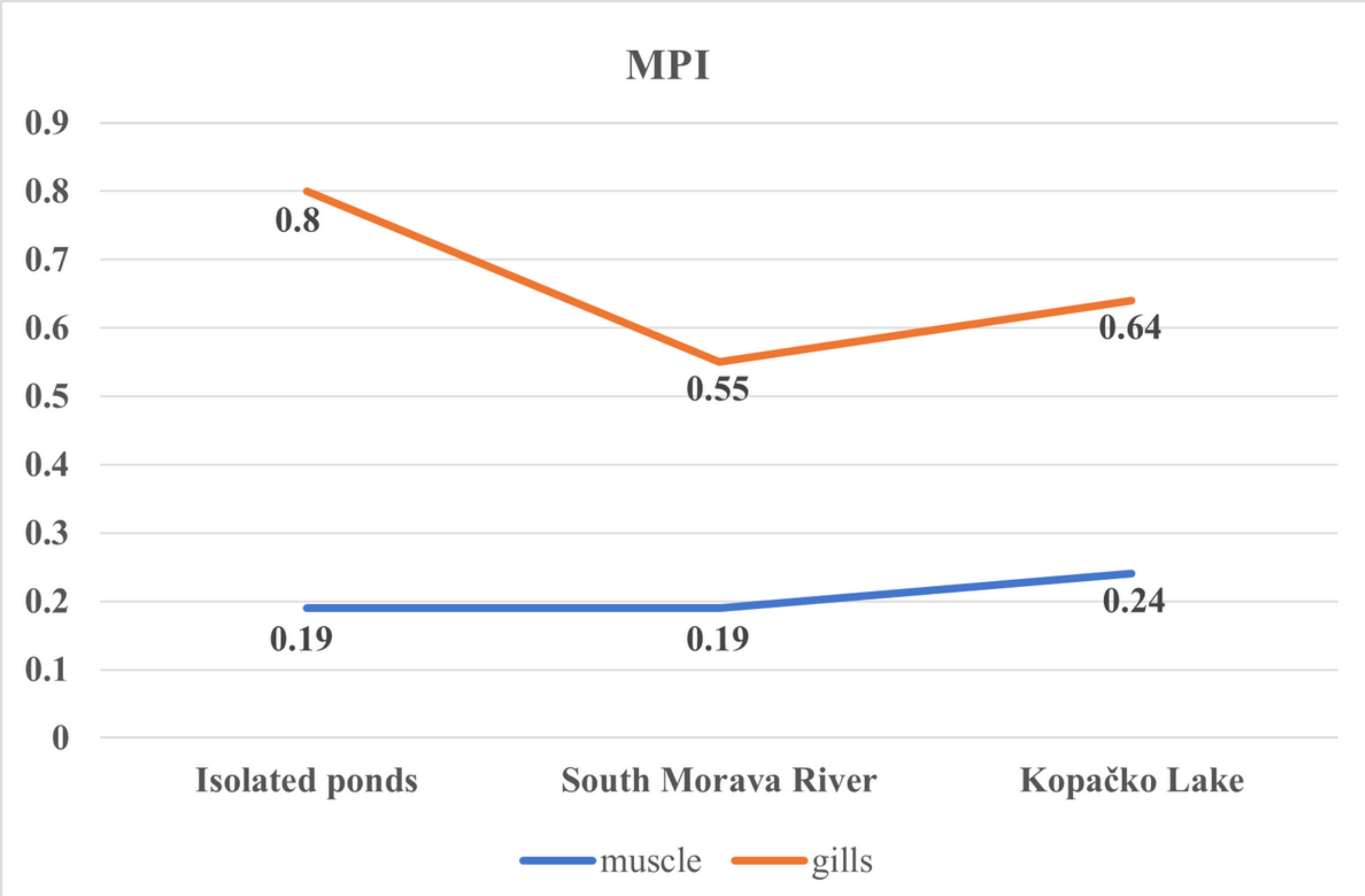

Figure 2

Metal pollution index (MPI) values of the total element accumulation levels in the muscle and gill tissues in Prussian carp examined per sampling site

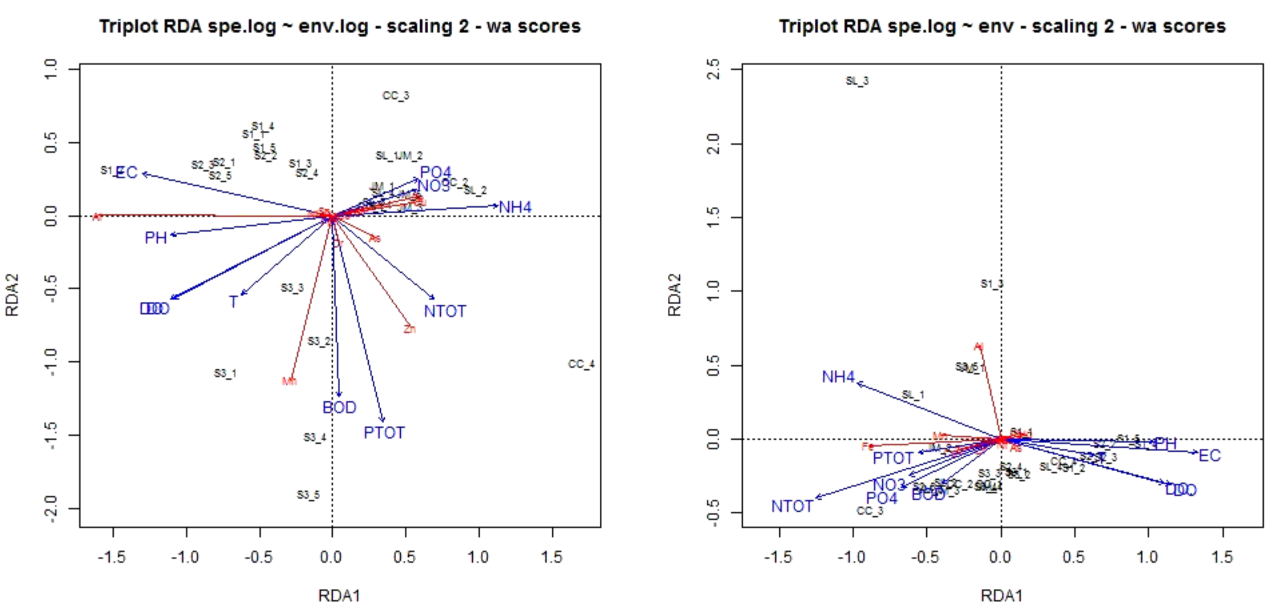

Figure 3

RDA analysis illustrating the relationship between the concentrations of PTEs in Prussian carp from different sampling sites and environmental variables (see Table S1); left - fish muscle, right - fish gills

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