Environmental Research 186 (2020) 109570

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Accumulation of trace elements in muscle, gill and liver of fish species (*Capoeta umbla* and *Luciobarbus mystaceus*) in the Tigris River (Turkey), and health risk assessment

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ARTICLE INFO

Keywords:
Trace elements
Tigris river
Fish tissues
Bioaccumulation
Fish size
Gender
Risk assessment

ABSTRACT

Two fish species (*Capoeta umbla* and *Luciobarbus mystaceus*) were collected from the Tigris River (Turkey), and tissues (gill, muscle and liver) of fish samples were analyzed for Cd, As, Mn, Cr, Cu, Co and Ni using the inductively coupled plasma-tandem mass spectrometry. Significant differences were present between fish species in regard to trace element (TE) concentrations in muscle for Mn, in liver for Cu, Cd and Mn, and in gill for As, Co, Cu and Cd ($p < 0.05$). Liver had the highest total TE concentration, followed by gill and muscle. Significant negative correlations were recorded between fish size (length and weight) and TE concentrations in the tissues. Both fish species showed no gender differences in TE accumulation in muscle, gill and liver ($p > 0.05$). The estimated daily intakes for all TEs were much lower than the tolerable daily intakes. The target hazard quotient (THQ) and total THQ values of all TEs did not exceed 1, which means that non-carcinogenic health effects are not expected for the consumers. Arsenic carcinogenic risk values were within acceptable range. Mean Cd concentrations in muscle tissue of both fish species exceeded the maximum permissible limit set by the European Commission Regulation, while mean concentrations of As, Cr and Cu were below the legislated limits.

1. Introduction

Industrialization and intensive agricultural activities have led to pollution of rivers, especially with trace elements (TEs) which have been identified as one of the most hazardous contaminants for aquatic organisms and humans due to their high bioaccumulation potential, toxicity and persistence (Yi and Zhang, 2012; Jia et al., 2017; Varol et al., 2019). TEs may occur naturally in river ecosystems. However, mining activities, industrial effluents, and agricultural activities are the main sources of TEs (Weber et al., 2013; Merciai et al., 2014; Karadede et al., 2004; Begum et al., 2013). On the other hand, certain TEs like Zn, Co, Fe and Cu are essential for the development of organisms. Arsenic, Pb, Hg and Cd are non-essential elements and toxic for organisms even at low concentrations. Also, high concentrations of essential TEs can be toxic (Varol et al., 2019; Subotic et al., 2013; Yi and Zhang, 2012; Rajeshkumar and Li, 2018; Rajkowska and Protasowicki, 2013). The elevated levels of TEs in fish may cause serious adverse health effects for people eating fish (Copat et al., 2013; Merciai et al., 2014; Alquezar et al., 2006). Therefore, the consumption of fish contaminated with TEs has become an important worldwide concern (Rajeshkumar and Li, 2018; Begum et al., 2013; Saha et al., 2016; Griboff et al., 2017).

Fish, at the top of the aquatic food chain, can uptake TEs by two main routes: directly from water through the gills, and indirectly from food through the digestive tract (Varol and Sünbül, 2019, 2020; Merciai et al., 2014; Alquezar et al., 2006; Rajkowska and Protasowicki, 2013; Jia et al., 2017). Accumulation patterns of TEs in fish depend both on uptake and elimination rates (Karadede et al., 2004). TE concentrations can show significant variability in different organs or tissues of fish. This variability is mainly depend on the concentrations of TEs in water and food (Rajkowska and Protasowicki, 2013; Squadrone et al., 2013). Also, feeding behaviour, fish size, habitat, gender, physiological conditions such as spawning status, and water chemistry such as pH and hardness can affect accumulation of TEs in tissues of fish (Çalta and Canpolat, 2006; Canli and Atli, 2003; Al-Yousuf et al., 2000; Merciai et al., 2014; Yi and Zhang, 2012; Rajkowska and Protasowicki, 2013). Therefore, fish can be serve as reliable bioindicators for monitoring of TE pollution in the rivers (Jia et al., 2017; Varol and Sünbül, 2019).

Determining the concentrations of TEs in edible parts of fish is

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https://doi.org/10.1016/j.envres.2020.109570
Received 22 January 2020; Received in revised form 10 April 2020; Accepted 20 April 2020
Available online 21 April 2020

important for human health, because the consumption of contaminated fish is a major route for transfer of TEs into humans (Rajeshkumar and Li, 2018). It is well known that fish containing high concentrations of TEs can pose serious risks to human health (Griboff et al., 2017; Varol et al., 2017; Yu et al., 2014). Therefore, in recent years, many studies have been performed to assess potential health risks from the consumption of fish exposed to TEs (Copat et al., 2013; Alamdar et al., 2017; Varol and Sünbül, 2017, 2018; 2019; Saha et al., 2016; Griboff et al., 2017).

The Tigris River is one of the largest rivers in Turkey with the length of 523 km. Also, it is a transboundary river, flowing through Turkey, Syria and Iraq. The river is used for fishing, recreation and irrigation. There are two hydroelectric dam reservoirs (Kralkızı and Dicle) on the river in Turkey (Varol, 2011). However, various anthropogenic activities in the basin have caused pollution problems in the river. Domestic and industrial wastewaters, and agricultural activities are the major sources of pollution for the river (Varol et al., 2012). Although there are some studies on the TE accumulation in fish species living in the river, these studies were limited to only a few elements and to the upstream region of the Tigris River (Karadede-Akin and Ünlü, 2007; Gümgüm et al., 1994). In this study, concentrations of seven trace elements were investigated in the liver, muscle and gill of two fish species collected in the downstream region of the Tigris River. Although there are many fish species inhabiting the Tigris River basin (Kaya et al., 2016), *Capoeta umbla* and *Luciobarbus mystaceus* that are highly consumed by the local population were considered in this study. The main aims of this study were to compare the concentrations of TEs between two fish species, among fish tissues (muscle, gill and liver), and between male and female fish, to reveal the relationships between TE concentrations in tissues and fish size (length and weight), and to assess carcinogenic and non-carcinogenic health risks from exposure to TEs through fish consumption.

2. Materials and methods

2.1. Sample collection and analysis

Two fish species (*Capoeta umbla* and *Luciobarbus mystaceus*) belonging to the Cyprinidae family were taken from Ilısu region of the Tigris River (Fig. S1). Fish samples were caught by fishermen from the sampling region in September 2014. All fish samples were immediately transferred to the laboratory. After total length and weight measurements (Table S1), fish samples were dissected, and muscle, gill and liver of each fish were removed. Gender was determined visually after dissection. All muscle, gill and liver samples were separately packed and then kept at −20 °C until analysis.

In this study, seven TEs (Cd, As, Cr, Cu, Ni, Co and Mn) were analyzed in the fish samples. These TEs were selected based on their significant contribution to health risk (Varol and Sünbül, 2018; Copat et al., 2013; Saha et al., 2016). For TE analysis, fish muscle, gill or liver sample (about 0.5 g) was placed into the digestion tubes, and 7 mL HNO_3 and 1 mL H_2O_2 were added to each tube. Digestion was performed using a microwave digestion system (Milestone Start D, USA). After digestion process, the tubes were allowed to cool to room temperature. Then, the caps of the tubes were opened, the solution was transferred to the falcon tubes and diluted with ultrapure water to 25 mL. Inductively coupled plasma-tandem mass spectrometry (ICP-MS/MS) (Agilent 8800, USA) was used to measure concentrations of trace elements in the digested samples. The instrument is equipped with two hyperbolic profile quadrupoles separated by the ORS (octopole reaction system) cell, allowing operation in MS/MS mode. The first quadrupole operates as a mass filter, allowing only the target analyte mass to pass through to the ORS cell and rejecting all other matrix-derived and plasma-based ions. Thus, this configuration provides the most efficient removal of polyatomic and isobaric interferences.

To check the accuracy of the method, certified reference material (DORM-2) for trace elements were digested with every nine samples and analyzed using the same procedure as the samples. The mean recoveries ranged from 90.7 to 107.3% (Table S2). The limit of detection (LOD) and limit of quantification (LOQ) for each element were calculated by multiplying the standard deviation of 10 independent measurements of the blank by 3 and 10, respectively (Table S2). According to the European Commission Regulation No 333/2007 (EFSA (European Food Safety Authority), 2009 EFSA (European Food Safety Authority), 2009), LOD should be less than 1/10 of the maximum permissible limit (MPL) set for Cd in foodstuffs, and LOQ should be less than 1/5 of the MPL. In our study, LOD and LOQ were adequate to reliably quantify Cd to establish whether fish species were safe for human consumption.

2.2. Data treatment and statistical analyses

Concentrations of TEs were expressed in mg/kg weight (ww). Levels of TEs in tissues of fish species were represented by mean values. Among TEs, only Co concentrations in two muscle samples of *L. mystaceus* (n = 10) were below LOD. Therefore, for calculations, Co concentrations below LOD were assumed to be half of the respective LOD (Noel et al., 2013).

Before statistical analyses, normality of data was tested by one-sample Kolmogorov-Smirnov test. Since variables had a normal distribution, parametric statistical tests were applied. One-way ANOVA was applied to determine differences in TE concentrations among muscle, liver and gill for each fish species ($p < 0.05$, Duncan's test). Also, Student' *t*-test was used to determine significant differences in TE concentrations between two fish species and between males and females ($p < 0.05$). Pearson correlation test was conducted to check for significant relationships between TE concentrations in tissues of fish species and fish size (length and weight). Statistical analyses were carried out using SPSS 11.5.

2.3. Risk assessment

In order to assess health risks of TEs for adults through fish consumption, target hazard quotient (THQ), estimated daily intake (EDI) and lifetime carcinogenic risk (CR) were calculated. Also, the concentrations of TEs found in each fish species were compared with maximum permissible limits for edible fish tissues. In this study, for all risk assessment methods (EDI, THQ and CR), inorganic arsenic was taken into account instead of total arsenic and it is assumed that 10% of the total arsenic is inorganic arsenic (Varol and Sünbül, 2018). Also, risk assessment methods were calculated using the concentrations of TEs determined in only muscle tissues of fish species because other parts of fish are not consumed by people in Turkey.

The EDI was calculated using the following equation (Griboff et al., 2017; Varol et al., 2017):

$$EDI = \frac{TEC \times IRd}{BW}$$

where, TEC is the trace element concentration in fish muscle (mg/kg wet weight). IRd is daily fish consumption (g/day). The average fish consumption was 15.069 g/day per adult in Turkey (GDFA, 2018). BW is the average adult body weight (70 kg). In this study, the EDI of each element was compared with the tolerable daily intake (TDI).

The non-cancer health risks were assesed using the target hazard quotient (THQ) and total THQ (or hazard index). The acceptable guideline for THQ and TTHQ is 1. The THQ and TTHQ >1 means that there may be a potential for adverse non–cancer health effects to occur. The THQ and TTHQ <1 means that non-cancer health effects are not expected (USEPA, 1989). The following equations were used to calculate the THQ and TTHQ (USEPA, 2019a):

$$THQ = \frac{EF \times ED \times IRd \times TEC}{RfDo \times BW \times ATnoncancer} \times 10 - 3$$

$$TTHQ \text{ (or HI)} = THQ(As) + THQ(Cd) + ... + THQ(Mn) + THQ(Ni)$$

where, RfD is the oral reference dose for TEs, ED is the exposure duration (26 years) (USEPA, 2011), EF is exposure frequency (350 days/year) (USEPA, 1991a) and AT is averaging time for non-carcinogens (365 days/year × 26 years) (USEPA, 1989).

Among the studied TEs, only inorganic arsenic is a human carcinogen (USEPA, 2019b). Therefore, the cancer risk (CR) is estimated for only inorganic arsenic. The following equation was used to calculate the CR (USEPA, 2019a):

$$CR = \frac{EF \times ED \times IRd \times TEC \times CSFo}{BW \times ATcancer} \times 10 - 3$$

where, CSF_o represents oral carcinogenic slope factor and is 1.5 $(mg/kg\text{-}day)^{-1}$ for inorganic As (USEPA, 2019b). AT is averaging time for carcinogens (365 days/year × 70 years) (USEPA, 1989). Acceptable levels for carcinogenic risks range from 10^{-4} to 10^{-6} (USEPA, 1991b).

All THQ, TTHQ and CR values obtained in our study were confirmed using Regional Screening Levels calculator (USEPA, 2019c).

3. Results and discussion

3.1. Trace element (TE) concentrations in fish species

Mean concentrations of TEs in muscle, gill and liver of *C. umbla* and *L. mystaceus* from the Tigris River are shown in Table 1.

Regarding the differences in TE concentrations between *C. umbla* and *L. mystaceus*, mean concentrations of As, Cd, Co, Cr, Ni and Cu in muscle, As, Co, Cr and Ni in liver, and Cr, Ni and Mn in gill were did not show statistically significant differences between the two fish species ($p > 0.05$) (Table S3). However, mean concentrations of Mn in muscle and Mn and Cu in liver of *C. umbla* were statistically higher than those of *L. mystaceus* ($p < 0.05$) (Table S3). Also, mean concentrations of Cd in liver and As, Co, Cu and Cd in gill of *L. mystaceus* were statistically higher than those of *C. umbla* ($p < 0.05$) (Table S3). The differences in TE concentrations between fish species collected in the same region can be a result of different feeding behaviours, ecological needs and physiologies of fish species (Varol et al., 2019; Rajeshkumar and Li, 2018; Fathi et al., 2013).

Regarding fish tissue (muscle, gill and liver), liver (78.49 and 51.98 mg/kg) of *C. umbla* and *L. mystaceus* had the highest total mean concentration of seven TEs, followed by gill (21.07 and 25.75 mg/kg) and muscle (4.62 and 4.39 mg/kg). For *C. umbla*, mean concentrations of As, Cu and Cd in liver were statistically higher than muscle and gill ($p < 0.05$), whereas mean concentration of Co in gill was comparable to liver ($p > 0.05$) and higher than muscle ($p < 0.05$) (Table 1). For *L. mystaceus*, mean concentrations of Cu and Cd in liver were statistically higher than muscle and gill ($p < 0.05$), while mean concentrations of As and Co in gill was comparable to liver ($p > 0.05$) and higher than muscle ($p < 0.05$) (Table 1). For both fish species, mean concentrations of Cr, Mn and Ni in gill were statistically higher than liver and muscle ($p < 0.05$) (Table 1). These results are consistent with previous studies, which found that TE concentrations are usually lowest in muscle and highest in liver and gill of fish species (Squadrone et al., 2013; Rajeshkumar and Li, 2018; Jia et al., 2017; Jaric et al., 2011; Karadede et al., 2004).

Gill and liver are the metabolically active organs in fish. Also, they are target organs of TEs due to their physiological roles in fish metabolism (Squadrone et al., 2013; Sow et al., 2019). For instance, the gill is an important site for the entry of TEs and is the first target organ for TE exposure. The concentrations of TEs in the gill show the concentrations of TEs in the water where the fish live. Liver plays an important role in the processes of accumulation, uptake, and storage of TEs. However, metabolic activity is relatively lower in muscles. Therefore, this tissue has lower levels of TEs than liver and gill (Rajeshkumar and Li, 2018; Fathi et al., 2013; Sow et al., 2019). For instance, in this study, mean concentrations of Cu in liver of *C. umbla* and *L. mystaceus* were 32 and 20 times higher than muscle and 23 and 10 times higher than gill, that is in agreement with the results of previous studies (Rashed, 2001; Karadede et al., 2004; Lenhardt et al., 2012; Yılmaz and Doğan, 2008) where Cu concentrations were considerably higher in fish liver than other tissues.

The mean concentrations of TEs in muscle and liver of both fish species followed this order: Cu > Mn > Ni > As > Cd > Cr > Co, while the order of elements in gill of the fish species was Mn > Cu > Ni > As > Cr > Co > Cd (Table 1). These results may be due to different concentrations of TEs in diet and water. It is well known that fish uptake TEs directly from water and diet (Varol and Sünbül, 2019). Gills mostly accumulate waterborne TEs, while liver accumulates food-associated TEs (Gagnaire et al., 2015; Golovanova, 2008).

The relationships between TE concentrations in gill, muscle and liver of the two fish species and fish size (length and weight) were given in Table 2. For *C. umbla*, all TEs in gill did not show significant correlations with both length and weight ($p > 0.05$). However, Cd in muscle and As, Co, Ni, Cu and Cd in liver showed negative correlations with length ($p < 0.05$). Also, there were significant negative correlations between weight and As, Mn, Co, Ni and Cd in liver ($p < 0.05$). For *L. mystaceus*, all TEs in muscle did not show significant correlations with

Table 1
Mean concentrations of trace elements in the muscle, gill and liver of the examined fish species (units mg/kg ww).

		N		As	Cr	Mn	Co	Ni	Cu	Cd
C. umbla										
	Muscle	9	Mean	0.331^a	0.056^a	1.146^a	0.021^a	0.795^a	2.203^a	0.065^a
			S.D.	0.087	0.023	0.263	0.028	0.307	0.473	0.017
	Gill	9	Mean	0.437^a	0.393^b	15.464^b	0.115^b	1.520^b	3.036^a	0.102^a
			S.D.	0.108	0.110	5.874	0.028	0.487	0.859	0.029
	Liver	9	Mean	0.760^b	0.198^c	6.254^c	0.113^b	0.989^a	69.966^b	0.207^b
			S.D.	0.272	0.084	2.252	0.040	0.381	21.471	0.093
L. mystaceus										
	Muscle	10	Mean	0.398^a	0.081^a	0.856^a	0.008^a	0.657^a	2.311^a	0.083^a
			S.D.	0.166	0.028	0.248	0.003	0.222	0.816	0.032
	Gill	10	Mean	0.777^b	0.423^b	17.449^b	0.192^b	2.323^b	4.438^a	0.156^a
			S.D.	0.232	0.159	5.418	0.079	1.101	1.257	0.046
	Liver	10	Mean	0.712^b	0.196^c	3.051^a	0.183^b	1.240^a	46.242^b	0.359^b
			S.D.	0.411	0.126	1.664	0.107	0.967	25.956	0.162

The different letters in the same column indicate statistical difference among tissues (muscle, gill and liver) for each fish species at $p < 0.05$ (Duncan test).
S.D., standard deviation.
N, number of samples.

Table 2
Pearson correlation coefficients between trace elements and fish size (length and weight).

Species	Tissue	Total length							Body weight						
		As	Cr	Mn	Co	Ni	Cu	Cd	As	Cr	Mn	Co	Ni	Cu	Cd
C. umbla	Muscle	0.074	−0.310	0.163	−0.539	−0.083	0.119	−0.766[a]	0.092	−0.344	0.103	−0.430	−0.062	0.208	−0.568
	Gill	0.124	0.172	0.175	−0.032	0.166	0.133	0.149	0.053	0.208	0.183	−0.004	0.074	0.088	0.094
	Liver	−0.911[a]	−0.467	−0.557	−0.885[a]	−0.756[a]	−0.657[b]	−0.867[a]	−0.909[a]	−0.371	−0.660[b]	−0.841[a]	−0.677[b]	−0.567	−0.750[a]
L. mystaceus	Muscle	−0.416	−0.101	−0.437	−0.503	−0.540	−0.533	−0.428	−0.753[a]	−0.497	−0.780[a]	−0.805[a]	−0.802[a]	−0.811[a]	−0.738[a]
	Gill	−0.550[b]	−0.528	−0.617[b]	−0.733[a]	−0.604[b]	−0.324	−0.306	−0.721[a]	−0.680[b]	−0.683[b]	−0.782[a]	−0.769[a]	−0.339	−0.294
	Liver	−0.643[b]	−0.386	−0.573[b]	−0.585[b]	−0.117	−0.442	−0.319	−0.599[b]	−0.364	−0.522	−0.543	−0.064	−0.425	−0.364

[a] Correlation is significant at the 0.01 level.
[b] Correlation is significant at the 0.05 level.

Table 3
Comparison of the concentrations of TEs in this study with those of fish from other rivers.

Tissue	Location	Species	As	Cr	Mn	Co	Ni	Cu	Cd	Reference
Muscle	Buriganga River, Bangladesh	*Heteropneustes fossilis*	0.3	1.56				8.05	0.36	Begum et al. (2013)[a]
	Tigris River, Turkey	*Silirus triostegus*			0.31		0.82	3.05		Karadede-Akin and Ünlü (2007)[b]
	Danube River, Serbia	*Cyprinus carpio*	0.66	0.01	0.12	0.0001		1.3	0.005	Subotic et al. (2013)[a]
	Asi River, Turkey	*Carasobarbus luteus*		0.16			0.29	5.23	0.15	Yılmaz and Doğan (2008)[b]
	Xiang River, China	*Carassius auratus*	0.042		0.215			0.118	0.021	Jia et al. (2017) [b]
	Neretva River, Bosnia and Herzegovina	*Cyprinus carpio*						0.807	0.013	Djedjibegovic et al. (2012) [b]
	Rivers, France	*Abramis brama*	0.109						0.004	Noel et al. (2013) [b]
	Sava River, Croatia	*Alburnus alburnus*	0.17	0.039			0.36	2.53	0.009	Zuliani et al. (2019) [a]
	Tigris River, Turkey	*Capoeta umbla*	0.331	0.056	1.146	0.021	0.795	2.203	0.065	This study[b]
	Tigris River, Turkey	*Luciobarbus mystaceus*	0.398	0.081	0.856	0.008	0.657	2.311	0.083	This study[b]
Gill	Buriganga River, Bangladesh	*Heteropneustes fossilis*	0.86	3.62				6.31	3.57	Begum et al. (2013) [a]
	Tigris River, Turkey	*Silirus triostegus*			10		1.23	2.79		Karadede-Akin and Ünlü (2007) [b]
	Danube River, Serbia	*Cyprinus carpio*	0.29	0.01	10.05	0.0001		1.9	0.03	Subotic et al. (2013) [a]
	Asi River, Turkey	*Carasobarbus luteus*		1.4			1.87	14.1	1.28	Yılmaz and Doğan (2008) [b]
	Xiang River, China	*Carassius auratus*	0.056		7.241			0.715	0.078	Jia et al. (2017) [b]
	Tigris River, Turkey	*Capoeta umbla*	0.437	0.393	15.464	0.115	1.520	3.036	0.102	This study[b]
	Tigris River, Turkey	*Luciobarbus mystaceus*	0.777	0.423	17.449	0.192	2.323	4.438	0.156	This study[b]
Liver	Buriganga River, Bangladesh	*Heteropneustes fossilis*	2.61	6.3				45.61	3.92	Begum et al. (2013) [a]
	Tigris River, Turkey	*Silirus triostegus*			1.1		0.74	6.81		Karadede-Akin and Ünlü (2007) [b]
	Danube River, Serbia	*Cyprinus carpio*	0.49	0.01	2.21	0.0001		33.49	0.28	Subotic et al. (2013) [a]
	Asi River, Turkey	*Carasobarbus luteus*		1.22			3.31	73.84	2.35	Yılmaz and Doğan (2008) [b]
	Xiang River, China	*Carassius auratus*	0.082		1.616			23.13	0.093	Jia et al. (2017) [b]
	Neretva River, Bosnia and Herzegovina	*Cyprinus carpio*						2.01	0.031	Djedjibegovic et al. (2012) [b]
	Tigris River, Turkey	*Capoeta umbla*	0.760	0.198	6.254	0.113	0.989	69.966	0.207	This study[b]
	Tigris River, Turkey	*Luciobarbus mystaceus*	0.712	0.196	3.051	0.183	1.240	46.242	0.359	This study[b]

[a] Units mg/kg dry weight.
[b] Units mg/kg wet weight.

length ($p > 0.05$), while all TEs except Cr in muscle showed significant negative correlations with weight ($p < 0.05$). Also, significant negative correlations were found between length and As, Mn, Co, and Ni in gill and As, Mn and Co in liver ($p < 0.05$). Arsenic, Cr, Mn, Co and Ni in gill and As in liver had significant negative correlations with weight ($p < 0.05$). These results indicated that all significant correlations between TE concentrations and fish size were negative. Similarly, there are many studies in the literature which report the negative correlations between TE concentrations in the fish tissues and fish sizes. For instance, Merciai et al. (2014) showed that significant correlations between fish size and TE concentrations were negative for all TEs and all fish species from the Llobregat River in Spain. Farkas et al. (2003) reported that Cu, Pb and Zn in muscle and gill and Zn and Pb in liver of *Abramis brama* from the Lake Balaton (Hungary) showed negative correlations with fish size. Another study by Canli and Atli (2003) also found significant negative correlations between TE concentrations and fish size, except in a few cases.

There are several mechanisms causing negative relationships between TE concentrations and fish size. One of them is size-specific metabolic rate of fish. Metabolic rate is higher in younger fish, with consequent higher food intake and the relative quantity of respiratory water passing through the gills per time unit (Canli and Atli, 2003; Merciai et al., 2014; Farkas et al., 2003). Second mechanism is the relative dilution effect of the lipid content of tissues. This assumption is supported well also by the fact that the percentage of fat tissue in young fish is lower than adult fish (Farkas et al., 2003; Merciai et al., 2014). Both mechanisms may explain a higher potential uptake of TEs in smaller fish than larger fish.

In this study, in terms of TE concentrations in muscle, gill and liver, statistically significant differences between female and male fish were not found ($p > 0.05$) (Table S4). Gender differences in TE concentrations of fish tissues can be affected by a combination of factors, such as physiological metabolism in relation to stage in reproductive cycle, dietary preferences or foraging behaviour (Alquezar et al., 2006; Rajkowska and Protasowicki, 2013). The spawning period of cyprinids in the Tigris River basin occurs from mid-spring through mid-summer (Varol and Sünbül, 2018). In this study, fish samples were collected in autumn, when gonads are in the recovery stage (no gonadal development). Thus, no significant differences in element tissue concentrations between males and females could be explained by this. Similarly, no gender differences in TE concentrations in tissues (muscle, liver, gill, kidney, spleen, digestive tract and skin) of *Esox lucius* and *Abramis*

Table 4
Mean concentrations trace elements detected in muscle of fish species in this study and maximum permissible limits for fish (units mg/kg ww).

	iAs	As	Cd	Cr	Cu	
C. umbla	0.0331	0.331	0.065	0.056	2.203	This study
L. mystaceus	0.0398	0.398	0.083	0.081	2.311	This study
Maximum permissible limits						
Chinese Health Ministry	0.1		0.1	2.0		MHPRC (2013)
European Commission Regulation			0.05			EC (2006)
Food and Agriculture Organization					30	FAO (1983)

iAs: inorganic As (10% of total arsenic).

brama in lakes in Poland were found (Rajkowska and Protasowicki, 2013). Also, Alkan et al. (2013) determined TE concentrations in muscle and gill of fish species from Black Sea and found no statistical differences between female and male fish. However, Al-Yousuf et al. (2000) found higher average TE concentrations in muscle, skin and liver of female fish than those in male fish.

Trace element concentrations detected in gill, muscle and liver of the fish species in this study were compared with other studies investigating TE concentrations in tissues of riverine fish (Table 3). In a previous study performed in the Tigris River (Turkey), compared to the two fish species in this study, Karadede-Akin and Ünlü (2007) reported higher mean Cu concentrations in liver, muscle and gill of *Silirus triostegus* and higher mean Ni concentrations in muscle (Table 3). However, mean Ni concentrations in liver and gill and mean Mn concentrations in muscle, gill and liver reported by Karadede-Akin and Ünlü (2007) were lower than those in this study (Table 3). In comparison to *Cyprinus carpio* samples from the Danube River (Serbia) (Subotic et al., 2013), both fish species in our study had higher mean concentrations of As, Cd, Co, Cr, Cu and Mn in liver, muscle and gill samples (Table 3). In the Buriganga River, Bangladesh (Begum et al., 2013), *Heteropneustes fossilis* had lower mean concentrations of As and Cu in liver, muscle and gill than those detected in our study, but had higher mean concentrations of Cr and Cd (Table 3). In comparison to *Carasobarbus luteus* samples collected in autumn from the Asi River, Turkey (Yılmaz and Doğan, 2008), the two fish species in our study had lower mean concentrations of Cd, Cr and Cu in liver, muscle and gill, and lower mean concentrations of Ni in liver, but higher in muscle (Table 3). Jia et al. (2017) reported lower mean concentrations of As, Cu, Cd and Mn in gill, muscle and liver of *Carassius auratus* from the Xiang River (China) than those in both fish species in this study (Table 3). Mean Cd and Cu concentrations in muscle and liver of both fish species in our study were higher than those in *Cyprinus carpio* from the Neretva River (Bosnia and Herzegovina) (Djedjibegovic et al., 2012). Also, Noel et al. (2013) reported lower mean concentrations of As and Cd for muscle tissue of *Abramis brama* in rivers in France. Similarly, mean Cu, As, Cr, Ni and Cd concentrations in muscle of *Alburnus alburnus* in the Sava River (Croatia) (Zuliani et al., 2019) were lower than those detected in this study.

3.2. Risk assessment

Trace element concentrations determined in muscle of fish species were compared with maximum permissible limits (MPLs) for edible fish tissues established by Chinese Health Ministry (MHPRC , 2013), European Commission Regulation (EC, 2006) and Food and Agriculture Organization (FAO, 1983) (Table 4). In the current study, mean concentrations of inorganic As (iAs), Cr and Cu in muscle of the two fish species were below MPLs. Also, no fish samples exceeded the MPLs set for iAs, Cr and Cu. Mean Cd concentrations were above MPL set by EC (2006), while they were below MPL established by (MHPRC , 2013) (Table 4). It should be noted that Cd concentrations in seven samples of *C. umbla* (n = 9) and nine samples of *L. mystaceus* (n = 10) exceeded MPL set by EC (2006). High Cd concentrations in muscle of the two fish species can be associated with chemical fertilizers used in agricultural areas in the upstream region of the river, because some fertilizers can contain high levels of Cd (Yanardağ et al., 2016).

The EDI values of TEs calculated for the two fish species and TDI values are given in Table 5. The estimated daily intakes of seven TEs represented 0.004–1.75% of TDI for *C. umbla*, and 0.006–2.23% for *L. mystaceus*. These results indicated that the contributions of TEs in fish species to the corresponding TDI were very small (Table 5). Therefore, it was assumed that the daily intakes of TEs via the consumption of fish species would not cause health problems for consumers.

The target hazard quotients (THQs) estimated for individual TEs through consumption of the two fish species are presented in Table 5. The THQs of TEs in each fish species from the Tigris River did not exceed 1. Also, TTHQs of combined metals for each fish species were lower than 1 (Table 5). According to the THQ and TTHQ results, non-carcinogenic health effects from the intake of individual or combined elements in both fish species are not expected for consumers.

The carcinogenic risk (CR) values estimated for inorganic As ranged from 3.81×10^{-6} in *C. umbla* to 4.58×10^{-6} in *L. mystaceus* (Table 5), suggesting that the CR values were within acceptable range of 10^{-4} and 10^{-6}. Thus, there was low carcinogenic risk for consumers due to the exposure to inorganic As through the consumption of the two fish species from the Tigris River.

Table 5
Target hazard quotient (THQ), total THQ (TTHQ), carcinogenic risk and estimated daily intake (EDI) values of trace elements from fish consumption, and their tolerable daily intake (TDI) values.

	C. umbla	*L. mystaceus*	Rfd (mg/kg/bw/day)	*C. umbla*	*L. mystaceus*	TDI (μg/kg bw/day)
	Target hazard quotient (THQ)			Estimated daily intakes (μg/kg bw/day)		
As[a]	2.28E-02	2.74E-02	0.0003 (USEPA, 2019b)	7.13E-03	8.57E-03	2.14 (JECFA, 1989)
Cd	1.34E-02	1.71E-02	0.001 (USEPA, 2019b)	1.40E-02	1.79E-02	0.8 (JECFA, 2011)
Co	1.44E-03	5.50E-04	0.003 (Finley et al., 2012)	4.52E-03	1.72E-03	8.6 (EFSA, 2009)
Cr	3.85E-03	5.57E-03	0.003 (USEPA, 2019b)	1.21E-02	1.74E-02	300 (EFSA, 2014)
Cu	1.14E-02	1.19E-02	0.04 (USEPA, 2019b)	4.74E-01	4.97E-01	500 (JECFA, 1982)
Mn	1.69E-03	1.26E-03	0.14 (USEPA, 2019b)	2.47E-01	1.84E-01	140 (USEPA, 2019b)
Ni	8.21E-02	6.78E-03	0.02 (USEPA, 2019b)	1.71E-01	1.41E-01	12 (WHO , 2011)
Total THQ (TTHQ)						
	6.28E-02	7.06E-02				
Carcinogenic risk						
As[a]	3.81E-06	4.58E-06				

[a] Inorganic arsenic.

4. Conclusions

This study has demonstrated that some TEs in tissues showed statistically significant differences between fish species. TE concentrations were lowest in muscle and highest in liver and gill of fish species. Fish gender did not influence TE accumulation in tissues. All significant correlations between TE concentrations in tissues and fish size were negative. The EDI value of each element was below the its TDI value. The THQ and TTHQ values were below 1, and the CR values in fish species were between 10^{-4} and 10^{-6}. The concentrations of all TEs except Cd were found below the maximum permissible limits, indicating that Cd in both fish species from the Tigris River may pose health risk to the consumers.

Acknowledgements

Special thanks are given to the editors Jose L. Domingo and Paul T.J. Scheepers and three anonymous reviewers for their constructive comments and suggestions for improving this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2020.109570.

Author contribution statement

Emel Kaçar and Hülya Karadede Akın concepted the study. Emel Kaçar collected fish samples and conducted laboratory analyses of the samples. Memet Varol completed data interpretation, performed health risk assessment, drafted and finalized the manuscript. All the authors contributed to the revision of manuscript.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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