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PERSISTENT ORGANIC POLLUTANTS (POPS): A GLOBAL ISSUE, A GLOBAL CHALLENGE

Metal pollution in biotic and abiotic samples of the Büyük Menderes River, Turkey

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Abstract The Büyük Menderes River (BMR) is one of the largest rivers in Turkey. This river irrigates efficient farmlands and includes tributaries of other rivers and streams and many populated towns within its limits in the Ege region. Both the estuary and Işıklı Lake serve as a sanctuary for various waterbirds. Therefore, the BMR plays a critical role both for the inhabitants and for the ecosystem organisms in its environs. In the present study, we analyzed levels of metals including iron, barium, zinc, vanadium, cobalt, chromium, cadmium, copper, nickel, aluminum, arsenic, manganese, antimony, silver, selenium, boron, mercury, titanium, and lead in river water, sediment, fish (*Cyprinus carpio*; common carp), and in various waterbird (*Fulica atra*, Euroasian coot; *Larus michahellis*, yellow-legged gull; *Ardea cinerea*, grey heron; *Larus melanocephalus*, Mediterranean gull; and *Pelecanus crispus*, pelican) samples. Analyses were performed using an inductively coupled plasma–mass spectrometry (ICP-MS) instrument after sample preparation. Comparing metal concentrations among different sample types, it was found that barium, aluminum, and zinc are the major metals in river water, and zinc in common carp muscle, while iron, aluminum, and manganese are the major metals in sediments. Iron, zinc, copper, and aluminum were the highest in waterbird muscle tissue. Iron and barium were found to be the major metals in eggshell, while iron and zinc are the major metals in egg samples. A simple "worst-case scenario" model of risk assessment revealed that some of the analyzed metals may pose a risk for human health through consuming fish.

Keywords Büyük Menderes River · Metal pollution · Sediment · Common carp · Waterbird · Egg · Biomonitoring

Introduction

Aquatic ecosystems are exposed to a mixture of inorganic and organic contaminants that impact on ecosystems and human health. Metals are the main group of inorganic contaminants that induce an accumulation in the environment resulting from urban sewage, industrial, and agricultural activities. Heavy metals in the environment have especially high risks due to their toxicity, long-term persistence, bioaccumulation, and biomagnification properties (Monroy et al. 2014). In terms of aquatic species, elements such as copper (Cu), zinc (Zn), cobalt (Co), and iron (Fe) cannot be considered a health risk unless they exceed certain threshold levels, as they are necessary for animal life (Canli and Atli 2003). In contrast, others such as cadmium (Cd), mercury (Hg), and lead (Pb) always pose a risk to the healthy status of organisms (Amundsen et al. 1997; Kojadinovic et al. 2007). Exposure to sublethal concentrations of metals results in reproductive dysfunction, diminished immunological defense, and behavioral changes in fishes and birds (Zhang and Ma 2011). Similar to other pollutants, metals in aqueous systems are associated with sediment in varying proportions, depending on their physicochemical properties. Sediment-associated metals exhibit equilibrium with water in a dynamic process, and their sedimental concentration is less variable than water in long-term monitoring (Alloway 2013). On the other hand, the metal concentration of water and sediment alone does not provide information on

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the potential adverse impact of metals on the environment (Maceda-Veiga et al. 2012). Supporting the measurements with different vertebrates provides information on the risk due to metal bioaccumulation (Monroy et al. 2014). Fish species are commonly used as a biomonitor of aquatic pollution (Karaca et al. 2014). In addition, waterbirds can also be utilized as indicators of short- and long-term pollutant exposure, as they are the top predators of the food chain (Kocagöz et al. 2014). In terms of human health, metal pollution in freshwaters or saltwaters (lakes, rivers, and sea, respectively) may have an adverse impact on health when reaching humans by several means: contamination of tap water by the running of the polluted rivers into dams; contamination of crops by irrigating lands with polluted water; and the consumption of seafood, especially fish and clams.

The Büyük Menderes River (BMR) is the largest stream in western Turkey discharging into the Aegean Sea. The levels of persistent organic pollutants (POPs) were recently reported both in abiotic and biotic media such as water and sediment, and fish and waterbird species, respectively (Karaca et al. 2014, Kocagöz et al. 2014, Çağdaş et al. 2015). Surprisingly, metal levels have not been studied and reported in this significant river in terms of agriculture, fishery, and environment. The present study has two objectives: the monitoring of metals in both biotic and abiotic samples in the BMR, and estimating the risk posed by metals to human health from consuming fish caught from the river. By doing this, levels of a large group of metals will be reported for the first time along the river.

Materials and methods

Chemicals

Acetone, nitric acid, and hydrofluoric acid were obtained from Fluka (Buchs, Switzerland), Sigma (MO, USA), and Merck (Darmstadt, Germany), respectively. Metal calibration standards were purchased from Agilent (CA, USA). All other chemicals were of analytical grade. Type 1 water with a resistivity of 18.2 MΩ cm at 25 °C was used for all experimental purposes and was produced using an Millipore - Direct Q water purification system (Millipore, MA, USA).

Study area and sampling sites

The BMR is located in Turkey between 37° 6′ and 38° 55′ north and 27° and 30° 36′ east. The river is contaminated by the domestic wastewaters produced from the residential areas along the basin and by the industrial discharge produced from extensive industrial activities. In addition, the contamination caused by agricultural activities is of concern along the whole river, especially in the middle and lower part of the basin. The BMR is the longest river of the Ege region, providing wildlife with a habitat and playing a critical role in the ecosystem. Biotic samples were collected in July 2009 at four stations: the BMR's source, which is the Işıklı Lake; Sarayköy, the area's most industrialized region; Söke, the river estuary; and the intense agricultural region, the Plains. Taşburun, as a sea-sampling station for abiotic samples at 6-km distance to the south of the estuary, was also included in the study (Fig. 1).

Sample collection and preparation

Abiotic and biotic samples were collected from five stations as indicated in Fig. 1, sited on the BMR and Ege Sea. Precautions were taken to minimize possible metal contamination throughout all sampling and analytical procedures. Sample types, numbers, and sampling locations are summarized in Table 1.

Water samples

Water samples were collected from 30 cm deep below the surface and filtered with plankton net of 55-μm mesh and stored at +4 °C until analyzed. Five milliliters of water samples was used for each analysis.

Sediment samples

The surface sediments were collected in a chemically cleaned borosilicate jar and were kept on ice until taken to the laboratory and stored in the dark at +4 °C.

After drying in the oven at 50 °C overnight, sediment samples were homogenized (Heidolph SC-M) and sieved through vibrating stainless steel sieves with a mesh size of 50 μm. Hundred milligrams of each sample were digested for 1 h in screw-cap teflon tubes in a 4-ml solution of high purity of 65 % nitric acid and 40 % hydrofluoric acid (3/1) and subsequently microwaved (CEM, MARS 5, MD 7840) at 200 °C-800 W with increasing 15 °C/min for 30 min. After cooling, samples were diluted to 10 ml with ultrapure water and kept in sealed tubes until analysis.

Common carp and waterbird muscle tissues

Common carp was chosen as a biomonitor of aquatic metal pollution in the BMR, as it consumes sediment-associated organisms and plants, both of which integrate sedimental metals. A total number of 34 carp specimens that reached sexual maturity were collected by professional fishermen using fishnet from the BMR in three sampling sites (Table 1).

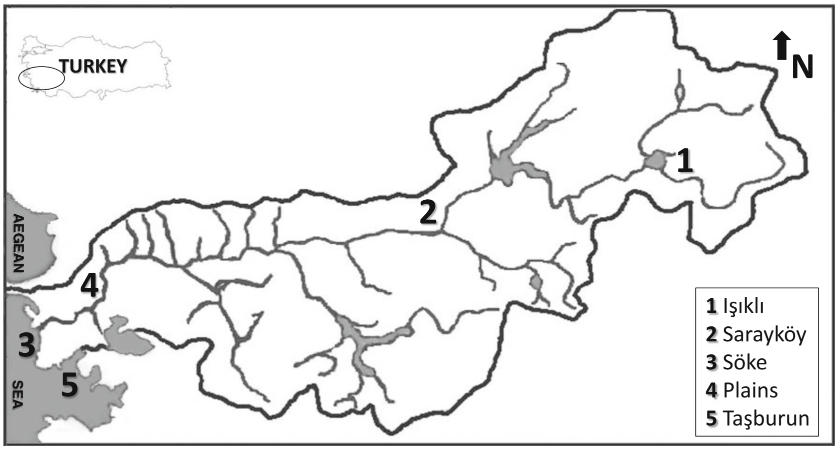

Fig. 1 Sampling sites in the basin of the BMR and Ege Sea. *1*, Işıklı station (origin); *2*, Sarayköy station (industry and agriculture); *3*, Söke station (estuary); *4*, Plains (intense agriculture); and *5*, Taşburun (Sea station). *Scale* 1/3,000,000

Grey heron and pelican were chosen because they are the species under risk of extinction. Eurasian coot and gull species (yellow-legged and Mediterranean gull) were chosen because they integrate pollutants over a broad area of the Işıklı Lake and the river estuary, and represent an ideal biomonitor for metals. Upon catching the waterbirds by net from two sampling sites (Table 1), muscle was taken as an invasive sample from six yellow-legged gulls and six Eurasian coots, but not from grey heron and pelican due to their risk of extinction. All samples were dissected within 2 h after collection and removed tissues stored in cryotubes at −86 °C. Muscle samples (0.3 g) (*wet weight*) digested in 3 ml high purity of 65 % nitric acid and microwave-assisted digestion was performed in at 200 °C-1200 W with increasing 15 °C/min for 30 min. After digestion process, the samples were diluted to 30 ml with ultrapure water and kept in sealed tubes until analysis.

Egg shells and content

Nonhatched eggs as noninvasive samples were obtained from all waterbirds except the yellow-legged gull (Table 1). Eggshells were rinsed with acetone, followed by cleaned with pure water, allowed to dry at 60 °C, and homogenized. Egg contents were homogenized with a blender followed by 0.3 g homogenized egg contents (*wet weight*), and eggshells (*dry weight*) were digested with 3 ml high purity of 65 % nitric acid and microwaved at 200 °C-1200 W for 30 min. After digestion process, the samples diluted to 30 ml with ultrapure water and kept in sealed tubes until analysis.

Ethical permission

Although only grey heron and pelican are endangered species and common carp and Eurasian coot are consumed by local inhabitants without restriction, the official ethical permission for all types of samples of all species for systematic sampling was provided by the Ministry of Environment prior to the study (approval no. B.12.0.KKG.0.17/106.01-11.01-540/24.03.2008). Other details of the methods have been reported previously (Karaca et al. 2014, Kocagöz et al. 2014, Çağdaş et al. 2015).

ICP-MS analysis

Levels of 16–19 metals were quantified in water and sediment samples, in the muscles of Eurasian coot, yellow-legged gull and common carp, as well as in the eggs and eggshells of all waterbirds, except the yellow-legged gull. Analyses were carried out by inductively coupled plasma–mass spectrometry (ICP-MS,

Table 1 Sample types, numbers, and sampling locations

Sample type	Common carp Işıklı/Sarayköy/Söke	Yellow-legged gull Işıklı/Söke	Eurasian coot Işıklı/Söke	Grey heron Işıklı/Söke	Pelican Işıklı/Söke	Mediterranean gull Işıklı/Söke
Muscle	13 / 11 / 10	3 / 3	3 / 3	– / –	– / –	– / –
Egg	– / –	– / –	3 / –	– / 3	– / 3	3 / 5
Eggshell	– / –	– / –	3 / –	– / 8	– / 4	3 / 5

Agilent 7500). Settings for the ICP-MS analysis are summarized as follows:

Plasma conditions		Ion lenses		Octopole parameters	
RF power	1500 W	Extract 1	−54.9 V	Octp RF	180 V
RF matching	1.8 V	Extract 1	−120 V	Octp bias	−6 V
Sample depth	8 mm	Omega bias	−30 V	*Reaction cell*	
Carrier gas	0.91 L/min	Omega lens	0.2 V	Reaction mode	On
Make-up gas	0.17 L/min	Cell entrance	−20 V	H2 gas	0 mL/min
Nebulizer pump	0.08 rps	QP focus	5 V	He gas	1 mL/min
S/C temperature	2 °C	Cell exit	−28 V	Ar gas	15 mL/min

Calibration curves that were generated automatically for each metal consist of a blank, 50, 100, 250, and 500 ppb. Limit of quantitation (LOQ) values for Al, As, Sb, Cu, Ba, Hg, Zn, Fe, Ag, Cd, Sn, Ca, Co, Cr, Mg, Mn, Cr, Pb, Mg, Mn, Ni, K, Se, Na, and Ti were 0.002, 0.001, 0.001, 0.012, 0.003, 0.003, 0.017, 0.049, 0.001, 0.001, 0.001, 0.221, 0.001, 0.007, 0.004, 0.032, 0.003, 0.004, 0.016, 0.003, 0.576, and 0.003 mg/kg, respectively. Dilution ratios of 1:100 were used to perform the analysis.

Environmental calibration standard (Agilent 5183-4688) for trace metals in 18.2 MΩ cm at 25 °C double deionized water were analyzed after every five samples to ensure the accuracy of the equipment and the experimental procedure. Other reference standards used were 8-93VY, 6-40GS, 36-12AS, and 13-166JB. The metal analysis produced recovery values ranging from 85.1 to 110.8 % for the entire analytical process. Number of replicates was two for each measurement. The in-house methods were accredited by TURKAK (No. AB-0040-T). Three blanks with ultrapure water were used as controls, and the analytical procedures were determined according to EPA 9056 and EPA 200.7. Blanks were under the detection limits for the elements.

Statistics

Results were considered in four groups according to characteristics of the region. Only in sediment and water samples obtained via Söke station stated in two parts (Söke and Plains) to observe the agricultural effects. The precise details of the muscle, egg, and eggshell sample numbers and origin samples from three stations Işıklı, Sarayköy, and Söke stations clearly set out in Table 1. Statistical analysis was performed using SPSS version 11.5 (SPSS, Chicago, IL). The Kolmogorov-Smirnov test was used to test normality and Levene, *F* test for homogeneity of variances. Differences between groups were tested using the Student's *t* test for independent samples, while for variables with skewed distributions, the Mann-Whitney *U* test was performed to compare metal concentrations between two sets. For multiple datasets, one-way analyses of variance (ANOVA) were then performed for each tissue to compare metal concentrations among sampling sites followed by the Tukey post hoc test for pairwise comparisons. The metal concentrations are presented as a mean ± standard error (SE) in micrograms per liter for water, micrograms per gram on a dry weight (d.w.) for eggshells, sediments or wet weight (w.w.) basis for muscle tissues, egg contents. $p \leq 0.05$ was considered to be statistically significant.

Results

Metal concentrations in water and sediment samples

Results showed that metal concentrations in water vary in a wide range among the five different stations (Table 2). Ba, Al, Zn, As, Ni, and Fe exhibited relatively higher water concentrations than the other metals. Interestingly, Cu concentration was significantly higher in Işıklı Lake water than in other stations, including Taşburun (sea station), while Al levels were comparable between Işıklı and Taşburun. Another remarkable result was for Se: its water concentrations in Söke and Taşburun were dramatically higher than the other sites, ranging from 29- to 940-fold and 73- to 15,640-fold, respectively. In water samples, Ag, Co, Ni, and V concentrations were found to be higher in Sarayköy, which is the most industrialized area compared to the other sampling sites. Mercury was found only in water samples from the Söke and Taşburun sites. Sb, Ba, Co, V, Ni, and As levels have a tendency to increase from Işıklı to Sarayköy and Plains and return to the starting levels in Taşburun (Table 2). As expected, the concentrations of all metals in the sediment samples were 3–100,000 times higher than water concentrations (Table 2). The pattern was different for the sediment: Fe, Al, Mn, Ba, Ni, Pb, Cr, and As concentrations were significantly higher than the other metals in all stations. Among the stations, Ba was the lowest in Işıklı with a factor of between 9- and 64-fold. For some metals—Ag, As, Cd, Co, Cr, Ni, Mn, Pb, Sb, Ti, and V—similar low concentrations in Işıklı compared to other sites, except Taşburun, were observed. Mercury was measurable in sediment samples from all sampling sites, while it was below the limit of detection in water samples from the first three sites (Table 2). Al, As, Ba, Cr, Fe, Mn, Pb, Sb, and V levels were found to be highest in the Plains station. Only Cd concentration was found to be highest in Sarayköy. Mn, Cr, Co V, Ni, and As levels increased

Table 2 Concentrations (μg/L for water and μg/g dry weight for sediment) of metals in water and sediment from five sampling sites

Metals	Işıklı		Sarayköy		Plains		Söke		Taşburun	
	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment
Ag	0.004	0.4	0.8	0.8	0.004	1.0	0.1	1.0	0.2	0.2
Al	17.3	44,340	6.5	10,530	6.5	54,760	6.7	12,220	15.2	13,610
As	6.7	15.2	6.9	22.8	10.6	32.1	8.6	24.8	1.4	29.5
B	3.1	22.6	2.7	11.2	2.7	4.5	4.4	7.0	5.1	30.2
Ba	13.0	12.5	21.9	154.6	34.5	807.8	33.4	782.8	8.0	113
Cd	0.4	1.0	0.5	3.9	0.2	2.6	0.7	3.7	0.3	0.6
Co	0.05	3.8	0.2	9.8	0.1	17.4	0.01	8.40	0.03	23.8
Cr	0.1	7.1	0.5	84.5	0.4	138.9	0.9	42.4	1.9	96.7
Cu	29.1	21.2	1.6	10.1	1.2	22.5	2.6	10.4	1.1	9.0
Fe	4.3	14,460	6.9	59,600	4.7	109,900	4.4	61,590	5.9	6622
Hg	LOD	1.26	LOD	0.8	LOD	1.46	0.2	1.0	0.01	0.004
Ni	4.4	26.8	13.2	91.2	8.0	127.5	8.2	58.3	5.5	187.4
Mn	0.7	54.6	0.6	156	1.1	416.5	0.7	126.8	0.3	345.3
Pb	1.5	14.1	3.6	48.8	2.1	104.6	4.7	110	3.1	14.7
Sb	0.4	1.1	1.5	4.6	1.4	11.4	1.2	5.2	0.4	0.4
Se	0.05	5.7	1.6	1.8	0.3	0.9	47.6	3.2	118.4	0.6
Tl	0.02	0.5	0.01	1.1	0.1	2.3	0.001	2.8	0.03	0.9
V	1.0	1.9	3.6	13.8	3.2	66.8	2.6	10.5	1.8	34.2
Zn	11.4	38.1	30.4	32.1	21.6	26.6	36.3	27.3	33.7	19.5

LOD limit of detection

downward toward the Plains, decreased in Söke, and increased again toward Taşburun.

Metal concentrations in muscle tissue of carp

Zinc has been found to be the highest metal in carp muscle tissue (Table 3). The others were Se, Cu, B, As, and Ni, in descending order. When comparing the sampling sites, Cu, Mn, Co, Ni, As, Se, and B concentrations were found to be higher in Sarayköy; Zn, Cr, V, and Ba concentrations were higher in Işıklı; and Fe, Cd, Sb, Hg, and Pb concentrations were higher in the Söke station. The levels of Zn, Cr, Ba, and Sb exhibited a similar pattern: all were decreased in Söke compared to Işıklı; however, concentrations in Söke were statistically significantly lower only for Cr and Ba compared to Işıklı. On the other hand, Hg levels were significantly higher in Işıklı than in Söke (Table 3).

Metal concentrations in muscle tissue of waterbirds

In contrast to carp muscle, the highest metal in muscle tissues of Eurasian coot and yellow-legged gull was Fe (Table 4). The metals in descending order were Zn, Cu, Al, and Ni. Observing the high concentrations independently from the species and sampling sites suggested that both species accumulate these metals through similar uptake and/accumulation parameters. In addition, environmental concentrations of these metals were comparable in the Işıklı and Söke stations. Waterbirds could not be sampled from Sarayköy; therefore, a comparison between muscle concentration and water and sediment concentrations was not possible in this sampling site. When checking the corresponding water and sediment concentrations of these metals, levels generally correlate to muscle concentrations except for Ba, although the descending order was slightly different in muscle concentrations compared with water and sediment concentrations. In the comparison of muscle concentrations of each species, levels were generally higher in Söke. Cd, Sb, Cr, and Ba levels were remarkably higher in Eurasian coot muscle from Söke, although differences were not statistically significant, due to relatively high standard errors (Table 4).

Metal concentrations in eggs and eggshells of waterbirds

Due to the absence of nonhatched eggs or eggs that had fallen out of the nest from all species, only the eggs from the species in the section "Sample collection and preparation" could be sampled. The eggs from the same species in both Işıklı and Söke were obtained only from Mediterranean gull (Table 5). Therefore, this species was the only one that allowed multiple

Table 3 Concentrations (mean ± SE, μg/g wet weight) of metals in muscle tissues of common carps

Metals	Işıklı (*n* 13)	Sarayköy (*n* 11)	Söke (*n* 10)
Ag	0.003 ± 0.002	0.12 ± 0.3	0.002 ± 0.006
Al	3.6 ± 3.2	4.9 ± 4.0	2.0 ± 4.0
As	4.6 ± 1.3	6.3 ± 4.2	3.3 ± 1.0
B	7.9 ± 2.2	10.4 ± 5.5	0.1 ± 0.1^{a,b}
Ba	0.1 ± 0.0	0.03 ± 0.0	0.02 ± 0.0^{a}
Cd	0.001 ± 0.0	0.0002 ± 0.0	0.003 ± 0.0
Co	0.4 ± 0.2	0.8 ± 0.7	0.1 ± 0.0
Cr	3.5 ± 0.8	2.2 ± 0.7	0.9 ± 0.3^{a}
Cu	15.3 ± 1.5	34.3 ± 9.9	15.8 ± 6.4
Fe	3.4 ± 0.3	3.6 ± 0.5	3.6 ± 1.5
Hg	0.01 ± 0.0	0.13 ± 0.05^{a,c}	0.2 ± 0.0^{a,b}
Ni	9.0 ± 3.2	14.2 ± 5.3	4.7 ± 1.8
Mn	14.0 ± 1.6	14.1 ± 3.1	7.8 ± 2.1
Pb	0.01 ± 0.0	0.01 ± 0.0	0.02 ± 0.0
Sb	nd	0.00	0.0003 ± 0.0
Se	11.0 ± 1.8	37.7 ± 22.3	8.1 ± 2.2
TI	0.001 ± 0.001	0.001 ± 0.002	0.0
V	5.9 ± 4.3	0.4 ± 0.1	0.6 ± 0.1
Zn	358.8 ± 36.9	302.3 ± 50.3	258.5 ± 64.8

nd not detected

[a] Significantly different than Işıklı ($p < 0.05$)

[b] Significantly different than Sarayköy ($p < 0.05$)

[c] Significantly different than Söke ($p < 0.05$)

comparisons between two stations. The most obvious result is Fe, which was the dominant element in both eggs and eggshells; this was followed by Zn > Ba > Mn > Cu > Hg > Ni in eggs, in descending order. This order was slightly different in eggshell: Fe > Ba > Zn > Mn > Cu > Ni > Hg. Interestingly, metal contents in the eggs and eggshells correlated well. When comparing the egg and eggshell metal contents in Mediterranean gull from two different sites, Söke levels were found to be higher than those of Işıklı, except for Cu, Fe, Ba, and B. However, differences were not significant due to high variations.

Discussion

The objectives of the present study were to monitor the levels of metals in biotic and abiotic samples of the BMR in order to evaluate the risk to aquatic species, and to evaluate whether the levels in edible tissues pose a risk to humans by consuming them as food. We aimed at evaluating the metal pollution in river water and sediment, as well as in tissues of aquatic species in a comparative manner to the literature. The present study presents the first inventory results of the metal burden of BMR both in abiotic and biotic media. However, there is no similar study in literature that reports metal levels in biotic or abiotic samples obtained directly from the BMR. Therefore, we compared our results to the studies performed in the proximity, as well as in different regions of Turkey and

Table 4 Concentrations (mean ± SE, μg/g wet weight) of metals in muscle tissues of waterbirds

Metals	Eurasian coot		Yellow-legged gull	
	Işıklı (*n* 3)	Söke (*n* 3)	Işıklı (*n* 3)	Söke (*n* 3)
Ag	2.1 ± 1.6	1.5 ± 0.7	0.3 ± 0.3	0.1 ± 0.1
Al	499.0 ± 141.2	856.8 ± 119.2	534.2 ± 90.0	745.0 ± 66.5
As	6.2 ± 5.2	18.7 ± 1.8	1.0 ± 0.6	48.0 ± 42.7
B	18.8 ± 8.4	20.6 ± 15.1	15.7 ± 9.4	6.6 ± 5.6
Ba	1.2 ± 0.9	14.9 ± 6.5	3.8 ± 1.2	3.8 ± 1.2
Cd	n.d.	0.04 ± 0.0	n.d.	n.d.
Co	0.4 ± 0.4	3.3 ± 1.9	0.4 ± 0.2	2.1 ± 0.7
Cr	4.3 ± 4.2	42.54 ± 29.9	23.5 ± 11.5	103.9 ± 78.2
Cu	1202.2 ± 238.3	988.9 ± 449.4	413.7 ± 56.0	407.5 ± 127.4
Fe	6396.3 ± 1817.6	4695.0 ± 1940.8	4779.7 ± 297.3	3945.3 ± 6.0
Hg	2.1 ± 1.5	13.1 ± 6.8	20.2 ± 8.1	9.6 ± 2.7
Ni	35.6 ± 12.7	58.1 ± 19.9	84.4 ± 70.3	98.6 ± 46.7
Mn	13.6 ± 11.7	46.9 ± 24.7	34.9 ± 9.6	49.3 ± 16.3
Pb	56.4 ± 53.1	6.4 ± 4.8	2.3 ± 1.1	87.5 ± 57.9
Sb	2.0 ± 1.8	43.5 ± 43.4	0.3 ± 0.1	1.8 ± 1.2
Se	62.6 ± 53.9	15.2 ± 8.3	168.8 ± 117.5	29.9 ± 20.7
V	0.3 ± 0.3	0.9 ± 0.6	0.5 ± 0.3	1.2 ± 0.7
Zn	708.3 ± 310.5	859.5 ± 199.2	1268.0 ± 407.9	1288.7 ± 404.9

Table 5 Concentrations of metals in eggshells (mean ± SE, μg/g dry weight) and eggs (mean ± SE, μg/g wet weight)

	Grey Heron-Işıklı		Eurasian coot-Işıklı		Pelican-Söke		Mediterranean gull-Işıklı		Mediterranean gull-Söke	
	Eggshell (*n* 8)	Egg (*n* 3)	Eggshell (*n* 3)	Egg (*n* 3)	Eggshell (*n* 4)	Egg (*n* 3)	Eggshell (*n* 3)	Egg (*n* 3)	Eggshell (*n* 5)	Egg (*n* 5)
As	0.6 ± 0.2	3.9 ± 1.5	2.6 ± 1.3	2.6 ± 1.3	3.3 ± 2.2	2.6 ± 1.3	0.2 ± 0.2	2.6 ± 1.3	1.45 ± 0.6	1.0 ± 0.2
B	n.d.	n.d.	n.d.	n.d.	0.8 ± 0.8	n.d.	14.3 ± 12.03	4.8 ± 4.8	n.d.	n.d.
Ba	194.9 ± 62.7	10.0 ± 0.2	4927 ± 2472	168.1 ± 46.5	389.5 ± 181.6	290.3 ± 283.4	805.7 ± 399.5	366.7 ± 346.8	726.50 ± 346	37.4 ± 5.4
Cd	0.005 ± 0.0	0.01 ± 0.01	n.d.	0.1 ± 0.1	0.01 ± 0.01	n.d.	n.d.	0.003 ± 0.0	1.43 ± 1.4	2.2 ± 1.6
Co	0.9 ± 0.3	1.1 ± 0.1	2.0 ± 1.3	0.2 ± 0.1	1.8 ± 1.8	0.6 ± 0.1	0.9 ± 0.5	1.0 ± 0.9	2.87 ± 1.3	1.3 ± 0.1
Cr	3.3 ± 1.2	3.9 ± 1.9	5.3 ± 2.7	4.3 ± 2.1	11.2 ± 9.0	5.1 ± 3.1	2.1 ± 0.7	3.2 ± 1.8	1.63 ± 0.5	5.4 ± 1.8
Cu	21.9 ± 6.2	65.3 ± 1.3	16.8 ± 8.8	71.6 ± 7.6	21.6 ± 7.2	57.3 ± 10.9	54.4 ± 23.2	84.3 ± 30.4	36.54 ± 12.0	52.7 ± 0.8
Fe	44,391 ± 31,234	9122 ± 7785	28,047 ± 27,432	1453 ± 758	3193 ± 2750	9968 ± 8092	3554 ± 2054	2165 ± 209	202.8 ± 58.3	30,627 ± 28,441
Hg	32.1 ± 10.5	43.9 ± 6.2	15.9 ± 10.9	6.7 ± 0.8	24.8 ± 7.9	46.5 ± 9.5	12.1 ± 8.4	21.4 ± 5.3	25.69 ± 17.7	44.0 ± 12.8
Ni	8.9 ± 2.6	16.1 ± 10.0	27.4 ± 18.9	4.6 ± 1.2	25.9 ± 1.2	18.9 ± 17.9	10.7 ± 4.7	12.5 ± 9.0	30.73 ± 14.9	6.8 ± 1.0
Mn	18.6 ± 7.4	27.2 ± 0.7	274.8 ± 179	46.4 ± 13.3	232.1 ± 179.9	73.5 ± 62.8	40.6 ± 8.2	35.5 ± 19.6	73.47 ± 62.8	109.0 ± 57.0
Pb	3.6 ± 1.4	3.4 ± 0.8	5.1 ± 1.4	6.7 ± 3.4	5.3 ± 2.7	3.5 ± 1.1	3.6 ± 0.8	3.4 ± 0.6	3.70 ± 0.8	7.5 ± 2.2
Sb	0.03 ± 0.02	0.02 ± 0.0	n.d.	0.04 ± 0.04	0.01 ± 0.0	0.34 ± 0.3	n.d.	0.03 ± 0.03	0.092 ± 0.07	0.2 ± 0.1
Se	12.6 ± 5.9	31.5 ± 1.9	6.4 ± 3.5	18.6 ± 6.2	5.2 ± 3.1	23.2 ± 5.6	12.6 ± 3.9	21.8 ± 11.9	29.73 ± 11.5	39.9 ± 3.9
V	0.1 ± 0.2	0.9 ± 0.4	1.0 ± 0.6	0.8 ± 0.3	5.5 ± 4.1	1.9 ± 1.8	0.6 ± 0.1	1.0 ± 0.5	4.91 ± 2.4	1.2 ± 0.3
Zn	131.2 ± 65.4	616.7 ± 72.1	40.6 ± 21.5	781.4 ± 295.5	322.6 ± 273.7	328.8 ± 132.9	32.4 ± 7.9	427.0 ± 393.5	259.32 ± 146	1172.3 ± 87.9

Europe. Atatanir et al. (2011) conducted a study in water, sediment, and soil samples from Dilek National Park, which is to the north of the BMR estuary. The Cu, Fe, Mn, and Zn levels they measured in seawater were significantly higher than the corresponding levels measured in river water in the present study. That study's samples were collected 2 years earlier than the present study, analyses were performed by atomic absorption spectrophotometer versus ICP-MS in the present study, and most importantly, both water and sediment samples were obtained from the sea, while the corresponding samples were obtained from the river in this study, except for the Taşburun sampling site. In contrast to the significantly higher water concentrations, Atatanir et al. (2011) measured significantly lower concentrations in sediment samples than in the present study. The reason for these low sedimental concentrations remains unclear. Another river in inner Anatolia is the Kizilirmak, which is suspected to be polluted by industrial chemicals as well as metals because of the heavy industries around the basin, and it exhibited comparable metal concentrations to the present study in water and sediment samples (Akbulut and Akbulut 2010). Kalyoncu et al. (2012) measured Zn, Pb, Cd, Ni, Co, Fe, Mn, Cu, and Cr in the muscle tissue of carp sampled from Işıklı Lake. Tissue concentrations are comparable to the concentrations measured in the present study, although they did not indicate the sampling date. When comparing present metal concentrations in carp muscle to the corresponding concentrations in carp sampled from Beyşehir Lake (inner Anatolia) (Özparlak et al. 2012), Sir Dam Lake (South-East Turkey) (Erdoğrul 2007) and Atatürk Dam Lake (East Turkey) (Mol et al. 2010), levels have been found to be significantly higher, similar and higher again in the present study, respectively. In a study from Greece, Goutner et al. (2000) measured a significant amount of Hg in the feathers of gull chicks in three islands in the Aegean Sea that are located in the proximity of the BMR estuary. We have found relatively higher Hg concentrations in water samples from the estuary and from Taşburun (sea sampling site) than in other sites in the river basin. This result, together with Goutner's finding (2000), suggests a relatively higher Hg contamination at this site. On the other hand, Goutner et al. (2000) concluded that their results did not support the prediction that mercury levels would be higher in the north Dodecanese area due to the proximity of the polluted Menderes Delta. Since this region of the Aegean Sea is on the international traffic route, including the Dardanelles and the Bosporus, washouts of tankers may be a possible source of pollution. The same group reported Pb and Cd concentrations in egg samples of Mediterranean gull sampled from Greece (Goutner et al. 2001). The concentrations were 0.02–0.06 μg/g w.w. and 0.003–0.007 μg/g w.w., respectively. The present study measured concentrations for the same metals as 3.4–7.5 μg/g d.w. and 0–2.2 μg/g d.w., respectively. The present concentrations are significantly higher than those obtained in Goutner's study. Ayaş (2007)) detected mean concentrations of Cd, Ni, Cu, and Pb as 0.93, 0.40, 6.75, and 6.83 μg/g d.w., respectively, in eggshells of grey herons. Ni and Cu levels in the present study in corresponding samples were measured as 8.9 and 21.9 μg/g w.w. and were relatively higher, although Ayas et al. reported the concentrations relative to dry weight, while we reported wet weight. In contrast, Cd and Pb levels in the present study (0.005 and 3.6 μg/g w.w.) were lower than those reported. Gasparik et al. (2010) measured muscle concentrations of Pb, Cd, Hg, and As in Eurasian coot sampled from Slovakia as 0.2, 0.07, 0.01, and 0.02 μg/g w.w., respectively. The corresponding concentrations in the present study were found to be significantly higher with a fold range of 10–435, 0.6, 200–1300, and 300–2400, respectively.

Table 6 The calculated exposures to metals by consuming carps caught from Işıklı, Sarayköy, and Söke stations, based on "worst-case scenario"

Metal	Tolerable intakes	Calculated exposures		
	(mg/kg b.w./day)			
		Işıklı	Sarayköy	Söke
Cu	0.5	0.13	0.27	0.13
Zn	1	*3.1*	*2.6*	*2.2*
Fe	0.8	0.04	0.05	0.05
Se	0.003	*0.03*	*0.32*	*0.07*
	(mg/kg b.w./week)			
As	0.015	*0.27*	*0.38*	*0.2*
Cd	0.007	0.00007	0.000007	0.0002
Pb	0.025	0.0007	0.0007	0.001
Hg	0.005	0.0007	*0.008*	*0.015*

The italicized figures exceed the tolerable intake values

In order to meet the second objective of the present study, we attempted to calculate the health risk to humans posed by consuming the carp caught from the BMR. There are

Table 7 US-EPA water criteria for chronic exposure to select inorganic priority pollutants

Pollutant	Freshwater (μg/L)	Saltwater (μg/L)
Cu	9	3.1
Zn	120	81
Cd	0.25	8.8
Pb	2.5	8.1

provisional tolerable daily and weekly intake values for some metals in food reported by JECFA, WHO, and EPA (JECFA 1982a, JECFA 1982b, JECFA 1983, WHO 1993, WHO 2003).

In order to calculate the amount of metals in humans who have been exposed by consuming carp caught from the BMR using a "worst-case scenario" approach, we considered the values in the following:

Weight of one portion of fish:	*300 g*
Body weight of an adult:	*60 kg*
Max. amount of daily fish consumed:	*2*
Sum of fish weight consumed daily:	*600 g*
Sum of fish weight consumed weekly:	*4200 g*

Table 6 represents the calculated exposures in a comparable manner to the provisional tolerable daily and weekly intakes, reported by JECFA and EPA.

Even in the exaggerated situation, Cu, Fe, Cd, and Pb levels, which humans may be exposed to by consuming carp caught from all three sampling sites, seem acceptable. However, the daily risk for Zn and Se and the weekly risk for As and Hg are above the tolerable levels in all sampling sites. It should be borne in mind that fish are not the only source of exposure to these toxic metals. Environmental Protection Agency (EPA) published chronic exposure criteria for some toxic metals in water (Table 7).

According to these criteria, Cu and Cd concentrations in Işıklı are above the limits. The maximum limits of Pb, Cd, and Hg in fish meat were reported as 0.3, 0.05, and 0.50 μg/g w.w., respectively, in the Turkish Food Codex (2011). Hg levels in common carp muscle caught from Sarayköy and Söke exceed these thresholds, in accordance with the estimated total intakes above. On the other hand, these assessments should be taken into consideration with some precaution, as the sample size was relatively small due to the restricted ethical permissions as well as risk of extinction for some species.

Altogether, results showed that fish metal concentrations, as well as water concentrations, in all sampling sites should be monitored periodically. In addition, the public should be informed about current threshold limits regarding fish consumption, according to its metal content, as a preventive measure.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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