



Heavy metal contamination in the muscle of Aegean chub (*Squalius fellowesii*) and potential risk assessment

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Received: 17 April 2017 / Accepted: 12 December 2017 / Published online: 22 December 2017
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Abstract

Especially after the industrial revolution, the amount of contaminants released in aquatic ecosystems has considerably increased. For this reason, the necessity to carry on research on the existence of contaminants, specifically heavy metals, has emerged. In this study, heavy metal concentrations in muscle tissues of Aegean chub, which was an endemic species of south western part of Turkey, gathered from Tersakan River were examined. Heavy metal concentrations of the samples were analyzed with ICP-MS. Estimated daily intakes (EDI), target hazard quotient (THQ), and carcinogenic risk (CR) of elements were calculated. The heavy metals detected in muscle tissues were Zn > Cu > Cr > Mn > Pb > Cd, consecutively. According to the results of the applied health risk assessment (EDI, THQ and CR) for heavy metal exposure from fish consumption in children and adults, it was determined that there was no any significant threat to human health.

Keywords Heavy metal concentrations · Aegean chub · Aquatic ecosystems

Introduction

In parallel to industrial developments, contaminants have spread into environment with ever increasing amounts. Domestic and industrial waste waters including toxic chemicals in great amounts and agricultural wastes are discharged into rivers without being treated or by being partially treated (Förstner and Prosi 2013). For this reason, it is very important to conduct research on the presence of contaminants, especially heavy metals in aquatic ecosystems. There have been a lot of studies about heavy metal contamination in rivers in recent years (Chabukdhara and Nema 2012, Varol and Şen 2012, Protano et al. 2014).

Heavy metals in water are disposed to be bioaccumulated in various tissues of aquatic organisms. These metals can reach humans via food chain. Even though the amount of heavy metals in water is not much, their concentrations increase with biomagnification in humans in upper trophic

levels of food chain. Thus, this situation threatens consumers' health (Gupta et al. 2009).

Aegean chub, *Squalius fellowesii* (Günther, 1868), is a cyprinid species endemic to the Gediz, Bakır, Madra, Büyük Menderes, Eşen, and Dalaman drainages in the Aegean region of Anatolia, Turkey (Özulug and Freyhof 2011). Initially described as *Leuciscus fellowesii*, the name of the species has been recently revised as *Squalius fellowesii* by Özulug and Freyhof (2011). Based on molecular evidence, Durand et al. (2000) suggested that the group of “short snouted chubs” from Western Anatolia, previously known as a subspecies of *Squalius cephalus*, should be considered as a separate species. According to this hypothesis, Özulug and Freyhof (2011) have recently re-defined six different valid species of the genus *Squalius* in Western and Central Anatolia, including *S. fellowesii*. As these species have been recently defined, very little information is available (e.g., Giannetto et al. 2012; Sülün et al. 2014, Şaşı and Giannetto 2016). In addition, the IUCN Red List of Threatened Species (IUCN 2012) has recently listed *S. fellowesii* as “Least Concern” (Freyhof 2014).

Although it is one of the most common freshwater fish species in its area of distribution and consumed by the local people (Balık et al. 2004; Şaşı 2004; Dirican and Barlas 2007, Top et al. 2016), there are not many studies regarding either its heavy metal contamination or its potential effects on human health after consumption.

Responsible editor: Philippe Garrigues

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It would be wrong to make some interpretations on this fish species without making a risk assessment in terms of human health who consume heavy metal-contaminated fish. Therefore, some risk assessment methods like target hazard quotient (THQ), hazard index (HI), etc. are also applied (Copat et al. 2013, Storelli and Barone 2013, Barone et al. 2015). Risk assessment method based on THQ is used by the US Environmental Protection Agency for determining potential risk which can be emerged from the digestion of food that may be contaminated with toxic including heavy metals (Petroczi and Naughton 2009). According to Environmental Protection Agency (EPA), human health risk assessment is defined as the process to estimate the nature and probability of adverse health effects in humans exposed to chemicals in contaminated environmental media, now or in the future (EPA 2017). Risk assessment for heavy metals is evaluated with parameters such as estimated daily intake (EDI), target hazard quotient (THQ), and carcinogenic risk (CR). These parameters are based on intake amount of contaminant, exposure frequency and duration, average body weight, and oral reference dose (RfD_o).

The aim of the present study is to increase awareness towards Aegean chub in terms of its potential risk to health of consumers and to examine its heavy metal accumulation in terms of various metals with the fish samples gathered from Tersakan River which is one of the major watercourses of Muğla (South Western Turkey).

In this study, heavy metal concentrations in muscle tissues of Aegean chub gathered from Tersakan River are determined and discussed. According to the obtained heavy metal concentrations, risk assessment based on THQ and CR values is evaluated with regard to consumers' health. As it is determined that Aegean chub is genetically different from its relatives and also it is a newly defined species, this study can be regarded as the first in its field.

Material and method

Study area and sampling

Samples of Aegean chub of different size, age, sex, and weight were collected by electro-fishing and seine net in winter and summer seasons from two different stations located along Tersakan River (Fig. 1). These two stations were selected as follows: station 1 was exposed to anthropogenic effects caused by the highway and it was situated under the bridge where the main road and the river were intersected; station 2 was located at the river mouth where Tersakan flowed to the Mediterranean Sea. Also, there is an airport close to the study area which is used intensely for both international and domestic flights especially during summer months. Gathered samples were brought to Mugla Sıtkı Kocman University Faculty

of Fisheries Laboratory under cool conditions and stored – 20 °C until analyses.

Analytical procedures

The muscle tissues of fish samples were separated by dissection. The fish samples were treated by deionized water (resistivity 18.0 MΩ cm) before dissection. Muscle tissues were homogenized thoroughly in a laboratory blender (Waring trade marker) with stainless steel cutters. Digestion of the homogenized samples was carried out using this procedure; around 0.5 g (wet weight) homogenized tissue sample was weighed and placed in polytetrafluorethylene (PTFE) vessel with 5 mL of 65% nitric acid (65% suprapur, Merck). Milestone ETHOS Easy® microwave digestion system was used to digest fish samples prior to metal analysis. The microwave digestion system parameters were given at Table 1. Digestion was finally made up with 2% nitric acid (65% suprapur, Merck), 0.5% hydrochloric acid (30% suprapur, Merck) solution to 30 mL in acid washed standard flasks and then placed in 50 mL polypropylene centrifuge tubes (Matek and Blanuša 1998).

Heavy metal concentrations of the samples were analyzed with ICP-MS (Agilent 7700 with auto-sampler) instrument in Applied Science Research Center (ONCE I) of Celal Bayar University. Operating conditions of the device were shown at Table 2.

NRCC TORT-2 (National Research Council Canada TORT-2 Lobster hepatopancreas reference material for trace metals) was used for testing reliability of ICP-MS procedure. Recoveries were found between 92 and 103% level which was within acceptable limits. Before the measurement, calibration of ICP-MS spectrometer using multielemental mixture of metal standards, 10 mg L⁻¹ mix element standard stock solution (AccuTrace MES-21-1), was carried out. Five multielement calibration solutions were prepared at different concentration levels (concentrations of 0, 5, 10, 50, 100 µg L⁻¹ for studied heavy metals) using Merck® suprapur purity nitric acid (HNO₃). The calibration curve for all the elements revealed a good linearity over the whole range of concentrations, with determination coefficients higher than 0.999. A five-sample blank replicate analysis was used to calculate the limit of quantitation (LOQ = standard deviation (SD) 10) and limit of detection (LOD = standard deviation (SD) 3). The blank consisted of deionized water with 2% HNO₃. For elements Cr, Mn, Cu, Zn, Cd, and Pb, the detection limits (LOD) were 0.038, 0.064, 0.016, 0.102, 0.0005, and 0.017 µg kg⁻¹, respectively (Table 3).

Estimated daily intakes of elements

The health risks associated with the consumption of heavy metal-contaminated seafood were assessed based on the EDI

Fig. 1 Sampling area and stations



of heavy metals, THQ, and carcinogenic risk (CR) (Nkpaa et al. 2016). EDI values for each one of the elements were calculated according to Islam et al. (2014) by the formula reported below;

EDI (mg analysed element kg⁻¹body weight⁻¹day⁻¹)

$$= \frac{FIR \times C}{BW}$$

where *C* is the average element concentration in fish muscle tissue (mg kg⁻¹), FIR the daily fish consumption rate (kg person⁻¹ day⁻¹), and BW the mean body weight (kg). In risk assessments, reference body weight is generally assessed as 70 kg for adults (Brodberg and Klasing 2003). As annual fish consumption rate per capita for 2015 in Turkey had not been announced yet, the mean values for the previous 5 years (2010–2014, TURKSTAT 2014) was used. According to this calculation, annual fish consumption rate per capita was calculated as 6.42 kg year⁻¹.

Cancer slope factor and carcinogenic risk

Cancer slope factor indicating carcinogenic risk and the related weight-of-evidence specification are the toxicity data which is commonly used for determining potential human

Table 1 Microwave digestion procedure

| Step | 1 | 2 | 3 | 4 |
|------------------|-----|-----|-----|-----|
| Temperature (°C) | 160 | 175 | 200 | 150 |
| Ramp (min) | 5 | 1 | 1 | 1 |
| Hold (min) | 5 | 5 | 10 | 10 |
| Pressure (bar) | 40 | 50 | 60 | 30 |

carcinogenic risks (Molina 2011). In general, slope factor is used in risk assessments to estimate an upper-limit lifetime probability of an individual developing cancer due to being exposed to a specific level of a potential carcinogen over a period of time (USEPA 1998b; USEPA 2011a).

Lifetime probability of contracting cancer due to exposure to site-related chemicals is calculated according to Nkpaa et al. (2016) by the formula below:

Carcinogenic risk (CR) or lifetime probability of cancer

$$= EDI \times CSF$$

In this formula, EDI is the estimated daily intake of each heavy metal (mg⁻¹ kg⁻¹ day⁻¹) and *cancer slope factor (CSF)* is the ingestion cancer slope factor (mg⁻¹ kg⁻¹ day⁻¹)⁻¹.

By comparing the daily intake (of the amount in fish consumed over a certain time) with the chronic oral reference

Table 2 Operating conditions of Agilent 7700× ICP-MS

| Instrument | Agilent 7700× ICP-MS |
|-------------------------|--------------------------|
| RF power | 1550 W |
| RF matching | 2.1 V |
| Sample depth | 8 mm |
| Sample uptake rate | 0.1 mL min ⁻¹ |
| Plasma gas flow rate | 15 L min ⁻¹ |
| Auxiliary gas flow rate | 1.0 L min ⁻¹ |
| Carrier gas flow rate | 0.95 L min ⁻¹ |
| He gas flow rate | 4.3 mL min ⁻¹ |
| Spray chamber temp. | 2 °C |
| Spray chamber | Soft double pass-type |
| Torch | Quartz glass torch |

Table 3 LOD of heavy metals in ICP-MS ($\mu\text{g kg}^{-1}$)

| | Cr | Mn | Cu | Zn | Cd | Pb |
|-----|-------|-------|-------|-------|--------|-------|
| LOD | 0.038 | 0.064 | 0.016 | 0.102 | 0.0005 | 0.017 |

dose (RfD_o), it is possible to determine whether exposure level of a person exceeds acceptable health guidance levels (Patrick et al. 2008). The calculations were made according to USEPA risk analysis, FAO/WHO, and USDOE standards as indicated in Table 4.

Determination of target hazard quotient

THQ is a ratio of determined dose of a pollutant to the dose level (RfD_o). It is used at health risk assessment in order to determine the carcinogenicity of the samples (Islam et al. 2014). The THQ-based risk assessment technique does not give a quantitative approximation of the likelihood of an exposed population reflecting an unfavorable health effect; on the other hand, it shows a sign of the risk level related to pollutant exposure (Yi et al. 2011). In other words, THQ is not a measure of risk but it reflects the level of concern (Harmanescu et al. 2011; Khan et al. 2009).

The rate of THQ was calculated using Chien et al. (2002) formula:

$$THQ = \frac{E_{fr} \times ED_{tot} \times FIR \times C}{RfD_o \times BW_a \times AT_n} \times 10^{-3}$$

In the formula, E_{fr} stands for frequency of exposure (365 days year^{-1}), ED_{tot} for period of exposure (average life expectancy: 70 years approx.), FIR for food intake rate ($\text{kg person}^{-1} \text{year}^{-1}$), C for the heavy metal concentration in fish muscle tissue (mg kg^{-1}), RfD_o for oral reference dose ($\text{mg kg}^{-1} \text{day}^{-1}$), BW_a for average body weight (*), and AT_n for period of average exposure for non-carcinogenic (365 days $\text{year}^{-1} \times$ number of exposure years 70 years). If the obtained THQ value is below “1,” an adverse effect for human health can be excluded considering the elements studied.

*A body weight of 10 kg was applied for 1-year-old children, 15 kg for 3-year-old children, 20 kg for 6-years-old children, and 44 kg for 12-years-old children (Yaman et al. 2014). In this risk assessment, 70 kg of body weight is usually

Table 4 RfD_o ($\text{mg kg}^{-1} \text{body weight}^{-1} \text{day}^{-1}$) and CSF ($\text{mg kg}^{-1} \text{body weight}^{-1} \text{day}^{-1}$) values (USDOE 2011; USEPA 2011a; WHO 1993)

| | Cr | Mn | Cu | Zn | Cd | Pb |
|----------------|--------------------|----------------------|--------------------|--------------------|----------------------|----------------------|
| RfD_o | 3×10^{-3} | 1.4×10^{-1} | 4×10^{-2} | 3×10^{-1} | 1×10^{-3} | 4×10^{-3} |
| CSF | 5×10^{-1} | n.a. | n.a. | n.a. | 3.8×10^{-1} | 8.5×10^{-3} |

applied for adults as aforementioned above (Brodberg and Klasing 2003).

Total THQ values (TTHQ) were calculated via the formula suggested by Chien et al. (2002);

$$TTHQ = THQ_{(toxicant\ 1)} + THQ_{(toxicant\ 2)} + \dots + THQ_{(toxicant\ n)}$$

Statistical analyses

Descriptive statistics (mean, standard deviation, and range), normality test (skewness and kurtosis), analysis of variance (ANOVA), and t test with post-hocs (Dunnnett and Tukey tests) were conducted with IBM SPSS Statistics V.20. A $p < 0.05$ value was assessed as statistically significant. In order to determine statistically significant differences within data set formed by only one group (e.g., the differences between stations in terms of heavy metal accumulations in fish muscle tissues), t test was applied. For the analyses of comparisons between two or more groups simultaneously (e.g., the comparison of mean heavy metal concentrations detected in muscle tissues in terms of summer and winter seasons or genders), ANOVA was applied.

Results and discussion

The total number of analyzed samples was composed of 32 fish specimens collected during winter and summer seasons of 2016 from two different stations on Tersakan River. The biometric characteristics of fish samples are shown in Table 5.

The mean heavy metal concentrations detected in fish muscle tissues are presented at Table 6. Mean heavy metal concentrations detected in muscle tissues from different stations were $Zn > Cu > Cr > Mn > Pb > Cd$, consecutively. In muscle tissues, the element with highest amount was Zn and the lowest was Cd. There were not any statistical differences in terms of ANOVA results. Also, it was found that mean concentrations of the studied metals in muscle tissues did not exceed the recognized and accepted limits.

The mean heavy metal concentrations detected in fish muscle tissues for different seasons are presented at Table 7. There were seasonal differences in terms of all the elements except

Table 5 Biometric characteristic of fish ($n = 32$ [19♂, 13 ♀])

| | Minimum | Maximum | Mean \pm sd^* |
|-------------------|---------|---------|-------------------|
| Weight (g) | 5.09 | 52.23 | 20.29 ± 10.95 |
| Total length (cm) | 7.90 | 16.30 | 11.86 ± 2.23 |

sd^* standard deviation

Table 6 The mean heavy metal concentrations detected in muscle tissues from different stations and these legal limits (mg kg⁻¹ ± sd*)

| Stations | Heavy metal concs. | | | | | |
|--------------|--------------------|-------------|--------------------|--------------------|------------------|------------------|
| | Cr | Mn | Cu | Zn | Cd | Pb |
| 1 | 0.37 ± 0.24 | 0.22 ± 0.16 | 1.13 ± 0.84 | 12.59 ± 2.37 | 0.03 ± 0.02 | 0.18 ± 0.07 |
| 2 | 0.46 ± 0.27 | 0.22 ± 0.12 | 1.44 ± 1.03 | 11.85 ± 1.72 | 0.03 ± 0.01 | 0.14 ± 0.05 |
| Legal limits | 8.00 ^a | ND. | 10.00 ^b | 30.00 ^b | 0.5 ^b | 0.5 ^b |

Non-detected (ND): The limit for manganese in Codex Alimentarius is not defined. But, the upper tolerable intake level of manganese for children and adults is 2 and 11 mg day⁻¹, respectively (Institute of Medicine 2003).

^a Tuzen (2009)

^b Nauen (1983)

* Mean ± sd (standard deviation)

Zinc. The reason for this result could be related to the increase in human population density in summer months with tourist arrivals. This leads to an increase in pollutant materials and also water level decreases with vaporization and with the increase in temperature, fish metabolism increases, too. Temperature is one of the most important environmental factors affecting metabolism of poikilotherms (Kargin 1998). A study on *Abramis brama* (Farkas et al. 2003) showed similar significant seasonal differences. Also, Tekin-Özan and Kir (2008) reported the highest Cu and Zn concentrations in summer for *Cyprinus carpio*.

Considering the effect of gender on heavy metal concentrations, it was found that gender was not statistically significant in terms of all the elements except Cd. Cadmium amount in muscle tissues of female samples were found at higher levels than other elements. In a previous research, the effect of gender on metal accumulation in the species *Lethrinus lentjan* was studied and it was determined that cadmium concentration was more in female samples than males just like in this study (Al-Yousuf et al. 2000). Also, in another study on *Leporinus obtusidens*, a statistically significant difference was found in terms of Cd (Costa and Hartz 2009). There were no any statistically significant effects of weight and total length of fish samples on heavy metal concentrations.

In addition to statistical analyses performed with only one variable, some tests were also conducted for heavy metal accumulation in individuals of different genders during summer

Table 7 The mean heavy metal concentrations detected in muscle tissues for season (mg kg⁻¹ ± sd*)

| | Summer mean * | Winter mean * | Total mean * |
|----|---------------|---------------|--------------|
| | Cr | 0.61 ± 0.23 | 0.22 ± 0.05 |
| Mn | 0.13 ± 0.10 | 0.30 ± 0.12 | 0.22 ± 0.14 |
| Cu | 2.04 ± 0.75 | 0.52 ± 0.17 | 1.28 ± 0.94 |
| Zn | 12.81 ± 1.85 | 11.63 ± 2.17 | 12.22 ± 2.07 |
| Cd | 0.03 ± 0.01 | 0.02 ± 0.01 | 0.03 ± 0.01 |
| Pb | 0.19 ± 0.05 | 0.13 ± 0.05 | 0.16 ± 0.06 |

* Mean ± sd (standard deviation)

and winter (Table 8). According to the results of ANOVA, bioaccumulations of Cr and Cu showed significant differences in both female and male fish during summer and winter. Except Zn, for all the metals, bioaccumulation values detected both in males in winter and in females in summer showed significant differences. These differences may be related to or caused by physiological changes between genders in spawning period or nutrient selectivity of male and female fish which change seasonally.

As *S. fellowesii* is among the newly entitled species, the current study can be regarded as one of the first studies in this field. Table 9 reports the results of previous studies on heavy metal bioaccumulation carried on chub species both from Turkey and other European territories.

In the table, there are also previous studies on heavy metal accumulation of chub from other watercourses in Mugla territory. However, in these studies even those carried on after the species was renamed (2011), the species was still reported as *Leuciscus cephalus* or *Squalius cephalus*. To this regard, it is important to underline that today, the only species of chub reported from Muğla region is *S. fellowesii*, endemic to Aegean region, whereas *S. cephalus* is native only in other European countries but not in Turkey. The use of the name *S. cephalus* could be wrongly considered as a record of this alien species in

Table 8 Comparison of the mean heavy metal concentrations detected in muscle tissues in terms of the seasons (summer/winter) and genders (male/female) (mg kg⁻¹ ± sd)

| | Male | | Female | |
|----|---------------------------|---------------------------|---------------------------|---------------------------|
| | Winter (± sd)* | Summer (± sd)* | Winter (± sd)* | Summer (± sd)* |
| Cr | 0.23 ± 0.05 ^b | 0.66 ± 0.21 ^a | 0.20 ± 0.04 ^b | 0.56 ± 0.25 ^a |
| Mn | 0.33 ± 0.14 ^b | 0.12 ± 0.07 ^a | 0.24 ± 0.06 ^{ab} | 0.15 ± 0.12 ^a |
| Cu | 0.55 ± 0.19 ^b | 2.14 ± 0.62 ^a | 0.47 ± 0.11 ^b | 1.95 ± 0.89 ^a |
| Zn | 11.46 ± 2.05 ^a | 13.05 ± 1.67 ^a | 12.01 ± 2.61 ^a | 12.57 ± 2.11 ^a |
| Cd | 0.02 ± 0.01 ^b | 0.03 ± 0.01 ^{ab} | 0.03 ± 0.01 ^{ab} | 0.03 ± 0.02 ^a |
| Pb | 0.12 ± 0.05 ^b | 0.19 ± 0.06 ^{ab} | 0.13 ± 0.06 ^{ab} | 0.20 ± 0.05 ^a |

Different superscripts refer to statistically significant differences

Table 9 Comparison of studied heavy metal concentrations in *Squalius fellowesii*, *Leuciscus cephalus*, and *Squalius cephalus* with other studies (mg kg⁻¹)

| Species | Site | Cr | Mn | Cu | Zn | Cd | Pb | References |
|----------------------|--------------------------------------|---------------|---------------|---------------|-----------------|----------------|---------------|-------------------------|
| <i>L. cephalus</i> | Çanakale (Turkey) | – | 2.08 ± 0.21 | 3.67 ± 0.47 | 29.86 ± 0.85 | 0.07 ± 0.01 | 0.23 ± 0.03 | Selvi et al. 2015 |
| | Çanakale (Turkey) | – | 4.72 ± 0.20 | 7.57 ± 0.36 | 54.59 ± 0.75 | 1.27 ± 0.34 | 3.22 ± 0.70 | Selvi et al. 2015 |
| | Brno (Czech republic) | – | – | 0.253 ± 0.093 | 4.916 ± 1.447 | 0.010 ± 0.002 | 0.115 ± 0.048 | Harkabusová et al. 2012 |
| | Salek Lake (Slovenia) | – | – | – | 7.93 | < 0.01 | 0.03 | Petkovšek et al. 2012 |
| | Sarıçay, Muğla (Turkey)* | – | 0.112 ± 0.012 | 0.193 ± 0.025 | 6.350 ± 2.345 | 0.023 ± 0.014 | 0.068 ± 0.008 | Yılmaz et al. 2007 |
| | Sarıçay, Muğla (Turkey)* | – | 1.201 ± 0.123 | 0.572 ± 0.078 | 9.663 ± 2.389 | 0.010 ± 0.009 | 0.299 ± 0.045 | Yılmaz et al. 2007 |
| <i>S. cephalus</i> | Dipsiz çay, Muğla (Turkey)* | 2.540 ± 1.180 | – | 0.790 ± 0.873 | 11.060 ± 12.610 | 0.012 ± 0.004 | 0.230 ± 0.250 | Demirak et al. 2006 |
| | Garasi (Serbia) | 0.01 ± 0.01 | 0.3 ± 0.09 | 1.31 ± 0.62 | 16.62 ± 10.42 | – | – | Sunjug et al. 2016 |
| | Uvac (Serbia) | 0.01 ± 0.02 | 0.59 ± 0.07 | 2.22 ± 0.64 | 61.09 ± 18.87 | – | – | Sunjug et al. 2016 |
| | Great Morava (Serbia) | 0.28 ± 0.15 | 0.2 ± 0.06 | 0.20 ± 0.08 | 4.67 ± 0.69 | 0.006 ± 0.0003 | 0.15 ± 0.03 | Milošević et al. 2016 |
| | Great Morava (Serbia) | 0.23 ± 0.05 | 0.11 ± 0.02 | 0.16 ± 0.04 | 4.6 ± 0.8 | 0.006 ± 0.0006 | 0.21 ± 0.05 | Milošević et al. 2016 |
| | Sarıçay, Muğla (Turkey)* | 0.97 ± 0.19 | 5.94 ± 0.57 | 1.72 ± 0.15 | 37.09 ± 6.10 | 0.01 ± 0.01 | 1.48 ± 0.24 | Ögütü et al. 2015 |
| <i>S. fellowesii</i> | Kayseri (Turkey) | 2.78 | 8.38 | 5.28 | 27.83 | 4.40 | 8.88 | Duman and Kar 2012 |
| | Bolu (Turkey) | 0.16 ± 0.05 | 2.50 ± 0.38 | 1.79 ± 0.55 | 57.81 ± 21.5 | LOD* | 0.46 ± 0.17 | Saygi and Yiğit 2012 |
| | Tersakan çayı, Muğla (Turkey) | 0.41 ± 0.26 | 0.22 ± 0.14 | 1.28 ± 0.94 | 12.22 ± 2.07 | 0.03 ± 0.01 | 0.16 ± 0.06 | Present study |
| | | | | | | | | |

* The fish species used in these studies are now called as *S. fellowesii*

Table 10 EDI, TDI, and CR rates calculated with regard to fish consumption

| | EDI (mg kg ⁻¹ day ⁻¹) | TDI (mg kg ⁻¹ day ⁻¹) | CR |
|----|--|--|-------------------------|
| Cr | 0.10 | 60 | 5 × 10 ⁻² |
| Mn | 0.06 | 0.11* | – |
| Cu | 0.32 | 30 | – |
| Zn | 3.07 | 60 | – |
| Cd | 0.007 | 60 | 2.66 × 10 ⁻³ |
| Pb | 0.04 | 0.2 | 3.4 × 10 ⁻⁴ |

* uTDI, for Mn

Muğla territory. For these reasons, we strongly encourage the use of the correct and worldwide accepted name *S. fellowesii* when referring to the chub populations of Muğla territory.

The estimated daily intake amounts of fish consumption were compared to tolerable daily intake (TDI) and tolerable upper daily intake (uTDI) amounts (Table 10). TDI and uTDI values were obtained from various studies (Nauen 1983; Trumbo et al. 2001; United Kingdom Department of Health 1991; WHO 1982a; WHO 1982b; Ysart et al. 2000). The risks related to exposure to the heavy metal were evaluated with the consideration of the EDI for consumers. The obtained EDI values and TDI values were compared with acceptable exposure levels for each element in order to estimate potential limit excesses.

According to New York State Department of Health (NYS DOH 2007), if the ratio of EDI of heavy metal to its RfD_o is equal to or less than the RfD_o, then the risk will be minimum, but if it is > 1–5 times the RfD_o, then risk will be low, if > 5–10 times the RfD_o, then risk will be moderate; however, if > 10 times the RfD_o, then the risk will be high (Javed and Usmani 2016). In the current study, the ratio obtained for Mn was found < z1 (0,42). The ratios of Cu, Cd, Zn, and Pb were found seven-, eight-, ten-, and tenfolds, respectively. The ratio for Cr was found 33 times higher than their RfD_o, indicating potential health hazard to the public.

According to USEPA, CR between 10⁻⁶ (1 in 1,000,000) and 10⁻⁴ (1 in 10,000) indicates a range of acceptable predicted lifetime risks for carcinogens (Valberg et al. 1996; USEPA

Table 11 THQ and TTHQ values determined for children and adults with regard to fish consumption

| Exposure Group | Cr | Mn | Cu | Zn | Cd | Pb | TTHQ |
|----------------|------|--------|-------|------|-------|------|-------|
| Children | | | | | | | |
| Age 1 | 0.24 | 0.003 | 0.06 | 0.07 | 0.05 | 0.07 | 0.493 |
| Age 3 | 0.16 | 0.002 | 0.04 | 0.05 | 0.04 | 0.05 | 0.342 |
| Age 6 | 0.12 | 0,001 | 0.03 | 0.04 | 0.03 | 0.04 | 0.261 |
| Age 12 | 0.05 | 0.0006 | 0.01 | 0.02 | 0.01 | 0.02 | 0.111 |
| Adult | 0.03 | 0.0003 | 0.008 | 0.01 | 0.008 | 0.01 | 0.066 |

2011b). In this study, all CR values for the studied heavy metals were found under permissible limits.

Health risk assessment studies of heavy metal potential depending on fish consumption has gained importance and showed an increase in number in recent years. In some of these studies, calculated THQ values of metals were found as < 1 in compliance with current study (Table 11). The results of THQ calculations indicated that consumers of *Squalius fellowesii* from the study area did not face with any significant health risk due to the intake of individual metals through the consumption of this species. THQ values of heavy metals were found as < 1 in human health risk assessments implemented for mussel consumption on tropical fish in Buriganga River (Bangladesh) (Ahmed et al. 2015). In heavy metal risk assessment for fish and vegetable consumption in Tianjin (China), THQ values were found under 1 in children and adults (Wang et al. 2005). After the health risk assessment made for the consumption of tilapia fish (*Oreochromis niloticus*) gathered from Langat River and Engineering Lake (Bangi, Malaysia), it was claimed that there was no any threat to overall population (Taweel et al. 2013).

On the other hand, in some human health risk assessment studies, the results showed that there were some risks for human health with regard to heavy metals due to fish consumption. THQ refers to one heavy metal only; however, edible samples have more than one heavy metal in general as in fish muscle. In accordance with this fact, in this study, six heavy metals were detected in the sampled fish muscles. Therefore, TTHQ values which were the numerical sum of all the THQs had to be calculated. Like THQ, these values also should be < 1 (Islam et al. 2014), if it exceeds 1, it could be an indicator of public health concern (Javed and Usmani 2016). TTHQ value related to fish consumption in Yangtze River (China) was determined as 1.13 for overall population (Yi et al. 2011). As a result of a risk assessment made with regard to the consumption of fish gathered from the Nile River, THQ values were found as > 1 in terms of some metals (Al, Fe, Pb, Zn, St) (Dahshan et al. 2013). In health risk assessment on the consumption of *Cyprinus carpio* gathered from the Rawal Lake (Pakistan), THQ values in terms of Cr, Cd, and Pb were determined as > 1 (Iqbal and Shah 2014). Li and Zn concentrations in muscle tissues of the species *Oreochromis leucostictus* caught from Naivasha Lake (Kenya) were found high and THQ values of these elements were > 1 (Otachi et al. 2014). THQ values calculated for children with regard to consumption of fish obtained from Gomti River (India) were found as > 1 in terms of Ni and Pb, but there were not any risks for adults (Gupta et al. 2015).

Conclusion

The purpose of this study was to reveal the effects of bioaccumulation and consumption of Aegean chub (*S. fellowesii*)

on human health. The species was endemic to the region and also extensively sold in local markets and consumed by the local community. The heavy metals detected in muscle tissues were $Zn > Cu > Cr > Mn > Pb > Cd$ consecutively. The results of the applied health risk assessment (EDI, THQ) for heavy metal exposure from fish consumption in children and adults did not indicate any threat to human health. The ratio for EDI of Cr to its RfD_0 was found much higher than acceptable limits, which showed that there could be a potential health hazard to the public and all CR values for the studied heavy metals were found under permissible limits.

In future studies, it is suggested that researchers may study the evaluation of heavy metal accumulation in different tissues and limbs of this species and its potential as a bio-indicator. As a species recently identified and still not well known, future studies may focus on other aspects of the biology of this species.

Acknowledgements The authors appreciate Assist. Prof. Daniela Giannetto (Mugla Sitki Kocman University, Mugla, Turkey) for her helpful comments. They would also like to thank Res. Assist. Nisan Yozukmaz (Mugla Sitki Kocman University) for her contribution to the editing of the manuscript.

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