Environmental Science and Pollution Research (2018) 25:6928–6936
https://doi.org/10.1007/s11356-017-1030-y

RESEARCH ARTICLE

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

Hüseyin Şaşi[1] · Aykut Yozukmaz[1] · Murat Yabanli[1]

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

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

✉ Aykut Yozukmaz
aykutyoz@gmail.com

[1] Department of Aquatic Sciences, Faculty of Fisheries, Mugla Sitki Kocman University, 48000 Mugla, Turkey

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 (HNO_3). 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% HNO_3. 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

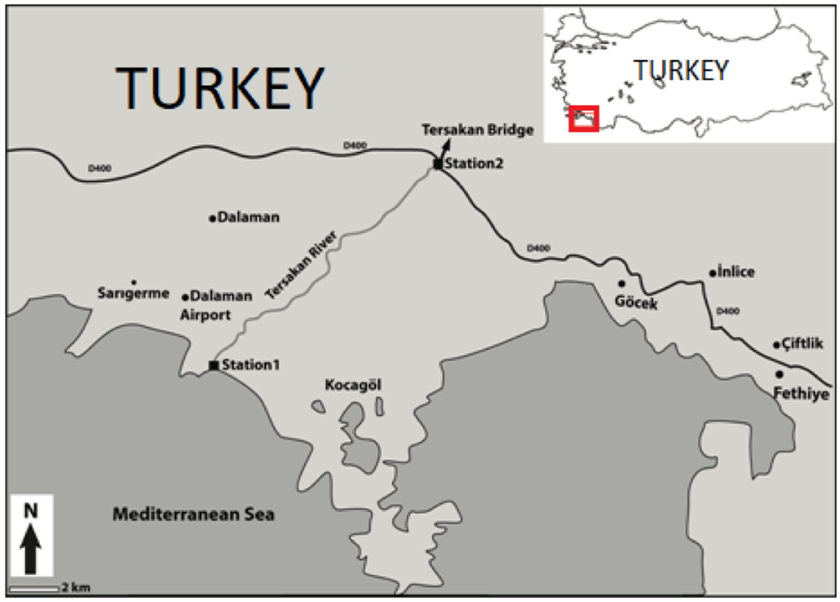

Fig. 1 Sampling area and stations

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;

$$\text{EDI}\left(\text{mg } \textit{analysed element}\ \text{kg}^{-1} \textit{body weight}^{-1} \text{day}^{-1}\right) = \frac{\text{FIR} \times C}{\text{BW}}$$

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 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:

$$\text{Carcinogenic risk (CR) or lifetime probability of cancer} = \text{EDI} \times \text{CSF}$$

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 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

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 (μg 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 (kg $person^{-1}$ $year^{-1}$), C for the heavy metal concentration in fish muscle tissue (mg kg^{-1}), RfD_o for oral reference dose (mg kg^{-1} day^{-1}), BW_a for average body weight (*), and AT_n for period of average exposure for non-carcinogenic (365 days $year^{-1}$ × 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 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);

$$TTHQ = THQ\left(_{toxicant\ 1}\right) + THQ\left(_{toxicant\ 2}\right) + \ldots + THQ\left(_{toxicant\ n}\right)$$

Statistical analyses

Descriptive statistics (mean, standard deviation, and range), normality test (skewness and kurtosis), analysis of variance (ANOVA), and *t* test with post-hocs (Dunnett 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 4 RfD_o (mg kg^{-1} body $weight^{-1}$ day^{-1}) and *CSF* (mg kg^{-1} body $weight^{-1}$ 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}

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

	Minimum	Maximum	Mean ± 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 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. fellowesi*, 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 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	0.41 ± 0.26
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)

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
L. cephalus	Çanakkale (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
	Çanakkale (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, Mugla (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, Mugla (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
	*Dipsiz cay, Mugla (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
S. cephalus	Garasi (Serbia)	0.01 ± 0.01	0.3 ± 0.09	1.31 ± 0.62	16.62 ± 10.42	–	–	Sunjog et al. 2016
	Uvac (Serbia)	0.01 ± 0.02	0.59 ± 0.07	2.22 ± 0.64	61.09 ± 18.87	–	–	Sunjog 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	Milošković 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	Milošković et al. 2016
	*Sarıçay, Mugla (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	Öğlü et al. 2015
	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	Saygı and Yiğit 2012
S. fellowesii	**Tersakan çayı, Mugla (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_o 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.

References

Ahmed MK, Baki MA, Islam MS, Kundu GK, Habibullah-Al-Mamun M, Sarkar SK, Hossain MM (2015) Human health risk assessment of heavy metals in tropical fish and shellfish collected from the river Buriganga, Bangladesh. Environ Sci Pollut Res 22(20):15880–15890. https://doi.org/10.1007/s11356-015-4813-z

Al-Yousuf MH, El-Shahawi MS, Al-Ghais SM (2000) Trace metals in liver, skin and muscle of Lethrinus lentjan fish species in relation to body length and sex. Sci Total Environ 256(2-3):87–94. https://doi.org/10.1016/S0048-9697(99)00363-0

Balık S, Sarı HM, Ustaoğlu MR, İlhan A (2004) Age and growth characteristics of chub (Leuciscus cephalus L., 1758) population in Işıklı Lake, Çivril, Denizli, Turkey. Ege J FAS 21:257–262

Barone G, Storelli A, Garofalo R, Busco VP, Quaglia NC, Centrone G, Storelli MM (2015) Assessment of mercury and cadmium via seafood consumption in Italy: estimated dietary intake (EWI) and target hazard quotient (THQ). Food Addit Contam A 32(8):1277–1286. https://doi.org/10.1080/19440049.2015.1055594

Brodberg R, Klasing S (2003) Evaluation of potential health effects of eating fish from black butte reservoir (Glenn and Tehama Counties): guidelines for sport fish consumption. Pesticide and Environmental Toxicology Section Office of Environmental Protection Agency, California

Chabukdhara M, Nema AK (2012) Assessment of heavy metal contamination in Hindon River sediments: a chemometric and geochemical approach. Chemosphere 87(8):945–953. https://doi.org/10.1016/j.chemosphere.2012.01.055

Chien LC, Hung TC, Choang KY, Yeh CY, Meng PJ, Shieh MJ, Han BC (2002) Daily intake of TBT, Cu, Zn, Cd and As for fishermen in Taiwan. Sci Total Environ 285(1-3):177–185. https://doi.org/10.1016/S0048-9697(01)00916-0

Copat C, Arena G, Fiore M, Ledda C, Fallico R, Sciacca S, Ferrante M (2013) Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: consumption advisories. Food Chem Toxicol 53:33–37. https://doi.org/10.1016/j.fct.2012.11.038

Costa SDC, Hartz SM (2009) Evaluation of trace metals (cadmium, chromium, copper and zinc) in tissues of a commercially important fish (Leporinus obtusidens) from Guaíba Lake, Southern Brazil. Braz Arch Biol Technol 52(1):241–250. https://doi.org/10.1590/S1516-89132009000100029

Dahshan H, Abd-Elall AMM, Megahed AM (2013) Trace metal levels in water, fish, and sediment from River Nile, Egypt: potential health risks assessment. J Toxicol Environ Health A 76(21):1183–1187. https://doi.org/10.1080/15287394.2013.848421

Demirak A, Yilmaz F, Tuna AL, Ozdemir N (2006) Heavy metals in water, sediment and tissues of Leuciscus cephalus from a stream in southwestern Turkey. Chemosphere 63(9):1451–1458. https://doi.org/10.1016/j.chemosphere.2005.09.033

Dirican S, Barlas M (2007) Dipsiz-Çine (Muğla-Aydin) Çayi'nda yaşayan *Leuciscus cephalus* (l., 1758) ve *Barbus capito pectoralis* Heckel, 1843 populasyonlarinin kondüsyon faktörleri üzerine bir çalişma. AKU-J Sci Eng 7:105–114

Duman F, Kar M (2012) Temporal variation of metals in water, sediment and tissues of the European Chup (*Squalius cephalus* L.) Bull Environ Contam Toxicol 89(2):428–433. https://doi.org/10.1007/s00128-012-0679-7

Durand JD, Erhan Ü, Doadrio I, Pipoyan S, Templeton AR (2000) Origin, radiation, dispersion and allopatric hybridization in the chub *Leuciscus cephalus*. Proc R Soc Lond B Biol Sci 267(1453):1687–1697. https://doi.org/10.1098/rspb.2000.1196

EPA (2017) Human Health Risk Assessment. Available at: https://www.epa.gov/risk/human-health-risk-assessment

Farkas A, Salánki J, Specziár A (2003) Age-and size-specific patterns of heavy metals in the organs of freshwater fish *Abramis brama* L. populating a low-contaminated site. Water Res 37(5):959–964. https://doi.org/10.1016/S0043-1354(02)00447-5

Förstner U, Prosi F (2013) Heavy metals pollution in freshwater ecosystems. In: Ravera O (ed) Biological aspects of freshwater pollution. Pergamon Press, Oxford, pp 129–150

Freyhof J (2014) *Squalius fellowesii*. The IUCN Red List of Threatened Species 2014: e.T19451307A19849380. https://doi.org/10.2305/IUCN.UK.2014.1.RLTS.T19451307A19849380.en. Downloaded 15 Feb 2017

Giannetto D, Pompei L, Lorenzoni M, Tarkan AS (2012) Empirical standard weight equation for the Aegean chub Squalius fellowesii, an endemic freshwater fish species of Western Anatolia, Turkey. North Am J Fish Manage 36:1102–1107

Gupta A, Rai DK, Pandey RS, Sharma B (2009) Analysis of some heavy metals in the riverine water, sediments and fish from river Ganges at Allahabad. Environ Monit Assess 157(1-4):449–458. https://doi.org/10.1007/s10661-008-0547-4

Gupta SK, Chabukdhara M, Singh J, Bux F (2015) Evaluation and potential health hazard of selected metals in water, sediments, and fish from the Gomti River. Hum Ecol Risk Assess 21(1):227–240. https://doi.org/10.1080/10807039.2014.902694

Harkabusová V, Čelechovská O, Lavičková A, Svobodová Z (2012) Monitoring of risk metals in chub (*Leuciscus cephalus* L.) from the Svitava and Svratka rivers in the urban area of Brno, Czech Republic. Acta Vet Brno 81(1):69–73. https://doi.org/10.2754/avb201281010069

Harmanescu M, Alda LM, Bordean DM, Gogoasa I, Gergen I (2011) Heavy metals health risk assessment for population via consumption of vegetables grown in old mining area; a case study: Banat County, Romania. Chem Central J 5(1):64. https://doi.org/10.1186/1752-153X-5-64

Institute of Medicine Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (2003) Dietary reference intakes: applications in dietary planning. Washington, DC, National Academies Press, p 248

Iqbal J, Shah MH (2014) Study of seasonal variations and health risk assessment of heavy metals in *Cyprinus carpio* from Rawal Lake, Pakistan. Environ Monit Assess 186(4):2025–2037. https://doi.org/10.1007/s10661-013-3515-6

Islam S, Ahmed K, Al Mamun H (2014) Heavy metals in cereals and pulses: health implications in Bangladesh. J Agric Food Chem 62(44):10828–10835. https://doi.org/10.1021/jf502486q

IUCN (2012) IUCN Guidelines for Reintroductions and Other Conservation Translocations (Gland, Switzerland). Updated from, IUCN, 2001. IUCN Red List Categories and Criteria: Version 3.1. IUCN Species Survival Commission, IUCN, Gland, Switzerland and Cambridge, UK

Javed M, Usmani N (2016) Accumulation of heavy metals and human health risk assessment via the consumption of freshwater fish Mastacembelus armatus inhabiting, thermal power plant effluent loaded canal. Springer Plus 5(1):776. https://doi.org/10.1186/s40064-016-2471-3

Kargin F (1998) Metal concentrations in tissues of the freshwater fish *Capoeta barroisi* from the Seyhan River (Turkey). Bull Environ Contam Toxicol 60(5):822–828. https://doi.org/10.1007/s001289900701

Khan S, Farooq R, Shahbaz S, Khan MA, Sadique M (2009) Health risk assessment of heavy metals for population via consumption of vegetables. World App Sci J 6:1602–1606

Matek M, Blanuša M (1998) Comparison of two methods for destruction of biological material for determination of selenium. Arh Hig Rada Toksikol 49:301–305

Milošković A, Dojčinović B, Kovačević S, Radojković N, Radenković M, Milošević D, Simić V (2016) Spatial monitoring of heavy metals in the inland waters of Serbia: a multispecies approach based on commercial fish. Environ Sci Pollut Res 23(10):9918–9933. https://doi.org/10.1007/s11356-016-6207-2

Molina VB (2011) Health risk assessment of heavy metals bioaccumulation in Laguna de Bay fish products, Philippines. 14th World Lake Conference, Austin Texas. pp 1–23

Nauen CE (1983) Compilation of legal limits for hazardous substances in fish and fishery products, Circular No. 764. FIRI/ C 764. Food and Agriculture Organization of the United Nations, Rome, p 102

Nkpaa KW, Patrick-Iwuanyanwu KC, Wegwu MO, Essien EB (2016) Health risk assessment of hazardous metals for population via consumption of seafood from Ogoniland, rivers state, Nigeria; a case study of Kaa, B-dere, and Bodo City. Environ Monit Assess 188:1–10

NYS DOH (New York State Department of Health) (2007) Hopewell precision area contamination: Appendix C-NYS DOH, In: Procedure for evaluating potential health risks for contaminants of concern. Available at: http://www.health.ny.gov/environmental/investigations/hopewell/appendc.htm

Öğlü B, Yorulmaz B, Genc TO, Yilmaz F (2015) The assessment of heavy metal content by using bioaccumulation indices in European chub, *Squalius cephalus* (Linnaeus, 1758). Carpath J Earth Env 10:85–94

Otachi EO, Körner W, Avenant-Oldewage A, Fellner-Frank C, Jirsa F (2014) Trace elements in sediments, blue spotted tilapia *Oreochromis leucostictus* (Trewavas, 1933) and its parasite *Contracaecum multipapillatum* from Lake Naivasha, Kenya, including a comprehensive health risk analysis. Environ Sci Pollut Res 21(12):7339–7349. https://doi.org/10.1007/s11356-014-2602-8

Özulug M, Freyhof J (2011) Revision of the genus Squalius in Western and Central Anatolia, with description of four new species (Teleostei: Cyprinidae*)*. Ichthyol Explor Fres 22:107–148

Patrick JW, Sharon LS, Fleming LE, Solo-Gabriele HM, Gerwick WH (2008) Oceans and human health. Risks and remedies from the seas. Elsevier Inc., Burlington

Petkovšek SAS, Grudnik ZM, Pokorny B (2012) Heavy metals and arsenic concentrations in ten fish species from the Šalek lakes (Slovenia): assessment of potential human health risk due to fish consumption. Environ Monit Assess 184(5):2647–2662. https://doi.org/10.1007/s10661-011-2141-4

Petroczi A, Naughton D (2009) Mercury, cadmium and lead contamination in seafood: a comparative study to evaluate the usefulness of Target Hazard Quotients. Food Chem Toxicol 47(2):298–302. https://doi.org/10.1016/j.fct.2008.11.007

Protano C, Zinnà L, Giampaoli S, Spica VR, Chiavarini S, Vitali M (2014) Heavy metal pollution and potential ecological risks in rivers: a case study from southern Italy. Bull Environ Contam Toxicol 92(1):75–80. https://doi.org/10.1007/s00128-013-1150-0

Şaşı H (2004) The reproduction biology of chub (*Leuciscus cephalus* L. 1758) in Topçam Dam Lake (Aydın, Turkey). Turk J Vet Anim Sci 28:693–699

Şaşı H, Giannetto D (2016) First record of adult Nematomorpha Gordius sp. from western Anatolia (Turkey). Turk J Zool 40:320–323. https://doi.org/10.3906/zoo-1506-44

Saygı Y, Yiğit SA (2012) Assessment of metal concentrations in two cyprinid fish species (*Leuciscus cephalus* and *Tinca tinca*) captured from Yeniçağa Lake, Turkey. Bull Environ Contam Toxicol 89(1): 86–90. https://doi.org/10.1007/s00128-012-0647-2

Selvi K, Kaya H, Akbulut M, Tulgar A (2015) Comparison of heavy metal concentrations on European chub (*Leuciscus Cephalus* L., 1758) from Sariçay Creek and Atikhisar reservoir (Çanakkale–Turkey). Fresenius Environ Bull 24:445–450

Storelli MM, Barone G (2013) Toxic metals (Hg, Pb, and Cd) in commercially important demersal fish from Mediterranean Sea: contamination levels and dietary exposure assessment. J Food Sci 78(2): T362–T366. https://doi.org/10.1111/j.1750-3841.2012.02976.x

Sülün Ş, Başkurt S, Emiroğlu Ö, Giannetto D, Tarkan AS, Ağdamar S, Gaygusuz Ö, Dorak Z, Aydin H, Cicek A (2014) Development of empirical standard weight equation for Pursak chub Squalius pursakensis, an endemic cyprinid species of Northwest Anatolia. Turk J Zoolss 38:582–589. https://doi.org/10.3906/zoo–1311-24

Sunjog K, Kolarević S, Kračun-Kolarević M, Višnjić-Jeftić Ž, Skorić S, Gačić Z, Lenhardt M, Vasić N, Vuković-Gačić B (2016) Assessment of status of three water bodies in Serbia based on tissue metal and metalloid concentration (ICP-OES) and genotoxicity (comet assay). Environ Pollut 213:600–607. https://doi.org/10.1016/j.envpol.2016.03.008

Taweel A, Shuhaimi-Othman M, Ahmad AK (2013) Assessment of heavy metals in tilapia fish (*Oreochromis niloticus*) from the Langat River and Engineering Lake in Bangi, Malaysia, and evaluation of the health risk from tilapia consumption. Ecotox Environ Safe 93:45–51. https://doi.org/10.1016/j.ecoenv.2013.03.031

Tekin-Özan S, Kir İ (2008) Seasonal variations of heavy metals in some organs of carp (*Cyprinus carpio* L., 1758) from Beyşehir Lake (Turkey). Environ Monit Assess 138(1-3):201–206. https://doi.org/10.1007/s10661-007-9765-4

Top N, Tarkan AS, Akbaş F, Karakuş U (2016) Growth and life history traits of Aegean chub, *Squalius fellowesii* (Günther, 1868) in streams in Muğla Province, Aegean coast, Turkey. J Appl Ichthyol 32(3): 532–537. https://doi.org/10.1111/jai.13040

Trumbo P, Yates AA, Schlicker S, Poos M (2001) Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. J Am Diet Assoc 101(3):294–301. https://doi.org/10.1016/S0002-8223(01)00078-5

TURKSTAT (2014) Fisheries Statistics (2013). Turkish Statistical Institute, Ankara

Tuzen M (2009) Toxic and essential trace elemental contents in fish species from the Black Sea, Turkey. Food Chem Toxicol 47(8): 1785–1790. https://doi.org/10.1016/j.fct.2009.04.029

United Kingdom Department of Health (1991) Dietary reference values for food, energy and nutrients for the United Kingdom. In: Report on health and social subjects 41. Panel on dietary reference values for food, energy and nutrients for the United Kingdom, London, pp 174–177

USDOE (2011) The risk assessment information system (RAIS), U.S. Department of Energy's Oak Ridge Operations Office (ORO)

USEPA (1998) USEPA Region 6. Human health risk assessment. Protocol Chapter 7: Risk and Hazard Characterization. Multimedia Planning and Permitting Division. Center for Combustion Science and Engineering. Office of Solid Waste, USEPA 7, pp 1–7

USEPA (2011a) Regional screening tables. Available at: http://www.epa.gov/reg3hwmd/risk/human/rb-concentration_table/index.htm

USEPA (2011b) Regional screening levels for chemical contaminants at superfund sites. Regional screening table. User's guide. http://www.epa.gov/reg3hwmd/risk/human/rb-concentration_table/usersguide.htm

Valberg PA, Drivas PJ, McCarthy S (1996) Evaluating the health impacts of incinerator emissions. J Hazard Mater 47(1-3):205–227. https://doi.org/10.1016/0304-3894(96)82225-4

Varol M, Şen B (2012) Assessment of nutrient and heavy metal contamination in surface water and sediments of the upper Tigris River, Turkey. Catena 92:1–10. https://doi.org/10.1016/j.catena.2011.11.011

Wang X, Sato T, Xing B, Tao S (2005) Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. Sci Total Environ 350(1-3):28–37. https://doi.org/10.1016/j.scitotenv.2004.09.044

WHO (1982a) Toxicological evaluation of certain food additives. Joint FAO/WHO expert committee on food additive. WHO Food Additive Series, Number 17. World Health Organization, Geneva

WHO (1982b) Evaluation of certain food additives and contaminants. WHO Technical Report Series, Number 683. World Health Organization, Geneva

WHO (1993) Evaluation of certain food additives and contaminants. WHO Technical Report Series, Number 837. World Health Organization, Geneva

Yaman M, Karaaslan NM, Yaman IH (2014) Seasonal variations in toxic metal levels of two fish species, Mugil cephalus and Mullus barbatus and estimation of risk for children. Bull Environ Contam Toxicol 93(3):344–349. https://doi.org/10.1007/s00128-014-1342-2

Yi Y, Yang Z, Zhang S (2011) Ecological risk assessment of heavy metals in sediment and human health risk assessment of heavy metals in fishes in the middle and lower reaches of the Yangtze River basin. Environ Pollut 159(10):2575–2585. https://doi.org/10.1016/j.envpol.2011.06.011

Yılmaz F, Özdemir N, Demirak A, Tuna AL (2007) Heavy metal levels in two fish species *Leuciscus cephalus* and *Lepomis gibbosus*. Food Chem 100(2):830–835. https://doi.org/10.1016/j.foodchem.2005.09.020

Ysart G, Miller P, Croasdale M, Crews H, Robb P, Baxter M, de L'Argy C, Harrison N (2000) 1997 UK Total diet study—dietary exposures to aluminium, arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, tin and zinc. Food Addit Contam 17(9):775–786. https://doi.org/10.1080/026520300415327