Turkish Journal of Fisheries and Aquatic Sciences 15: 399-405 (2015)

www.trjfas.org
ISSN 1303-2712
DOI: 10.4194/1303-2712-v15_2_25

PROOF

Heavy Metal Levels in Four Commercial Fishes Caught in Sinop Coasts of the Black Sea, Turkey

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Received 23 December 2014
Accepted 22 April 2015

Abstract

The aim of this study is to evaluate the levelsof eight heavy metals (aluminium, arsenic, copper, zinc, mercury, iron, cadmium and lead) in the muscle tissue of Red mullet (*Mullus barbatus*), Whiting (*Merlangius merlangus*), Mediterranean horse mackerel (*Trachurus mediterraneus*) and Golden grey mullet (*Liza aurata*)caught from Sinop coast of the Black Sea during fishing season in 2012 and 2013.Metal analyses in fish were performed using ICP-MS (Inductively Coupled Plasma – Mass Spectrometer).The levels of Al, Hg, Cd, Pb and Cu in all fish species except *T. mediterraneus* for Cu were below the limit of detections. Mean Cu level in Mediterranean horse mackerel was 0.67 mg/kg wet wt.Highest value of Zn(24.7 mg/kg wet wt.) wasalso found in the *T. mediterraneus*which were about 7-8.5 times more than the other species.*L. aurata* have the highest Fe value (3.2 mg/kg wet wt.), but have the lowest As value (0.25 mg/kgwet wt.) compared with the other fish species. The levels of As for *M. barbatus* were high (1.3 mg/kgwet wt.) and followed by *M. merlangus* (1.24 mg/kgwet wt).The concentrations of thestudied heavy metals werefar below the established values by the Turkish Food Codex and Commission Regulation (EC). Therefore, theircontribution to the total body burden of these heavymetals can be considered as negligibly small.

Keywords: Black Sea, heavy metal, *Mullusbarbatus, Merlangiusmerlangus, Trachurusmediterraneus, Liza aurata.*

Karadeniz'in Sinop Kıyılarında (Türkiye) Yakalanan Dört Ticari Balıkta Ağır Metal Düzeyleri

Özet

Bu çalışmanın amacı 2012-2013 balıkçılık sezonunda Karadeniz'in Sinop kıyılarından yakalanan barbun (*Mullusbarbatus*), mezgit (*Merlangiusmerlangus*), istavrit (*Trachurusmediterraneus*) ve altınbaş kefal (*Lizaaurata*) balıklarının kas dokularında sekiz ağır metalin (alüminyum, arsenik, bakır, çinko, cıva, demir, kadmiyum ve kurşun) düzeylerini değerlendirmektir. Balıklardaki metal analizleri ICP-MS (endüktif eşleşmiş plazma – kütle spektrometresi) kullanılarak gerçekleştirilmiştir. *T. mediterraneus* türündeki Cu miktarı hariç tüm balık türlerindeki Al, Hg, Cd, Pb ve Cu düzeyleri ölçüm düzeylerinin altında bulunmuştur. İstavritteki ortalama Cu miktarı 0.67 mg/kg yaş ağırlıktır. En yüksek Zn (24,7 mg/kg yaş ağırlık) yine *T. mediterraneus* türünde bulunmuş olup diğer türlerdeki değerlerden 7-8,5 kez daha fazladır. Diğer türler ile karşılaştırıldığında *L. aurata* türü en yüksek Fe (3,2 mg/kg yaş ağırlık) ancak en düşük As (0,25 mg/kg yaş ağırlık) değerine sahiptir. *M. barbatus* türündeki As düzeyleri yüksek (1,3 mg/kg yaş ağırlık) olup bunu *M. merlangus*türü (1,24 mg/kg yaş ağırlık) izlemiştir. Çalışılan ağır metal konsantrasyonları Türk Gıda Kodeksi ve Avrupa Birliği Tüzüğü'nce belirlenen değerlerin oldukça altında bulunmuştur. Bu nedenle, bu ağır metallerin toplam vücut yükündeki payının önemsenmeyecek kadar küçük olarak nitelendirilebilir

Anahtar Kelimeler: Karadeniz, ağır metal, *Mullus barbatus, Merlangius merlangus, Trachurus mediterraneus, Liza aurata.*

Introduction

The pollution of marine environment with chemical contaminants especially heavy metals has become one of the most critical environmental problems of the century. As a result of the heavy metals transport from industrial areas into the environment and their chemical persistence, many marine ecosystems like the Black Sea are faced with spatially or temporally serious high levels of heavy metals. The Black Sea is one of the youngest seas in the world and it is nearly-enclosed basin between southern Europe and the Anatolian peninsula and has historically been one of the most biologically productive regions in the world(Özsoy and Ünlüata, 1997).The Black Sea ecosystem has been seriously damaged as a result of pollution (Bat, 2014). In terms of heavy metal, the Black Sea is vulnerable to pollution from untreated industrial effluents and municipal wastewater, runoff from chemical fertilizers in the coastal area and rivers.Bakan *et al.* (1996) pointed out that Ordu and Giresun city centres have separate sewerage systems where Sinop, Trabzon and Zonguldak have combined systems and Samsun city centre have both combined and separate sewerage system.The dumping of wastes via rivers in the Black Sea provides a significant source of metal input(Bakan and Büyükgüngör, 2000; Altas and Büyükgüngör, 2007; Bat *et al.*, 2009).

Some of essential heavy metals are required by humans and quickly decay into harmless or less harmful forms, while non-essentials are non-biodegradable and remain dangerous for a long time. Now, there is a growing concern worldwide over the indiscriminate use of such heavy metals, resulting in environmental pollution and toxicity risk to marine organisms. Thus, ecological damage of the environment caused by anthropogenic factors as well as the presence of heavy metals may affect people. Heavy metals tend to accumulate in advanced organisms through bio-magnification effects in the food chain. Thus they can enter into thehuman body and accumulate in the human tissues to pose chronic toxicity. Chronic assimilation of heavy metals is a known cause of cancer (The Earth Report 3, 1992).

The Marine Strategy Framework Directive (MSFD, 2008/56/EC)establishes a framework for the development of marine strategies designed to achieve Good Ecological Status (GES) in the marine environment, by the year 2020, using 11qualitative descriptors (Official Journal of the European Union (2008).The concentration of contaminants including heavy metals in the marine environment and their effects needs to be assessed taking into account the impacts and threats to the ecosystem in Directive 2008/56/EC. Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards (Descriptor 9; Directive 2000/60/EC) (Official Journal of the European Communities (2000).

Fish is a healthy food because of its nutritional benefits related to its proteins of highbiological quality, desirable lipid composition, valuable mineral compounds and vitamins. The particular composition of its lipid fraction, rich in essential ω-3 polyunsaturated fatty acids(PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and low cholesterol levels makes it an important item in human food. Thus, it is very important to determine the heavy metals concentrations in commercial fish in order to evaluate the possible risk of fish consumption for human health.

Total fish production in Turkey was reported as 644.852 tons in 2012 and 607.515 tons in 2013, where 315.636,5 tons in 2012 and 295.167,9 tons in 2013 of this total production came from sea fish (TUIK, 2014). Catches of Red mullet, Whiting, Mediterranean horse mackerel and Golden grey mullet from Turkish waters in 2013 were 2.055,4 tons, 9.396,9 tons, 21.817,8 tons and 2.504,9 tons, respectively (TUIK, 2014). These data show that these species of fish are economically important for human consumption and there is a need for more information on contaminant levels in fish of Turkey. To protect and develop fishery resources, it is important to undertake research work on this environmental issue.

The present study provides information on eight heavy metal concentrations in dorsal muscle tissue of four commercial fish species (*M. barbatus, M. merlangus, T. mediterraneus*and *L. aurata*)from theSinop coast of the Black Sea, Turkey during fishing season(i.e. autumn, winter) in 2012 and 2013. The heavy metals investigated include aluminium, arsenic, copper, zinc, mercury, iron, cadmium and lead because they are known to accumulate in the environment and in fish, and are known to cause adverse health effects if consumed in sufficient quantities.

The aims of this study are to determine and to compare the concentrations of aluminium, arsenic, copper, zinc, mercury, iron, cadmium and lead in edible parts of four selected commercial Sinop coast of the Black Sea fish species and to compare with the guidelines set down by the Ministry of Agriculture, Fisheries and Food (MAFF), the Turkish Food Codex, Commission Regulation (EC) for the safe consumption limits of fish and the other studies.

Materials and Methods

Sampling

The fish species selected were; Red mullet (*M. barbatus*), Whiting (*M. merlangus*), Mediterranean horse mackerel (*T. mediterraneus*) and Golden grey mullet (*L. aurata*). Red mullet, Whiting and Mediterranean horse mackerel were sampled during fish season in 2012; Golden grey mullet were sampled during fish season in 2012 and 2013directly from the Turkish fishing vessels in Sinop coats of the Black Sea. The total length was measured from snout to the tip of the caudal fin and the body weight (g) was taken on a top loading electronic balance to the nearest 0.1g.

Study Area

To understand the bioavailability of heavy metals and assess their potential impact on marine biota, fish samples were obtained during the fishing period of 2012 and 2103. The study area is presented in Figure 1.

Preparation Samples and Determination of Heavy Metals

Twenty fish individuals from each species taken randomly were rinsed in uncontaminated sea water then were washed with double distilled water. The muscle tissues of the fish were prepared for analysis according to the method described by Bernhard (1976).All samples were stored deep frozen at -21°C until their analysis.Metal analysis in fish was performed using m-AOAC 999.10- ICP/MS (Inductively Coupled Plasma – Mass Spectrometer) method by accredited ÇEVRE Industrial Analysis Laboratory Services Trade Company (TÜRKAK Test TS EN ISO IEC 17025 AB-0364-T). EN 15763 European Standard methods was applied. The limits of detection used for analysis of aluminium, arsenic, copper, zinc, mercury, iron, cadmium and lead were 0.5, 0.05, 0.5, 0.5, 0.05, 0.5, 0.02 and 0.05, respectively.

Statistical Analysis

A one- way analysis of variance (ANOVA) was performed, followed by Tukey post hoc comparisons for the source of statistically significant difference. The significance was set at 0.05 and P-values less of 0.05 were considered statistically significant (Zar, 1984). All values were being expressed on an mg/kg wet wt. basis.

Results

The mean lengths (cm) and weights (g) of the fish samples are given in Table 1. The analytical data for these metals also showed that there was difference in heavy metal content in various fish. Figure 2 shows the mean concentrations of eight heavy metals in the muscle of fish species (*M. barbatus*, *M. merlangus*, *T. mediterraneus* and *L. aurata*) from the Sinop coast of the Black Sea. The levels of Al, Hg, Cd and Pb in all fish species were below the limit of detection. The levels of Cu in Red mullet, Whiting and Golden grey mullet were also below the limit of detection. However, Cu level in Mediterranean horse mackerel

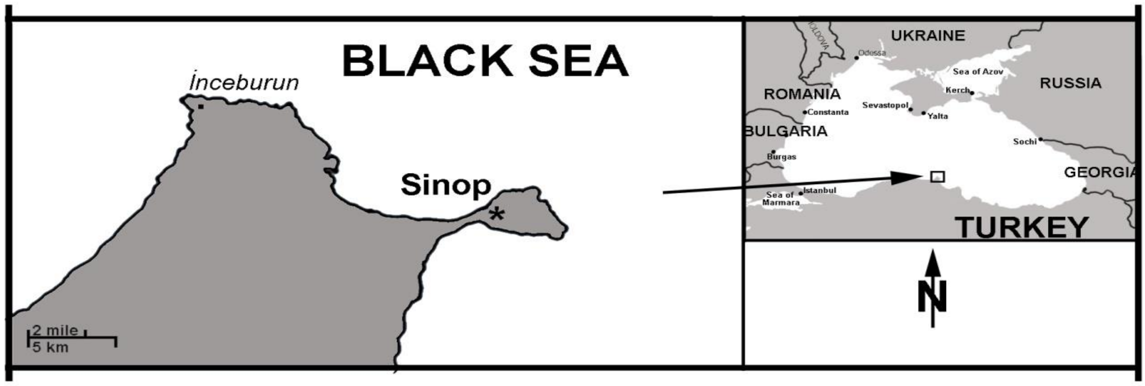

Figure 1. Fish sampling area from Sinop coasts of the Black Sea, Turkey.

Table 1. The mean and standard deviations of total length (cm) and total weight (g) of fish species from Sinop coasts of the Black Sea

Fish species	Mean ±SD Lengths (cm)	Mean ±SD Weights (g)
Mullus barbatus	13±3	25±9
Merlangius merlangus	12±2	21±5
Trachurus mediterraneus	11±1	17±3
Liza aurata	25±4	185±14

Table 2. Metal concentrations (mean mg/kg wet wt.) in the muscle tissues of the fish species from the Black Sea. Vertically, letters a, b and c show statistically significant differences (P<0.05)

Species	Heavy metals							
	Cd	Hg	Pb	Al	Cu	As	Fe	Zn
Mullus barbatus	<0.02[a]	<0.05[a]	<0.05[a]	<0.5[a]	<0.5[a]	1.30[a]	2.3[a]	3.2[a]
Merlangius merlangus	<0.02[a]	<0.05[a]	<0.05[a]	<0.5[a]	<0.5[a]	1.24[a]	0.87[b]	3.4[a]
Liza aurata	<0.02[a]	<0.05[a]	<0.05[a]	<0.5[a]	<0.5[a]	0.25[b]	3.2[c]	2.9[b]
Trachurus mediterraneus	<0.02[a]	<0.05[a]	<0.05[a]	<0.5[a]	0.67[b]	0.39[c]	2.2[a]	24.7[c]

was 0.67 mg/kg. In general all the species Zn is the most abundant of the metals examined. Highest values were found in the *T. mediterraneus* which were about 7-8.5 times more than the other species.*L. aurata* have the highest Fe values (3.2 mg/kg), but have the lowest As values (0.25 mg/kg) compared with the other fish species. The levels of As for *M. barbatus* were high (1.3 mg/kg) and followed by *M. merlangus*(1.24 mg/kg).Metal concentrations in the different fish species by ANOVA and Tukey test are shown in Table 2. The results indicated that there were significant differences within fish species ($P<0.05$).

Discussion

Recently, Bat (2014) reviewed the heavy metals in biota from the Black Sea coast of Turkey and suggested that fish can be considered good bioindicators for heavy metal contamination in marine ecosystems because they occupy different trophic levels and are widely consumed in many parts of the world by humans. Heavy metals whether essential or not may be toxic to living organisms including fish andthis can lead to deleterious effects on fisheries and, potentially, public health. In this study, the metal concentrations decreased in the order Zn>Fe>As>Cu>Al>Pb>Hg>Cd. Zn and Cu accumulated in higher concentrations in *T. mediterraneus* than inother species studied. The highest Fe level was in *L. aurata*. Similar results were found by other studies (Tepe *et al.*, 2007; Türkmen *et al.*, 2008 and 2009). Differences in metal concentrations were related to diet and feeding habits of benthic and pelagic fish species (Bustamente *et al.*, 2003). They show that benthic fish generally accumulate higher concentrations of heavy metals than pelagic fish. Topping (1973) suggested that mainly plankton feeding fish contain much higher concentrations of some heavy metals than bottom feeding fish. Metals, such as Fe, Cu and Zn, are essential metals since they play important roles in biological systems (Türkmen *et al.*, 2009) and the contaminant uptake rate is positively linked to the metabolic rate in marine organisms, it can be supposed that metal accumulation would be high (Kojadinovic *et al.*, 2007).Among the four species, thehighest As was found in *M. barbatus* followed by *M. merlangus*. The red mullet is a carnivorous species, found mostly on muddy bottoms and feed on Polychaeta, Lamellibranchiata and Crustacea. Whiting is benthopelagic and is also a carnivorous species, found mainly on mud and gravel bottoms, but also on sand and rock. They feed on shrimps, crabs, molluscs, small fish and polychaetes. Kojadinovic *et al.* (2007) suggested that that fish muscle impregnation is the result of the accumulation of heavy metals from preys living in different environments than where the fish were caught and which contained high metal levels.The remaining metals including Al, Pb, Hg and Cd were below the limit of detection in all fish samples. Hg, Pb and Cd belong to the group of non-essential and toxic metals and they have no known function in biochemical processes and are accumulated in multiple organs (Phillips, 1977).

Studies on the distribution of heavy metals show that there exists a certainvariation in accumulation patterns among fish species. The metal concentrations in these fish species were compared among the Turkish Black Sea and other coasts and presented in Table 3.The maximum concentrations of Pb (6.86±0.26 mg/kg dry wt.) and Cu (9.10±5.9 mg/kg dry wt.) were found to be highest in *M. barbatus* of Trabzon coasts (Topçuoğlu *et al.*, 1990). Fe (163±12 mg/kg dry wt.) and Zn (106±9.1 mg/kg dry wt.) concentrations were found to be highest in *M. barbatus* (Uluozlu *et al.*, 2007). Highest As level (2.375 mg/kg dry wt.) was also found in *M. barbatus* of Sinop coast (Das *et al.*, 2009). The maximum concentrations of Al and Hg were 86.3±11 mg/kg dry wt. (Turan *et al.*, 2009) and 0.084±0.005 mg/kg wet wt. (Tüzen, 2009) in *M. merlangus*, respectively. Cd concentrations were found to be highest (0.81-1.61 mg/kg dry wt.) in *L. aurata* from Egypt and it was followed by *M. merlangus* with 0.55 ± 0.04 mg/kg dry wt. (Türkmen *et al.*, 2008). Boran and Altinok (2010) concluded that heavy metal pollution in living organisms of the Black Sea has attracted considerable research attention since last 20 years.

In this study the levels of Al, Hg, Cd and Pb in all fish species were below the limit of detection.However, the average concentrations of other metals were slight lower than the maximum tolerance levels for human consumptionestablished by the Turkish Food Codex, Commission Regulation (EC) and MAFF (TGK, 2002; Council of Europe, 2001; MAFF, 1995). Furthermore, the tolerable weekly intakes were estimated by means of references for muscles tissues of fishes consumed by people. The average yearly fish consumption in Turkey is 7.1 kg per person(TUIK, 2014),which are equivalent to 19.45 g/day for Turkey.

Highest Cu level in Mediterranean horse mackerel was 0.67 mg/kg.The Joint FAO/WHO Expert Committee on Food Additives established a PTWI for Cu of 3.5 mg/kg body weight/week which was equivalent to 245 mg/week for a 70 kg adult (WHO, 1996; Council of Europe, 2001). By using the means of weekly fish consumption in Turkey of 136.15 g per person and intake of Cu in fish was calculated as 0.09 mg (136.15 g x 0.67 mg/1000 g) per person.*L. aurata* have the highest Fe values (3.2 mg/kg),the Joint FAO/WHO Expert Committee on Food Additives established a PTWI for Fe of 5.6 mg/kg body weight/week which was equivalent to 392 mg/week for a 70 kg adult (FAO/WHO, 2010). Intake of Fe in fish was calculated as 0.44 mg(136.15

Table 3. Heavy metal levels in fish from the Black Sea coasts and other country expressed in mg/kg wet wt. (*= dry wt., BS= Black Sea)

Species	Region	Heavy metals								References
		Cd	Hg	Pb	Al	Cu	As	Fe	Zn	
M. barbatus	Sinop	<0.02	<0.05	<0.05	<0.5	<0.5	1.30	2.3	3.2	Present study
*M. barbatus**	Trabzon	<0.1	-	6.86±0.26	-	9.10±5.9	-	39±1	11.5±3.5	Topçuoğlu *et al.*, 1990
M. barbatus	Sinop	0.023±0.002	-	0.28±0.06	-	0.76±0.07	-	4.18±0.81	2.42±0.27	Bat *et al.*, 1996
M. barbatus	Sinop	0.076	-	0.424	-	8.968	-	21.33	9.90	Bat *et al.*, 2006
*M. barbatus**	BS	0.017	-	0.077	-	0.01	-	4.5	4.3	Dalman *et al.*, 2006
*M. barbatus**	Sinop	-	-	-	-	0.38-2.714	-	-	1.424-63.29	Turk Culha *et al.*, 2007
*M. barbatus**	BS	0.45±0.04	-	0.84±0.07	-	0.98±0.07	-	163±12	106±9.1	Uluozlu *et al.*, 2007
*M. barbatus**	BS	0.208±0.017	-	0.727±0.141	9.85±14	-	-	21.2 ±1.47	7.573±0.389	Turan *et al.*, 2009
M. barbatus	BS	0.17 ± 0.02	0.036±0.002	0.36 ± 0.03	-	0.96±0.08	0.11±0.01	53.2 ± 2.8	75.5 ± 5.3	Tuzen, 2009
*M. barbatus**	Samsun	<0.02	<0.050	0.0815±0.003	-	-	1.33±0.02	-	-	Das *et al.*, 2009
*M. barbatus**	Sinop	<0.02	<0.050	0.051±0.0005	-	-	2.375	-	-	Das *et al.*, 2009
*M. barbatus**	BS	0.020±0.002	low	0.92±0.12	-	3.14±0.31	-	29.17±2.18	23.71±0.71	Nisbet *et al.*, 2010
*M. barbatus**	Sinop	0.08-0.18	-	0.02	-	2.85-5.26	-	-	22.82-34.33	Bat *et al.*, 2013
M. merlangus	Sinop	<0.02	<0.05	<0.05	<0.5	<0.5	1.24	0.87	3.4	Present study
M. merlangus	Kastamonu	-	0.01-0.22	0.02-0.11	-	0.62-3.25	-	-	-	Ünsal *et al.*, 1993
M. merlangus	Zonguldak	-	0.006-0.04	0.05-2.26	-	0.37-7.72	-	-	-	Ünsal *et al.*, 1993
*M. merlangus**	Perşembe	<0.02	-	<0.05	-	1.86±0.04	-	57±1	43.1±0.1	Topçuoğlu *et al.*, 2002
*M. merlangus**	Rize	<0.02	-	<0.05	-	4.54±0.11	-	46±1	30.2±0.1	Topçuoğlu *et al.*, 2002
*M. merlangus**	BS	0.0131	-	0.088	-	1.3	-	2.5	3.3	Dalman *et al.*, 2006
*M. merlangus**	BS	0.55 ± 0.04	-	0.93 ± 0.07		1.25±0.10		104±9.8	48.6 ± 3.9	Türkmen *et al.*, 2008
*M. merlangus**	BS	0.192 ±0.02	-	0.502 ± 0.104	86.3±11	-	-	4.48 ±0.441	6.03 ± 0.54	Turan *et al.*, 2009
M. merlangus	BS	0.21 ± 0.02	0.084±0.005	0.53 ± 004	-	1.32±0.11	0.17±0.01	98.1±7.7	65.4 ± 4.2	Tuzen, 2009
*M. merlangus**	BS	0.18 ± 0.02	-	0.46 ± 0.05	-	1.8 ± 0.2	-	27.7±2.8	20.6 ± 2.1	Mendil *et al.*, 2010
L. aurata	Sinop	<0.02	<0.05	<0.05	<0.5	<0.5	0.25	3.2	2.9	Present study
*L. aurata**	Egypt	0.81-1.61	-	1.41-2.66	-	3.43-5.49	-	-	12.98-35.45	Bahnasawy *et al.*,
*L. aurata**	Caspian Sea	0.35±0.23	-	1.50±0.53	-	4.54±1.07	-	67.52±33.53	13.69±7.23	Taghavi Jelodar *et al.*, 2011
T. mediterraneus	Sinop	0.03±0.00	-	0.63±0.25	-	16.7±2.08	-	57.2±17.9	42.6±9.14	Türkmen *et al.*, 2008
T. mediterraneus	Bartın	0.30±0.04	-	1.31±0.34	-	1.68±0.11	-	57.6±3.63	8.15±1.81	Türkmen *et al.*, 2008
T. mediterraneus	Sinop	0.043-0.048	-	0.17-0.23	-	2.22-6.21	-	-	17.89-32.38	Bat *et al.*, 2012
T. mediterraneus	Sinop	<0.02	<0.05	<0.05	<0.5	0.67	0.39	2.2	24.7	Present study

g x 3.2 mg/1000 g) per person. Highest values of Zn were found in the *T. mediterraneus* (27.4 mg/kg wet wt.) which were about 7-8.5 times more than the other species. The Joint FAO/WHO Expert Committee on Food Additives established a PTWI for Zn of 7 mg/kg body weight/week which was equivalent to 490 mg/week for a 70 kg adult (WHO, 1996; Council of Europe, 2001). Intake of Zn in fish was calculated as 3.36 mg (136.15 g x 24.7 mg/1000 g) per person. The highest levels of As were found in *M. barbatus* (1.3 mg/kg), but there is no available data on the As levels in fish to compare. However, the EU Scientific Committee for Food reports indicated that large consumption of fish had as intakes of about 0.050 mg/kg body weight, which corresponds to a daily intake of 3.5 mg for a 70 kg adult. Moreover, it is allowed that fish and seafood consumers may reach an intake of 1 mg/day from these foods alone (Directorate-General Health and Consumer Protection, 2004).By using the means of weekly intake calculated as 0.177 mg (136.15g x 1.3 mg/1000 g) per person for As in muscles of the fishes.As it can be seen that, the concentration of metals in the muscle tissues weregenerally low and within the ranges expected for the metals in muscle tissues of fish from Sinop coasts of the Black Sea. The concentrations of these metals found in the examined fish species were well belowthe acceptable levels for human consumptionrecommended by the Turkish Food Codex and Commission Regulation (EC) which means that they are not toxic to the health of consumers.

Conclusion

Based on the results, heavy metal concentrations found in the edible tissues of the fish samples were considerably lower than the maximum levels set by the Ministry of Agriculture, Fisheries and Food (MAFF), the Turkish Food Codex, Commission Regulation (EC). Even though, there is no risk from toxic heavy metals in fish, a possible hazard may develop in the future depending on the industrial, agricultural, urbanand fishing development in the Black Sea coasts. These data provide a useful baseline to measure any future changes in metal pollution of the Black Sea coasts. Furthermore, constant monitoring of the marine ecosystems in the Black Sea is required bythe concerned agency due to increase in anthropogenic activities for safe supply of fish to the consumers.

Acknowledgments

This work was supported in the Sinop University for Scientific Research Project; project number SÜF-1901-12-02.

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