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Heavy Metal Levels in the Black Sea Anchovy (*Engraulis encrasicolus*) as Biomonitor and Potential Risk of Human Health

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Abstract

The concentrations of heavy metals arsenic, copper, zinc, mercury, cadmium and lead were estimated in muscle tissue of commercially the most important marine fish *Engraulis encrasicolus* collected from the south coast of the Black Sea, Turkey and Georgia during fish season in 2013. Cu, Hg, Cd and Pb were not detected in the edible part of anchovy. Zinc shows negative correlation with fat content while no effect of fat content was observed for arsenic accumulation in anchovy. The ranges of Zn and As concentrations were found between 8.6-13 and 0.41-0.69 mg/kg, respectively. Concentrations of As and Zn in the anchovy samples were not elevated the tolerance levels of national and international guidelines. None of the values in edible tissues exceeded the standard guideline values and would pose no health hazards for consumers.

Keywords: Black Sea, *Engraulis encrasicolus*, heavy metals, Provisional Tolerable Weekly Intake (PTWI).

Biyomonitör Olarak Karadeniz Hamsisinin (*Engraulis encrasicolus*) Ağır Metal Düzeyleri ve İnsan Sağlığına Potansiyel Riski

Özet

Çalışmada 2013 yılı avcılık sezonunda güney Karadeniz'in Türkiye ve Gürcistan kıyılarından toplanan en önemli ticari deniz balığı *Engraulis encrasicolus* türünün kas dokusunda ağır metallerden arsenik, bakır, çinko, cıva, kadmiyum ve kurşun konsantrasyonları tahmin edilmiştir. Hamsinin yenilebilir dokusunda Cu, Hg, Cd ve Pb tespit edilememiştir. Hamside biriken çinko yağ miktarı ile negatif korelasyon gösterirken arsenik birikiminin yağ miktarı ile herhangi bir etkisi gözlenmemiştir. Zn ve As miktarları sırasıyla 8.6-13 ve 0.41-0.69 mg/kg arasında bulunmuştur. Hamsi örneklerindeki As ve Zn konsantrasyonları ulusal ve uluslararası yönergelerce verilen tolerans düzeylerinden daha yüksek değerlerde bulunmamıştır. Yenilebilir dokulardaki hiçbir değer standart yönetmeliklerde belirtilen değerleri aşmamış ve tüketiciler için hiçbir sağlık tehlikesi oluşturmamıştır.

Anahtar Kelimeler: Karadeniz, *Engraulis encrasicolus*, ağır metaller, Haftalık Tolere Edilebilir Miktar (PTWI).

Introduction

The heavy metal concentrations can be affected by anthropogenic activities such as human industrial and mining activity, as well as agricultural and domestic wastes, the combustion of fossil fuels and also by natural factors such as erosion and volcanic and tectonic activities. Heavy metals are widely dispersed in the marine fish through several different routes: solution uptake via the gills, absorption through the skin, ingestion of sea water and food. Among them, fish are generally considered accumulators of environmental contaminants such as heavy metals due to their longevity and their elevated position in marine foods chains. It is well known that, most marine organisms including fish concentrate heavy metals at higher concentrations than those occurring in their environment.

Fish accumulate heavy metals which enter the food web naturally, or as a result of marine pollution, accumulation may be correlated with fat content, and depends on food sources, the physiological state of the individual and the toxicological dynamics of the specific metal, consequently, a high variability in the heavy metal concentration exists among fish and their habitats. Hence, the fish appear to be potentially valuable indicator of the level of heavy metals accumulated in the marine environment: according to their position in the trophic level and their long biological half-time of elimination of contaminants.

It is reported that, presently the major environmental problems in the Black Sea area involve the decline of commercial fish stocks, loss of habitats and species, replacement of indigenous species with exotic ones, and undesirable changes caused by eutrophication and chemical pollution (BSC (SOE), 2008). The Black Sea coast of Turkey is mainly due to major land-based sources such as untreated wastewater from domestic and industrial settlements, pollutants brought from major rivers Danube, Dniester, Dnieper, Yesilirmak, Kizilirmak and Sakarya run through to the sea, agriculture, urbanisation of the coastal zone and tourism, leading to pollution, changes in biodiversity and risk to human health.

It seems that heavy metals do not pollute the whole Black Sea but appear in 'hot spots' near certain well-identified sources (Beyazli *et al*., 2010). Nut and tea-plant factories, variable food manufactures, fishing activities are the main industry types in the Turkish Black Sea Region. However, coal mining, fertilizer factories and copper facilities are rare but important industries (Bakan and Büyükgüngör, 2000). Altas and Büyükgüngör (2007) pointed out that there are a lot of big and little industries such as food, cement, fertilizer, pesticide, resin, plastic, textile and cigarette manufacturing exist in the Turkish middle Black Sea region. Most of them have no treatment plant and they have potential to create local pollution problem. There are no important industrial factories in the eastern part of the Black Sea Region of Turkey, but only hazelnut facilities, floor manufacturing and fish meal and-oil factories. Besides small industrial activities, pulp and paper factory present in this region is one of the important industries (Altas and Büyükgüngör, 2007).

The environmental changes and anthropogenic impact must influence on upper ecosystem trophic level: on stock and condition Black sea small pelagic fish. The Black Sea fisheries have a special importance in Turkish fisheries. The largest proportion of national fish yields were from the Black Sea where anchovy (*Engraulis encrasicolus*) was the dominant fish in caught with about 51 percent of total between 2003 and 2012 (TUIK, 2012). Having short life span, earlier maturation, high fecundity, feeding on plankton, moving actively both species integrate changes taken place on all lower trophic levels and may indicate ecosystem wellbeing degree. Priority Actions Programme Regional Activity Centre (PAP/RAC) of the Mediterranean Action Plan (MAP-UNEP) (2005) reported that the breeding grounds of anchovy, which is economically the most important fish species of the Black Sea, have shifted from the north-western shelf (due to very high level of pollution brought by the Danube), to the southeast shores.Therefore, the present study was undertaken to study the concentration levels of selected metals (arsenic, copper, zinc, mercury, cadmium and lead) in the Black Sea anchovy (*E. encrasicolus*) as biomonitor, and correlate the concentration of metals with respect to fat content.

The aims of this study are to:

1) Determine and compare the concentrations of arsenic, copper, zinc, mercury, cadmium and lead in edible parts anchovy species from the Turkish and Georgian coasts of the Black Sea; 2) Describe relationship with the concentrations of these heavy metals and fat content in anchovy; 3) Compare with the guidelines set down by the Ministry of Agriculture, Fisheries and Food (MAFF), the Turkish Food Codex, the Georgian Food Safety Rules, Commission Regulation (EC) for the safe consumption limits of fish and the other studies.

Materials and Methods

Study Area

To understand the bioavailability of heavy metals and assess their potential impact on marine biota, samples of anchovy (*E. encrasicolus*) were obtained during the period mainly in January, September and November in 2013 directly from the Turkish fishing vessels in the Black Sea coast of Turkey and Georgia. The study area is presented in Figure 1.

Cohort of 100 fishes from the catch was measured to the nearest 0.5 cm and divided to 2 size groups (no less than 20 individuals from each one) for each location.

Fat analysis was carried out according to the Soxhlet method (AOAC, 2005).

For metal analysis, sampled anchovy individuals from each species were measured and rinsed in clean sea water. 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 method by accredited ÇEVRE Industrial Analysis Laboratory Services Trade Company (TÜRKAK Test TS EN ISO IEC 17025 AB-0364-T). The method for determination of heavy metals, used acid, standard reference material, wet digestion was used by European Standard method with number EN 15763.

The limits of detection used for analysis of arsenic, copper, zinc, mercury, cadmium and lead were 0.05, 0.5, 0.5, 0.05, 0.03 and 0.05, respectively.

Statistical Analysis

Statistical analysis (one-way ANOVA) was performed, followed by Duncan comparisons for the source of statistically significant differences of metal concentrations between locations. The paired samples *t*-test was performed using a computer package MS-Excel for windows 8.1 software to compare differences between samples. Descriptive statistics for each trait were expressed as Mean ± Standard Error

Figure 1. Sampling localities of anchovy in the Black Sea.

and P-values less of 0.05 were considered statistically significant.

Results and Discussion

Metal Concentrations in *Engraulis encrasicolus*

The Black Sea anchovy from the south coast of the Black Sea, Turkey and Georgia during fish season in 2013 were analysed for arsenic, copper, zinc, mercury, cadmium and lead. Cu, Hg, Cd and Pb were not detected in the edible part of anchovy. The ranges of Zn and As concentrations were found between 8.6-13 and 0.41-0.69 mg/kg wet weight, respectively. The concentrations of examined metals in fish tissues from the Black Sea demonstrated regional differences. The concentrations of zinc and arsenic in *E. encrasicolus* are presented in Figures 2 and 3.

In anchovy with 105-110 mm length from Fatsa coasts As concentration was lower than those in other regions. In the region of Samsun As levels were significantly higher for both 105-110 and 115-120 mm lengths group.

Comparison of Heavy Metal Levels in Anchovy with those in Other Regions of the Black Sea

Heavy metal concentrations in the Black Sea anchovy were compared with the other studies and summarised in Table 1.

The metal concentrations in *E. encrasicolus* from the Black Sea coasts decrease in the order Zn>Cu>Pb>As>Cd>Hg (Table 1). Metals accumulated in higher concentrations in tissue (liver) and less in muscle. When the metal concentrations were compared among the Black Sea coasts, Cu and Pb concentrations were found to be highest in Bartın (Türkmen *et al*., 2008). Zn concentrations were found to be highest in Samsun (Aygün and Abanoz, 2011). In terms of As and Hg concentrations in anchovy from Turkish coast of the Black Sea were low (Table 1).

Relationship between Fat Contents and Metals in Anchovy

Density of populations, condition of fish stocks, and fish availability for trawling depend on characteristics of its environment. The fat contents (%) of muscles of anchovy from the Black Sea coasts are represented in Figure 4. The food supply of fish populations is the most important among them. Indicator of food supply for anchovy, as well as sprat is lipid reserves accumulated in the body at the end of feeding period (Shulman *et al*., 2007; Nikolsky *et al*., 2007). Shulman *et al*. (2008) showed that the most important physiological factor which determines the degree to which the anchovy are prepared for the wintering migration to the Black Sea is the level of energy (fat deposits) accumulated during summer and autumn feeding. Populations which accumulate significant level of fat over 20% carry out migrations with great intensity in dense schools. Therefore fat contents were different for locations and seasons. Statistical analysis by ANOVA one way showed that there was no significant length variation in fat content levels for Sinop and Fatsa fish samples ($P>0.05$), whereas there were significant for Samsun and Batumi anchovies ($P<0.05$). Regressions were performed for fat contents with metal concentrations. Zinc shows negative correlation with fat content while no effect of fat content was observed for arsenic accumulation in anchovy (Figure 5A, B).

Human Health Risk Effects

Copper has been introduced into the marine environment with the several industries such as

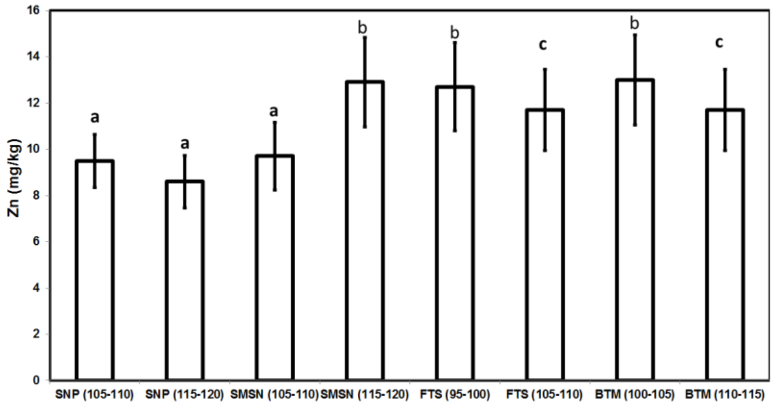

Figure 2. The means with standard errors (vertical line) of Zn concentrations (µg/g wet wt.) in the dorsal muscle tissues of *Engraulis encrasicolus* from the Black Sea coasts during fishing season in 2013. a, b, c = The same letters beside the vertical bars indicate the values are not significantly different (P>0.05). SNP (Sinop), SMSN (Samsun), FTS (Fatsa), BTM (Batumi).

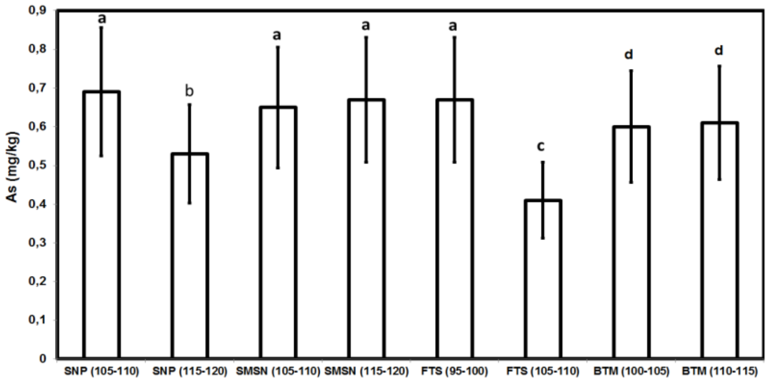

Figure 3. The means with standard errors (vertical line) of As concentrations (µg/g wet wt.) in the dorsal muscle tissues of *Engraulis encrasicolus* from the Black Sea coasts during fishing season in 2013. a, b, c, d = The same letters beside the vertical bars indicate the values are not significantly different (P>0.05). SNP (Sinop), SMSN (Samsun), FTS (Fatsa), BTM (Batumi).

mining, electroplating, paint, chemical and agricultural effluents. Complex form of copper is biologically unavailable but living organisms may absorb some copper in the environment. Although copper is an essential element for which in fish were not found. Whereas, essential element zinc is found in anchovy samples but the mean concentration was below than the permissible limits. The maximum Zn level is 50 and 40 mg/kg wet weight in fish in Turkish Food Codex (TGK, 2002) and Georgian Food Safety Rules (2001). Zinc involves in many enzymes and it plays an important role in the cell division, metabolism, reproduction and etc. The sources of zinc in marine waters may be from geological rock weathering and from anthropogenic activity such as industrial, agricultural and domestic effluents.

Arsenic is a ubiquitous element with metalloid properties and enters the marine environment from natural diffuse sources and from anthropogenic point and diffuse sources. The ambient level of arsenic in the marine environment is generally accepted as being in the range 2-3 mg l^{-1} (Mance and Yates, 1984). There is no information about maximum As levels in fish in the Commission Regulation (EC, 2006). The maximum As concentration (0.69±0.166 mg/kg wet weight) in the present study was considerably lower than the maximum level (1.0 mg/kg wet weight) set by Turkish Legislation (Anonymous, 1995). Moreover, the maximum As level reported by Georgian Food Safety Rules (2001) for fish is 2.0 mg/kg wet weight.

Among the different metals analysed mercury, cadmium and lead are classified in fish as chemical hazards and maximum residual levels have been prescribed for human by various organizations (MAFF, 1995; EC, 2001; EC, 2006). In the present study, Hg, Cd and Pb levels in the samples were not detected.

In the present study, the lowest and highest zinc levels in anchovy were found as 7.2 mg/kg in SNP and 15 mg/kg in BTM for muscles. The Provisional Tolerable Weekly Intake (PTWI) value is an estimate of the amount of a contaminant that can be consumed by human over a lifetime without appreciable risk. PTWI is established by the Joint Food and Agricultural Organization for the United Nations (FAO) / World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA).

Table 1. Heavy metal concentrations (µg metal g^{-1} wet wt.) in muscle tissues of *Engraulis encrasicolus* from Inebolu (INBL), Sinop (SNP), Amasra (AMSR), Persembe (PRS), Samsun (SMSN), Trabzon (TRBZN), Bartin (BRTN), Fatsa (FTS) and Batumi (BTM) coasts of the Black Sea. ***:** expressed in µg metal g^{-1} dry wt. ND: Not Determined; --: no data, BS: Black Sea.

Area	As	Cu	Zn	Hg	Cd	Pb	References
INBL	--	0.68-1.33	--	0.04 ± 0.02	--	0.06-0.06	Ünsal *et al.*, 1993
BS*	--	3.39±0.49	50.7±8.3	--	0.27±0.06	2.51±0.09	Topçuoğlu *et al.*, 1995
SNP	--	0.69±0.06	3.55±0.68	--	0.025±0.005	0.78±0.04	Bat *et al.*, 1996
BS (1997-1998)*	--	2.2-2.8	30-40	--	0.1-0.2	<0.01	Topçuoğlu, 2000
AMSR (1997)*	--	2.21±0.11	35.7±0.4	--	0.10±0.01	<0.05	Topçuoğlu *et al.*, 2002
PRS* (Dec.,1997)	--	2.81±0.02	40.3 ± 0.1	--	0.16 ± 0.04	0.6 ± 0.1	Topçuoğlu *et al.*, 2002
PRS*(Dec.,1998)	--	3.09±0.05	44.2 ± 0.2	--	0.15 ± 0.03	< 0.05	Topçuoğlu *et al.*, 2002
SMSN*	--	1.94±0.10	17.38±2.01	--	0.20±0.03	0.38±0.02	Tüzen, 2003
SMSN	--	1.96±0.17	18.85±1.72	--	0.18±0.02	0.39±0.07	Tüzen, 2003
BS*	--	0.95±0.08	40.2 ± 3.2	--	0.65 ± 0.04	0.33 ± 0.01	Uluozlu *et al.*, 2007
TRBZN	--	0.88±0.10	10.8±1.29	--	0.03±0.01	0.12±0.03	Türkmen *et al.*, 2008
SNP	--	1.12±0.16	10.6±0.88	--	0.02±0.00	0.27±0.05	Türkmen *et al.*, 2008
BRTN	--	8.58±2.15	45.6±22.1	--	0.06±0.02	0.87±0.40	Türkmen *et al.*, 2008
BS	0.25 ± 0.02	1.96±0.14	38.8 ± 3.2	0.055 ± 0.003	0.27 ± 0.02	0.30 ± 0.02	Tüzen, 2009
BS*	--	NM	25.416±3.664	--	0.124 ± 0.018	0.329 ± 0.302	Turan *et al.*, 2009
BS (winter)*	--	2.78±0.21	26.59±1.95	ND	0.039±0.008	0.57±0.04	Nisbet *et al.*, 2010
BS (spring)*	--	2.68±0.25	25.91±0.71	ND	0.031±0.003	0.83±0.08	Nisbet *et al.*, 2010
SMSN (2009)*	--	3.7±1.6	129.3±15.0	--	0.2±0.05	0.4±0.2	Aygun and Abanoz, 2011
SMSN (2010)*	--	3.8±1.9	221.0±0.5	--	ND	ND	Aygun and Abanoz, 2011
SNP (2010)	--	1.88-4.16	8.12-16.41	--	0.09-0.17	0.09-0.26	Bat *et al.*, 2013
SNP (Sep.) 105-110 mm	0.69±0.166	ND	9.5±1.43	ND	ND	ND	The Present Study
SNP (Sep.) 115-120 mm	0.53±0.127	ND	8.6±1.29	ND	ND	ND	The Present Study
SMSN (Nov.) 105-110 mm	0.65±0.156	ND	9.7±1.46	ND	ND	ND	The Present Study
SMSN (Nov.) 115-120 mm	0.67±0.161	ND	12.9±1.94	ND	ND	ND	The Present Study
FTS (Jan.) 95-100 mm	0.67±0.161	ND	12.7±1.91	ND	ND	ND	The Present Study
FTS (Jan.) 105-110 mm	0.41±0.098	ND	11.7±1.76	ND	ND	ND	The Present Study
BTM (Jan.) 100-105 mm	0.60±0.144	ND	13.0±1.95	ND	ND	ND	The Present Study
BTM (Jan.) 110-115 mm	0.61±0.146	ND	11.7±1.76	ND	ND	ND	The Present Study

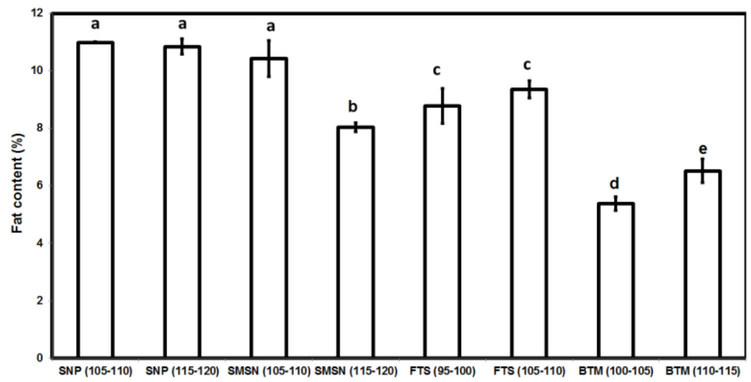

Figure 4. Fat content (%) of the dorsal muscle tissues of *Engraulis encrasicolus* from the Black Sea coasts during fishing season in 2013. Values are averages of three replicates ± standard error.a, b, c, d = The same letters beside the vertical bars indicate the values are not significantly different (P>0.05).SNP (Sinop), SMSN (Samsun), FTS (Fatsa), BTM (Batumi).

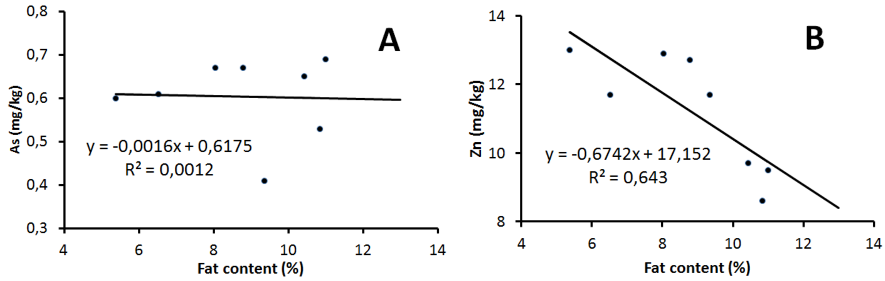

Figure 5. Arsenic (A) and zinc (B) (mg/kg wet weight) concentrations *vs* total fat content (%) in the Black Sea anchovy in SNP (Sinop), SMSN (Samsun), FTS (Fatsa), BTM (Batumi) areas in 2013.

PTWI value for Zn is 7 mg per kg body weight per week (WHO, 1996; Council of Europe, 2001). The Joint FAO/WHO (2010) Expert Committee on Food Additives established PTWI for Zn was equivalent to 490mg/week for a person weighing 70kg.The average daily pelagic fish consumption in Turkey and Georgia are11and 13 g per person, which are equivalent to 77 and 91 g/week for Turkey and Georgia, respectively (FAO, 2010). The weekly intake calculated ranged from 0.6 mg (77 g x 7.2 mg/1000 g) for Sinop coasts to 1.4 mg (91 g x 15 mg/1000 g) for Batumi coasts per person for zinc in muscles of fish.

No data on the arsenic levels in fish is presently available. However, The EU Scientific Committee for Food reports indicated that large consumption of fish had arsenic intakes (in the form of organo arsenicals) of about 0.050 mg/kg body weight, without any report of ill effects. This corresponds to a daily intake of 3.5 mg fora 70 kg adult. Moreover, it is reported that fish and other seafood contribute more than 50% of the dietary arsenic. The mean daily intake of arsenic from fish and other seafood is below 0.35 mg. Therefore it is assumed that the total daily intake of arsenic by the mean adult population is below 1 mg. As results 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).

In the present study, the lowest and highest arsenic levels in anchovy were found as 0.3 mg/kg in FTS and 0.9 mg/kg in Sinop for muscles. By using the means of weekly pelagic fish consumption in Turkey of 77 g per person and minimum and maximum lead levels in fish, weekly intake calculated ranged from 0.023 mg (77 g x0.3 mg/1000 g) to 0.069 mg (77 g x 0.9 mg/1000 g) per person for arsenic in muscles of anchovy.

As it can be seen that, the estimated PTWIs of Zn and As in the present study is quite below the established PTWIs; thereby there is no health threatening concern due to the consumption of edible parts of *E. encrasicolus* from coastal waters of the Black Sea, Turkey and Georgia.

Overall Conclusions

Cu, Hg, Cd and Pb concentrations in the muscle tissue of the anchovy from the Turkish and Georgian coast of the Black Sea were not detected. No significant correlation ($p>0.05$) was observed between the levels of fat content (%) and the concentrations of heavy metals in the present study. There were negative correlation between the fat content (%) and the zinc concentrations in anchovy. However, this cannot be said for arsenic.

Concentrations of As and Zn in the anchovy samples were not elevated the tolerance levels of national and international guidelines and were below Provisional Tolerable Weekly Intake (PTWI) limits set by FAO/WHO (2010).

Metal concentrations in the edible parts of fish were assessed for human consumption by comparison with Provisional Tolerable Weekly Intake (PTWI) values. The values of all metals in muscles of analysed fish in the present study stayed below the established limit values. Consequently, it might be concluded that these metals in the edible parts of the examined species should not pose any public health problems.

Acknowledgments

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