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Heavy Metal Accummulation in Muscles and Total Bodies of *Mullus barbatus*, *Trachurus trachurus* and *Engraulis encrasicolus* Captured from the Coast of Sinop, Black Sea

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AG and STC collected samples, performed laboratory analyses and wrote the article. FK and MC performed samples analysis with ICP-AES.

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Heavy metal accumulation, muscle, ICP-AES.

A B S T R A C T

The levels of heavy metals *viz*., Cd, Cu, Fe, Ni, Pb, Zn and Al were determined by ICP-AES in the muscles and total bodies of *Mullus barbatus* (Linnaeus 1758), *Trachurus trachurus* (Linnaeus 1758), *Engraulis encrasicolus* (Linnaeus 1758) captured from the coast of Sinop. The order of the levels of the heavy metals in the total fish samples was Fe > Zn > Al > Pb > Cu > Ni > Cd, whereas in the muscles, the order was Zn > Fe > Cu > Al > Pb > Ni > Cd. The experiment results are discussed by comparison with literature values.

INTRODUCTION

About 71 percent of the Earth's surface is covered by water, and the oceans, one of mankind's great hopes for future food supplies, hold about 96.5 percent of all Earth's water. Fish, other aquatic products and meat products are important for human diet in many parts of the world because they contribute to solve the global nutritional problem and provide the well known proteins, minerals and vitamins (Alturiqi and Albedair, 2012). In order to sustain normal life of aquatic organisms or fish species, the essential metals are taken up from sediment, water or nutrients (Canlı and Atlı 2003), but they can also have poisonous effect when taken in large amounts (Celik and Oehlenschlager, 2007). For biomonitoring trace metals, it is essential to determine the ecosystem wellness (Mico *et al.*, 2006). Heavy metals can be categorized as potentially toxic (As, Sb, Cd, Hg, Pb and Al), semi-essential like Ni, Co, V and essential like Zn, Cu, Cr, Fe, Mn and Se (Munoz-Olivas and Camara 2001; Szentmihalyi and Then, 2007).

Aquatic organisms are constantly exposed to chemicals in contaminated and polluted waters. Many of them have been found to be good indicators of heavy metal contamination in aquatic systems because they occupy different trophic levels and are of different ages and sizes (Burger *et al.*, 2002). Heavy metals are regarded as the most important form of pollution in the aquatic surroundings because of their toxicity and bioaccumulation by aquatic organisms, such as fish (Emami-Khansari *et al.*, 2005). The concern about the effects of anthropogenic pollution of water ecosystems is growing. Heavy metals from man-made pollution sources are continually released into the aquatic ecosystems. Contamination with heavy metals is a serious threat because of their toxicity, accumulation and magnifications in the food chain (Eisler, 1988). In recent years, much attention has been focused on the concentrations of heavy metals in fish and other nutrients in order to check for those hazardous to human health (Farkas *et al.*, 2003).

Economic growth and fast industrialization across the world has caused the increase of water pollution in the coastal regions. Pollutants deposited in water led to very serious changes that directly or indirectly influence the ecological balance of the environment, causing extensive damage of aquatic organisms that results in mass deaths and cessation of activities due to their high poisoning and bioaccumulation effect (Matta *et al.*, 1999). As human populations proliferate and industrialization increases, the problems of environmental pollution become more critical (Raja *et al.*, 2009). Many pollutants are discharged into the environment every day. Among these, heavy metals are regarded as one of the most serious pollutants of aquatic life. Their contamination of aquatic ecosystems has been put as a serious pollution problem. All heavy metals are potentially harmful to most organisms at some level of adsorption and exposure.

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Contamination of aquatic environments with potentially harmful subtances occurs, especially when they are near industrial and settlement areas (Marcovecchio, 2004). During the last few decades, in some areas of Black Sea, metal concentrations in sea waters, sediments and organisms with a wide range of pollutants has become a matter of great concern. It is clear from many studies conducted that the heavy metal pollution should be taken into account in the Black Sea, as it is highly remarkable (Boran and Altınok, 2010). The purpose of this study is to determine ranges and variations of potentially toxic heavy metals (Cd, Cu, Zn, Al, Fe, Ni and Pb) in the fish collected by means of fishing in the Black Sea as they are an important component of the human diet. The species we investigated are commonly consumed by the local population in Turkey, thus, it is also aimed to predict the health risks for the fish consumers.

MATERIALS AND METHODS

The fish species were obtained from commercial fish caught randomly from the coast of Sinop in the Middle Black Sea (Turkey) between October and December 2007. Three species of fish, namely Red Mullet (*M. barbatus;* L. 1758*)*, Horse Mackerel (*T. trachurus;* L. 1758) and Anchovy (*E. encrasicolus;* L. 1758*)*, were included in the study. Fish samples were kept at -21°C until the time of evaluation. Total length (cm) and weight (g) of the species were determined before dissection. Before analyses, the fish samples were divided into two groups. The first group was evaluated in terms of total body (head, bones, muscles, internal organs, skin, tail and fins), while the second group was evaluated in terms of muscle tissue. The total body and muscle of the fish were separated and dried in an oven at 105°C until constant weight was reached. Then, 0.5 g of the homogenized samples of muscle and total body of the fish were digested. All samples were completely digested in concentrated HNO_3 (65%). They were placed in a hot block digester first at low temperature for 1 h, and then fully digested at high temperature (140°C) for at least 3 h (Yap *et al.*, 2004; Turk Culha *et al.*, 2011). The digested samples were then diluted to volume with double-distilled water. After filtration, the prepared samples were measured for Cd, Cu, Fe, Ni, Pb, Zn and Al by ICP-AES. The detection limits were; Cd: 2.0 ng/mL, Pb: 40.0 ng/mL, Cu: 1.2 ng/mL, Al: 2.0 ng/mL, Fe: 1.3 ng/mL, Ni: 6.5 ng/mL, Zn: 1.8 ng/mL. The accuracy of the analysis was verified by analyzing the European certified reference material (ERM-C278, mussel tissue), by the same procedure used for the samples. The average recover of all analyzed metals to the reference material was 94.6%.

Statistical analysis

Statistical analysis of data was carried out using Minitab 16.0 statistical package program. Two-way ANOVA was applied to identify the differences between three species. The P-value was found as statistically significant at $P<0.05$. Correlation matrices were created to evaluate the relationships between the examined heavy metal concentrations of the samples. The values of correlation coefficients between metal concentrations in fish species were also given as statistically significant if $P<0.05$, $P<0.01$ and $P<0.001$.

RESULTS AND DISCUSSION

This study aimed to determine heavy metals (Cd, Cu, Fe, Ni, Pb, Zn and Al) in *M. barbatus*, *T. trachurus* and *E. encrasicolus* bought from local salesmen in Sinop Peninsula in the Black Sea Region. The body length and weight of each of 997 fish specimens fish measured and evaluated in the study (Table I).

As the total body concentrations were taken into account, the contents of investigated heavy metals in fish species were found to be in the range of 0.08-1.25 $\mu g\ g^{-1}$ dry wt. for Cd, 2.68-21.00 $\mu g\ g^{-1}$ dry wt. for Cu, 41.26-629.19 $\mu g\ g^{-1}$ dry wt. for Fe, 3.07-12.89 $\mu g\ g^{-1}$ dry wt. for Ni, 2.39-24.18 $\mu g\ g^{-1}$ dry wt. for Pb, 46.13-88.45 $\mu g\ g^{-1}$ dry wt. for Zn and 5.01-47.30 $\mu g\ g^{-1}$ dry wt. for Al. The order of the levels of the heavy elements obtained from the three different fish species in Sinop Peninsula is Fe > Zn > Al > Pb > Cu > Ni > Cd. The highest metal accumulation levels among the species were determined as Cd, Cu, Fe, Ni and Pb for *M. barbatus*; Zn and Al for *E. encrasicolus*, while the lowest metal accumulation levels among the species were determined as Cd, Cu, Fe, Ni and Pb for *T. trachurus*; Al and Zn for *M. barbatus*, respectively (Table II). *M. barbatus* is a bottom-dwelling fish, hence, its heavy metal accumulation is more than the other fish (Kalay *et al.*, 2004). The lowest heavy metal accumulation levels were usually determined in *T. trachurus.* The obtained results suggested that significant differences existed in the concentrations of metals, which is largely associated with the organism mobility and metabolic differences (Gündoğdu *et al.*, 2011), or with other characteristics of the fish. Bustamante *et al.* (2003) reported that the extreme environmental conditions might play a key role on the processes of uptake, deposition and elimination of the metals by organisms. Furthermore, differences in heavy metal concentrations were associated with diet and nutritional habits of pelagic and benthic fish species. They demonstrated that lower concentrations of heavy metals, usually accumulated in pelagic fish compared to the benthic fish (Bustamante *et al.*, 2003). Topping (1973) suggested that mainly pelagic fish

Table I.- The number, average size and weight of *M. barbatus, T. trachurus* and *E. encrasicolus* samples from Black Sea.

Species	Sample No.	N	Weight (g) (X±SD)	Min-Max (g)	Length(cm) (X±SD)	Min- Max (cm)
M. barbatus	1	31	20.59±1.78	8.87-44.00	10.49±0.27	8.20-14.00
	2	31	26.51±1.32	16.20-46.21	11.90±0.17	10.50-14.30
	3	19	30.88±1.37	19.32-43.38	12.51±0.19	10.90-14.40
	4	28	20.29±2.18	8.34-51.52	10.95±0.30	8.50-14.50
	5	32	17.27±1.25	7.66-30.45	10.36±0.24	8.00-13.00
	6	33	17.24±1.09	9.10-41.15	10.38±0.20	8.50-14.00
	7	27	21.72±1.21	14.12-32.63	11.45±0.17	10.20-12.80
	8	30	19.73±1.17	12.13-49.12	10.84±0.18	9.30-12.10
T. trachurus	1	33	17.30±0.68	11.78-27.73	11.12±0.18	9.60-12.90
	2	40	18.23±0.80	13.42-27.76	11.40±0.16	9.90-13.00
	3	39	15.02±059	11.19-28.81	11.31±0.16	10.50-13.30
	4	33	21.53±1.39	12.93-37.85	12.50±0.26	10.60-15.70
	5	35	15.84±0.81	11.71-29-57	11.56±0.18	10.50-14.90
	6	33	20.50±1.11	11.45-3356	12.38±0.23	10.40-14.50
	7	34	14.41±0.43	10.20-20.29	11.04±0.10	10.20-12.30
	8	18	14.52±0.36	12.63-18-38	11.19±0.09	12.20-11.90
E. encrasicolus	1	64	6.87±0.18	3.48-10.54	8.72±0.10	7.00-10.50
	2	70	7.51±0.16	5.03-12.21	10.06±0.09	8.50-12.00
	3	70	7.49±0.15	5.39-10.73	10.00±0.09	8.50-12.00
	4	59	10.35±0.31	5.91-16.22	10.83±0.09	9.50-12.50
	5	70	8.95±0.27	5.31-14.15	10.26±0.08	9.20-11.80
	6	67	8.76±0.22	6.32-13.77	10.29±0.10	7.70-11.80
	7	69	7.58±0.18	5.44-12.02	9.82±0.07	9.00-11.30
	8	32	8.84±0.25	6.09-12.05	10.44±0.11	8.70-11.60

N = Number of fishes; (X±SD) mean ± Standard deviation values are given in parentheses, and minimum, maximum values.

contain much higher concentrations of several heavy metals than bottom fish. The results are different from our values, which is attributable to environmental and regional differences.

The heavy metals were found to be higher in the total fish than the muscle tissue (Tables II, III). This can be because of the fact that total tissue of the fish involves liver, kidney, digestive tract, etc. and these accumulate heavy metals highly easily. The highest concentrations of Zn, Pb, Cu, Fe, Al and Cd were found in internal organs and concentrations were much higher compared to the muscle tissues (Yılmaz *et al.,* 2007; Gundogdu *et al.,* 2009a). Experiments have shown that metal accumulation is considerably higher in liver, kidney, digestive tract, and in gills, whereas it is low in muscles in many species (Yılmaz, 2009; Benzer *et al.,* 2013; Karadede and Ünlü, 2000; Gündoğdu *et al.,* 2009b; Alhas *et al.,* 2009). Muscle is not an active tissue in respect of the accumulation of metals (Mansour and Sidky, 2002; Zlatka *et al.,* 2005).

When the structure of fish muscles were taken into consideration, the contents of investigated heavy metals in fish species were determined to be in the range of 0.01-0.27 μg g^{-1} dry wt. for Cd, 2.79-6.88 μg g^{-1} dry wt. for Cu, 12.87-31.28 μg g^{-1} dry wt. for Fe, 0.29-0.91 μg g^{-1} dry wt. for Ni, 0.01-1.42 μg g^{-1} dry wt. for Pb, 10.64-44.82 μg g^{-1} dry wt. for Zn, 0.51-1.72 μg g^{-1} dry wt. for Al. The order of the levels of the heavy metals obtained from the three different fish species in Sinop is Zn > Fe > Cu > Al > Pb > Ni > Cd. The maximum metal accumulation levels in the species were found in *E. encrasicolus for* Zn, Cu, Pb, Ni and Cd; *T. trachurus* for Fe; *M. barbatus* for Al, while the minumum metal accumulation levels in the species were found in *T. trachurus* for Cd, Pb, Ni and Al; *M. barbatus* for Cu, Zn and Fe, respectively (Table III). *E. encrasicolus* is a fish fed with zooplankton. According to their ecology and food habits, fish continuously migrate from one region to the other. It is also known that metal concentrations in fish tissues are related to the pollution level of environmental conditions. It was found that plankton feeding fish contain much higher concentrations of heavy metals than bottom feeding fish (Topping, 1973).

Table II.- Heavy metal contents (µg g^{-1} dry weight) in total tissues of *M. barbatus, T. trachurus* and *E. encrasicolus* from Black Sea.

Species	Samples No	Cd (X±SD)	Cu (X±SD)	Fe (X±SD)	Ni (X±SD)	Pb (X±SD)	Zn (X±SD)	Al (X±SD)
M. barbatus	1	0.36±0.01^{abB}	11.02±0.24^{abC}	402.16±2.90^{abB}	9.38±0.24^{abC}	12.84±1.07^{abB}	54.42±5.18^{aA}	18.47±1.93^{bA}
	2	0.35±0.19^{abB}	6.87±0.99^{aC}	144.82±1.53^{aB}	5.48±0.25^{aC}	24.18±1.73^{bB}	50.21±1.20^{aA}	5.01±0.20^{abA}
	3	0.61±0.32^{bB}	10.32±0.98^{aC}	312.48±1.93^{abB}	9.34±0.64^{bC}	12.81±0,92^{bB}	54.66±3.20^{aA}	18.35±1.61^{bA}
	4	0.53±0.09^{abB}	12.40±1.22^{abC}	621.93±5.29^{bB}	8.75±1.08^{abC}	17.64±0.03^{bB}	55.60±1.91^{aA}	31.27±3.52^{abA}
	5	1.25±0.00^{abB}	11.90±0.38^{abC}	424.71±5.17^{abB}	9.59±1.03^{abC}	14.77±0.00^{abB}	52.46±068^{aA}	20.64±0.55^{abA}
	6	0.79±0.13^{abB}	10.70±0.51^{abC}	629.19±3.78^{bB}	12.89±1.40^{bC}	4.88±0.06^{abB}	51.79±4.27^{aA}	25.96±0.93^{abA}
	7	0.33±0.05^{aB}	21.00±2.37^{bC}	343.40±1.69^{abB}	10.97±0.03^{bC}	7.85±0.82^{abB}	48.76±2.29^{aA}	14.39±0.30^{abA}
	8	0.38±0.04^{abB}	12.69±1.74^{abC}	364.68±3.54^{abB}	10.38±0.59^{bC}	10.29±2.49^{aB}	46.13±0.31^{aA}	15.56±2.68^{aA}
T. trachurus	1	0.52±0.00^{abA}	2.68±0.68^{abA}	45.70±0.88^{abA}	4.71±0.65^{abA}	5.44±1.37^{abA}	57.88±1.29^{aB}	8.04± 0.73^{bA}
	2	0.13±0.06^{abA}	3.34±0.10^{aA}	41.26±3.49^{aA}	3.66±0.79^{aA}	2.39±0.67^{bA}	66.29±1.00^{aB}	8.83±0.46^{abA}
	3	0.27±0.01^{Ba}	3.96±0.44^{aA}	48.87±2.78^{abA}	6.56±0.00^{bA}	3.98±0.00^{bA}	76.14±2.26^{aB}	20.42±0.88^{bA}
	4	0.35±0.00^{abA}	3.57±0.7^{abA}	47.15±5.54^{bA}	3.20±0.65^{abA}	6.96±0.09^{bA}	64.29±2.41^{aB}	10.22±1.70^{abA}
	5	0.08±0.06^{abA}	4.07±0.24^{abA}	49.65±3.33^{abA}	4.59±0.31^{abA}	4.30±0.76^{abA}	68.49±3.66^{aB}	11.53±0.67^{abA}
	6	0.36±0.01^{abA}	3.81±0.05^{abA}	51.29±0.04^{bA}	3.07±0.21^{bA}	9.11±0.97^{abA}	72.12±3.83^{aB}	11.78±0.11^{abA}
	7	0.10±0.04^{aA}	3.88±0.16^{bA}	48.19±1.09^{abA}	3.92±0.12^{bA}	4.66±1.18^{abA}	60.06±2.90^{aB}	11.14±1.98^{abA}
	8	0.19±0.00^{abA}	4.03±0.40^{abA}	57.03±6.09^{abA}	4.49±0.67^{bA}	2.65±0.00^{aA}	66.58±3.26^{aB}	12.61±0.54^{aA}
E. encrasicolus	1	0.55±0.01^{abAB}	7.67±0.88^{abB}	72.62±4.94^{abA}	7.03±0.58^{abB}	8.66±0.02^{abB}	78.05±3.25^{aB}	61.11±0.10^{bB}
	2	0.51±0.08^{abAB}	4.75±0.03^{aB}	58.06±3.44^{aA}	7.15±0.42^{aB}	21.53±0.04^{bB}	72.90±2.61^{aB}	46.67±1.27^{abB}
	3	0.84±0.21^{abAB}	5.04±0.62^{abB}	82.00±6.48^{abA}	6.95±0.57^{bB}	20.80±1.75^{bB}	68.64±3.65^{aB}	47.30±1.93^{bB}
	4	0.75±0.10^{abAB}	5.65±0.19^{abB}	53.28±2.39^{bA}	6.70±0.25^{abB}	23.82±0.96^{bB}	54.91±3.62^{aB}	20.92±1.47^{abB}
	5	0.25±0.01^{abAB}	6.75±0.94^{abB}	55.53±4.44^{abA}	7.69±0.55^{abB}	16.15±0.58^{abB}	57.87±3.95^{aB}	25.59±2.57^{abB}
	6	0.08±0.03^{abAB}	6.95±0.08^{abB}	73.17±4.34^{bA}	7.95±0.44^{bB}	5.42±0.05^{abB}	79.67±2.07^{aB}	19.32±2.80^{abB}
	7	0.12±0.01^{aAB}	6.17±0.50^{bB}	80.00±1.62^{abA}	8.09±1.24^{bB}	17.84±0.58^{abB}	88.45±3.14^{aB}	22.99±3.7^{abB}
	8	0.17±0.03^{abAB}	7.01±0.02 ^{abB}	70.01±0.20^{abA}	7.43±0.00^{bB}	4.80±0.12^{aB}	76.19±0.00^{aB}	15.64±0.06^{aB}

a, b in samples; A,B and C in fish species = The same letters beside the means within columns for fish species and heavy metals indicate the values are not significantly different ($P>0.05$); The means within columns for fish species and heavy metals with different letters significantly differ ($P < 0.05$), [expressed as mean concentration (X) ± standard deviation(SD)].

Table III.- Heavy metal contents (µg g^{-1} dry weight) in muscle tissues of *M. barbatus, T. trachurus* and *E. encrasicolus* from Black Sea.

Species	Samples No:	Cd (X±SD)	Cu (X±SD)	Fe (X±SD)	Ni (X±SD)	Pb (X±SD)	Zn (X±SD)	Al (X±SD)
M. barbatus	1	0.10±0.03^{aAB}	3.36±0.51^{aA}	17.80±2.28^{aB}	0.39±0.01^{aC}	0.22±0.04^{aB}	13.00±0.07^{aA}	1.72±0.22^{aA}
	2	0.13±0.03^{aAB}	2.79±0.13^{abA}	12.87±2.13^{aB}	0.54±0.10^{abC}	0.14±0.03^{abB}	11.93±0.77^{aA}	1.12±0.22^{aA}
	3	0.04±0.00^{aAB}	2.85±0.07^{aA}	16.41±2.59^{aB}	0.44±0.02^{abC}	0.45±0.01^{abB}	10.64±0.45^{aA}	1.16±0.16^{aA}
	4	0.18±0.03^{aAB}	5.45±0.35^{bA}	20.81±2.62^{aB}	0.59±0.07^{abC}	0.21±0.02^{aB}	19.53±1.77^{aA}	1.71±0.01^{aA}
	5	0.12±0.01^{aAB}	3.15±0.21^{abA}	19.09±2.79^{aB}	0.44±0.00^{abC}	0.29±0.00^{bB}	12.97±0.86^{aA}	0.68±0.01^{aA}
	6	0.03±0.01^{aAB}	2.91±0.22^{abA}	20.31±0.02^{aB}	0.42±0.05^{abC}	0.01±0.00^{aB}	12.45±0.34^{aA}	1.32±0.07^{aA}
	7	0.19±0.01^{aAB}	3.96±0.30^{bA}	18.86±3.52^{aB}	0.51±0.05^{bC}	0.01±0.00^{abB}	13.81±0.57^{aA}	0.96±0.10^{aA}
	8	0.06±0.01^{aAB}	4.43±0.63^{bA}	19.10±1.45^{aB}	0.45±0.02^{abC}	0.44±0.04^{abB}	13.79±0.02^{aA}	0.71±0.03^{aA}
T. trachurus	1	0.01±0.00^{aA}	3.48±0.01^{aA}	19.65±0.20^{aA}	0.49±0.01^{aA}	0.04±0.00^{aA}	24.52±0.17^{aB}	0.99±0.00^{aA}
	2	0.06±0.02^{aA}	4.50±0.13^{abA}	21.13±2.86^{aA}	0.36±0.02^{abA}	0.02±0.00^{abA}	21.09±2.18^{aB}	0.81±0.02^{aA}
	3	0.08±0.02^{aA}	3.25±0.12^{aA}	24.34±0.32^{aA}	0.67±0.06^{abA}	0.71±0.05^{abA}	19.83±0.48^{aB}	0.51±0.06^{aA}
	4	0.04±0.00^{aA}	4.61±0.36^{bA}	31.28±2.84^{aA}	0.40±0.06^{abA}	0.53±0.05^{aA}	24.17±0.39^{aB}	0.81±0.01^{aA}
	5	0.04±0.01^{aA}	3.88±0.55^{abA}	23.85±2.20^{aA}	0.30±0.02^{abA}	1.05±0.22^{bA}	20.45±1.44^{aB}	0.94±0.05^{aA}
	6	0.13±0.02^{aA}	4.80±0.08^{abA}	30.73±1.59^{aA}	0.29±0.03^{abA}	0.44±0.01^{aA}	25.10±0.71^{aB}	0.82±0.02^{aA}
	7	0.05±0.01^{aA}	4.64±0.13^{bA}	30.71±1.01^{aA}	0.64±0.12^{bA}	1.08±0.00^{abA}	19.98±1.67^{aB}	0.76±0.02^{aA}
	8	0.01±0.00^{aA}	4.58±0.17^{bA}	30.72±0.82^{aA}	0.45±0.04^{abA}	0.15±0.01^{abA}	27.39±0.97^{aB}	0.80±0.03^{aA}
E. encrasicolus	1	0.05±0.01^{aB}	3.86±0.02^{aB}	22.18±1.16^{aA}	0.47±0.02^{aB}	0.88±0.00^{aB}	40.28±0.67^{aB}	0.59±0.05^{aB}
	2	0.16±0.00^{aB}	3.10±0.39^{abB}	23.90±3.77^{aA}	0.70±0.09^{abB}	1.23±0.06^{abB}	44.82±3.04^{aB}	0.86±0.08^{aB}
	3	0.27±0.00^{aB}	5.48±0.18^{aB}	18.69±0.03^{aA}	0.49±0.07^{abB}	0.76±0.04^{abB}	40.01±1.91^{aB}	1.08±0.06^{aB}
	4	0.09±0.02^{aB}	5.85±0.24^{bB}	23.20±0.54^{aA}	0.72±0.06^{abB}	0.48±0.04^{aB}	31.96±0.76^{aB}	0.94±0.04^{aB}
	5	0.02±0.01^{aB}	5.37±0.21^{abB}	19.22±0.52^{aA}	0.66±0.06^{abB}	1.27±0.01^{bB}	36.07±1.05^{aB}	0.98±0.04^{aB}
	6	0.23±0.04^{aB}	5.38±0.80^{abB}	18.33±0.59^{aA}	0.76±0.00^{abB}	0.45±0.02^{aB}	42.23±1.17^{aB}	0.84±0.01^{aB}
	7	0.19±0.07^{aB}	6.88±0.81^{bB}	17.27±2.09^{aA}	0.78±0.05^{bB}	0.39±0.00^{abB}	40.79±1.13^{aB}	0.89±0.02^{aB}
	8	0.24±0.04^{aB}	5.61±0.00^{bB}	17.86±0.11^{aA}	0.91±0.00^{abB}	1.42±0.06^{abB}	35.65±1.62^{aB}	0.90±0.03^{aB}

a, b in samples; A,B and C in fish species = The same letters beside the means within columns for fish species and heavy metals indicate the values are not significantly different ($P>0.05$); The means within columns for fish species and heavy metals with different letters significantly differ ($P < 0.05$), [expressed as mean concentration (X) ± standard deviation(SD)].

Table IV.- Heavy metal contents (µg g^{-1} dry weight) in tissues of *M. barbatus, T. trachurus* and *E. encrasicolus* in Turkey.

Species	Cd	Cu	Fe	Ni	Pb	Zn	Al	References
M. barbatus	0.45	0.98	-	-	0.84	106.0	-	Uluozlu *et al*. (2007)
M. barbatus	0.17	0.96	53.2	1.55	0.36	75.5	-	Tuzen (2009)
M. barbatus	0.208	-	21.901*	-	0.727	7.573	-	Turan *et al*. (2009)
M. barbatus	0.02	3.14	-	-	0.92	23.71	-	Nisbet *et al*. (2010)
M. barbatus	0.23	1.4	41.4	-	0.40	17.8	-	Durali *et al*. (2010)
M. barbatus	0.03-0.19	2.79-5.45	12.87-20.81	0.39-0.59	0.11-0.45	10.64-19.53	0.68-1.72	Present study
T. trachurus	0.012	1.79	-	-	0.60	27.70	-	Nisbet *et al*. (2010)
T. trachurus	0.32	0.65	75.7	1.50	0.82	52.7	-	Tuzen (2009)
T. trachurus	0.47	1.52	32.40	-	0.85	12.05	-	Tuzen (2003)
T. trachurus	0.25	1.1	12.8	0.5	0.16	13.2	1.5	Tuzen and Soylak (2007)
T. trachurus	0.50	0.95	-	-	0.68	37.4	-	Uluozlu *et al*. (2007
T. trachurus	0.22	2.4	36.4	-	0.64	25.7	-	Durali *et al*. (2010)
T. trachurus	0.01-0.13	3.25-4.80	19.65-31.28	0.30-0.64	0.02-1.08	19.98-27.39	0.51-0.94	Present study
E. encrasicolus	0.20	1.94	10.45	-	0.38	17.38	-	Tuzen (2003)
E. encrasicolus	0.65	0.95	-	-	0.35	40.2	-	Uluozlu *et al*. (2007)
*E. encrasicolus***	0.12	1.77	30.3	0.64	0.40	34.4	0.80	Tuzen and Soylak (2007)
E. encrasicolus	0.124	-	-	-	0.329	25.416	95.313	Turan *et al*. (2009)
E. encrasicolus	0.27	0.96	75.7	1.93	0.30	38.8	-	Tuzen (2009)
E. encrasicolus	0.035	2.73	-	-	0.70	26.25	-	Nisbet *et al*. (2010)
E. encrasicolus	0.02-0.27	3.10-6.88	17.27-23.90	0.47-0.91	0.39-1.42	31.96-44.82	0.59-1.08	Present study

*from Mediterranean Sea, **metals in canned fish marketed.

Table V. Correlations between heavy metal contents in fish species.

	Al	Cd	Cu	Fe	Ni	Pb
Cd	0.097					
Cu	-0.005	0.300**				
Fe	-0.243*	-0.280*	0.194			
Ni	-0.122	0.410***	0.431***	-0.166		
Pb	-0.295**	0.121	0.210	0.168	0.399***	
Zn	-0.260*	0.376***	0.538***	0.126	0.471***	0.535***

(*)P ≤0,05 , (**)P ≤0,01 , (***)P ≤0,001

The lowest and the highest Cd levels of muscles in the fish species were 0.03-0.19 µg g^{-1} dry wt., 0.01-0.13 µg g^{-1} dry wt., 0.02-0.27 µg g^{-1} dry wt. in *M. barbatus*, *T. trachurus* and *E. encrasicolus*, respectively. Statistical analysis of Cd concentrations in tissues displayed a significant difference between *T. trachurus* and *E. encrasicolus* (P<0.05). The Cd concentration of muscle in *E. encrasicolus* was below the detection limit. Turkish Food Codex (2011) indicate that maximum level is 0.30 mg/kg wet wt. for Cd, whereas the Cd concentration in muscle of other fish samples was higher than the detection limit. Commission Regulation of the European Union [Official Journal of the European Union (EC), 2006] and Turkish Food Codex (2011) indicate that maximum level is 0.05 mg/kg wet wt for Cd. However, the maximum Cd level permitted is 0.5 mg/kg for Food and Agriculture Organization (FAO, 1983), and all Cd levels in analyzed fish samples were found to be lower than the permissible legal limits.

The minimum and maximum Cu accumulation levels were found in *M. barbatus*, *T. trachurus* and *E. encrasicolus*, as 2.79-5.45 µg g^{-1}, 3.25-4.80 µg g^{-1} and 3.10-6.88 µg g^{-1}, respectively. Statistical analysis of Cu concentrations in muscle tissues found a significant difference among the three fish (P<0.05). Cu is essential for good health, but a very high absorption can create health problems, such as the damage of internal organs (Ikem and Egiebor, 2005). The maximum Cu level permitted for fish is 10 mg/kg, 20 mg/kg and 30 mg/kg according to the Commission Regulation (EC, 2006), Turkish Food Codex (2011), and FAO (1983)/ World Health Organization (WHO, 1996) limits, respectively. Cu levels in analyzed fish samples were found to be lower than internationally accepted limits. Furthermore, the values of these metals found in the investigated fish species are below the acceptable levels for human consumption. Mean Fe concentrations in the fish muscles accumulated in the order of *T. trachurus* > *E. encrasicolus* > *M. barbatus,* whereas, mean Fe concentrations in the fish samples accumulated in the order of *M. barbatus* > *E. encrasicolus* > *T. trachurus* (Tables II, III). Although *M. barbatus* has a lot of heavy metal deposition in total body, Fe accumulation in muscle tissues are lower than in the other fish. *T. trachurus* generally has less heavy metal accumulation in the whole body but Fe accumulation in its muscle tissues is higher than in other fish. These results are explained by the metabolic differences of fish species. There is no information about maximum Fe levels in fish samples in Turkish standards (Anonymous, 2008). The present study showed that the concentration levels of Fe were determined to be 20.81-12.87 µg g^{-1} dry wt. in *M. barbatus,* 31.28-19.65 µg g^{-1} dry wt. in *T. trachurus* and 23.90-17.27 µg g^{-1} dry wt. in *E. encrasicolus.* These Fe values are generally lower than data reported in the literature (Table IV). However, results obtained from this study for Fe are similar to those recorded by Tuzen (2003), Tuzen and Soylak (2007) and Turan *et al.* (2009). It is noticed that Fe contents in the muscles (*T. trachurus)* were significantly (p< 0.05) higher compared to those in the other muscles (*M. barbatus* and *E. encrasicolus).* The level of Fe that can easily be tolerated is likely to decrease because Fe is a metal that is used active in animals.

Mean Pb and Zn concentrations in fish muscles accumulated in the order of *E. encrasicolus* > *T. trachurus* > *M. barbatus,* and the amount of zinc concentrations in the whole body of the fish is ordered in the same way.

Mean Cd (for fish muscles) and Al (total of fish) concentrations in the fish accumulated in the order of *E. encrasicolus* > *M. barbatus* > *T. trachurus,* whereas, the concentration levels of Ni in the total of fish samples accumulated in the order of *M. barbatus* > *E. encrasicolus* > *T. trachurus* (Tables II, III). Statistical analysis of Pb and Zn concentrations in muscle tissues found a significant difference among the three species (P<0.05). It is noticed that Ni contents in the muscles (*E. encrasicolus)* were significantly (p< 0.05) higher compared to those in the other muscles (*M. barbatus* and *T. trachurus*) (Table III). The maximum level permitted for fish is 0.3 mg/kg, 0.5 mg/kg for Pb and 50 mg/kg, 30 mg/kg for Zn according to Turkish Food Codex (2011) and FAO (1983) limits, respectively. However, the maximum Pb level permitted is 2.0 mg/kg for WHO (1996), and all Pb levels in analyzed fish samples were found to be lower than legal limits. The present study showed that Zn concentrations in muscle tissues were below determining limits. The lowest and highest Al contents of muscle in fish species were found to be 0.68 - 1.72 µg g^{-1} dry wt. in *M. barbatus*, 0.51- 0.94 µg g^{-1} dry wt. in *T. trachurus* and 0.59 – 1.08 µg g^{-1} dry wt. It is reported that maximum Al levels in several food samples are 15 mg/kg (Anonymous, 2002). As shown in Table IV, Al contents of fish species have been determined by some researchers in Turkey. There was significant difference in Ni and Zn concentrations in muscle tissues among the three fish (P<0.05). The bottom-dwelling fish is *M. barbatus*. Hence, it can be expected that the accumulation of heavy metal is higher in *M. barbatus* than in the other fish (*T. trachurus* and *E. encrasicolus). M. barbatus* had the highest metal accumulation in the whole body. The heavy metal concentration in sediments is several times higher than the water column (Mendil and Uluozlu, 2007). Large amount of metals accumulate in sediments but because of the solvent quality and mobility of water, they can pass through the water column again. *M. barbatus* being a bottom dweller fish has high contact with sediments and thus has the highest accumulation of metals in its tissue (Tabinda *et al*., 2013). On the contrary, *E. encrasicolus* had the highest level of metal accumulation in muscle tissues except for Al and Fe. Yılmaz (2003) reported that concentrations of heavy metals was higher in fish skin than in muscles tissues. The reason for high metal concentrations in small fish could be due to the metal complex with the mucus in skin that is impossible to be purified completely from fish tissue before the analysis. Thus, for small fish the skin may be an important site for the uptake of metals due to their high surface area to body ratio.

Table V shows values of correlation coefficients among metal concentrations. The negative correlations between Fe and Al, Cd; Zn and Al were r values of -0.243, -0.280-0.260 ($P \leq 0{,}05$), respectively. There is a positive correlation between Cu and Cd (r = 0. 300), whereas a negative correlation for Pb and Al (r = -0. 295) at the $P \leq 0.01$ level. Ni is correlated strongly and positively with Cd, Cu and Pb ($P \leq 0.001$), with corresponding r values of 0.410, 0.431 and 0.399, respectively. Zn is also correlated strongly and positively with Cd, Cu, Ni and Pb ($P \leq 0.001$), with corresponding r values of 0.376, 0.538, 0.471 and 0.535, respectively. Uluturhan and Kücüksezgin (2007) reported that there was a strong positive correlation of Zn with Cu and Cd in Aegean Sea. Also, Turkmen *et al.* (2004) have stated that there is a positive correlation between water pollution and the heavy metal concentration in Iskenderun bay. They thought that this was because of the fact that these metals came from the same sources.

CONCLUSIONS

Heavy metal pollution in the aquatic environment is determined by measuring its concentration in water, sediment and the organisms. The present study showed that heavy metal content in the whole fish body is high, compared to muscle tissue alone. The concentration of heavy metals in the muscle tissues were generally low and within the expected ranges. This finding shows that these heavy metals are all within the limits, as these fishes are extensively used for human consumption. Both the aquatic environment and sediment in the Black Sea are under the influence of anthropogenic metals, therefore metal concentrations have been increasing in the natural surroundings day by day.

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