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Metal Levels in Tissues of the European Anchovy, *Engraulis encrasicolus* L., 1758, and Picarel, *Spicara smaris* L., 1758, from Black, Marmara and Aegean Seas

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Abstract Levels of metals in commercially important two species, *Engraulis encrasicolus* L., 1758 and *Spicara smaris* L, 1758 from Black, Marmara and Aegean seas, Turkey was evaluated. The concentration ranges of cadmium, cobalt, chromium, copper, iron, manganese, nickel, lead and zinc in the samples were 0.01–0.07, 0.01–0.08, 0.09–0.51, 0.21–8.58, 11.9–169, 0.14–2.82, 0.06–1.51, 0.12–0.87, 7.12–45.6 mg kg^{-1} for muscles, and 0.06–0.69, 0.06–0.53, 0.28–2.97, 0.99–30.7, 55.2–316, 0.72–9.67, 0.47–11.6, 0.26–3.38, 12.5–145 mg kg^{-1} for livers, respectively. The present study show that although different metals were present in the samples at different levels but within the maximum residual levels prescribed by the TKB and FAO the specimens from these areas, in general, are safe for human consumption.

Keywords European anchovy · Picarel · Metals · Turkish coastal waters

Heavy metals can be accumulated by marine organisms through a variety of pathways, including respiration, adsorption and ingestion (Zhou et al. 2001). Over the last few decades the marine environment has been contaminated by persistent pollutants of agriculture and industrial origin. Heavy metal contamination has been identified as a concern in coastal environment, due to discharges from industrial wastes, agricultural and urban sewage. Fish are good indicators for the long term monitoring of metal accumulation in the marine environment. Therefore, numerous studies have been carried out on metal accumulation in different fish species (Andres et al. 2000; Türkmen et al. 2005; Türkmen at al. 2006; Dural et al. 2007; Uluozlu et al. 2007). In addition, many species of marine benthic fish have been shown to reflect ambient metal concentrations (Hornung and Ramelow 1987; Romeoa et al. 1999). The aim of this study is to determine the levels of metals (Cd, Cu, Cr, Pb, Co, Zn, Fe, Ni and Mn) in the muscle and liver tissues of two commercially valuable fish species *Engraulis encrasicolus* and *Spicara smaris* from coastal waters of the Black, Marmara and Aegean seas in order to assess spatial trends of the fish habitat quality.

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Materials and Methods

Fish samples were collected from six stations from the coastal waters of Black, Marmara and Aegean seas, Turkey between January and May 2005. These sampling sites are Trabzon coasts (TRB), Sinop coasts (SNP) and Bartın coasts (BRT) in the Black Sea; Yalova coasts (YLV) in Marmara Sea, northern Aegean Sea (NAS) and central Aegean Sea (CAS) in Aegean Sea (Fig. 1). Two fish species (66 specimens) were examined in this study. These species are European anchovy (*Engraulis encrasicolus*) and picarel (*Spicara smaris*). Total length and weight of the samples brought to laboratory on ice after collection were measured to the nearest millimeter and gram before dissection. Approximately 0.5 g sample of muscle and entire liver (for small fish, the livers of 3–4 samples were pooled) from each fish were dissected, washed with distilled water,

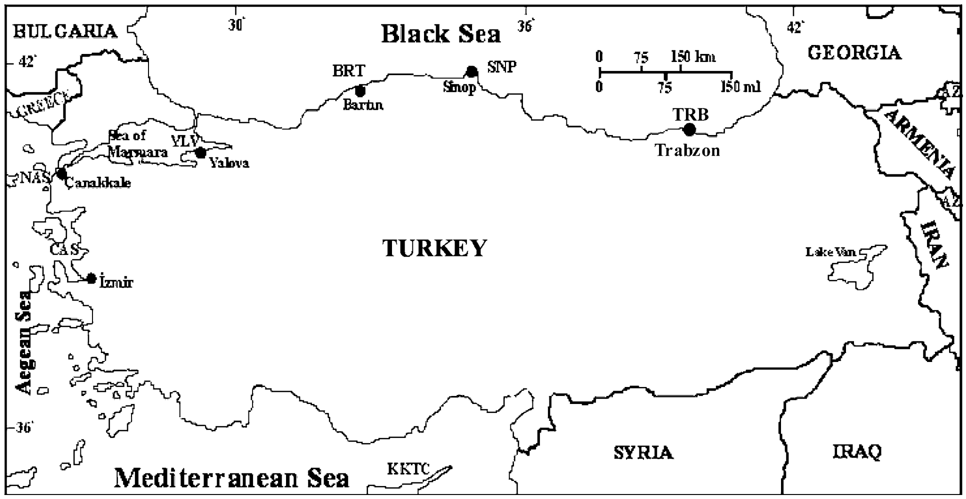

Fig. 1 The sampling locations from the Black, Marmara and Aegean seas (Sampling locations are TRB: Trabzon, SNP: Sinop, BRT: Bartın, YLV: Yalova, NAS: North Aegean Sea, CAS: Central Aegean Sea)

weighed, packed in polyethylene bags and stored at −18°C until chemical analysis. Tissues were homogenized and muscle samples (0.5 g) were digested with 10 mL of 1 N HNO_3 in Teflon vessels in microwave oven (CEM MARS-5 Closed Vessel Microwave Digestion System) using the following microwave digestion program; pressure 200 psi, ramp time 25 min., temperature 210°C, maximum power 300 W, hold time 10 min. After cooling, residue was transferred to 25 mL volumetric flasks and diluted to level with deionized water. Because of low weight, liver samples were digested with 4 mL of 1 N HNO_3 in glass vessels on a hot plate at 100°C for 12 h. The residue was transferred in to 10 mL volumetric flasks and diluted to level with deionized water. Before analysis, the samples were filtered through a 0.45 µm membrane filter. Sample blanks were prepared in the laboratory in a similar manner to the field samples. Metal contents were expressed as mg kg^{-1} wet weight. All samples were analyzed three times for Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) (Varian Model-Liberty Series II). Standard solutions were prepared from stock solutions (Merck, multi element standard). A Dorm-2 certified dogfish tissue was used as the calibration verification standard. Recoveries between 90% and 110% were accepted to validate the calibration. The results showed good agreement between the certified and the analytical values, the recovery of elements being partially complete for most of them. To test the differences between stations, one way ANOVA and following Post hoc test (Tukey) was performed ($p < 0.05$).

Results and Discussion

This study examined the concentrations of Cd, Cu, Cr, Pb, Co, Zn, Fe, Ni and Mn in European anchovy, *Engraulis encrasicolus*, and picarel, *Spicara smaris* from five sites in the coastal waters of the Black, Marmara and Aegean seas. The metal levels in muscle and liver tissues of totally 66 fish analyzed in this study were presented in Tables 1 and 2. As shown in tables, iron has the highest levels in both muscle and liver of both species (except zinc in tissues of *Engraulis encrasicolus* from BRT). Since, one of the purposes of this study is to compare metal levels at several sampling sites, levels of metal concentrations (mg kg^{-1} wet weight) and their comparisons in fish species from different sites were done and shown in Tables 1 and 2.

The lowest and highest Cd levels as mg kg^{-1} in *E. encrasicolus* were 0.02 in SNP and 0.07 in NAS for muscles, and 0.06 in SNP and 0.24 in BRT for livers, in *S. smaris*, 0.01 in YLV and 0.03 in NAS for muscles, and 0.07 in NAS and 0.69 in CAS. Cd levels in the literature have been reported as 0.20–0.55 for muscles in İskenderun Bay, north eastern Mediterranean sea (Türkmen et al. 2006), 0.03–0.12 for muscles and 0.02–0.35 for livers of fish in Tuzla Lagoon, Mediterranean region, Turkey (Dural et al. 2007), 0.1–0.48 for muscles of fish from Black Sea, Turkey (Tüzen 2003). Cadmium levels in livers of *E. encrasicolus* from BRT and of *S. smaris* from TRB and CAS were found to be higher than Turkish permissible limit that is 0.1 mg kg^{-1} for cadmium (TKB 2002). On the other hand, according to Nauen (1983) the maximum permissible cadmium levels for fish are 0.05–5.5 mg kg^{-1}. Our values were in agreement with literature and Nauen (1983).

The minimum and maximum Co levels as mg kg^{-1} in *E. encrasicolus* were 0.01 in YLV and 0.08 in BRT for muscles, and 0.06 in YLV and 0.53 in BRT for livers, in *S. smaris*, 0.01 in NAS and 0.04 in TRB and CAS for muscles, and 0.07 in NAS and 0.23 in CAS for livers. Co levels reported in the literature were in the range of 0.006–0.244 for muscles of fish from the coastal waters of the Caspian Sea (Anan et al. 2005), 0.003–0.015 for livers of

Table 1 Mean metal levels with standard errors in the tissues of the European anchovy, *Engraulis encrasicolus* and comparison of the sites (μg g^{-1} wet wt)*

Metal	Tissues	Sites				
		TRB	SNP	BRT	YLV	NAS
Cd	Muscle	0.03 ± 0.01[ab]	0.02 ± 0.00[a]	0.06 ± 0.02[b]	0.03 ± 0.01[ab]	0.07 ± 0.02[b]
	Liver	0.07 ± 0.02[a]	0.06 ± 0.01[a]	0.24 ± 0.09[b]	0.07 ± 0.01[a]	0.10 ± 0.01[a]
Co	Muscle	0.07 ± 0.03[a]	0.06 ± 0.01[a]	0.08 ± 0.01[a]	0.01 ± 0.00[b]	0.05 ± 0.02[ab]
	Liver	0.19 ± 0.05[a]	0.11 ± 0.02[a]	0.53 ± 0.19[b]	0.06 ± 0.02[a]	0.10 ± 0.03[a]
Cr	Muscle	0.17 ± 0.05[a]	0.15 ± 0.03[a]	0.09 ± 0.02[a]	0.22 ± 0.05[ab]	0.36 ± 0.05[b]
	Liver	0.28 ± 0.06[a]	0.29 ± 0.06[a]	0.51 ± 0.05[ab]	0.60 ± 0.11[b]	0.64 ± 0.12[b]
Cu	Muscle	0.88 ± 0.10[a]	1.12 ± 0.16[a]	8.58 ± 2.15[b]	0.60 ± 0.09[a]	0.21 ± 0.10[a]
	Liver	1.08 ± 0.20[a]	1.27 ± 0.20[a]	30.7 ± 7.54[b]	0.99 ± 0.14[a]	3.60 ± 1.06[a]
Fe	Muscle	44.4 ± 9.23[a]	35.7 ± 9.81[a]	35.9 ± 12.1[a]	11.9 ± 1.16[a]	20.1 ± 7.74[a]
	Liver	188 ± 76.9[a]	78.0 ± 11.5[ab]	124 ± 19.9[ab]	55.2 ± 11.7[b]	75.9 ± 11.2[ab]
Mn	Muscle	0.76 ± 0.13[a]	0.70 ± 0.12[a]	2.82 ± 1.12[b]	0.49 ± 0.03[a]	0.62 ± 0.17[a]
	Liver	1.11 ± 0.20[a]	1.53 ± 0.36[a]	9.67 ± 2.65[b]	0.72 ± 0.02[a]	1.15 ± 0.18[a]
Ni	Muscle	1.51 ± 0.26[a]	0.63 ± 0.19[ab]	0.51 ± 0.12[b]	0.14 ± 0.03[b]	1.49 ± 0.53[a]
	Liver	2.87 ± 0.78[ab]	5.10 ± 0.59[a]	1.19 ± 0.10[b]	0.47 ± 0.12[b]	4.36 ± 1.32[a]
Pb	Muscle	0.12 ± 0.03[a]	0.27 ± 0.05[a]	0.87 ± 0.40[b]	0.37 ± 0.04[ab]	0.36 ± 0.08[ab]
	Liver	0.47 ± 0.13[a]	0.74 ± 0.19[a]	3.38 ± 0.55[b]	0.88 ± 0.20[a]	0.44 ± 0.05[a]
Zn	Muscle	10.8 ± 1.29[a]	10.6 ± 0.88[a]	45.6 ± 22.1[b]	9.29 ± 1.17[a]	12.9 ± 1.40[a]
	Liver	14.1 ± 2.31[a]	12.5 ± 0.96[a]	145 ± 38.0[b]	17.0 ± 1.20[a]	21.0 ± 2.26[a]

*Horizontally, letters a, b and c show differences among sites. Means with the same letter are not statistically significant, $p > 0.05$

Table 2 Mean metal levels with standard errors in the tissues of the picarel, *Spicara smaris* and comparison of the sites (μg g^{-1} wet wt)*

Metal	Tissues	Sites			
		TRB	YLV	NAS	CAS
Cd	Muscle	0.02 ± 0.00[a]	0.01 ± 0.00[a]	0.03 ± 0.02[a]	0.02 ± 0.00[a]
	Liver	0.23 ± 0.07[a]	0.09 ± 0.02[a]	0.07 ± 0.03[a]	0.69 ± 0.18[b]
Co	Muscle	0.04 ± 0.01[a]	0.03 ± 0.01[a]	0.01 ± 0.00[a]	0.04 ± 0.01[a]
	Liver	0.08 ± 0.03[a]	0.11 ± 0.01[a]	0.07 ± 0.01[a]	0.23 ± 0.03[b]
Cr	Muscle	0.09 ± 0.04[a]	0.15 ± 0.04[a]	0.51 ± 0.07[b]	0.16 ± 0.04[a]
	Liver	0.33 ± 0.08[a]	1.43 ± 0.35[b]	0.63 ± 0.08[a]	2.97 ± 0.17[c]
Cu	Muscle	0.83 ± 0.10[ab]	0.35 ± 0.06[a]	0.60 ± 0.08[ab]	1.35 ± 0.58[b]
	Liver	1.86 ± 0.22[a]	1.45 ± 0.47[a]	21.7 ± 8.61[b]	12.5 ± 4.66[c]
Fe	Muscle	32.2 ± 8.00[a]	17.3 ± 3.53[a]	14.3 ± 4.62[a]	169 ± 34.1[b]
	Liver	75.7 ± 14.8[a]	151 ± 28.8[b]	51.2 ± 9.52[a]	316 ± 31.5[c]
Mn	Muscle	0.39 ± 0.05[a]	0.14 ± 0.03[b]	0.18 ± 0.06[b]	0.75 ± 0.08[c]
	Liver	0.72 ± 0.09[a]	0.32 ± 0.03[a]	1.78 ± 0.88[a]	4.46 ± 0.87[b]
Ni	Muscle	0.25 ± 0.07[ab]	0.06 ± 0.02[a]	1.32 ± 0.26[c]	0.70 ± 0.12[b]
	Liver	5.71 ± 1.04[a]	0.20 ± 0.02[b]	10.2 ± 5.28[c]	11.6 ± 4.69[c]
Pb	Muscle	0.15 ± 0.04[a]	0.35 ± 0.09[a]	0.45 ± 0.17[a]	0.38 ± 0.06[a]
	Liver	1.01 ± 0.19[a]	0.90 ± 0.31[a]	0.26 ± 0.06[b]	2.48 ± 0.47[c]
Zn	Muscle	12.2 ± 2.63[a]	7.25 ± 0.61[b]	10.8 ± 0.89[ab]	7.12 ± 1.20[b]
	Liver	18.5 ± 2.38[a]	24.2 ± 5.85[a]	17.9 ± 0.76[a]	45.8 ± 3.13[b]

* Horizontally, letters a, b and c show differences among sites. Means with the same letter are not statistically significant, $p > 0.05$

fish from Mediterranean Sea region (Türkmen and Ciminli 2007). Our cobalt levels were in agreement with literature values. There is no information about cobalt limits in fish tissues in Turkish standards (TKB 2002).

The lowest and highest Cr levels as mg kg^{-1} in *E. encrasicolus* were 0.09 in BRT and 0.36 in NAS for muscles, and 0.28 in TRB and 0.64 in NAS for livers, in *S. smaris*, 0.09 in TRB and 0.51 in NAS for muscles, and 0.33 in TRB and 2.97 in CAS. Cr levels reported for fish in the literature were 0.08–1.4 for muscles of fish from Caspian Sea (Anan et al. 2005), 0.59–1.69 for muscles of fish from the İskenderun bay (Türkmen et al. 2006), 0.95–1.98 for muscles of fish from the Black and Aegean seas (Uluozlu et al. 2007). Although there is no information about maximum Cr levels in fish in Turkish standards (TKB 2002), the maximum level reported by Nauen (1983) for fish are 1.0 mg kg^{-1}. Cr levels in YLV and CAS for livers were found to be higher than permissible limits reported by Nauen (1983).

The minimum and maximum Cu levels as mg kg^{-1} in *E. encrasicolus* were 0.21 in NAS and 8.58 in BRT for muscles, and 0.99 in YLV and 30.7 in BRT for livers, in *S. smaris*, 0.35 in YLV and 1.35 in CAS for muscles, and 1.45 in YLV and 21.7 NAS for livers. Co levels in the literature were 1.57 in muscles of fish from İskenderun bay (Türkmen et al. 2005), 0.26–0.82 in muscles and 0.35–12.0 in livers of fish from Tuzla Lagoon, Mediterranean sea region (Dural et al. 2007), 0.84–1.83 for muscles of fish from Black and Aegean seas (Uluozlu et al. 2007). Our results were generally in agreement with literature. The permissible Cu level for fish is 20 mg kg^{-1} according to TKB (2002). Although maximum Cu levels in muscles of both species were found to be lower than those in TKB (2002), the level in liver of *E. encrasicolus* in BRT were higher than it. On the other hand, according to Nauen (1983) the maximum permissible Cu levels are 10.0–100 mg kg^{-1} for fish, and our results were in agreement with it.

The lowest and highest Fe levels as mg kg^{-1} in *E. encrasicolus* were 11.9 in YLV and 44.4 in TRB for muscles, and 55.2 in YLV and 188 in TRB for livers, in *S. smaris*, 14.3 in NAS and 169 in CAS for muscles, and 51.2 in NAS and 316 in CAS. Fe contents were in the range of 8.87–18.8 in muscles of fish from İskenderun bay (Türkmen et al. 2006), 7.16–16.5 in muscles and 48.1–384 in livers of fish from Tuzla Lagoon, Mediterranean sea region (Dural et al. 2007), 1.49–3.69 in muscles and 19.5–21.6 in livers of fish from Mediterranean sea region (Türkmen and Ciminli 2007). Fe levels were generally in agreement with literature. There is no information about maximum permissible iron concentrations in fish tissues in Turkish standards (TKB 2002).

The minimum and maximum Mn contents as mg kg^{-1} in *E. encrasicolus* were found as 0.49 in YLV and 2.82 in BRT for muscles, and 0.72 in YLV and 9.67 in BRT for livers, in *S. smaris*, 0.14 in YLV and 0.75 in CAS for muscles, and 0.32 in YLV and 4.46 in CAS for livers. Mn levels in the literature have been reported in the range of 0.09–9.23 in muscles of fish from coastal waters of Caspian sea (Anan et al. 2005), 1.3–3.1 in muscles of fish from İskenderun Bay, north eastern Mediterranean sea (Türkmen et al. 2006), 0.07–0.45 in muscles and 0.89–3.32 in livers (Türkmen and Ciminli 2007). There is no information about maximum permissible Mn concentrations for fish tissues in Turkish standards (TKB 2002).

The lowest and highest Ni levels as mg kg^{-1} in *E. encrasicolus* were found as 0.14 in YLV and 1.51 in TRB for muscles, and 0.47 in YLV and 5.10 in SNP for livers, in *S. smaris*, 0.06 in YLV and 1.32 in NAS for muscles, and 0.20 in YLV and 11.6 in CAS for livers. Ni contents in the literature have been reported in the range of 0.06–0.39 in muscles of fish Ria de Averio, Portugal (Perez Cid et al. 2001), 0.66–1.59 in muscles of fish from İskenderun bay, Mediterranean sea (Türkmen et al. 2006), 0.009–0.011 in muscles and 0.07–0.10 in livers of fish from Mediterranean region (Türkmen and Ciminli, 2007). There is no information about maximum permissible nickel concentrations in fish tissues in Turkish standards (TKB 2002).

The minimum and maximum Pb contents as mg kg^{-1} in *E. encrasicolus* were 0.12 in TRB and 0.87 in BRT for muscles, and 0.44 in NAS and 3.38 in BRT for livers, in *S. smaris*, 0.15 in TRB and 0.45 in NAS for muscles, and 0.26 in NAS and 2.48 in CAS for livers. Pb levels in the literature have been reported in the range of 0.01–0.15 in muscles of fish from Ria de Averio (Perez Cid et al. 2001), 0.40–2.44 in muscles and 1.41–3.92 in livers of fish from Tuzla Lagoon (Dural et al. 2007), 0.33–0.93 in muscles of fish from Black and Aegean seas (Uluozlu et al. 2007), 0.008–0.014 in muscles and 1.35–4.39 in livers of fish from Mediterranean sea region (Türkmen and Ciminli 2007). The permissible lead concentration for fish is 1.0 mg kg^{-1} according to TKB (2002). Although lead levels in muscles for both species were lower than Turkish permissible limit, lead levels in livers of *E. encrasicolus* in BRT and *S. smaris* in CAS were higher than it (TKB 2002). On the other hand, according to Nauen (1983) the maximum permissible lead levels are 0.5–6.0 mg kg^{-1} for fish, and our results were in agreement with it.

The lowest and highest zinc levels as mg kg^{-1} in *E. encrasicolus* were in the range of 9.29 in YLV and 45.6 in BRT for muscles, and 12.5 SNP and 145 in BRT for livers, in *S. smaris*, 7.12 in CAS and 12.2 in TRB for muscles, and 17.9 in NAS and 45.8 in CAS for livers. Zinc contents in the literature have been reported in the range of 4.71–23.1 for muscles of fish from Ria de Averio, Portugal (Perez Cid et al. 2001), 12.5–201 for muscles of fish from Caspian Sea (Anan et al. 2005), 4.36 for muscles of fish from İskenderun bay, Turkey (Türkmen et al. 2005), 35.4–201 for muscles of fish from Black and Aegean seas (Uluozlu et al. 2007). Although zinc levels in muscles of both species from all sites were lower than Turkish permissible limit, zinc levels in livers of *E. encrasicolus* from BRT in this study were found to be higher than Turkish permissible limit which is 50 mg kg^{-1} for fish. On the other hand, according to Nauen (1983) the maximum permissible lead levels are 30–100 mg kg^{-1} for fish, zinc levels in livers of *E. encrasicolus* from BRT in this study were also higher than it. In *E. encrasicolus*, the differences between stations were not statistically significant for Fe in muscles, in *S. smaris*, for Cd, Co and Pb in muscles ($p > 0.05$).

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References

Anan Y, Kunito T, Tanabe S, Mitrofanov I, Aubrey DG (2005) Trace element accumulation in fishes collected from coastal water of the Caspian Sea. Mar Pollut Bull 51:882–888

Andres S, Ribeyre F, Tourencq JN, Boudou A (2000) Interspecific comparison of cadmium and zinc contamination in the organs of four fish species along a polymetallic pollution gradient (Lot River, France). Sci Total Environ 248:11–25

Dural M, Göksu MZL, Özak AA (2007) Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. Food Chem 102:415–421

Hornung H, Ramelow G (1987) Distribution of Cd, Cr, Cu and Zn in Eastern Mediterranean fish. Mar Pollut Bull 18:45–49

Nauen CE (1983) Compilation of legal limits for hazardous substances in fish and fishery products. FAO Fish Circular 764:102

Perez Cid B, Boia C, Pombo L, Rebelo E (2001) Determination of trace metals in fish species of the Ria de Aveiro (Portugal) by electrothermal atomic absorption spectrometry. Food Chem 75:93–100

Romeoa M, Siaub Y, Sidoumou Z, Gnassia-Barelli M (1999) Heavy metals distribution in different fish species from the Mauritania coast. Sci Total Environ 232:169–175

TKB (2002) Fisheries laws and regulations. Ministry of agriculture and rural affairs, conservation and control general management. Ankara, Turkey

Türkmen M, Ciminli C (2007) Determination of metals in fish and mussel species by inductively coupled plasma-atomic emission spectrometry. Food Chem 103:670–675

Türkmen A, Türkmen M, Tepe Y, Akyurt İ (2005) Heavy metals in three commercially valuable fish species from İskenderun Bay, Northern East Mediterranean Sea, Turkey Food Chem 91:167–172

Türkmen A, Türkmen M, Tepe Y, Mazlum Y, Oymael S (2006) Heavy Metal levels in blue crab (*Callinectes sapidus)* and Mullet (*Mugil cephalus*) in İskenderun Bay (North Eastern Mediterranean, Turkey). B Environ Contam Tox 77:186–193

Tüzen M (2003) Determination of heavy metals in fish samples of the middle Black Sea (Turkey) by graphite furnace atomic absorption spectrometry. Food Chem 27:521–526

Uluozlu OD, Tuzen M, Mendil D, Soylak M (2007) Trace metal content in nine species of fish from the Black and Aegean Seas, Turkey. Food Chem 104:835–840

Zhou JL, Salvador SM, Liu YP, Sequeria M (2001) Heavy metals in the tissues of dolphins (*Delphinus delphis*) stranded on the Portuguese coast. Sci Total Environ 273:61–76