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Trace metal content in nine species of fish from the Black and Aegean Seas, Turkey

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Abstract

Trace metal content of nine fish species harvested from the Black and Aegean Seas were determined by microwave digestion and atomic absorption spectroscopy (MD–AAS). Verification of the MD–AAS method was demonstrated by analysis of standard reference material (NRCC-DORM-2 dogfish muscle). Trace metal content in fish samples were 0.73–1.83 μg/g for copper, 0.45–0.90 μg/g for cadmium, 0.33–0.93 μg/g for lead, 35.4–106 μg/g for zinc, 1.28–7.40 μg/g for manganese, 68.6–163 μg/g for iron, 0.95–1.98 μg/g for chromium, and 1.92–5.68 μg/g for nickel. The levels of lead and cadmium in fish samples were higher than the recommended legal limits for human consumption.

Keywords: Trace metal; Fish; Atomic absorption spectroscopy; Black Sea; Aegean Sea; Turkey

1. Introduction

The effects of heavy metals on human health and the environment is of great interest today, especially for aquatic food products (Sasmaz & Yaman, 2006; Kornekova, Skalicka, & Nad, 2006; Orak, Altun, & Ercag, 2005; Ranau, Oehlenschlager, & Steinhart, 2001). Heavy metals can be classified as potentially toxic (arsenic, cadmium, lead, mercury, etc.), probably essential (nickel, vanadium, cobalt) and essential (copper, zinc, iron, manganese) (Munoz-Olivas & Camara, 2001; Kheradmand, Kamali, Fathipour, Barzegar, & Goltapeh, 2006). Toxic elements can be very harmful even at low concentration when ingested over a long time period. The essential metals can also produce toxic effects when their intake is excessive (Celik & Oehlenschlager, 2007).

Heavy metal pollution of the marine environment has long been recognised as a serious environmental concern. In the sea, pollutants are potentially accumulated in marine organisms and sediments, and subsequently transferred to man through the food chain (Tuzen, 2003). For this reason, determination of the chemical quality of aquatic organisms, particularly the contents of trace metals in fish is extremely important for human health (Dural, Göksu, & Ozak, 2007). Levels of heavy metals in fish samples have been widely reported in the literature (Celik & Oehlenschlager, 2004; Ikem & Egiebor, 2005; Yilmaz, Ozdemir, Demirak, & Tuna, 2007; Turkmen, Turkmen, Tepe, & Akyurt, 2005). There is limited information on trace metal content of fish from the Black and Aegean Seas. Therefore, the aim of this study was to determine the trace metal contents found in the more popular fish species consumed in this region of Turkey i.e., copper, cadmium, lead, zinc, manganese, iron, chromium, nickel.

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2. Materials and methods

2.1. Sampling

Nine different fish species (54 samples) were collected from random commercial catches: European anchovy (*Engraulis encrasicholus*), Whiting (*Merlangius merlangus*), red mullet (*Mullus barbatus*), bluefish (*Pomatomus saltor*), Atlantic horse mackerel (*Trachurus trachurus*), flathead mullet (*Mugil cephalus*), and Atlantic bonito (*Sarda sarda*) from the Black Sea, and black scorpionfish (*Scorpaena porcus*), and gilthead seabream (*Sparus aurata*) from the Aegean Sea caught during 2005.

2.2. Reagents

All reagents were of analytical reagent grade, unless otherwise stated. Double deionised water (Milli-Q Millipore 18.2 $M\Omega\,cm^{-1}$ resistivity) was used for all dilutions. HNO_3, H_2O_2, and HCI were of Suprapur quality (E. Merck, Darmstadt, Germany). All the plastic and glassware were cleaned by soaking in dilute HNO_3 (1/9, v/v) and were rinsed with distilled water prior to use. The element standard solutions used for calibration were produced by diluting a stock solution of 1000 mg/l of the given element, supplied by Sigma Chemical Co. (St. Louis, MO).

2.3. Apparatus

A Perkin Elmer AAnalyst 700 atomic absorption spectrometer equipped with HGA graphite furnace and with deuterium background correction was used. Copper, zinc, manganese and iron were determined in an air/acetylene flame. The operating parameters for working elements were set as recommended by the manufacturer (Table 1). For flame measurements, a 10 cm long slot-burner head, a lamp and an air/acetylene flame were used. Chromium, nickel, lead and cadmium were determined in a graphite furnace. For graphite furnace measurements, argon was used as inert gas. Pyrolytic-coated graphite tubes with a platform were used. Samples were injected into the graphite furnace using a Perkin Elmer AS-800 autosampler. The atomic absorption signal was measured in peak height mode against a calibration curve.

A Milestone Ethos D microwave closed system (maximum pressure 1450 psi, maximum temperature 300 °C) was used for digestion.

2.4. Microwave digestion

One gram of sample was digested with 6 ml of concentrated HNO_3 (Suprapure, Merck, Darmstadt, Germany) and 2 ml of concentrated H_2O_2 (Merck) in a microwave digestion system and diluted to 10 ml with double deionised water. A blank digest was carried out in the same way (digestion conditions for microwave system were applied as 2 min at 250 W, 2 min at 0 W, 6 min at 250 W, 5 min at 400 W, 8 min at 550 W, vent: 8 min).

The accuracy of our microwave digestion/atomic absorption method was verified by analysis of certified reference materials (NRCC-DORM-2 dogfish muscle).

2.5. Statistical analysis

The whole data were subjected to a statistical analysis and correlation matrices were produced to examine the inter-relationships between the investigated trace metal concentrations of the samples. Student's *t*-test was employed to estimate the significance of values.

3. Results and discussion

Trace metal concentrations were determined on a dry weight basis with relative standard deviations of less than

Table 1
Instrumental analytical conditions of investigated elements

Element	Acetylene (L/min)	Air (L/min)	Wavelength (nm)	Slit width (nm)
Conditions for atomic absorption spectrometry				
Fe	2.0	17.0	248.3	0.2
Cu	2.0	17.0	324.8	0.7
Zn	2.0	17.0	213.9	0.7
Mn	2.0	17.0	279.5	0.2
Instrumental conditions	Pb	Cd	Ni	Cr
Conditions for graphite furnace atomic absorption spectrometry				
Argon flow (ml/min)	250	250	250	250
Sample volume (μl)	20	20	20	20
Modifier (μl)	5	10	5	5
Heating program temperature °C (ramp time (s), hold time (s))				
Drying 1	100 (5, 20)	100 (5, 20)	100 (5, 20)	100 (5, 20)
Drying 2	140 (15, 15)	140 (15, 15)	140 (15, 15)	140 (15, 15)
Ashing	700 (10, 20)	850 (10, 20)	1300 (10, 20)	1600 (10, 20)
Atomisation	1800 (0, 5)	1650 (0, 5)	2500 (0, 5)	2500 (0, 5)
Cleaning	2600 (1, 3)	2600 (1, 3)	2600 (1, 3)	2600 (1, 3)

10%. We demonstrated accuracy of 95–101% in our method by means of trace metal determination in the standard reference material, within 95% confidence levels. The results are shown in Table 2. Lead, cadmium, iron, copper, manganese, zinc, chromium, and nickel were chosen as representative trace metals indices of environmental pollution. A summary of the trace metal content of nine species of fish is given in Table 3. The contents of investigated trace metals in fish species were found to be in the range of 0.73–1.83 μg/g for copper, 0.45–0.90 μg/g for cadmium, 0.33–0.93 μg/g for lead, 35.4–106 μg/g for zinc, 1.28–7.40 μg/g for manganese, 68.6–163 μg/g for iron, 0.95–1.98 μg/g for chromium, and 1.92–5.68 μg/g for nickel.

The lowest and highest copper levels in fish species were 0.73 μg/g in *S. porcus* and 1.83 μg/g in *P. saltor* (Fig. 1). In the literature copper levels in fish samples have been reported in the range of 0.11–0.97 μg/g in the northeast Atlantic (Celik & Oehlenschlager, 2004), 0.065–4.36 μg/g (Yilmaz et al., 2007), 0.04–5.43 μg/g in Iskenderun Bay, northeast Mediterranean Sea, Turkey (Turkmen et al., 2005). Our copper values are in agreement with literature values. The maximum copper level permitted for fishes is 20 mg/kg according to Turkish Food Codex (Anonymous, 2002). Copper levels in analysed fish samples were found to be lower than the legal limit. The FAO/WHO has set a limit for heavy metal intake based on body weight. For an average adult (60 kg body weight), the provisional tolerable daily intakes (PTDI) for lead, iron, copper and zinc are 214 μg, 48 mg, 3 mg and 60 mg, respectively (Joint FAO/WHO Expert Committee on Food Additives, 1999).

Table 2
Trace metal concentrations in certified reference material (NRCC-DORM-2 Dogfish Muscle; mean of 4 analyses)

Element	Certified value (μg/g)	Our value (μg/g)	Recovery (%)
Fe	142	139 ± 5.4	98
Cu	2.34	2.32 ± 0.10	99
Mn	3.66	3.55 ± 0.12	97
Zn	25.6	25.9 ± 1.6	101
Pb	0.065	0.063 ± 0.004	97
Cd	0.043	0.041 ± 0.003	95
Cr	34.7	34.9 ± 1.4	101
Ni	19.4	19.2 ± 1.1	99

The lowest cadmium content was 0.45 μg/g in *M. cephalus* and *M. barbatus*, while the highest cadmium content was 0.90 μg/g in *S. sarda* (Fig. 1). Cadmium levels have been reported in the range of 0.01–4.16 μg/g dry weight in fish species from Iskenderun Bay (Turkmen et al., 2005), 0.09–0.48 μg/g dry weight in fish samples of the middle Black Sea (Turkey) (Tuzen, 2003), and 0.010–0.084 μg/g (Yilmaz et al., 2007). The maximum cadmium level permitted for sea fishes is 0.1 mg/kg, according to Turkish Food Codex (Anonymous, 2002). Cadmium levels in the analysed fish samples were found to be higher than legal limits. The maximum permissible doses for an adult are 3 mg Pb and 0.5 mg Cd per week, but the recommended doses are only one-fifth of those quantities.

The minimum and maximum lead levels observed were 0.33 μg/g in *E. encrasicholus* and 0.93 μg/g in *M. merlangus* (Fig. 1). Lead contents in the literature have been reported in the range of 0.09–6.95 μg/g dry weight in fish species from Iskenderun Bay (Turkmen et al., 2005), 0.22–0.85 μg/g dry weight in fish samples of the middle Black Sea (Turkey) (Tuzen, 2003), 0.068–0.874 μg/g (Yilmaz et al., 2007). The maximum lead level permitted for sea fishes is 0.4 mg/kg according to Turkish Food Codex (Anonymous, 2002). Generally, lead levels in the analysed fish samples were found to be higher than legal limits.

The lowest and highest zinc levels in fish species were 35.4 μg/g in *P. saltor* and 106 μg/g in *M. barbatus* (Fig. 1). Zinc contents in the literature have been reported in the range of 0.60–11.6 μg/g dry weight in fish species from Iskenderun Bay (Turkmen et al., 2005), 9.50–22.9 μg/g dry weight in fish samples of the middle Black Sea (Turkey) (Tuzen, 2003), 47.2–73.4 μg/g dry weight in fish samples from the freshwater Dhanmondi Lake in Bangladesh (Begum, Amin, Kaneco, & Ohta, 2005), and 2.1–8.7 μg/g in northeast Atlantic (Celik & Oehlenschlager, 2004). The maximum zinc level permitted for fishes is 50 mg/kg, according to Turkish Food Codex (Anonymous, 2002). Zinc levels in *S. aurata*, *S. porcus*, *M. barbatus* fish species were found to be higher than legal limits.

The lowest manganese content was 1.28 μg/g in *P. saltor* while the highest manganese content was 7.40 μg/g in *T. trachurus* (Fig. 1). Manganese contents in the literature

Table 3
Trace metal contents of fish species from Turkey (μg/g); mean of 4 analyses

Fish species	Cu	Cd	Pb	Zn	Mn	Fe	Cr	Ni
European anchovy (*Engraulis encrasicholus*)	0.95 ± 0.08	0.65 ± 0.04	0.33 ± 0.01	40.2 ± 3.2	5.61 ± 0.40	95.6 ± 8.1	1.98 ± 0.10	2.63 ± 0.15
Gilthead seabream (*Sparus aurata*)	0.86 ± 0.05	0.50 ± 0.03	0.62 ± 0.05	56.3 ± 4.5	3.98 ± 0.32	69.7 ± 5.6	1.03 ± 0.10	3.19 ± 0.20
Whiting (*Merlangius merlangus*)	1.25 ± 0.10	0.55 ± 0.04	0.93 ± 0.07	48.6 ± 3.9	1.96 ± 0.10	104 ± 9.8	0.97 ± 0.06	1.92 ± 0.10
Black scorpionfish (*Scorpaena porcus*)	0.73 ± 0.06	0.80 ± 0.06	0.66 ± 0.06	95.3 ± 8.7	4.80 ± 0.40	81.5 ± 7.1	1.47 ± 0.11	3.63 ± 0.25
Red mullet (*Mullus barbatus*)	0.98 ± 0.07	0.45 ± 0.04	0.84 ± 0.07	106 ± 9.1	6.54 ± 0.50	163 ± 12	1.63 ± 0.12	4.34 ± 0.35
Bluefish (*Pomatomus saltor*)	1.83 ± 0.10	0.60 ± 0.05	0.38 ± 0.02	35.4 ± 3.2	1.28 ± 0.10	68.6 ± 5.3	1.92 ± 0.10	3.89 ± 0.30
Atlantic horse mackerel (*Trachurus trachurus*)	0.95 ± 0.04	0.50 ± 0.03	0.68 ± 0.05	37.4 ± 2.9	7.40 ± 0.60	74.3 ± 6.1	0.95 ± 0.07	3.93 ± 0.25
Flathead mullet (*Mugil cephalus*)	1.26 ± 0.10	0.45 ± 0.03	0.61 ± 0.04	40.2 ± 3.3	4.21 ± 0.24	82.7 ± 5.6	0.98 ± 0.08	5.68 ± 0.40
Atlantic bonito (*Sarda sarda*)	0.84 ± 0.05	0.90 ± 0.07	0.76 ± 0.05	48.7 ± 3.7	2.68 ± 0.22	73.5 ± 6.3	1.06 ± 0.10	4.96 ± 0.33

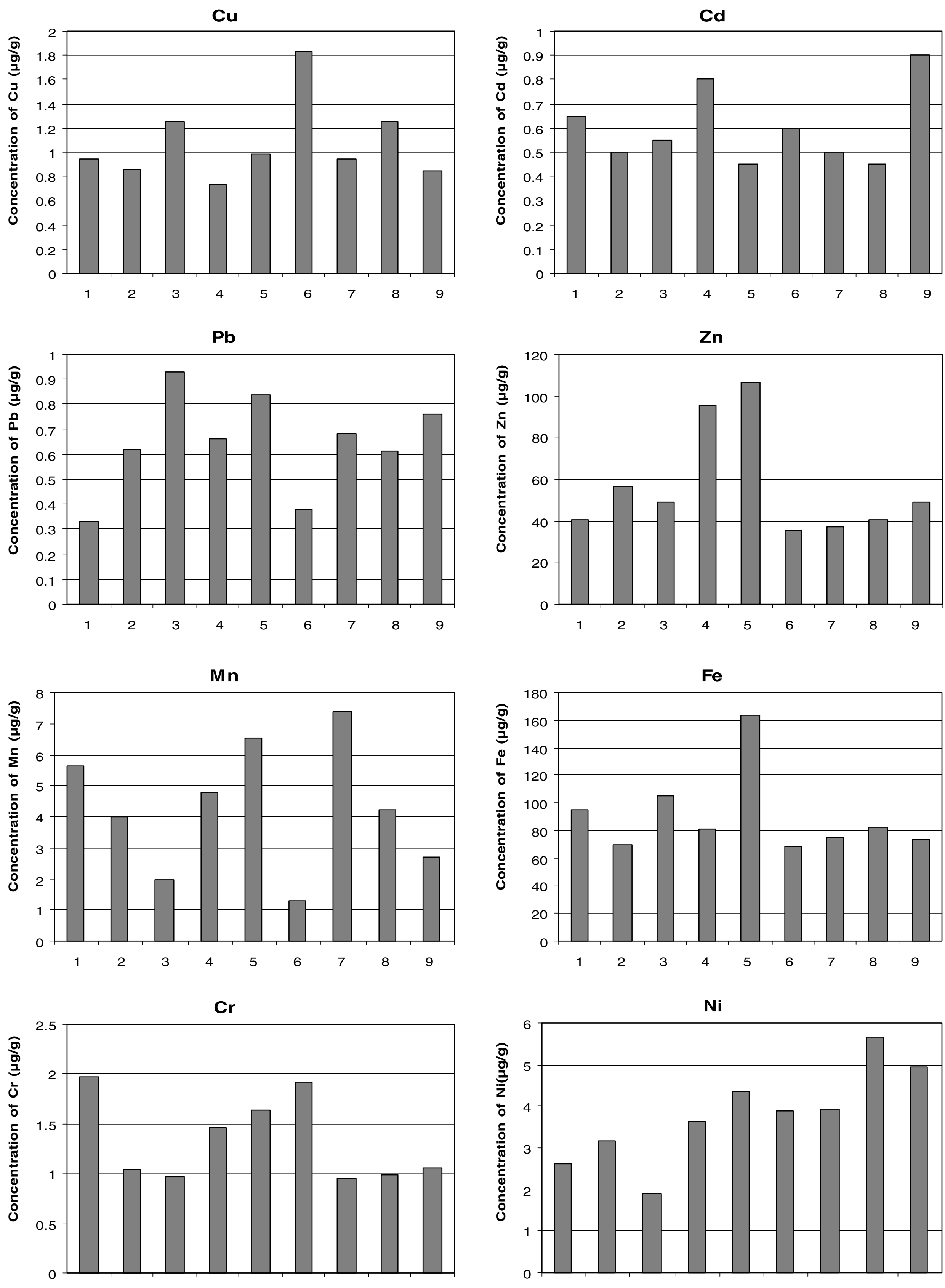

Fig. 1. Copper, cadmium, lead, zinc, manganese, iron, chromium and nickel concentrations in (1) *Engraulis encrasicholus*, (2) *Sparus aurata*, (3) *Merlangius merlangus*, (4) *Scorpaena porcus*, (5) *Mullus barbatus*, (6) *Pomatomus saltor*, (7) *Trachurus trachurus*, (8) *Mugil cephalus* and (9) *Sarda sarda*.

Table 4
Correlations between metal concentrations in fish species

	Cu	Cd	Pb	Zn	Mn	Fe	Cr
Cd	−0.317						
Pb	−0.319	−0.071					
Zn	−0.460	0.058	0.436				
Mn	−0.580	−0.309	0.031	0.341			
Fe	−0.118	−0.355	0.442	0.659	0.371		
Cr	0.295	0.136	−0.645	0.173	0.006	0.252	
Ni	0.027	0.024	−0.001	0.043	0.120	−0.065	−0.188

have been reported in the range of 1.56–3.76 μg/g dry weight in fish samples of the middle Black Sea (Turkey) (Tuzen, 2003), 0.05–4.64 μg/g dry weight in fish species from Iskenderun Bay (Turkmen et al., 2005), 8.8–23.5 μg/g dry weight in fish samples from Dhanmondi Lake (Begum et al., 2005). There is no information about maximum manganese levels in fish samples in Turkish standards (Anonymous, 2002). The US National Academy of Sciences (1980) recommend 2.5–5 mg per day manganese and the WHO (World Health Organization, 1994) recommend 2–9 mg per day for an adult.

The lowest and highest iron levels in fish species were as 68.6 μg/g in *P. saltor* and 163 μg/g in *M. barbatus* (Fig. 1). Iron contents in the literature have been reported in the range of 0.82–27.4 μg/g dry weight in fish species from Iskenderun Bay (Turkmen et al., 2005), 9.52–32.4 μg/g dry weight in fish samples of the middle Black Sea (Turkey) (Tuzen, 2003), 71–186 μg/g dry weight in fish samples from Dhanmondi Lake (Begum et al., 2005). There is no information about maximum iron levels in fish samples in Turkish standards (Anonymous, 2002). It is known that adequate iron in a diet is very important for decreasing the incidence of anaemia. Iron deficiency occurs when the demand for iron is high, e.g., in growth, high menstrual loss, and pregnancy, and the intake is quantitatively inadequate or contains elements that render the iron unavailable for absorption (Lynch & Baynes, 1996). Poor bioavailability is considered to be an important factor leading to iron deficiency in many countries.

The lower chromium content was 0.95 μg/g in *T. trachurus* while the highest chromium content was 1.98 μg/g in *E. encrasicholus* (Fig. 1). Chromium contents in the literature have been reported in the range of 0.07–6.46 μg/g dry weight in fish species from Iskenderun Bay (Turkmen et al., 2005), 0.97–1.70 μg/g in canned fish (Tuzen & Soylak, 2007). There is no information about maximum chromium levels in fish samples in Turkish standards (Anonymous, 2002). Chromium is an essential mineral in humans and has been related to carbohydrate, lipid, and protein metabolism. The recommended daily intake is 50–200 μg (RDA, 1989).

The lowest and highest nickel levels in fish species were 1.92 μg/g in *M. merlangus* and 5.68 μg/g in *M. cephalus* (Fig. 1). Nickel contents have been reported in the range of 0.11–12.9 μg/g dry weight in fish species from Iskenderun Bay (Turkmen et al., 2005), 0.93–2.77 μg/g dry weight in fish samples from Dhanmondi Lake (Begum et al., 2005), 0.42–0.85 μg/g in canned fish (Tuzen & Soylak, 2007). There is no information about maximum chromium levels in fish samples in Turkish standards. The WHO (World Health Organization, 1994) recommends 100–300 μg nickel for daily intake.

The concentration of copper, zinc, manganese, iron, chromium, and nickel were low in comparison with concentrations reported in other studies from the Black and Aegean Seas, but the lead and cadmium levels in some species exceeded the guideline values for food. Fish samples should be analysed more often in Turkey with respect to toxic elements. Generally, *M. merlangus* and *M. barbatus* accumulated trace metals in high concentrations.

A linear regression correlation test was performed to investigate correlations between metal concentrations. The values of correlation coefficients between metal concentrations are given in Table 4. There is a good correlation between iron and zinc ($r = 0.659$), and positive correlations for iron and lead, zinc and lead, manganese and zinc, and iron and manganese, with corresponding r values of 0.442, 0.436, 0.341, and 0.371, respectively. The negative correlations between manganese and copper, zinc and copper, and chromium and lead were −0.580, −0.460, and −0.645, respectively.

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