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Metal Concentrations in Two Bioindicator Fish Species, *Merlangius merlangus*, *Mullus Barbatus*, Captured from the West Black Sea Coasts (Bartin) of Turkey

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Abstract The Black Sea is very vulnerable to originating from land based human activities and its health is equally dependent on the coastal and non-coastal states of its basin. Total concentrations of cadmium, copper, zinc, lead, nickel, aluminum, iron, manganese, boron and chromium concentrations were determined in *Merlangius merlangus* (whiting) and *Mullus barbatus* found in Amasra in the West Coast of the Black Sea (Turkey). The metal contents that were measured in head and muscle was expressed in $\mu g\ g^{-1}$ wet weight. On average, while the highest Fe (344.25 $\mu g\ g^{-1}$), Mn (10.35 $\mu g\ g^{-1}$), Cr (0.96 $\mu g\ g^{-1}$) and Al (76.77 $\mu g\ g^{-1}$) concentrations were measured in the heads of *M. merlangus* and the highest Zn (77.99 $\mu g\ g^{-1}$), Cu(8.53 $\mu g\ g^{-1}$), B (44.83 $\mu g\ g^{-1}$), Ni (1.96 $\mu g\ g^{-1}$), Cd (0.40 $\mu g\ g^{-1}$) and Pb (6.80 $\mu g\ g^{-1}$) concentrations were detected in the muscles of *M. merlangus*. There were significant differences between metal levels of muscles in these two species. In terms of permissible levels reported by WHO FAO and TSE, there is not any risk for human consumption for both *M. merlangus* and *M. barbatus,* for Pb.

Keywords West Black Sea · Metals · Bioindicator · Fish

The Black Sea is the most isolated sea from the World Oceans by being connected to the Oceans via the Mediterranean Sea through the Bosporus, Dardanelle and Gibraltar straits and via the Sea of Azov in the northeast through the Kerch Strait in the northeast. Therefore, the Black Sea is very vulnerable to pressure from land based human activity and its health is equally dependent on the coastal and non-coastal states of its basin. In the Black Sea fluvial discharges, municipal waste waters discharges, industrial wastewater discharges, maritime traffic and offshore oil activities influence the water quality and environment of the Black Sea's coastal waters (Anonymous 2008).

Mean annual catch value is 1,542,593 tons in the Mediterranean and Black Sea Fishing Area (FAO Area Code: 37) in the last 7 years (FAO 2011). Nearly 70% of the total figure has been caught from the Black Sea in this fishing area.

Fish constitute an important source of protein for many people throughout the world (Çiçek et al. 2008). Trace elements may be beneficial or harmful to plants, animals and humanbeings depending on their concentration. There are natural trace components of the aquatic environment but in recent years. Their levels have increased due to industrial, agricultural and mining activities (Turan et al. 2009; Mendil and Uluözlü 2007). Knowledge as regards to the metal concentrations in fish species is of critical importance with respect to both nature management and human consumption of fish (Papagiannis et al. 2004; Amundsen et al. 1997).

Metals available in our environment represent a permanent threat to human health. Due to their characteristics of high toxicity, persistence, and tendency to accumulate in water and sediment, metals and metalloids, when occurring in higher concentrations, become severe poisons for all living organisms when they exist in higher concentrations. The frequency and intensity of water contamination by

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metals mainly depend on human activities. However, impurities may occasionally originate from geological factors. The contamination chain of metals almost always follows the cyclic order: industry, atmosphere, soil, water, phytoplankton, zooplankton, fish, and human (Kadar et al. 2000).

Metals have long been recognized as one of the most important pollutants in Black Sea for a long time. Several studies have been carried out to determine the metal levels in the fish species of Black Sea so far. Recently, the data obtained through these studies have been reviewed by Tüzen (2003), Uluozlu et al. (2007), Tepe et al. (2008), Turan et al. (2009).

Some fish organs and tissues tend to accumulate metals and therefore, they must be omitted from human diets. Muscle is not always the best indicator of body contamination in the whole fish (Schön et al. 2006). As there can be considerable variations in metal concentration between organs (tissues) it is necessary to discuss how the analyzed fish species are prepared and eaten (Has-Schön et al. 2006).

As for some fish species, the whole body of the fish is consumed. Therefore it is important to determine metal concentrations in the different parts of their bodies. Both of the species examined in this study can be consumed with head. Therefore the main goal of our study was to determine total concentrations of total cadmium (Cd), copper (Cu), zinc (Zn), lead (Pb), nickel (Ni), aluminum (Al), iron (Fe), manganese (Mn), boron (B) and chromium (Cr) in the muscles and heads of *M. merlangus* and *M. barbatus* which are two fish species caught in the coasts of Bartin, at the West Black Sea coast of Turkey.

Materials and Methods

In this study conducted on *M. merlangus* and *M. barbatus* these species are described as bioindicator by UNEP and RAC/SPA (1999). They are also benthopelagic and have carnivorous feeding habits. They are mainly fed by fishes, crustaceans, annelids and mollusks (Demirsoy 1993). The lengths and weights of the examined individual samples varied from 10.4–15.3 cm to 13.1–41.3 g for *M. barbatus* and from 12.9–20.5 cm to 16.0–62.3 g for *M. merlangus*.

The fish species were collected from commercial catches in the West Black Sea Coast of Amasra (Turkey, 41°74′90″N, 32°38′9″E) during 2009 fishing season. A total of 31 fish samples of *M. merlangus* and 20 samples of *M. barbatus* were received from commercial fishing boats in February and March. The samples were preserved in plastic boxes filled with ice and transferred to the laboratory. The fishes were washed with distilled water, dried in filter paper and then frozen at −25°C until dissection. Lengths and weights of the fish were measured to the nearest g and mm and the measurements are given in Table 1.

Table 1 Mean wet weight and total length of fish used in this study

Species	n	L ± SD	W ± SD
Merlangius merlangus (whiting)	31	11.94 ± 0.97	19.11 ± 5.20
Mullus barbatus (red mullet)	20	15.04 ± 2.16	27.68 ± 14.01

L Mean length, *W* Mean weight, *SD* Standard deviation

An approximately 2 g of muscle and head sample were dissected from each fish which was examined, all samples were washed with deionized water and digested with concentrated nitric acid and percholoric acid (2:1, v/v) at 60°C for 3 days. All samples were diluted with deionized water and filtered through a 0.45-lm nitrocellulose membrane filter. Some blanks were prepared in the laboratory in a similar manner to the field samples (Alam et al. 2002). The sample preparation and analysis were carried out according to the procedure described by UNEP/FAO/IOC/IAEA reference methods (1984).

All metal concentrations were determined by Perkin Elmer 2100 DV models ICP-OES. The following absorption wavelengths were used; Fe 259.9 nm, Zn 213.9 nm, Cu 324.8 nm, Mn 257.6 nm, B 249.8 nm, Cr 367.7 nm, Ni 221,6 nm, Cd 228.8 nm, Pb 220,4 nm and Al 396.2 nm. The metal concentration detected in fish species were recorded as μg g^{-1} wet weight. All working standard solutions were made from stock solutions (1,000 mg L^{-1}) of all elements, which were supplied by Inorganic Ventures crop, USA. A high quality water, obtained using a Human UP 900 system, was used exclusively. Each sample was analyzed in triplicate. ICP-OES parameters used are summarized in Table 2. Detection limits of elements are Cd 0.0012 mg g^{-1}, Cr 0.0027 mg g^{-1}, Cu 0.0069 mg g^{-1}, Fe 0.0381 mg g^{-1}, Mn 0.001 mg g^{-1}, Pb 0.0078 mg g^{-1}, Zn 0.0015 mg g^{-1}, Al 0.0057 mg g^{-1} and Ni 0.0048 mg g^{-1}.

Table 2 Instrumental parameters of the ICP-OES

Parameter	Value
Power (watt)	1,450
Plasma argon flow rate (l/min)	15
Auxiliary argon flow rate(l/min)	0.2
Nebulizer argon flow rate (l/min)	0.55
Plasma aerosol type	Wet
Nebulizer type	Meinhard type A
Nebulizer set up	Instant
Sprey chember	Perkin Elmer
Sample flow rate (ml/min)	1.5
Replicate	3

The accuracy of analytical procedure was checked through the analysis of standard reference materials. Recovery rates ranged from 99% to 100% for all elements that were examined.

Mean values and standard deviations were calculated for each group. The differences between the metal concentrations detected in the same and different parts of the two different species were analyzed by Student's *t* test (Glantz 1997; Wayne 2005). Probabilities less than 0.05 were considered statistically significant ($p < 0.05$). Statistical analysis of data was carried out using SPSS 19.0 statistical package programs for Windows (Serial number: 10241512).

Results and Discussion

The mean values, standard deviations and range of metals concentrations in studied fish species are given in the Table 3. In both of these species Fe, Mn and Al concentrations in head were statistically higher than muscle ($p < 0.001$); Cu, B, Cd and Pb concentrations in muscle were statistically higher than head ($p < 0.001$). Concentrations of other metals such as Zn, Cr and Ni were not significantly different between head and muscle. All of the analyzed metal concentrations in *M. merluccius* were significantly higher than *M. barbatus* ($p < 0.001$). Muscle of *M. merluccius* displayed 2 (Cu) to 7 times (B, Cr and Al) higher metal concentrations than those detected in muscle of *M. barbatus*. Likewise, head of *M. merluccius* displayed 2 (Mn) to 20 times (Al) higher concentrations than those observed in the head of *M. barbatus*.

It was determined that there were statistically significant differences between the individual samples examined in this study. The metal accumulation in different samples depends on various factors such as level of pollutants in water, sediment and diet, size, sex, life history, behavior and feeding habits etc. (Clearwater et al. 2002; Al-Yousuf et al. 2000; Canlı and Atlı 2003). Fish can potentially accumulate metals by absorption through gills or by consumption of contaminated food and sediment.

In this study it was determined that *M. merluccius* has a higher mean of metal concentration level when compared to *M. barbatus*. Metal levels considerably varied among the species even within the individuals of the same species (Wong et al. 2001). Essential elements are carefully regulated by physiological mechanisms in most organisms (Papagiannis et al. 2004; Eisler 1993). It is reported that bioavailability and toxicity of metals in aquatic organisms depend on the total concentrations of metal in the water and diet of the organisms (Papagiannis et al. 2004; Chen et al. 2000; Rashed 2001). Bioaccumulation occurs both through water and diet transferred to tissues of the organisms. Previous studies have demonstrated that some of the metal concentrations are inversely related to trophic status and diet is the most important route of accumulation in organisms (Papagiannis et al. 2004; Eisler 1993; Fisher and Reinfelder 1995).

Table 3 Concentrations of metals in muscle and head of *M. merluccius* and *M. barbatus* expressed by mean value ± standard deviation and range (μg g^{-1})

Species	Metals	Muscle	Head
Merlangius merlangus	Fe*	83.01 ± 38.14 (59.97–150.74)	344.25 ± 222.37 (104.92–705.72)
	Zn***	77.99 ± 46.91 (37.96–152.67)	51.19 ± 24.37 (28.78–101.72)
	Cu**	8.53 ± 2.14 (6.30–11.83)	4.69 ± 2.01 (2.50–9.19)
	Mn*	2.13 ± 0.30 (1.85–2.60)	10.35 ± 5.65 (5.45–22.69)
	B**	44.83 ± 40.29 (11.98–108.19)	12.04 ± 9.21 (2.13–26.62)
	Cr***	0.92 ± 0.40 (0.63–1.62)	0.96 ± 0.75 (0.21–2.82)
	Ni**	1.96 ± 0.66 (1.25–2.87)	0.69 ± 0.33 (0.23–1.34)
	Cd**	0.40 ± 0.29 (0.15–0.89)	0.17 ± 0.09 (0.07–0.33)
	Pb**	6.80 ± 5.88 (2.69–17.11)	2.00 ± 1.14 (0.54–4.21)
	Al*	17.36 ± 5.21 (11.67–25.88)	76.77 ± 75.84 (15.16–260.59)
Mullus barbatus	Fe*	21.20 ± 12,99 (7.90–39.88)	124.80 ± 84.49 ((19.54–295.61)
	Zn***	16.03 ± 14.05 (3.48–40.72)	18.16 ± 10.08 (3.46–30.23)
	Cu**	4.08 ± 2.79 (1.23–9.21)	1.86 ± 0.93 (0.84–3.54)
	Mn*	0.77 ± 0.47 (0.31–1.53)	4.44 ± 2.51 (0.79–8.10)
	B**	6.73 ± 2.74 (1.35–10.61)	3.09 ± 3.49 (0.52–14.97)
	Cr***	0.14 ± 0.16 (0.02–0.65)	0.10 ± 0.05 (0.02–0.19)
	Ni**	0.63 ± 0.85 (0.09–2.84)	0.07 ± 0.06 (0.01–0.21)
	Cd**	0.11 ± 0.13 (0.02–0.55)	0.03 ± 0.01 (0.01–0.07)
	Pb**	1.11 ± 1.60 (0.09–7.00)	0.29 ± 0.39 (0.02–1.43)
	Al***	2.60 ± 2.79 (0.9–10.98)	3.98 ± 3.76 (0.27–11.35)

* Significantly higher metal concentrations in head than muscle

** Significantly higher metal concentrations in muscle than head

*** No significant differences between metal concentrations in the head and muscle

Table 4 Comparative metal concentrations in the muscle of *M. merluccius* and *M. barbatus* from West Black Sea as measured in previous studies (μg g^{-1})

Species	Cu	Cd	Pb	Zn	Mn	Fe	Cr	Ni	Al	B	References
Merlangius merlangus	1.25	0.55	0.93	48.6	1.96	104	0.97	1.92	–	–	Uluozlu et al. (2007)
	2.26	0.16	1.32	24.2	1.62	259	1.21	5.62	–	–	Tepe et al. (2008)
	–	0.19	0.5	6.03	0.08	4.48	0.14	1.36	86.3	–	Turan et al. (2009)
	8.53	0.40	6.80	77.99	2.13	83.0	0.92	1.96	17.36	44.83	This study
Mullus barbatus	0.98	0.45	0.84	106	6.54	163	1.63	4.34	–	–	Uluozlu et al. (2007)
	–	–	–	–	–	–	–	–	–	–	Tepe et al. (2008)
	–	0.21	0.73	7.57	0.005	21.27	1.06	0.66	9.85	–	Turan et al. (2009)
	4.08	0.11	1.11	16.03	0.77	21.2	0.14	0.63	2.60	6.73	This study

There were statistically significant differences between muscle and head concentrations except for Zn, Cr and Al. In general, different tissues showed different capacities for accumulating metals and may differences may come out when age, feeding habits, locality and even maturity stage are taken into consideration (Has-Schön et al. 2006; Wong et al. 2001).

Generally, metal concentrations were found lower in muscle and skin compared to gills, liver, intestines, gonad etc. (Wong et al. 2001). Therefore it is expected that the head including gills and contains more metals with respect to muscle. However, it was detected in this study only two metal (Fe and Mn) levels of the head were significantly higher than in muscle, while five metals (Cu, B, N, Cd and Pb) concentrations detected in muscle were higher than in the head. In this case, the site of accumulation appears to vary among metals (Wong et al. 2001). Has-Schön et al. (2006) suggested that gills are not suitable as a human food constituent and recommended that the gills must be cut off provided that the head is included in the meal.

The maximum acceptable metal concentration levels laid down in Turkish Food Codex for Cd, Pb, Zn and Cu are 0.05, 0.2, 50, 20 μg g^{-1} (Anonymous 2002) as laid down in Turkish Food Codex and the limits set by FAO (Food and Agricultural Organization) are 0.5, 0.5, 30, 30 μg g^{-1}, respectively (FAO 1983). While Cd level is above acceptable limit of Turkish Food Codex, it is below the level set by FAO. However, Pb level determined in the study was at least two times higher than the acceptable level of FAO and 6 times higher than that given in the Turkish Food Codex for *M. barbatus* and 13.6 times to 34 times for *M. merluccius*, respectively. High Pb concentration is a great risk for human consumption and natural environment. Therefore, firstly the source of the Pb pollution must be determined and then measures should be taken to prevent pollution. On the other hand, attention should also be paid to the high Cd concentrations found in muscle.

Metal concentrations had been previously reported from West Black Sea in several studies listed in Table 4. As can be seen in the table, Cu, Pb, Zn and Mn concentrations of *M. merluccius* were determined to be at the highest levels in this study when compared to the previous ones. In contrast to *M. merluccius*, all metal concentrations of *M. barbatus* were at the lowest levels in this study except for Cu.

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