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Comparison of Metal Accumulation in Fish Species from the Southeastern Black Sea

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Abstract In this study, it was aimed to determine accumulation of the metal concentrations in fish species in different regions which are Sürmene and Yomra bay at southeastern Black sea in Trabzon-Turkey. For this purpose, concentrations of metals (Cr, Co, Ni, Cu, Zn, As, Se, Cd, Sb, Hg, Pb) were measured by inductively coupled plasma mass spectrometry (ICP-MS) in muscle of red mullet (*Mullus barbatus ponticus*) and whiting (*Merlangius merlangus euxinus*). Metal concentrations were found for whiting ranged as follows; 0.35–1.64 (Cr), 0.01–0.09 (Co), 0.01–0.71 (Ni), 0.71–1.45 (Cu), 18.64–68.06 (Zn), 2.05–9.66 (As), 0.15–14.16 (Se), 0.01–0.22 (Cd), 0.01–0.01 (Sb), 0.01–0.15 (Hg), 0.01–0.25 (Pb) and for red mullet as 0.43–1.04 (Cr), 0.05–0.23 (Co), 0.02–0.67 (Ni), 0.74–2.32 (Cu), 19.89–43.50 (Zn), 6.04–24.82 (As), 0.02–9.83 (Se), 0.01–0.11 (Cd), 0.01–0.01 (Sb), 0.07–0.18 (Hg), 0.01–0.20 (Pb) μg g^{-1} dry weight. Metal concentrations in samples were found to be lower than legal limits proposed by Turkish Food Codex (2008), Food and Agricultural Organization (1983), and European Commission (Off J Eur Union L364-5–L364-24, 2006) for human consumption.

Keywords Black sea · Metals · Red mullet · Whiting

Effects of metals in ecosystems and their behavior are quite complicated. In our age the use of metals has been increasing in parallel with industrial development. Metals are discharged into the marine environment through domestic, agriculture, mining, and industrial waste water (Bilandzic et al. 2011; Mendil et al. 2010). They have a great ecological significance due to their toxicity and accumulative behavior (Matta et al. 1999). Aquatic organisms may represent a risk for human health since metal species can be mobilized from the environment and accumulated in aquatic organisms' tissues and subsequently transferred to humans through the food chain (Heier et al. 2009; Matta et al. 1999; Medeiros et al. 2012). For these reasons, determination of metals of aquatic organisms, particularly in fish is extremely important (Dural et al. 2007; Erkan et al. 2011; Jaric et al. 2011; Matta et al. 1999; Ozden et al. 2010).

In the southeastern Black sea main sources of metals are the agriculture, mining and anthropogenic activities (Ergul et al. 2008). Some of the mining activities in the southeastern Black Sea region have been continued but some of was run out. Sürmene bay was exposed to the effect of copper factory waste between years of 1985–1997 and two major streams are carrying anthropogenic and agricultural waste in this area. Because of these features, it can tend to create local pollution. Yomra bay just has been affected by domestic waste and agricultural activities.

Black sea includes a number of economically valuable fish species such as *Mullus barbatus ponticus* (red mullet) and *Merlangius merlangus euxinus* (whiting) (Mendil et al. 2010). Red mullet, a representative benthic fish recommended as a bio indicator is feeding on bottom (demersal), and schooling benthic species. Whiting is a semi pelagic species (Bănaru and Harmelin-Vivien 2009; UNEP and RAC/SPA 1999). Previous studies show that metal concentrations in fish muscles vary widely, depending on the location of capture (Bilandzic et al. 2011). Therefore, the

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objective of the study was to determine of metal concentrations (Cr, Co, Ni, Cu, Zn, As, Se, Cd, Sb, Hg, Pb) in two different fish species caught from two regions that have different characteristic property.

Materials and Methods

Fish samples were collected from Sürmene and Yomra bay at southeastern Black sea in Trabzon-Turkey (Fig. 1). The varied sizes of the fish were collected seasonally from November 2010 to November 2011. Immediately after collection, fish samples were transported to the laboratory in ice-cooled containers. To minimize contamination, all the materials used in the experiments were washed in ultra-pure water. The samples were put in sterile polythene bags and kept in the laboratory deep freezer (−20°C) to prevent deterioration until analyzed. Size, weight and length of the fish samples were measured (Table 1). All fish were classified as large (15 cm), medium (10–15 cm) and small (10 cm). For analysis, muscles on dorsal surface of each fish were taken out. Skin was separated muscle tissue with plastic knife and samples dried with freeze dryer (EYELA-FD5 N, Japan) under vacuum until constant mass weight and homogenized. The temperature control microwave heating device (Milestone Ethosplus, Italy) was used for digestion of the dried fish meat. About 0.5 g homogenized samples were taken and placed into digestion flasks. Ultra-pure concentration HNO_3 and H_2O_2 (7:1 v/v) was added on the samples and heated to 200°C until dissolution. The completely digested samples were allowed to cool to room temperature. Sample preparation was carried out according to the procedure described by Richard (2002) and MILESTONE (2011). After dissolution, samples diluted with ultra-pure water. Cr, Co, Ni, Cu, Zn, As, Se, Cd, Sb, Hg and Pb were determined by ICP-MS (inductively coupled plasma mass spectrometry) (820-MS Varian, Australia). All samples were analyzed three times. Accuracy and reproducibility of the preparation were tested by the analysis of standard reference material (DORM-3 National Research Council, Canada) and blanks along with each set of samples. Certified value and observed value are presented in Table 2. All reagents were used analytical grade.

All data were tested with normality (Shapiro–Wilk) and equal variance test. The data passed and failed from these tests were applied parametric and nonparametric tests, respectively. Parametric (one way ANOVA and *t* test) and nonparametric tests (Kruskal–Wallis and Mann–Whitney

Fig. 1 Fish sampling sites

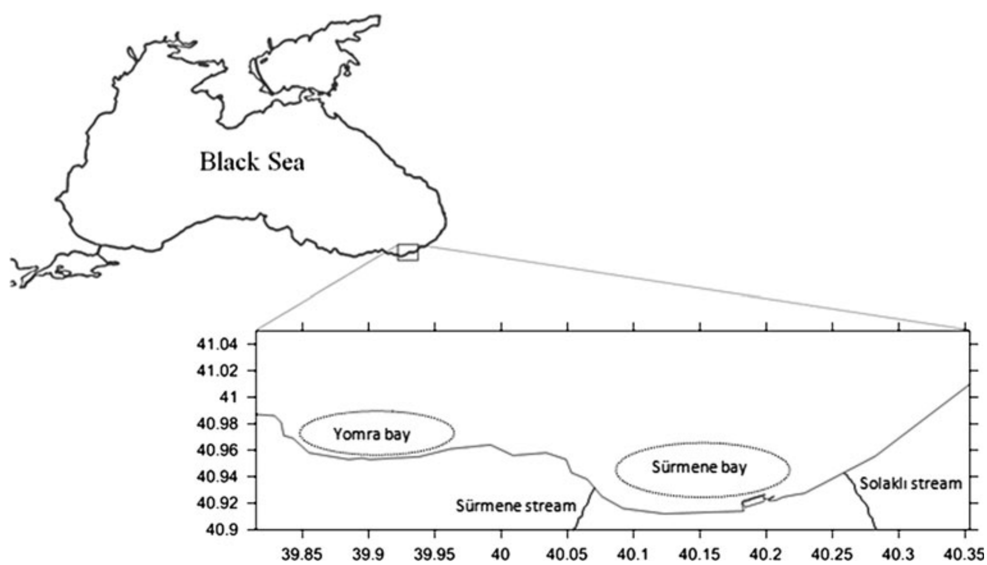

Table 1 Statistic results of fish biometry with standard error (SE)

Species	n		Length (cm)*		Weight (g)*	
	Y	S	Y	S	Y	S
M. merlangus euxinus	144	144	15.2 ± 0.75	15.6 ± 0.83	31.0 ± 4.91	32.5 ± 5.28
Mullus barbatus ponticus	96	96	12.5 ± 0.55	12.9 ± 0.45	21.4 ± 3.20	19.9 ± 2.25

n number of fish, *Y* Yomra, *S* Sürmene

* Mean ± SE

Table 2 Metal concentrations (μg g^{-1} dry wt) in certified reference material (DORM-3)

Metals	Certified value	Observed value	Recovery (%)
Cr	1.89 ± 0.17	1.85 ± 0.19[a]	97.88
Ni	1.28 ± 0.24	1.24 ± 0.22	96.88
Cu	15.5 ± 0.63	14.8 ± 0.43	95.48
Zn	51.3 ± 3.10	49.18 ± 0.89	95.87
As	6.88 ± 0.30	6.70 ± 0.36	97.38
Cd	0.29 ± 0.02	0.28 ± 0.04	96.55
Hg	0.38 ± 0.06	0.35 ± 0.06	91.62
Pb	0.40 ± 0.05	0.38 ± 0.05	95.00

[a] Mean ± standard errors

Rank Sum test) were performed to determine whether there were any significant differences in metal concentrations among sizes and species of fish collected at different sampling locations. In addition, Pearson correlation was carried out to determine relationship between not only metals but also metals and fish sizes. Statistical significance was set to a level of 5 % ($p < 0.05$) (Miller and Miller 2010). Statistical analyses were performed using the SigmaPlot 12 software (Systat Software Inc., Chicago, USA). Additionally, concentration of metals in fish samples were evaluated with permitted limits by TFC (Turkish Food Codex) (2008), FAO (Food and Agricultural Organization) (1983), and EC (European Commission) (2006). All data were given μg g^{-1} dry weight (dry wt).

Results and Discussion

The average of metal contents for whiting and red mullet was given in Table 3. Concentrations of metal values were

Table 3 Concentrations of metals (μg g^{-1} dry wt) in fish species

Metals	Whiting	Red mullet
Cr	0.80 ± 0.07[a]	0.62 ± 0.04[a]
Co	0.03 ± 0.01[a]	0.12 ± 0.01[a]
Ni	0.27 ± 0.06[a]	0.18 ± 0.05[a]
Cu	1.02 ± 0.05[a]	1.12 ± 0.09[a]
Zn	22.76 ± 2.01[a]	27.36 ± 1.95[b]
As	5.65 ± 0.38[a]	13.95 ± 1.37[b]
Se	4.32 ± 0.82[a]	2.83 ± 0.75[a]
Cd	0.04 ± 0.01[a]	0.02 ± 0.01[a]
Sb	nd	nd
Hg	0.05 ± 0.01[a]	0.11 ± 0.01[b]
Pb	0.08 ± 0.03[a]	0.10 ± 0.04[a]

[a,b] Values in the same line followed by different letters are significantly different, mean ± SE

nd Not determined

Zn > As > Se > Cu > Cr > Ni > Pb > Hg > Cd > Co > Sb in whiting while metal values were Zn > As > Se > Cu > Cr > Ni > Co > Hg > Pb > Cd > Sb in red mullet. There were statistically significant differences among species in Co, Zn, As, and Hg concentrations ($p < 0.05$). Co, Zn, As, and Hg concentrations in red mullet were higher than whiting.

In Sürmene and Yomra stations, average concentrations of metals in species were given Table 4. Concentrations of Cr, Co, Cu, Zn, Se, Cd, Hg, and Pb values in whiting of Yomra station were higher than Sürmene station. For whiting, there were not statistically differences except for Co among stations. Concentrations of Cr, Co, Cu, Zn, As, Se, Cd, Hg, and Pb values in red mullet of Sürmene stations were higher than Yomra station. There were statistically differences among stations for Se and Cd for red mullet.

There were statistically differences among species for Co, Zn, As, and Hg. This situation can be associated with the feeding of red mullet at the sea bottom. Sürmene is dirtier due to currently closed but earlier active copper mine.

The results in Table 5 for whiting show that a significant correlation was observed between Co ($r = -0.71$) and Cr ($r = 0.71$) with Ni ($p < 0.05$). Hg correlated with Cr ($r = 0.77$), As ($r = -0.66$), Cd ($r = 0.75$), and Zn ($r = 0.77$). Cd strongly correlated with Cr ($r = 0.71$) and Zn ($r = 0.95$). The results show significant correlations of Cr ($r = 0.65$) and Se ($r = 0.66$) with Zn. Cobalt strongly correlated with Cu ($r = 0.60$). Association of As with fish size show a significant correlation (r = -0.69). As a result of Table 6 for red mullet show that there was strongly and significant correlation between Cd and Cu ($r = 1.00$).

When metal concentrations of whiting were evaluated according to their size groups, there was not any significant difference among metals concentrations except for Cu ($p < 0.05$). Concentration of Cu were small (1.036 μg/g) > medium (1.248 μg/g) > large (0.908 μg/g). When metal concentrations of red mullet were evaluated according to their size groups, there was not any significant difference among metals concentrations ($p < 0.05$).

In this study metal concentration levels compared with reported in other studies from Black sea (Table 7). The concentrations of Ni were lower than compared the other studies (Findik and Cicek 2011; Nisbet et al. 2010; Turan et al. 2009; Tuzen 2009; Uluozlu et al. 2007). In present study, copper values in fish species were lower than earlier reported studies in Black sea coast (Findik and Cicek 2011; Nisbet et al. 2010). Copper concentrations in red mullet were higher but lower than in whiting than other studies (Tuzen 2009; Uluozlu et al. 2007). The maximum copper level permitted for fish is 30 μg g^{-1} according to FAO (1983). Copper levels in samples were found to be lower than legal limits. The Zn concentrations in the species were lower than previous studies (Tuzen 2009; Uluozlu et al.

Table 4 Concentrations of metals (μg g^{-1} dry wt) in fish species of different stations

Metals	Whiting		Red mullet	
	Yomra	Sürmene	Yomra	Sürmene
Cr	0.83 ± 0.12[a]	0.76 ± 0.09[a]	0.54 ± 0.04[a]	0.69 ± 0.06[a]
Co	0.05 ± 0.01[a]	0.02 ± 0.01[a]	0.11 ± 0.01[a]	0.13 ± 0.02[a]
Ni	0.25 ± 0.07[a]	0.29 ± 0.10[a]	0.24 ± 0.91[a]	0.14 ± 0.06[a]
Cu	1.05 ± 0.06[a]	0.99 ± 0.08[a]	1.10 ± 0.02[a]	1.14 ± 0.19[a]
Zn	24.86 ± 3.98[a]	20.67 ± 0.46[a]	26.13 ± 2.13[a]	28.58 ± 3.36[a]
As	5.02 ± 0.53[a]	6.28 ± 0.48[a]	13.43 ± 1.46[a]	14.47 ± 2.42[a]
Se	5.32 ± 1.41[a]	3.48 ± 0.93[a]	1.87 ± 1.21[a]	3.54 ± 0.94[b]
Cd	0.05 ± 0.02[a]	0.03 ± 0.07[a]	0.01 ± 0.01[a]	0.04 ± 0.01[b]
Sb	nd	nd	nd	nd
Hg	0.07 ± 0.01[a]	0.04 ± 0.01[a]	0.11 ± 0.01[a]	0.11 ± 0.01[a]
Pb	0.11 ± 0.04[a]	0.04 ± 0.01[a]	0.09 ± 0.05[a]	0.12 ± 0.08[a]

[a,b] Values in the same line followed by different letters are significantly different, mean ± SE

nd Not determined

Table 5 Correlations between metal concentrations and length in whiting

	Cr	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb
Mean length	0.33	−0.37	0.37	−0.43	0.31	−0.69*	0.01	0.27	0.59	0.21
Cr		−0.32	0.71*	−0.23	0.65*	−0.53	0.28	0.71*	0.77*	−0.42
Co			−0.71*	0.60*	−0.13	0.50	−0.19	0.02	−0.19	0.41
Ni				−0.37	0.48	−0.51	0.46	0.39	0.54	−0.35
Cu					0.04	0.29	−0.19	0.10	−0.38	−0.18
Zn						−0.60	0.66*	0.95*	0.77*	−0.15
As							−0.22	−0.50	−0.66*	0.16
Se								0.45	0.50	0.27
Cd									0.75*	−0.25
Sb									−0.36	−0.42
Hg										0.09

* Correlation is significant at the 0.05 level

Table 6 Correlations between metal concentrations and length in red mullet

	Cr	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb
Mean length	−0.98	−0.75	0.62	−0.58	−0.65	−0.22	−0.49	−0.56	0.95	−0.05
Cr		0.86	−0.46	0.72	0.79	0.02	0.31	0.71	−0.87	−0.14
Co			0.05	0.97	0.99	−0.49	−0.21	0.97	−0.51	−0.62
Ni				0.28	0.19	−0.90	−0.99	0.30	0.84	−0.81
Cu					1.00	−0.68	−0.43	1.00*	−0.29	−0.79
Zn						−0.60	−0.34	0.99	−0.38	−0.72
As							0.96	−0.69	−0.51	0.99
Se								−0.46	−0.73	0.90
Cd									−0.27	−0.80
Sb									−0.34	−0.75
Hg										−0.36

* Correlation is significant at the 0.05 level

2007) but higher than reported study (Turan et al. 2009). This study was compared with reported study (Findik and Cicek 2011), Zn values were higher in red mullet and lower in whiting. The maximum acceptable Zn concentration level for fish is 30 μg g^{-1} in FAO (1983). Zinc levels in samples were found to be lower than legal limits. The As

Table 7 Comparison of the metal concentrations (μg g^{-1} dry weight) in fish from the Turkish Coast of the Black Sea

	Cr	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb	References
Whiting	0.92	–	1.96	8.53	77.99	–	–	0.40	–	6.80	1*
	0.14		1.36	–	6.03		–	0.19	–	0.50	2
	–	–	3.78	3.72	31.34	–	–	0.002	nd	0.58	3
	0.86	–	1.14	1.32	65.40	0.17	0.29	0.21	0.84	0.53	4*
	0.97	–	1.92	1.25	48.60	–	–	0.55	–	0.93	5
	0.80	0.03	0.22	1.02	22.76	5.65	4.32	0.04	0.05	0.08	6
Red mullet	0.14	–	0.63	4.08	16.03	–	–	0.11	–	1.11	1*
	1.06	–	0.66	–	7.57	–	–	0.21	–	0.73	2
	–	–	2.47	3.14	23.71	–	–	0.02	nd	0.92	3
	1.35	–	1.55	0.96	75.50	0.11	0.45	0.17	0.36	0.36	4*
	1.63	–	4.34	0.98	106.00	–	–	0.45	–	0.84	5
	0.62	0.12	0.18	1.12	27.36	13.95	2.83	0.02	0.11	0.10	6
Codex	–	–	–	–	–	–	–	0.05	0.50	0.30	TFC (2008)*
	–	–	–	30.00	30.00	–	–	0.50	0.50	0.50	FAO (1983)*
	–	–	–	–	–	–	–	0.05	0.50	0.30	EC (2006)*

[1] Findik and Cicek (2011); [2] Turan et al. (2009); [3] Nisbet et al. (2010); [4] Tuzen (2009); [5] Uluozlu et al. (2007); [6] This study, * wet weight

values were found to be 5.65 and 13.95 μg g^{-1} in whiting and red mullet respectively. These values are lower than those earlier reported by (Tuzen 2009). Cadmium contents in this study lower than those earlier study (Findik and Cicek 2011; Turan et al. 2009; Tuzen 2009; Uluozlu et al. 2007). The cadmium value is similar to the value (0.02 μg g^{-1}) for red mullet, higher than for whiting (Nisbet et al. 2010). The maximum acceptable cadmium concentration levels are 0.05, 0.5, and 0.05 μg g^{-1} according to TFC (2008), FAO (1983), and EC (2006) respectively. Cadmium levels in analyzed fish samples were found to be lower than legal limits. Concentrations of mercury in fish species were found lower than previous study (Tuzen 2009). The maximum acceptable Hg concentration levels for fish are 0.5, 0.5 (1 for red mullet), 0.5 (1 for red mullet) μg g^{-1} according to FAO (1983), Turkish Food Codex (2008), and EC (2006) respectively. Mercury levels in fish samples were found to be lower than legal limits. Mean lead concentrations were found to be 0.08 and 0.10 μg g^{-1} for whiting and red mullet respectively. These values are lower the earlier reported values (Findik and Cicek 2011; Nisbet et al. 2010; Turan et al. 2009; Tuzen 2009; Uluozlu et al. 2007). The maximum lead levels permitted for fish are 0.3, 0.5, and 0.3 μg g^{-1} according to Turkish Food Codex (2008), FAO (1983), and EC (2006) respectively. Lead levels in fish samples were found to be lower than legal limits.

Accumulation metals in fish muscle are important for human health. In this study, freeze dryer and microwave digestion procedure were chosen for fish sample for more accurate results. The present study shows that average metal concentrations of red mullet in Sürmene bay were higher than Yomra bays samples but there were not found to be statistically differences. Metal levels of the investigated fish were in the permissible safety levels for human consumption.

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