

Metal Accumulations in Water, Sediment, Crab (*Callinectes sapidus*) and Two Fish Species (*Mugil cephalus* and *Anguilla anguilla*) from the Köyceğiz Lagoon System–Turkey: An Index Analysis Approach

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Abstract The concentrations of six metals (Hg, Cd, Cu, Pb, Cr and Zn) individual total metal load (IMBI) values and its relation to condition index were determined in water, sediment and tissues of crab (Callinectes sapidus) and two fish species (Mugil cephalus and Anguilla anguilla) inhabiting Köyceğiz Lagoon System. The average distribution of the IMBI values ranged from 0.033 to 0.265. Distribution patterns of IMBI in species follow the sequence: A. anguilla > M. cephalus > C. sapidus. Results showed that there are positive relationships between species sizes and metal levels in most cases. The concentrations of Pb in muscle in the three studied species were in all cases considerably higher than the maximum levels set by law. Average Cd, Cu and Zn values in M. cephalus were also higher than the limits proposed for fish by FAO/WHO, EC and TFC. Therefore, the human consumption of all analysed species is not recommended.

Keywords Metal index · Pollution · Callinectes sapidus · Mugil cephalus · Anguilla anguilla · Turkey

Metal pollution in aquatic ecosystems has been of great concern due to the increased anthropogenic release of metals into the environment. Metals can be easily accumulated into the aquatic organisms from the water and sediment by the means of food web (Alibabić et al. 2007; Burger

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Fevzi Yilmaz yfevzi@mu.edu.tr et al. 2007). Fishes play a major role in aquatic food-webs dynamics and interaction. They occupy different habitats in the same ecosystem and have different feeding behaviors (Van der Oost et al. 2003). Because of the increasing concern of their nutritional and therapeutic benefits, fish consumption in the world has grown recently. Besides being a good source of protein, fishes are also rich in necessary minerals, vitamins and unsaturated fatty acids (Medeiros et al. 2012).

The European eel (Anguilla anguilla) is a fish species that lives predominantly in freshwater but returns to the Sargasso Sea to reproduce. They spend most of their lifetime in freshwater environments where they become "yellow eels" passing through a growing phase of at least 6 (males) to 8 years (females) A. anguilla is generally considered to be a good biomonitor of freshwater systems because of its various ecological and physiological traits (Esteve et al. 2012). Mullets (e.g. Liza aurata, Liza saliens and Liza ramada), have been considered as key indicators and are generally used in pollution and risk assessment in aquatic systems (Guilherme et al. 2008; Zorita et al. 2008). In addition, M. cephalus is considered as one of the fish species commonly consumed by the local population (Yilmaz 2009). Despite the determination of alterations in the different tissues of crabs starting to receive more attention, crabs are not widely employed as fish species. Blue crabs, Callinectes sapidus are important members of the estuarine food chain due to their high abundance and their multiple role as scavengers, predators and prey. In addition, crabs of the genus Callinectes (Crustacea: Portunidae) are abundant in Köyceğiz Lagoon System. These are the basis for a significant commercial harvest and recreational fishery. The contaminated crab from this area may become a public health concern (Genç and Yilmaz 2015).

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These species, considered the most favorite species by consumers, have different biological needs and show different feeding habits. Therefore, it becomes important to determine the levels of metals commercial species in order to evaluate the possible risk to sea food consumption for human health. The objectives of this study were therefore: (1) To provide a better understanding of the cause-effect relationships to the metal accumulation in several target tissues of eels, flathead mullet and blue crab inhabiting Köyceğiz Lagoon System (2) To quantify the relationship between metal bioaccumulation and well-being (condition) of fishes and crab. (3) To asses individual level of bioaccumulation index (IMBI) (4) to compare the results to maximum acceptable levels established by Turkish and international regulatory agencies.

Materials and Methods

The Köyceğiz Lake is placed at the coast side of the province of Muğla(Southwest Turkey) and connected to the Mediterranean Sea by Dalyan Delta. By and after 1988, the area has been intensely subjected to a rapid increase of touristic activities, mainly related to the case of Iztuzu and loggerhead sea turtle *Caretta caretta* that have mobilized economic demand in the region. The great natural interest of Köyceğiz Lake was also emphasized by the designation of the area as a National Natural Reserve administrated by the Authority of Special Protected Areas (Bayari et al. 1995).

Sampling was conducted seasonally between 2010 and 2011 in Köyceğiz Lagoon system (Fig. 1). M. cephalus is currently the main commercial fish harvested by some of the cooperatives, representing 90% of the total catch. The fish migrate from the Lake to the Mediterranean Sea to breed. As they travel to the sea they are trapped in the delta by the barriers. These migrations happen twice a year in the Summer (late June/beginning of July) and Winter (October), with the Winter migration being the most significant in terms of productivity. During the research, M. cephalus were sampled alive by means of 22 mm mesh size nvlon monofilament gillnets from sampling point (N36°51' 52.48", 28° 38' 05.87"E). Küçük et al. (2005) field work has shown that elvers start entering Muğla Bay in the last week of February until June- July. Research conducted earlier by Küçük et al. (2005) in the Antalya Bay (Neighboring city of Muğla) rivers showed that the first elvers entered the rivers during the last week of March and that recruitment continued until June. A. anguilla samplings were collected with drift nets (vertical mouth opening



Fig. 1 Köyceğiz Lagoon System and sample points

60 cm, total wing length 5 m and mesh size 1 mm), trap nets made out of window screens and fabric fly nets (1 mm mesh net, mouth opening 10 cm, three sections and total wing length 3 m) (N36°49' 22.20", 28°37' 35.66"E). *C. sapidus* has been widely recorded in Mediterranean, especially in its eastern basin (Nehring 2011) though selected as one of the 100 "worst invasive" species in the Mediterranean (Streftaris and Zenetos 2006). *C. sapidus* individuals were sampled by local fishermen with a star trap or a dip net (N36°47'60.02", 28°37'30.63"E).

Samples were taken from every distance in lake and analyzed independently. Water samples were collected at the surface in 40 mL acid-washed polyethylene sample bottles, taking care not to incorporate sediment into the samples and during the sampling, 0.5% concentrated nitric acid was added to the water samples. Water samples were taken from the surface of the stream for metal analyses. At each point, composite sediment samples were collected using standard protocol (U.S. EPA 2001). The sediments were dried at 105°C for 24 h. The dried sediments were passed through a 60 mesh stainless screen to remove larger particles. Ultrapure (Direct-O 8UV Germany) water was used for solution preparation. After caught, the samples were placed into an ice box for transportation to the laboratory, they were then washed with distilled water and kept in a freezer $(-20^{\circ}C)$ before analysis. The liver, muscle and gill tissues were dissected using stainless steel knife which had been cleaned with acetone and distilled water prior to use. The samples were then oven dried to constant weight at 90°C. The Teflon vessel were cleaned, soaked in %5 HNO₃ for more than 1 day than rinsed with ultrapure water and dried. For metal analysis, 0.5 g of sediment sample and 20 mL water sample was treated with 7 mL 70% HNO₃ acid and 3 mL 30% H₂O₂ in a closed Teflon vessel and then digested in a microwave digestion system (Berghof speedway MWS-3+). The digestion flasks were then put on a microwave digestion unit to 120°C (gradually increased) until all the materials were dissolved. The digested solution was then filtered by using Filter papers (Sartorius-Stedim, particle retention = $2-3 \mu m$) and stored in 25 mL polypropylene tubes. All samples were analyzed simultaneously two times for Hg, Cd, Cu, Pb, Cr and Zn by ICP-AES Optima 2000-Perkin Elmer (Inductively Coupled Plasma-Atomic Emission Spectrometry). Detection limits ($\mu g L^{-1}$) were as follows: Hg (0.061), Cd (0.001), Cu (0.014), Pb (0.001), Cr (0.007), Zn (0.006). Standard reference National Research Council Canada SPS-SW1 (for water) and WQB1 (for sediment) - National Water Research Institute were analyzed for metals. Replicate analysis of these reference materials showed good accuracy, with recovery rates for metals between 90%-97% for water and sediments. Standard reference materials for fish DORM- 3 (National Research Council Canada, Ottawa Ontario, Canada) were used and replicate analysis of these reference materials showed good accuracy, with recovery rates for metals between 91% and 109% for fish.

Condition index (CI) was calculated using the following formula as: $CF = W/L^{3*}100$ Where W = fish weight (g) and L = total length (cm) (Hung and Deng 2002).

The bioaccumulation factors (BAF) are commonly used as the criteria for bioaccumulation in the context of identifying and classifying substances that are hazardous to the aquatic environment. The BAF was calculated using the formula: BAF = Concentration of metal in the organism/ Concentration of metal in water.

BSAF were calculated to assess the net bioaccumulation of chemicals by an organism as a result of uptake from environmental sources and processes (Burkhard et al. 2005). Efficiency of metal accumulation in the fish species was evaluated to determine BSAF using the formula (Thomann et al. 1995): BSAF=Concentration of metal in the organism/Concentration of metal in sediments.

Spearman's rho test was also used to correlate metals accumulation. All statistical calculations were performed with SPSS 20.0 for Windows while Origin 8.0 was used to draw graphs. The individual mean (multi-metal) bioaccumulation index (IMBI) was calculated as:

$$IMBI = \frac{\sum_{i=1}^{n} = 1Ci/Cimax}{n}$$

With N the total member of metals, C_i the individual metal concentration of heavy metal, C_{imax} the maximal observed concentration of heavy metal and 0 < IMBI < 1 (Maes et al. 2005).

Results and Discussion

The results of metal concentrations on the water, sediment, fish and blue crab tissues are presented in Table 1.

Water contained the lowest concentration of Hg, Cu, Cr and Zn. Concentrations of metals in the sediment were 100-10000 times higher than those in the water. Some of studies have reported a similar result (Demirak et al. 2006; Kir et al. 2007). The results showed that among all tissues of fish and crab under study liver or hepatopancreas concentrate and accumulate the highest concentration of Hg and Pb. This can be demonstrated by the fact that the liver is generally considered to have a strong metal accumulative potential owing to the activity of metal-binding proteins such as metallothioneins. These proteins can bind Cu, Cd, and Zn, but not Pb, resulting in elevated levels of metals in liver (Uysal et al. 2009; Tapia et al. 2012). Blue crab gill exhibited higher levels of Cu and Cr than in the other fish tissues. Gills are

Table 1 Mean concentrations of metal (µg g	⁻¹) in tissues of fishes and blue crab from	n Köyceğiz Lagoon System (mean \pm SE)
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	Hg	Cd	Cu	Pb	Cr	Zn
Water $(n=32)$	0.001 ± 0.00	1.149±0.34	0.030 ± 0.00	2.059 ± 0.69	0.706 ± 0.28	0.218 ± 0.17
Sediment $(n=32)$	0.169 ± 0.04	1.016 ± 0.10	29.694 ± 3.15	6.805 ± 0.72	367.972±39.64	102.590 ± 31.63
<i>M. cephalus</i> muscle $(n=70)$	0.012 ± 0.00	0.476 ± 0.06	29.901 ± 5.69	0.626 ± 0.11	2.220 ± 0.46	78.897 ± 8.42
<i>M. cephalus</i> gill $(n=70)$	0.011 ± 0.00	0.061 ± 0.01	1.270 ± 0.16	0.682 ± 0.11	2.432 ± 0.53	21.780 ± 3.69
<i>M. cephalus</i> liver $(n = 70)$	0.029 ± 0.00	0.109 ± 0.01	5.435 ± 1.13	0.788 ± 0.10	3.035 ± 0.52	39.301 ± 4.23
A. anguilla Muscle (n=76)	0.14 ± 0.03	0.22 ± 0.03	8.56 ± 3.35	1.07 ± 0.11	1.39 ± 0.16	32.92 ± 4.70
<i>A. anguilla</i> Gill (n=76)	0.09 ± 0.03	0.21 ± 002	6.32 ± 2.02	1.22 ± 0.12	2.00 ± 0.31	41.44 ± 5.55
<i>A. anguilla</i> Liver (n=76)	0.16 ± 0.04	0.24 ± 0.02	22.11 ± 2.99	1.30 ± 0.14	1.50 ± 0.22	53 ± 5.74
C. sapidus muscle $(n=60)$	0.090 ± 0.01	0.161 ± 0.24	18.214 ± 2.60	1.208 ± 0.13	1.813 ± 0.41	43.981 ± 3.44
C. sapidus gill $(n=60)$	0.111 ± 0.02	0.189 ± 0.01	73.495 ± 11.62	2.230 ± 0.29	4.012 ± 1.03	25.852 ± 2.36
C. sapidus hepatopancreas $(n=60)$	0.126 ± 0.03	0.893 ± 0.12	50.169 ± 13.04	1.386 ± 0.13	2.856 ± 0.74	$55.202 \pm 13,21$

an important organ of interest in terms of their ability to uptake heavy metals from the water due to the metalbinding sites located at the tissue's surface (Wang and Rainbow 2008). Muscle tissue is commonly regarded to have low accumulation ability for heavy metals (Tapia et al. 2012). However, this is not always the case. For example, in the present study, Zn concentrations in M. cephalus were higher than those in gill and liver. The concentrations of metals of the gill reflect the concentrations of metals in the waters where the fish live; whereas, the concentrations in liver represent storage of metals (Rao and Padmaja 2000). Thus, the liver and gill in fish are more often recommended as environmental indicator organs of water pollution than any other fish organs (Karadede et al. 2004; Yilmaz et al. 2007). One of the interesting results was that the highest concentration of Cd and Zn is found in the muscle of *M. cephalus* from the Köyceğiz Lake. This is probably related with the trophic characteristics of this species, which being iliophagous fish (Marcovecchio 2004) reflect the metal concentrations in surface and suspended particulate matter, showing high metal concentrations. Blue crabs live on sediments on which they bury from where they mainly feed (Genç and Yilmaz 2015). The high bioaccumulation level of crab could be related to several factors such as, anthropogenic activities, physico-chemical parameters of the aquatic environment. Moreover crabs are less active and their feeding habits, ecological needs, other characteristic behaviors and metabolism are different than other organisms (Türkmen et al. 2006).

We calculated correlation coefficients between metals and allometric parameters (Table 2) to assess pollutant impact on well-being. In A. anguilla significant negative correlations between the CI and metal concentrations were determined only for Zn (r = -0.359, p < 0.01). Length, weight and CI showed significant positive correlations with Cr (r=0.291, p < 0.05, r=0.481 and r=0.339, p < 0.01respectively). There was no significant correlation between CI and metals (except Zn and Cr). We found positive relationships between Hg, Cd, Cr and weight of M. cepha*lus* (r=0.310, r=0.281, p < 0.05 and r=0.390, p < 0.01). Similarly, positive correlations were found between Hg (r=0.329, p<0.01), Pb (r=0.256 p<0.05) and length of M. cephalus. The significant positive correlations between the CI of M. cephalus and metal concentrations were determined only for Cd (r=0.283, p<0.05). Cd and Pb levels of C. sapidus showed positive relationships between length (r=0.588, r=0.341, p<0.01) while weight did not show any significant relationship. The CI of C. sapidus showed positive relationship with Cr (r=0.341, p<0.01) while Cd (r=0.363, p<0.01) showed negative relationship.

Tekin-Özan and Aktan (2012) found positive relationships between Cr, Cu, Fe, Mn, Zn and weight, length of Scomber japonicus (chub mackerel). Canli and Atli

Table 2 Allometric parameters and condition index	Species	Weight (g) (±SD)	Weight (g) (min–max)	Length (cm) (±SD)	Length (cm) (min–max)	CI±SE
	M. cephalus	235.46±93.99	95.5–478	33.30 ± 4.46	25.5–42	0.68 ± 0.40
	A. anguilla	192.24 ± 106.51	38–513	48.06 ± 12.31	25-74.5	0.20 ± 0.16
	C. sapidus	225.55 ± 76.79	121.5-426	17.85 ± 1.65	15-21.5	4.08 ± 1.59

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(2003) reported a positive relationships between Zn and Pb levels in the gill of *M. cephalus* and fish size. Yi and Zhang (2012) found positive correlations between fish size and Zn, Cd, Pb in grass carp, *Coreius heterodom* and *Cyprinus carpio* (common carp), while negative correlations were found in *Silurus asotus* (catfish) and *Pelteoba-grus fulvidraco* (yellow-head catfish). In our study a positive relationships between fish sizes and metal levels was found in most cases. Mean concentrations of both essential (Zn, Cr and Cu) and nonessential (Hg, Pb and Cd) metals in tissues of each species showed great variations. This may be related to the differences in ecological

needs, swimming behaviors and the metabolic activities (Canli and Atli 2003).

Correlation was applied to determine the relative importance of the different environmental compartments contributing to the variation in metal levels in the water, sediment, fishes and crab. The quantitative analyses of the possible relationships in water, sediments, fishes and crab between element pairs were carried out among six variables such as Hg, Cd, Cu, Pb, Cr and Zn (Table 3).

The positive relationship between Pb vs Cd (r=0.766), Cr versus Cd (r=0.415), Zn versus Cu (r=0.741) in water indicates high similarities in the distribution

	Hg	Cd	Cu	Pb	Cr	Zn
Water (n	=32)					
Hg	-	-	-	-	-	-
Cd	-	1	-0.109	0.766**	0.415*	-0.266
Cu	-		1	0.011	-0.863**	0.741**
Pb	-			1	0.273	-0.146
Cr	-				1	-0.753*
Zn	-					1
Sedimen	t (n=32)					
Hg	1	0.379*	0.227	0.455**	0.142	0.093
Cd		1	0.266	0.757**	-0.104	0.267
Cu			1	0.463**	-	0.244
Pb				1	-0.024	0.284
Cr					1	-0.315
Zn						1
Mugil ce	phalus (n = 7)	70)				
Hg	1	0.088	0.197*	0.384**	0.133	0.207**
Cd		1	0.331*	-0.008	0.062	0.413**
Cu			1	-0.047	0.135	0.441**
Pb				1	0.082	0.256**
Cr					1	0.126
Zn						1
Anguilla	<i>anguilla</i> (n=	=76)				
Hg	1	0.461**	0.096	0.169*	0.231**	-0.076
Cd		1	0.084	0.536**	0.074	-0.045
Cu			1	0.132*	0.119	0.420**
Pb				1	-0.167	0.073
Cr					1	-0.170
Zn						1
Callinect	tes sapidus (1	n = 60)				
Hg	1	0.056	0.148*	0.082	0.081	0.091
Cd		1	0.274**	0.380**	0.085	0.290
Cu			1	0.275**	0.199**	-0.226
Pb				1	0.157*	-0.158*
Cr					1	0.270
Zn						1

Table 3Correlation matrix ofwater, sediment, fish and crabfrom Köyceğiz Lagoon System

**p* < 0.05

***p* < 0.001

and their behavior in the lagoon mainly due to external inputs. Association of Cd vs Hg (r=0.379), Pb versus Hg (r=0.455), Pb versus Cd (r=0.757), Pb versus Cu (r=0.463) in sediments suggest that the region experiences considerable amount of external input from agricultural activities, untreated boat traffic disposal, into the lagoon system (Yilmaz 2006; Genç and Yilmaz 2016a). In fishes the data showed very high levels of correlation (p < 0.001) with mostly positive values except very few negative values among different pairs of variable. There was any significant negative correlation in tissues of fishes. Significantly negative relationships were found in crab only between Pb-Zn, while positive relationships were found between Cu-Cd, Pb-Cd, Pb-Cu, Cr-Cu (p < 0.001) and Hg–Cu, Pb–Cr (p < 0.05). Other trace metals showed weaker relationship. These strong positive correlations between the elements given above revealed that they have similar, anthropogenic sources, mainly represented by the touristic, agriculture and domestic effects.

The bio accumulation factor of metals (Hg, Cd, Cu, Pb, Cr and Zn) for individual species was also calculated. Based on the calculated average BAF values, the metals were ranked as follows: Cu (798,05)>Zn (199,98)>Hg (8,33)>Cr (3,34)>Pb (0,56)>Cd (0,24). It is evident that the average highest BAF of Hg (10) and Cd (0,36) is observed in the liver/hepatopancreas, whereas Cu (900,94), Pb (0,66) and Cr (3,98) is determined in the gill. The highest BAF value of Zn (238,22) was detected in muscle. Zn and Cu were found to be highly accumulated in the fish and blue crab species of the present study.

According to Dallinger (1993), the fish species can be classified based on the BSAF values which include the macroconcentrator (BSAF>2), microconcentrator (1 < BSAF < 2) and deconcentrator (BSAF < 1). In accordance with this, the studied species can be assessed as deconcentrators (Dallinger 1993). According to the BSAF values, the species could be ordered as C. sapidus > A. anguilla > M. cephalus. Thus the species C. sapidus with the highest (0.549) BSAF would be considered as a potential bio-indicator in the Köyceğiz Lagoon system for the assessment of environmental pollution status. Based on the BSAF calculated average values for the present study, the metals are ordered as Cu (0.806)>Hg (0.505)>Zn (0.424) > Cd (0.279) > Pb (0.171) > Cr (0.006). The BAFs and BSAFs tend to vary depending on the food web structure, the trophic level and life history of organism (Burkhard 2003).

The individual mean (multi-metal) bioaccumulation index (IMBI) was calculated according to Maes et al. (2005). Briefly, this index consists in dividing the individual concentration of each metal by the maximum observed concentration (standardizing) and averaging over the number of metals in study (Maes et al. 2005). IMBI were useful for determining metal accumulations in many researchers for *Anguilla anguilla, Capoeta bergamae* and *Squalius cephalus* (Maes et al. 2008; Oglu et al. 2015; Genç et al. 2016b). According to Maes et al. (2005) index value assessment was defined before 0.22 "low" and after 0.25 "high" polluted individuals. Increase level of IMBI shows that individuals are more polluted. The distribution of the

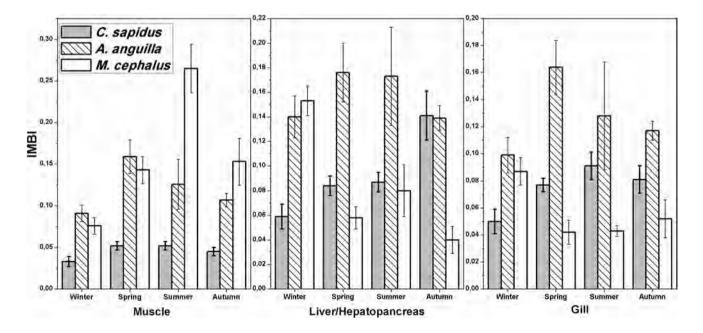


Fig. 2 Comparison individual mean bioaccumulation index values of species in different season (n = 70 for *M. cephalus*, n = 76 for *A. anguilla* and n = 60 for *C. sapidus*)

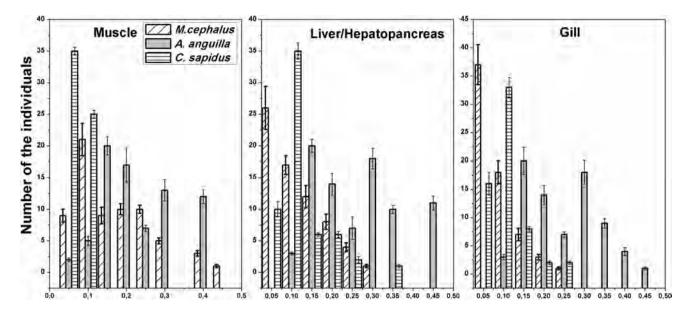


Fig. 3 The distribution of individual mean bioaccumulation index (IMBI) values in tissue of fishes and crab. Increase of *x*-axis defines the pollution of individuals

results of individual mean (multi-metal) bioaccumulation index (IMBI) according to species, seasons and tissues were given in the Figs. 2 and 3. The average distribution of the IMBI values ranged from 0.033 to 0.265. The highest metal concentrations were found in summer, on muscle of *M. cephalus*. Distribution patterns of IMBI in species follow the sequence: *A. anguilla* > *M. cephalus* > *C. sapidus*.

Result of this study showed that different fish species and crab contained different IMBI values in their tissues. Due to variations in feeding habits and behavior of the three species IMBI values of liver and gill in *A. anguilla* were generally higher than those found in other species throughout the year except winter. On the other hand IMBI value in muscle was found higher in *M. cephalus* in summer and autumn. Differences noted in the IMBI values in different organism between seasons could be the result of local pollution of lagoon. Seasonal changes of IMBI values in fish and crab may be due to intrinsic factors such as growth cycle and reproductive cycle and from changes in water temperature. In addition, we did not find any significant relationship between individual bioaccumulation and CI for three species.

Yilmaz (2009) investigated some heavy metals in liver, muscle and gills of three fish species (*A. anguilla*, *M. cephalus*, *Oreochromis niloticus*) caught from the Köyceğiz Lake between June–2005 and May–2006. Accumulation of Pb in muscle of *A. anguilla* at both studies is higher than the limits recommended for human consumption by international guidelines. Their study reported the highest concentration of Cd (0.43 µg g⁻¹ metal/wet w) and Cu (73.91 µg g⁻¹) in liver of *A. anguilla* whereas the concentration of Cd (0.16 and 0.15 μ g g⁻¹) and Cu (2.21 and 2.54 $\mu g \; g^{-1})$ in muscle and gill reported in our study is higher than their findings. Pb (0.78 μ g g⁻¹) concentrations are similar to those reported earlier in liver of *M. cephalus*. Cd (0.12 μ g g⁻¹), Cu (6.34 μ g g⁻¹) and Pb (0.43 μ g g⁻¹) concentrations in muscle of M. cephalus in present study were determined higher than earlier while these metal concentrations were found lower in gill (0.37, 5.68, 1.96 $\mu g g^{-1}$) and liver (3.32, 749.76, 0.78 $\mu g g^{-1}$ respectively). Engin (2015) measured concentrations of trace metals such as Cr, Mn, Fe, Ni, Cu, As, Se, Ag, Cd and Pb in M. cephalus of the middle Black Sea coasts. Cr concentrations in all tissues on their study were higher than M. cephalus inhabiting Köyceğiz Lagoon while Cd concentrations in all tissues were lower than our study. Canli and Atli (2003) investigated heavy metals (Cd, Cr, Cu, Fe, Pb, Zn) concentrations in the muscle, gill and liver of M. cephalus from the northeast Mediterranean Sea. In their study, Cd (0.66, 1.64 and 2.08 µg metal/g d.w.) and Pb (5.32, 12.59 and 8.95 µg metal/g d.w) accumulations in all tissues (muscle liver and gill respectively) were determined higher than our study. They also found higher Cr (4.58 and 4.85 µg metal/g d.w), Cu (202.80 and 13.48 $\mu g~g^{-1})$ and Zn (110.03 and 71.21 μ g g⁻¹) accumulation in liver and gill whereas these metal concentrations were lower in muscle (1.56 μ g g⁻¹ for Cr, 4.41 μ g g⁻¹ for Cu and 37.39 μ g g⁻¹for Zn) than our study. Usero et al. (2003) measured some metals in muscle and livers of A. anguilla of fish caught in four seawater reservoirs (the Odiel estuary and the Bay of Cadiz). Our results showed that Cr, Pb, Cd, Zn and Hg accumulation in liver and muscle are higher than their findings. Türkmen

	Sp	Hg	Cd	Cu	Pb	Cr	Zn
Present study	M. cephalus muscle	0.012 ± 0.00	0.476 ± 0.06	29.901 ± 5.69	0.626 ± 0.11	2.220 ± 0.46	78.897 ± 8.42
	A. anguilla Muscle	0.14 ± 0.03	0.22 ± 0.03	8.56 ± 3.35	1.07 ± 0.11	1.39 ± 0.16	32.92 ± 4.70
	C. sapidus muscle	0.090 ± 0.01	0.161 ± 0.24	18.214 ± 2.60	1.208 ± 0.13	1.813 ± 0.41	43.981 ± 3.44
TFC 2002	Fish	0.5	0.2	20	0.20	-	50
	Crab	0.5	0.5	20	0.20	-	50
FAO/WHO 2011	Fish	0.5 for Methylmercury	-	-	0.3	-	-
	Crab	-	2	-	-	-	-
EC 2006	Fish	0.5	0.05	-	0.30	-	-
		For Anguilla $sp = 1$					
	Crab	0.5	0.50	-	0.50	-	-

Table 4 Comparison of metal accumulation in muscle of species with guidelines ($\mu g g^{-1}$)

et al. (2006) investigation regarding muscle of blue crabs of Mediterranean Sea of Turkey revealed that the crabs contain Cd 1.06–2.51 μ g g⁻¹, Cr 2.82–6.56 μ g g⁻¹, Cu 3.88–9.39 μ g g⁻¹, Pb 2.67–4.30 μ g g⁻¹, Zn 6.67–11.5 μ g g⁻¹. These metal concentrations are relatively high for Cd, Cr, Pb, but less for Cu and Zn compared to those in the Köyceğiz Lagoon System.

Unfortunately, there is no uniform source of guidance or standards for contaminants and toxin residues in aquatic ecosystems. Metal values in muscle were compiled from documents of the Codex Alimentarius Commission assembled under the aegis of the United Nations Food and Agriculture Organization and the World Health Organization (2011), the European Community Commission (2006) and the Turkish Food Codex Regulation (2002) (Table 4). Hg was found below-the-limit values in the muscle tissues. The concentrations of Pb in muscle in the three studied species were in all cases considerably higher than the maximum levels set by law and, average Cd, Cu and Zn values in *M. cephalus* were also higher than the limits for fish proposed by FAO/WHO, EC and TFC. Therefore, the muscle of all analysed fish species and crab collected from Köyceğiz Lagoon System is not recommended for human consumption.

Consequently, the current study further showed that *C. sapidus, M. cephalus* and *A. anguilla* can be used in practical field monitoring for metal contamination in lagoon system, despite that crab may not necessarily take up the same concentration of metals as the fish in the environment. The three species with different ecological needs from the Köyceğiz Lagoon System showed different metal concentrations in their tissues. Results generally showed that metal concentrations were lowest in the muscle and highest in the gill and liver. The disparity in IMBI value found between crab and fish in the present study suggest that the use of both crab and fish can provide complementary information and therefore a better coverage and estimate on metals in

the environment. This study emphasizes that some metal levels are higher than the acceptable values for human consumption set by various health organizations.

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