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ANALYSIS OF METAL CONCENTRATION AND HEALTH RISK ASSESSMENT FOR CONSUMPTION OF FOUR ECONOMICALLY IMPORTANT FISH SPECIES FROM GÖKOVA BAY (TURKEY)

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Abstract Metals (Cd, Pb, Cu, Fe and Ni) accumulation in the tissues of four fish species, flathead grey mullet (*Mugil cephalus*), gilthead seabream (*Sparus auratus*), common pandora (*Pagellus erythrinus*) and goldblotch grouper (*Epinephelus costae*), inhabiting Gökova Bay were analysed by ICP-OES. Concentrations of all metals were significantly higher in *M. cephalus* compared with the other three examined species. The distribution of the individual total metal load (IMBI) values varied from 0.08 to 0.90, 0.10 to 0.85 and 0.13 to 0.95 in gill, muscle and liver tissue, respectively. During spring, IMBI values in *M. cephalus* and *E. costae* are of high concentration when compared to other seasons. The estimated daily intake (EDI) results for Cu, Fe, Cd, Ni and Pb ranged between 0.33 and 0.59 mg/day, 7.72 and 10.56 mg/day, 0.01 and 0.03 mg/day, 0.02 and 0.069 mg/day, 0.002 and 0.004 mg/day, respectively. The carcinogenic risk (CR) values for Ni and Pb from fish consumption varied from 0.014 to 0.114 and from 6.42E-06 to 4.76E-05. As a result, the mean total target hazard quotient (TTHQ) was relatively lower than value 1.00 in all the examined specimens, suggesting low health risks.

Keyword: Bioaccumulation, Metals, Fish, Risk assessment, Aegean Sea

1. INTRODUCTION

Fish vitally contribute to a healthy and balanced diet. Containing abundant essential amino acids and fatty acids, fish are clearly important for human health. In terms of nutritional physiology, fish, along with meat and milk, are an important source of animal protein. Metals affect fish through increased toxicity and bioaccumulation. While trace metals are found in natural waters, their concentration increases in water because of human activities, especially when industrial wastewater is mixed with drinking water or when particles contaminated with metal pass through the atmosphere to soil and water. Metals accumulate in the upper levels within the food chain (Garnero et al., 2018). The concentration of metals accumulated in the sediment in aquatic environments also damages firstly benthic organisms and then other organisms throughout the food chain. As in all ecosystems, substance and energy transitions between living organisms are provided through the food chain. The species in the upper steps of the food pyramid tend to accumulate more of the many pollutants, as they feed on the species in the lower steps that have deposited pollutants in their tissues. Among the bio-indicators of the marine ecosystem, fish are generally considered to be the most suitable species because they are an important protein source and have a high trophic level. Metals discharged into the freshwater and marine environment can damage the diversity of fish species and ecosystems because of their long persistence and toxicity (Saha & Zaman, 2011).

When reaching a significantly higher concentration, metals in the aquatic food chain can lethally affect the local fish populations. Therefore, metal pollution threatens not only aquatic species but also human health (Nambatingar et al., 2017). Metals such as Fe, Cu and Ni are essential elements and play an important role in living organisms. However, metals such as Pb and Cd are not essential elements and can produce toxic effects even in trace amounts. In addition, essential elements can be toxic when taken excessively (Genç & Yılmaz, 2017; Barone et al., 2018). Fe is required to produce red blood cells. High Fe and Mn accumulation in the body may cause iron oxide accumulation in Parkinson's disease (Matusch et al., 2010). Liver disease may occur because of excessive Cu accumulation. Zn accumulation with Cu may lead to negative food interactions. However, lipoprotein levels and immune resistance function may be reduced because of Zn (Spears, 2000). Ni, which is highly toxic at high concentrations, is important in the formation of enzymes necessary for DNA synthesis. Nonetheless, Ni can cause reduced lung function and gastrointestinal distress (Lu et al., 2005). Fish assimilate metals via different pathways, including food that affects the metabolic organs and water passing through the gill. Gills are the main organ for processing dissolved substances from water, but the liver can accumulate more metal due to blood flow from gills. Thus, gills and liver can accumulate different amounts of metal, which plays an important role in both organs as an indicator of pollution. Fish are known for their ability to accumulate metals also into their muscles. Because fish are an important part of the human diet, we chose the muscles as the most important tissue in metal intake.

Many studies in Turkey have aimed to determine the accumulation of metals in aquatic environments and fish tissue. Nevertheless, no previous study has explored metal accumulation in the tissues of fish occurring in Gökova Bay. Therefore, the objectives of this study are to: (1) provide a better understanding of the cause-effect relationships regarding metal accumulation in the tissues of some target species inhabiting Gökova Bay: flathead grey mullet *(Mugil cephalus)*, gilthead seabream *(Sparus auratus)*, common pandora *(Pagellus erythrinus)*, goldblotch grouper *(Epinephelus costae)*; (2) quantify the relationship between metal bioaccumulation and well-being (condition) of the fish; (3) assess individual levels of bioaccumulation index (IMBI) and metal pollution index (MPI) and (4) estimate health risk of heavy metals in the fish.

2. MATERIALS AND METHODS

2.1 Study Area

Gökova Bay is located at the intersection of the Aegean and the Mediterranean Sea on the southwestern coast of Turkey (37^{o} 01’ 25.92” N 28^{o} 15’ 04.58” E), and with an extension of 52000 hectares, Gökova Bay is one of the largest and richest gulfs of Turkey as well as one of the most important marine protected areas in the country (Cihangir et al., 1998). The sea area of Gökova Bay, located in the provincial borders of Muğla, is 1851 km^2 with an entire coastal length of approximately 500 km (Kıraç et al., 2010). Gökova Bay also represents an example of a closed area for fisheries in Turkey (Fig. 1). The density of tourists in the area has considerably increased in recent years, especially during the summer months. The gulf has begun to exhibit signs of environmental pollution. Small-scale fisheries are dominant because of the prohibition of trawling in a significant part of the bay. Approximately 100 active small-scale fishing boats are present here.

Figure 1. Study area (source: Google Earth, 2021).

2.2. Sample Preparation, Tissue Digestion and Analytical Procedures

Ninety-six sexually mature fish individuals were collected by Akyaka Aquaculture Cooperative, these were among the highly selected species for consumption by the local population. In 2019, six fish of every species were bought from the Akyaka Aquaculture Cooperative in every season. The caught fish were wrapped in polyethylene bags, kept in an ice box and transferred to the hydrobiology laboratory of Muğla Sıtkı Koçman University where they were measured by total length, weighed and kept frozen at -20 °C until further analysis. All the collected fish specimens were measured for length (standard length, SL ± 1 mm) and weight (total weight, TW ± 0.001 g). Over time, the caudal fins of collected and stored fish tend to become brittle and break off. In this case, the measurement of standard length instead of total length is preferred (Önsoy et al., 2011). Approximately 0.2–0.5 g of each dried tissue sample was placed in Teflon vessels containing 7 mL of HNO_3 (65%) and 3 mL of H_2O_2 (30%) (Merck). After standing for 10 min at room temperature, samples were digested in a microwave system (Berghof Speedwave MWS-3, Germany) as according by Genç et al., (2015). Compared with the classical method, microwave digestion offers benefits such as less time, less acid consumption and keeping volatile compounds in the solution (Sures et al., 1995). All samples were processed two times for Cu, Fe, Cd, Ni and Pb by Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP/OES Agilent 5100), which is a fast multi-element technique with a dynamic linear range and moderate-low detection limits. Analyses were carried out by the Muğla Sıtkı Koçman University soil, plant and irrigation water analysis laboratory. Standard solutions were prepared from stock solutions (Merck, multi-element standard). The quality of the analytical process was also controlled by the analysis of the Community Bureau of Reference; DORM-3 (National Research Council Canada, Ottawa Ontario, Canada) certified standard reference materials of fish.

2.3 Statistical Analysis and Metal Data

Spearman's rho test was also used to correlate metal accumulation. All statistical calculations were performed with SPSS 20.0 for Windows, and Graphpad Prism 7 was used to draw graphs. PCA of log-transformed and mean-centred data was employed using 'factoextra' and 'FactoMineR' packages in R software (ver. 3.6.3). To estimate the risk of heavy metals associated with fish consumption, the target hazard quotient (THQ) is widely calculated in new studies (Genç & Yilmaz, 2018; Quattara et al., 2020).

The models for estimating THQ are as follows (Chien et al., 2002):

$$(1)\ THQ = \frac{EFr \times EDtot \times FIR \times C}{RfDo \times BWa \times ATn} \times 10^{-3}$$

$$(2)\ \text{Total THQ (TTHQ)} = \text{THQ (toxicant 1)} + \text{THQ (toxicant 2)} + \text{THQ (toxicant 3)} + \ldots + \text{THQ (toxicant n)}$$

where EFr – exposure frequency (365 days/year); EDtot – the exposure duration (77 years, average lifetime); FIR – the food ingestion rate (g/day); C – the heavy metal concentration in fish (mg/g); RfDo – the oral reference dose (mg/kg/ day, Table 1); BWa – the average adult body weight (71.5 kg); ATn – the average exposure time for non-carcinogens (365 days/ year) number of exposure years, assuming 77 years (TSI, 2010; TSI, 2013). The THQ, which is the ratio between the exposure and the reference doses (a Reference Dose or RfD), is a formula used to calculate the risk of non-carcinogenic effects. According to this calculation, a rate value of less than one indicates no obvious risk. If the dose is equal to or higher than the RfD, health risks will arise for the population exposed to the metal. The THQ for individual metals and the total THQ were estimated for Gökova Bay using the data available regarding the consumption of fish for the total population of Turkey, which was 20 g/day according to FAO (2008).

The estimated daily intake (EDI) of metals (Cu, Zn, Pbi Ni and Cd) is calculated based on the amount of fish muscle consumption. The EDI of metals for adults was determined using the following equation:

$$(3)\ EDI = C_{metal}l \times W_{food} / B_w$$

The variable C_{metal} (μg·g^{-1}, on fresh weight basis) is the concentration of heavy metals in contaminated fish; W_{food} represents the daily average consumption of fish in this region; and B_w is the body weight.

Carcinogenic risk (CR) indicates an incremental probability of an individual developing cancer over a lifetime because of exposure to a potential carcinogen. CR over lifetime exposure to Pb and Ni were obtained using the cancer slope factor (CSF) (mg/kg/day, Table 1), provided by USEPA (2000). The equation used for estimation of the carcinogenic risk is as follows:

$$(4)\ CR = CSF \times EDI$$

The CSF is the carcinogenic slope factor of 0.0085 μg·mg^{-1}/day) for Pb (USEPA, 2010) and 1.7 μg·mg^{-1} /day) for Ni (USEPA, 2012). Because Fe and Cu do not cause any carcinogenic effects, their CSF have

Table 1. Reference dose for target hazard quotient and cancer slope factor for carcinogenic risk

	Cd	Pb	Cu	Fe	Ni
RfDo (μg·mg^{-1}/day)	1×10^{-3}	4×10^{-3}	4×10^{-2}	7×10^{-1}	2×10^{-2}
CSF (μg·mg^{-1}/day)		0.0085			1.7

RfDo – oral reference dose; CSF – carcinogenic slope factor

Table 2. Length, weight and condition factor in fish

Species	Average length (cm)	Min-Max length (cm)	Average weight (g)	Min-Max weight (g)	Condition factor (K)
Mugil cephalus (n=24)	38.645 ± 5.77	27–50	754.150 ± 421.04	212.77– 1782	1.176 ± 0.22
Pagellus. erythrinus (n=24)	21.270 ± 1.20	18.50–23.50	129.376 ± 22.54	93.79–189.01	1.355 ± 0.27
Epinephelus costae (n=24)	26.291 ± 3.39	16.50–32	208.047 ± 60.48	56.34–276.68	1.116 ± 0.13
Sparus auratus (n=24)	24.125 ± 1.79	21.50–27.50	185.734 ± 33.91	140,36–250,44	1.318 ± 0.12

not yet been established (USEPA, 2012). The EDI is the estimated daily intake of heavy metals. Acceptable risk levels for carcinogens range from 10^{-4} (where the risk of developing cancer over a human lifetime is 1 in 10000) to 10^{-6} (where the risk of developing cancer over a human lifetime is 1 in 1000000).

We calculated a relative bioaccumulation index by dividing (standardising) the individual concentration of heavy metal *i* (C_i) by the maximum observed concentration (C_{imax}) and averaging over all metals. Thus, the individual mean (multi-metal) bioaccumulation index (IMBI) was defined as:

$$(5)\ \text{IMBI} = \frac{\sum_{i=1}^{n} C_i/C_{imax}}{n}$$

The variable *n* is the total number of metals; C_i is the individual metal concentration of heavy metal *i*; C_{imax} is the maximal observed concentration of heavy metal *i* and $0 < \text{IMBI} < 1$ (Maes et al., 2008). An increase in IMBI indicates that individuals are more polluted. According to Maes et al., (2005), the index value assesses 'low polluted individuals', those with a value smaller than 0.22, and 'high polluted individuals', those with values higher than 0.25.

The MPI was used according to the following equation (Usero et al., 1997):

$$(6)\ \text{MPI} = (\text{Cf1 x Cf2,...Cfn})^{1/n}$$

The variable *Cfi* indicates concentration for the metal 'i' in the sample.

3. RESULTS AND DISCUSSION

3.1 Condition Factor and Metal Accumulation in Tissue

The length–weight relationship can provide an estimation of fish condition assuming that a heavier fish of a given length is in better condition. Mean length, weight and condition factor are reported in Table 2. During spring, the maximum fish weight was determined as 1782 g for *M. cephalus*. In autumn, the minimum fish weight was 56.34 g for *E. costae*. The mean condition factor for *P. erythrinus* was higher than that of other fish but the condition factor oscillated throughout the sampling months.

Mean concentrations of Cu, Fe, Cd, Ni and Pb in tissues of fish from Gökova Bay are shown in Table 3. Concentrations of Cu and Fe in all tissues were 100–1000 times higher than other metals. The metal concentrations were higher in gill than that in the muscle, but lower than that in the liver. Nevertheless, Fe accumulation in the gill tissue of *P. erythrinus* is higher in the summer season. In addition, the accumulation of Cd, except for the spring season, as well as Ni accumulation in *E. costae* in autumn and winter was also higher in muscle tissue than in gill. Among the analysed fish species, the most metal-accumulating organ is the liver. The liver, which is the detoxification organ of fish, contains numerous metallothionein proteins. Therefore, the liver especially accumulates toxic metals much more than other tissues (Hamilton & Mehrle, 1986); hence, the liver has been identified as the best environmental indicator for detecting metal contamination. However, Ni accumulation in all seasons for *M. cephalus* and Cd accumulation in all seasons for *P. erythrinus* were calculated more in the gill tissue than that in the liver. The gills of the fish have various functions in direct interaction with water and pollutants, such as gas exchange, ion transport and ammonia and urea excretion (Lawrance & Hemingway, 2003). Thus, the gill is an indicator of aquatic metal concentration, especially at the beginning of exposure (Dural et al., 2007).

According to several studies, Mendil & Uluözlü (2007) reported that the most accumulated metal in sediment and fish tissues was Fe, which is one of the most common elements in the earth's crust and usually the most abundant metal in all reservoirs (Usero et al., 2003). The high concentration of metal in the liver can be attributed to the role the liver plays to bind pollutants to its metallothionein. Conversely, the reason for the low concentration of metal in muscle is because of the presence of high calcium, which reduces the relationship of this tissue with metals (Copaja et al., 2017). However, in this study, the levels of all metals in each tissue were generally higher in *M. cephalus* than in the other three examined species. This can be explained by the differences in metabolic activity, migration and swimming behaviour among different fish species. As in other study results, metal concentrations in tissues varied greatly for different fish species. The following paragraph explains examples of studies related to metal accumulation in the tissues of fish species in different world regions.

In the study on *Chondrostoma nasus* and *Barbus capito pectoralis* living in the Büyük Menderes River-Turkey, the highest metal concentrations (Zn, Co, Cd, Cu and Pb) were calculated in the liver of both the fish species (Koca et al., 2008). Concentrations of metals (Cd, Pb, B, Cu, Co, Ni and Zn) in muscle, liver and gill of *Lepomis gibbosus* inhabiting Çine stream, Turkey, were analysed. Zn was the most concentrated metal and the highest accumulation was found in both the liver and gill (Koca et al., 2005). Yilmaz et al., (2007) investigated the accumulation of metals (Co, Ni, Mn, Cd, Pb, Fe and Zn) in the liver, gill and muscle of *L.*

Table 3. Average concentrations (µg·mg^{-1} dry wt) of heavy metals in the organs of the four fish species (mean±SEM)

		Mugil cephalus (n = 24)			*Pagellus erythrinus* (n = 24)			*Epinephelus costae* (n = 24)			*Sparus auratus* (n = 24)		
		Liver	Muscle	Gill	Liver	Muscle	Gill	Liver	Muscle	Gill	Liver	Muscle	Gill
Cu	1	16.17± 6.22	1.82 ±0.47	5.65± 3.24	5.08 ± 3.15	2.35 ± 2.89	3.93 ±1.72	8.78±8 .69	1.45± 0.35	2.05± 0.61	3.58± 1.15	0.76± 0.16	1.99± 0.48
	2	29.12± 13.10	2.43± 1.04	9.62± 5.49	5.17± 1.89	1.60± 0.68	3.44± 0.92	16.10± 14.13	1.95± 1.43	1.31± 0.63	3.91± 1.03	1.31± 0.65	2.31± 0.93
	3	14.22± 5.60	1.98± 0.42	4.15±1 .45	4.10±2 .18	1.30±0 .6	3.50±1 .68	8.48±9 .48	1.48±0 .60	1.48±0 .27	3.23±1 .42	1.34±0 .42	1.61± 0.53
	4	15.79± 4.57	2.27± 1.03	2.83± 0.31	5.35± 3.64	1.56± 1.39	2.99± 2.10	4.97± 1.59	0.93± 0.31	1.42± 1.01	3.86± 1.97	1.28± 0.84	1.85± 0.78
Fe	1	574.14 ± 28.39	43.76± 23.02	289.63 ± 111.37	373.23 ± 241.76	36.72± 16.02	383.42 ±205.2	265.08 ±74.12	17.67± 13.33	92.32± 18.13	387.12 ± 195.94	22.87± 12.71	194.37 ±39.12
	2	581.83 ± 190.17	65.08± 38.78	419.66 ± 123.28	424.84 ± 126.87	44.81± 14.83	430.26 ± 205.30	516.86 ± 134.32	49.11± 22.17	69.09± 10.74	309.15 ± 133.44	46.07± 21.75	214.18 ±70.56
	3	552.98 ± 181.16	21.65± 6.67	302.94 ± 138.41	395.02 ± 120.16	20.58± 12.81	236.72 ±90.21	321.79 ± 173.53	16.38± 5.52	61.38± 17.98	325.42 ± 247.91	32.38± 8.32	149.27 ±45.87
	4	385.85 ± 177.18	20.64± 7.30	274.24 ± 128.89	250.68 ±65.65	26.93± 15.53	299.90 ±2 31.06	146.10 ±63.30	27.29± 3.64	52.45± 6.06	282.44 ± 120.38	29.14± 13.61	207.50 ± 108.09
Cd	1	0.30± 0.03	0.14± 0.04	0.19± 0.06	0.18± 0.16	0.12± 0.09	0.20± 0.03	0.18± 0.05	0.06± 0.02	0.10± 0.05	0.27± 0.21	0.03± 0.02	0.04± 0.01
	2	0.33± 0.03	0.15± 0.03	0.17± 0.02	0.17± 0.06	0.16± 0.08	0.28± 0.04	0.35± 0.29	0.10± 0.03	0.06± 0.04	0.06± 0.03	0.045± 0.02	0.06± 0.05
	3	0.19± 0.07	0.1± 0.04	0.14± 0.03	0.15± 0.02	0.07± 0.03	0.23± 0.14	0.17± 0.05	0.08± 0.02	0.05± 0.03	0.12± 0.08	0.04± 0.02	0.05± 0.03
	4	0.14± 0.02	0.06± 0.01	0.11± 0.04	0.12± 0.05	0.07± 0.03	0.168± 0.18	0.16± 0.12	0.06± 0.01	0.03± 0.01	0.09± 0.07	0.03± 0.02	0.02± 0.01
Ni	1	0.70 ± 0.26	0.27± 0.11	0.71± 0.34	0.83± 0.84	0.30± 0.20	0.81± 0.36	0.41± 0.16	0.15± 0.04	0.30± 0.15	0.47± 0.28	0.06± 0.03	0.09± 0.04
	2	1.49 ± 1.05	0.36± 0.12	1.85± 1.12	1.13± 0.63	0.39± 0.14	1.19± 1.02	0.78± 0.35	0.26± 0.10	0.20± 0.08	0.87± 0.99	0.12± 0.06	0.31± 0.21
	3	0.44 ± 0.24	0.16± 0.04	0.72± 0.57	0.88± 0.64	0.13± 0.04	0.64± 0.33	0.39± 0.13	0.22± 0.10	0.20± 0.05	0.25± 0.28	0.11± 0.06	0.11± 0.03
	4	0.39 ± 0.07	0.18± 0.03	0.63± 0.46	0.41± 0.24	0.17± 0.10	0.74± 0.70	0.28± 0.07	0.24± 0.06	0.14± 0.02	0.59± 0.37	0.12± 0.05	0.39± 0.33
Pb	1	0.3± 0.006	0.01 ± 0.004	0.03± 0.017	0.2± 0.2	0.01± 0.007	0.02± 0.01	003± 0.004	0.01± 0.004	0.02± 0.008	0.04± 0.03	0.005± 0.002	0.009± 0.002
	2	0.03± 0.008	0.01± 0.004	0.02± 0.007	0.3± 0.1	0.01± 0.01	0.03± 0.007	0.03± 0.01	0.01± 0.007	0.01± 0.006	0.02± 0.01	0.009± 0.006	0.01± 0.004
	3	0.02± 0.01	0.1± 0.007	0.02± 0.002	0.18± 0.08	0.006± 0.001	0.2± 0.1	0.03± 0.01	0.01± 0.006	0.01± 0.007	0.02± 0.008	0.009± 0.003	0.01± 0.002
	4	0.01± 0.003	0.006± 0.0006	0.01± 0.005	0.01± 0.007	0.005± 0.001	0.017 ± 0.01	0.01± 0.003	0.008± 0.001	0.005± 0.0002	0.01± 0.004	0.008± 0.003	0.008± 0.003

1 = Spring, 2 = Summer, 3 = Autumn, 4 = Winter

gibbosus and *Leuciscus cephalus* inhabiting Saricay in the South–West of Turkey. In *L. cephalus*, concentrations of Mn and Fe were higher in the liver than that in gill and muscle. However, in *L. gibbosus*, the highest concentrations of metals were Mn and Zn in the gill and Fe in the liver. Coetzee et al., (2002) analysed metal concentrations in skin, muscle, liver and gill of *Clarias gariepinus* and *Labeo umbratus* inhabiting the river Olifants, South Africa. In both species, the liver and gill had accumulated higher concentrations of Fe, Zn, Al, Cr, Mn, Ni and Pb. Ahmad et al., (2015) investigated the concentrations of metals (Hg, Cr, Cu and Pb) in some tissues of *Oreochromis niloticus* and *Poecilia latipinna* inhabiting Wadi Namar, Saudi Arabia. They found that metal accumulation was higher in the liver than that in the gill in both species. Mohamed (2008) estimated bioaccumulation of Co, Cu, Fe, Zn, Pb and Cd in target tissues (muscle, gills, liver, heart, intestine and testis) of *Lates niloticus* and *O. niloticus* obtained from Lake Nasser, Egypt. In both species, the accumulation was found to be higher in both gills and liver than that in other tissues. Similarly to our study, they found that liver and gills were the most metal-accumulating organs. In a few studies, metal accumulation in muscle was found to be higher. Genç and Yılmaz (2017) were determined concentration of six metals in tissues of two fish species (*M. cephalus* and *Anguilla anguilla*. They found that the highest concentration of Cd and Zn in the muscle of *M. cephalus* from the Köyceğiz Lake. Türkmen et al., (2011) were performed to examined the metal concentrations in some tissues (gill, gonad, mucle and liver) of *S. aurata*, *M. cephalus*, *Dicentrarchus labrax*, *Liza saliens*. They reported that muscles were higher than those in livers (Cr for *L. saliens*, Cr, Ni and Pb in *D. labrax*)

3.2 Metal Correlation Coefficient in Fish

The correlation coefficient among the metals in fish living in Gökova Bay is presented in Table 4. Strong positive correlation coefficients between all metals in all four fish species were determined. No significant correlation coefficient was found between the metals and the condition factor (K) for the fish species except for the positive correlation determined between Pb and K (r = 0.236) in *S. auratus*. The highest positive correlation coefficient was analysed between Fe and Cu in *M. cephalus* (r = 0.844) and *S. auratus* (r = 0.791), and between Cd and Pb in *P. erythrinus* (r = 0.861) and *E. costae* (r = 0.774). In their study, Tekin-Özan & Aktan (2012) calculated positive relationships between Cr, Cu, Fe, Mn, Zn, and the length and weight of chub mackerel, *Scomber japonicus*. Yi & Zhang (2012) found a negative correlation between the fish size of *Silurus asotus* and *Pelteobagrus fulvidraco* and Zn, Cd and Pb and calculated a positive correlation in *Coreius heterodom* and *Cyprinus carpio*. Mean concentrations of both nonessential (Cd and Pb) and

Table 4. Correlation coefficients of heavy metals and condition factor in fish

	Cu	Fe	Cd	Ni	Pb	K
Mugil cephalus						
Cu	1.000					
Fe	.844(**)	1.000				
Cd	.605(**)	.613(**)	1.000			
Ni	.614(**)	.730(**)	.594(**)	1.000		
Pb	.455(**)	.551(**)	.822(**)	.621(**)	1.000	
K	-.077	-.106	-.053	-.165	-.116	1.000
Pagellus erythrinus						
Cu	1.000					
Fe	.725(**)	1.000				
Cd	.692(**)	.611(**)	1.000			
Ni	.763(**)	.828(**)	.774(**)	1.000		
Pb	.754(**)	.764(**)	.861(**)	.857(**)	1.000	
K	.051	-.049	-.023	.013	-.042	1.000
Epinephelus costae						
Cu	1.000					
Fe	.768(**)	1.000				
Cd	.655(**)	.577(**)	1.000			
Ni	.584(**)	.636(**)	.727(**)	1.000		
Pb	.560(**)	.510(**)	.774(**)	.658(**)	1.000	
K	.081	.119	.127	.165	.125	1.000
Sparus auratus						
Cu	1.000					
Fe	.791(**)	1.000				
Cd	.454(**)	.420(**)	1.000			
Ni	.647(**)	.669(**)	.266(*)	1.000		
Pb	.707(**)	.711(**)	.672(**)	.630(**)	1.000	
K	.039	.027	.050	-.037	.236(*)	1.000

** Correlation is significant at the 0.01 level (2-tailed).

essential (Cu, Fe and Ni) metals in the tissues of the four fish species varied greatly. This variation may be because of the ecological needs and differences in metabolic activities (Canli & Atli, 2003).

Results of the PCA on the metal profiles of fish are shown in Fig. 2. Notably, the metal profiles of the fish are different. In the spring, 85.8% of the total variance could be explained by the first three components (PC1: 59.6%, PC2: 15.2%, PC3: 11). Results of the PCA further indicated that the metal profiles of *S. auratus* muscle during the spring could be separated from the tissues of other fish. The results of the PCA analysis in the summer indicated that the first three components explained 87.8% (PC1: 61.9 %, PC2: 15.3%, PC3: 10.6%) of the total variance in the data. In the autumn, 82.2% of the total variance could be explained by the first three components (PC1: 48.2%, PC2: 19.1%, PC3: 14.9). The PCA scores plot in the autumn revealed that gill of *E. costae* is clearly separated from the other tissues of fish. In addition, PCA analysis results in winter were 84.6% (PC1: 48.5%, PC2: 22.3%, PC3:13.8%). The metal profiles in muscle of *S. auratus* also exhibited a clear separation in winter. PCA analysis signified that the metal profiles of fish are different, which may be attributed to several factors. Metal accumulation in fish may depend not only on the total metal concentration, but also on the proportion of specific metal forms that prevail in the natural environment. Also, the metal uptake of fish can differ because of food selection and metal regulation, which are often specific to species.

3.3 Multi-Metal Bioaccumulation Index

This index includes the average of the number of metals in the study, as well as the individual concentration divided by the maximum concentration analysed (standardisation). As a useful monitoring tool, IMBI concentrations have been assessed in many articles for the European chub, *Squalus cephalus*; the flathead grey mullet, *M. cephalus*; and the European eel, *A. anguilla* (Esteve et al., 2012; Öğlü et al., 2015; Genç & Yilmaz, 2017). The distribution of the IMBI results according to species, seasons and tissues were given in Figures. 3 and 4. The distribution of IMBI values varied from 0.08 to 0.90, 0.10 to 0.85 and 0.13 to 0.95 in gill, muscle and liver, respectively. During the spring, average IMBI values in *M. cephalus* and *E. costae* were of high concentration when compared with other seasons. In contrast, the average highest total metal loads were determined during the summer (0.47) in *P. erythrinus* and the winter (0.36) in *S. auratus*. Seasonal variations in IMBI values may be a

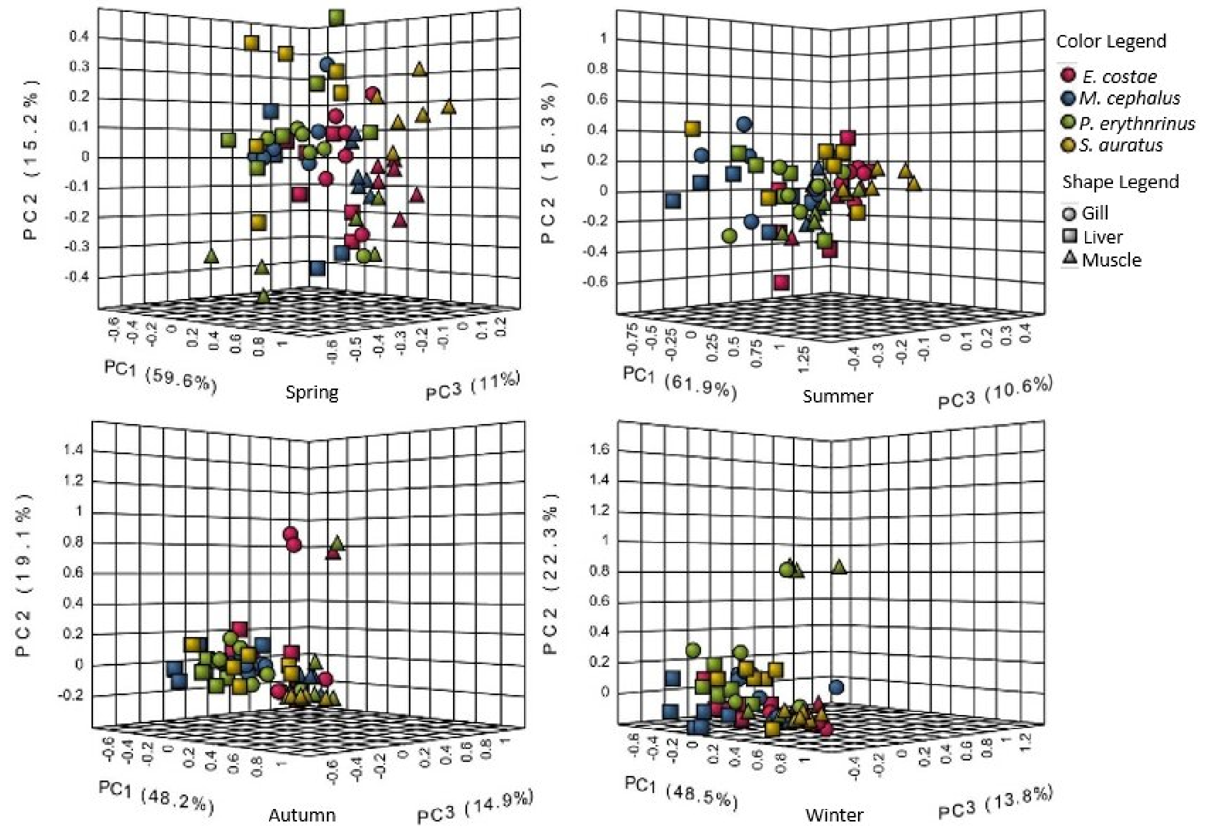

Figure 2. Principal components analysis of metal profiles in fish for each season. PC1, principal component 1; PC2, principal component 2; PC3, principal component 3.

result of local pollution in Gökova Bay. The reasons for seasonal variation in IMBI values in fish include factors such as changes in water temperature and growth and reproductive cycles. The average lowest IMBI value was determined in the liver tissue of *E. costae* (0.34), whereas the average highest value was determined in the liver tissue of *M. cephalus* (0.51). Generally, IMBI values calculated in gill were higher than that in muscle and liver in fish except *M. cephalus*. In *M. cephalus*, the IMBI value in the liver tissue was higher than that in the muscle and gill.

In this study, different IMBI values were calculated in different fish species. The reason for this can be explained by the differences in behaviour and feeding habits among fish. Consequently, the current study further showed that *M. cephalus*, *E. costae*, *P. erythrinus* and *S. auratus* can be used in practical field monitoring for metal contamination in Gökova Bay. IMBI may explain distribution of total metal load seasonally but cannot elucidate the metals affecting these values. IMBI values of most of the metals varied notably depending on the MPI values seasonally (Fig. 5). The distribution patterns of MPI in species throughout all seasons follow the sequence: Fe > Cu > Ni > Cd > Pb. The results of high IMBI values in all seasons may explain the increased MPI values of Fe (183.93, 104.52, 70.17, 109.97 for spring; 224.05, 139.61, 81.60, 133.19 for summer; 144.45, 82.56, 64.46, 102.65 for autumn; 118.64, 106.79, 42.01, 107.76 for winter) in *M. cephalus*, *P. erythrinus*, *E. costae* and *S. auratus*, respectively.

3.4 Health Risk from Consuming Fish

Metal accumulation studies in fish are important for evaluating the effects of fish consumption on human health. The four fish species studied are the most commercially caught fish in Gökova Bay. The analysis of metal accumulation in muscle tissue is especially important for food safety (Quattara et al., 2020). The EDI, recommended limits, THQ, TTHQ and CR for heavy metals are presented in Table 5. In particular, EDI through fish consumption was calculated using all fish species' metal concentrations in muscle tissue. The EDI results for Cu, Fe, Cd, Ni and Pb ranged between 0.33 and 0.59 mg/day, 7.72 and 10.56 mg/day, 0.01 and 0.03 mg/day, 0.02 and 0.069 mg/day, and 0.002 and 0.004 mg/day, respectively. According to the analysed metals, THQ value was calculated as less than one for all fish. The

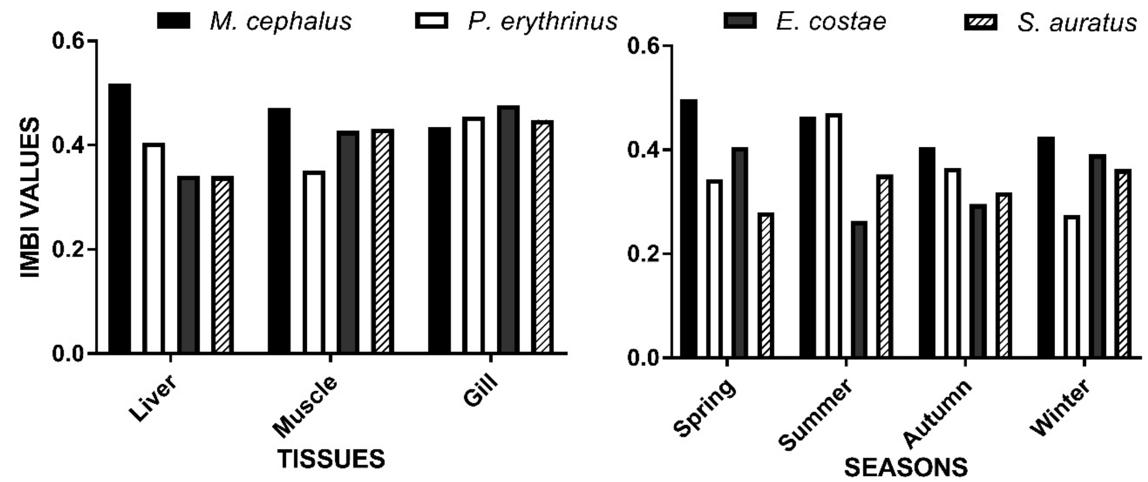

Figure 3. Comparison of individual mean bioaccumulation index values in tissues and different seasons.

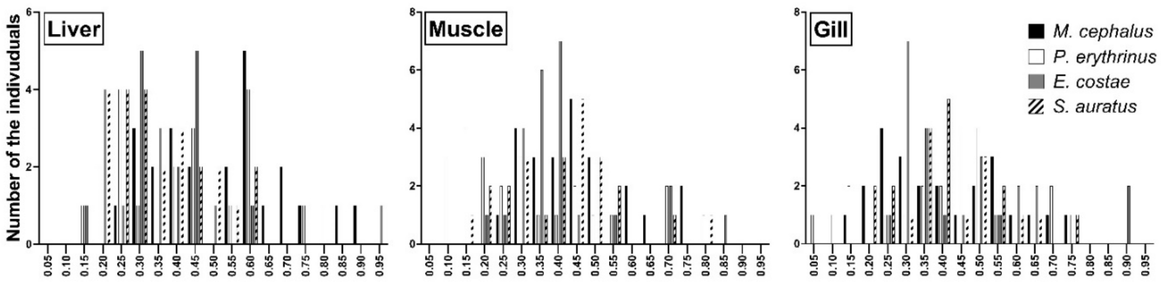

Figure 4. The distribution of individual mean bioaccumulation index values in tissue of fishes. Increase of *x-axis* defines the pollution of individuals.

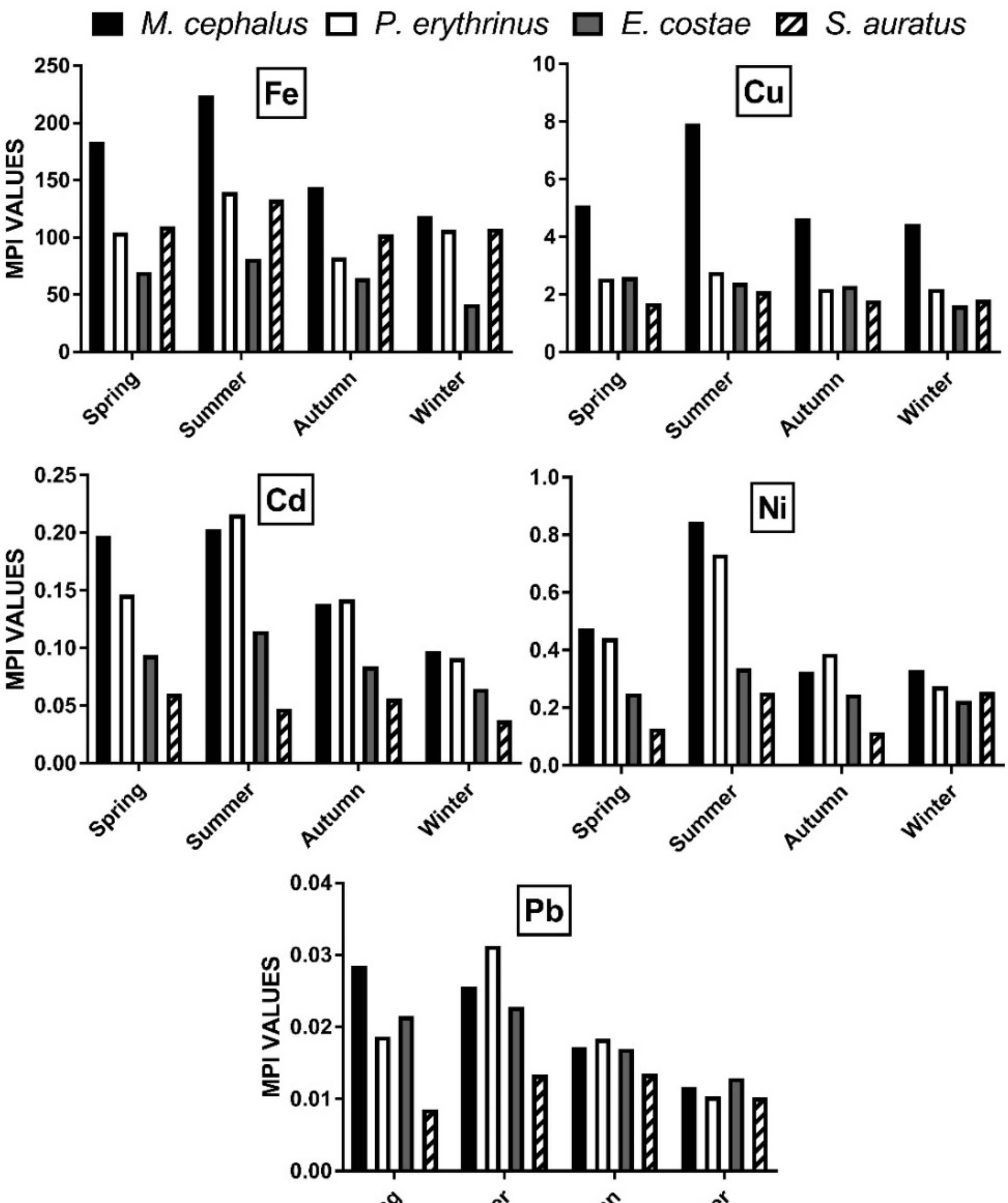

Figure 5. Comparison of metal pollution index values in different seasons.

average TTHQ values calculated for all fish muscles were lower than one for all analysed metals. The CR values for Ni and Pb from fish consumption varied from 0.014 to 0.114 and from 6.42E-06 to 4.76E-05. The THQ value was assessed as less than one, which indicates that the fish species examined in this study do not pose any health hazard.

Similarly, the average TTHQ values were calculated to be relatively lower than one in all fish and were understood to have low health risks. Cd, the major risk contributor for the population in Gökova Bay, accounted for 48.62%, 51.04%, 45.72% and 32.49% of the TTHQ, and Fe, the other highest risk contributor, accounted for approximately 22.62%, 21.78%, 23.73% and 37.84% of the TTHQ in *M. cephalus*, *P. erythrinus*, *E. costae*, and *S. auratus*, respectively. This result suggests that Fe, Cu and Cd are the dominant contributors whereas relatively

Table 5. Comparison of the estimated daily intake of heavy metals, carcinogenic and non-carcinogenic risks from fish species

		EDI (mg/day/person)	Recommended limits (mg/day/person)	THQ	TTHQ	CR
Mugil cephalus	Cu	0.5951	30 (FAO/WHO 1983)	0.0148		
	Fe	10.569	100 (FAO/WHO 1989)	0.0150		
	Cd	0.0326	0.06 (FAO/WHO 2009)	0.0324	0.0667	
	Ni	0.0682	70–80 (USFDA 1993)	0.0034		0.1161
	Pb	0.0044	0.21 (FAO/WHO 2009)	0.0009		3.76E-05
Pagellus erythrinus	Cu	0.4770	30 (FAO/WHO 1983)	0.0118		
	Fe	9.0247	100 (FAO/WHO 1989)	0.0128		
	Cd	0.0304	0.06 (FAO/WHO 2009)	0.0302	0.0591	
	Ni	0.0699	70–80 (USFDA 1993)	0.0034		0.1188
	Pb	0.0030	0.21 (FAO/WHO 2009)	0.0006		2.61E-05
Epinephelus costae	Cu	0.4073	30 (FAO/WHO 1983)	0.0101		
	Fe	7.7252	100 (FAO/WHO 1989)	0.0110		
	Cd	0.0213	0.06 (FAO/WHO 2009)	0.0212	0.0922	
	Ni	0.0623	70–80 (USFDA 1993)	0.0030		0.1060
	Pb	0.0040	0.21 (FAO/WHO 2009)	0.0009		3.40E-05
Sparus auratus	Cu	0.3300	30 (FAO/WHO 1983)	0.0082		
	Fe	9.1238	100 (FAO/WHO 1989)	0.0130		
	Cd	0.0114	0.06 (FAO/WHO 2009)	0.0111	0.0344	
	Ni	0.0294	70–80 (USFDA 1993)	0.0013		0.0501
	Pb	0.0026	0.21 (FAO/WHO 2009)	0.0005		2.22E-05

minor risk derives from Ni and Pb for fish species of Gökova Bay. The next highest risk contributor element was Cu with 22.21%, 20.08%, 21.81% and 23.96% in *M. cephalus*, *P. erythrinus*, *E. costae* and *S. auratus*, respectively. For all fish species, the risk contributions of Ni and Pb were relatively low at about 7% and 3%, respectively. In general, the CR lower than 10^{-6} are considered negligible. The CR above 10^{-4} are considered unacceptable and CR between 10^{-6} and 10^{-4} are considered acceptable (Chien et al., 2002; Wang et al., 2005). The CR for Ni and Pb were calculated below the negligible value. Therefore, explaining about a situation that will pose a potential health risk for the residents is not possible.

4. CONCLUSION

The paper presents original results on health risk assessment for consumption of four economically important fish species from Gökova Bay and explains the potential regional impacts of these metals in details. The results of present study indicate that the highest concentration of metals was determined in the liver followed by the gills and muscles. Generally, *Mugil cephalus* are more able to accumulate all analysed metals than other fishes. The estimated daily intake of the analysed metals through fish consumption is below the provisional tolerable values. The THQ-average of each metal from fish consumption based on the average concentration was less than 1, suggesting that people would not experience significant health risks from the intake of individual metals through fish consumption. Human health risk assessments related to fish consumption are important and sources of toxic metals such as Cd and Pb should be controlled.

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