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INVESTIGATION OF METAL TOXICITY RESPONSE AND HEALTH RISK ASSESSMENT OF COMMONLY CONSUMED MARINE FISH SPECIES ALONG THE TURKISH COAST

ABSTRACT

Marine environments are suffered from several types of pollution. Marine organisms take pollutants into their bodies which result in heavy metal accumulation in their tissues. Therefore, consumption of marine organisms may pose a risk to human health. This study was undertaken to estimate metal accumulation and metal toxicity responses of *M. barbatus*, *B.boops*, *T. trachurus* from Marmara Sea, Aegean Sea and Mediterranean Sea. Also, since these species were commonly consumed by Turkish people, health risk assessment employing The Target Hazard Quotient (THQ) and lifetime cancer risk (CR) was conducted. For that purpose, aluminum (Al), chrome (Cr), cobalt (Co), nickel (Ni), cadmium (Cd), lead (Pb), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn) concentrations in the muscle, gill and liver. To test the impact of metal toxicity on studies species, oxidative stress biomarkers (catalase-CAT, Malondialdehyde-MDA) in muscle and liver were investigated. Metal accumulation levels showed differences depending on tissues, species and/or bays. Also, strong correlation was observed between biostress parameters and metal accumulation levels. Both, THQ and CR calculations showed safe consumption depending on the consumption of *M. barbatus*, *B.boops*, *T. trachurus* at all stations. Results obtained in this study showed that biostress parameters could be effectively used to monitor environmental pollution and contribute to the knowledge of metal toxicity along Turkish coast.

Keywords: Health Risk, Marmara Sea, Aegean Sea, Mediterranean Sea, MDA

1. INTRODUCTION

Marine environments were contaminated due to natural or anthropogenic activities, such as air pollution, untreated wastewater discharge, agricultural and urban diffuse pollution [1]. Marine organisms living in these polluted environments may accumulate pollutants in their tissues. Due to the connection between marine organisms and humans via the food chain, this condition may pose a risk to human health [2 and 3]. Metals are the most studied pollutant in the marine

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environment due to long return cycles, unsuitability for decomposition and increase in accumulation along the food chain [4]. Some metals like iron, chromium, manganese, cobalt, nickel, copper, selenium are essential in trace amounts for aquatic microorganisms and may become toxic in excess amounts; on the other hand, others like mercury, cadmium and lead are non-essential and toxic [5]. Metal toxicity ends up with the formation of oxidative stress in aquatic organisms [6]. Organisms increase the antioxidant enzymes like catalase (CAT) and bind metals with metallothioneins to deal with oxidative stress [7]. Therefore, catalase enzyme activity and Malondialdehyde activity (MDA) was used as biomarkers to evaluate metal pollution in marine environments [8-10]. This study was designed to understand the impact of metal concentrations in the ambient environment on the marine fish by examining metal accumulation levels and stress biomarkers (MDA and CAT). For that purpose, *Mullus barbatus* Linnaeus, 1758, *Boops boops* (Linnaeus, 1758) and *Trachurus trachurus* (Linnaeus, 1758) samples were collected from Turkish coastal regions. *Mullus barbatus* is a demersal species and usually fed on small benthic crustaceans, worms and mollusks [79]. *Boops boops* is a demersal, omnivorous species, and mainly fed on crustaceans [79]. *Trachurus trachurus* is a pelagic-neritic species, and usually fed on fish, crustaceans, and cephalopods [79].

2. RESEARCH SIGNIFICANCE

There are several studies evaluating the metal accumulation levels in marine fish from Turkish coastal regions [4, 11, 12 and 13]. Yet, this study is, as far as we know, the first study designed to assess impact of metal toxicity by examining stress biomarkers (MDA and CAT). Also, since these species are intensively consumed by Turkish people, health risk assessments prior to the consumption of these species from Turkish coastal waters were evaluated.

Highlights

- Results obtained in this study showed that highest Co, Ni, Mn, Fe accumulation was observed in the muscle of demersal *M. barbatus*.
- Highest Al, Cr, Cd, Cu, Zn accumulation were found in the muscle of pelagic *T. trachurus*.
- MDA and CAT activity levels in the muscle and liver were ranked as *T. trachurus* > *B. boops* > *M. barbatus* and *M. barbatus* > *T. trachurus* > *B. boops*, respectively.

3. EXPERIMENTAL METHOD-PROCESS

3.1. Sampling and Analysis

Mullus barbatus, *Boops boops* and *Trachurus trachurus* samples were collected in January-February 2021 along Turkish coastal including Aegean Sea, Marmara Sea, and Mediterranean Sea of Turkey (Figure 1). Morphologically unharmed specimens were selected for the analysis. Target fish length and fish weight were selected as at least 15cm and 30g which was appropriate size for consumption. Morphological characteristics of examined individuals were given in Table 1. The samples were immediately brought to the laboratory in cold chain, and total fish length and weight were measured to the nearest millimeter (mm) and gram (g). Samples were digested as soon as they arrived in the laboratory.



Figure 1. Study area

Table 1. Morphological characteristics (length in cm, weight in g) of examined individuals (x:mean, s:standard error)

Station	n	<i>Mullus barbatus</i>		<i>Boops boops</i>		<i>Trachurus trachurus</i>	
		Total length (x ± s)	Weight (x ± s)	Total length (x ± s)	Weight (x ± s)	Total length (x ± s)	Weight (x ± s)
İskenderun	39	12.05±1.10	26.8±5.52	12.43±2.39	27.55±16.39	15.78±1.24	43.28±9.92
Antalya	38	13.92±0.72	29.30±4.38	17.62±1.15	61.41±14.69	14.96±1.07	28.67±6.13
Bodrum	41	14.13±0.83	35.03±5.98	18.32±1.20	70.62±15.55	28.8±1.22	214.38±31.97
Aydin	40	17.66±0.72	57.62±10.07	21.88±1.56	113.91±27.35	14.2±1.88	22.64±8.18
Çanakkale	40	14.28±1.50	30.36±7.89	17.84±1.07	54.36±7.47	11.95±0.96	14.40±3.68
Tekirdağ	38	13.36±0.26	27.09±2.73	17.96±1.11	53.09±9.31	12.62±0.69	16.05±2.78
Bandırma	40	14.44±0.54	32.66±3.68	14.97±1.19	28.79±6.19	11.27±0.71	12.84±1.43

3.2. Digestion Procedure

For metal accumulation analysis, digestion procedure was modified from Tüzen [14]. A homogenized 2g sample from epaxial muscle and gill. For liver, organ was completely separated and at least 0.5g of tissue was digested. Each tissue samples was placed in a 20ml digestion tube, and 5ml of high purity nitric acid (Merck) were added. Samples were heated and kept at 60°C for 7 days until they dissolve completely. After digestion, the samples were filtered through Whatman-Quantitative (No:42, 110mm£) filter paper. The digested portion was then diluted to a final volume of 20ml. A blank digest was carried out in the same way. All metals were determined against aqueous standards. Digested samples were analyzed with three replicates for each metal. For MDA and CAT analysis, digestion procedure were completed as fast as possible. 0.5g tissue samples were placed in falcon tubes and frozen at -80°C until further analysis.

3.3. Analytical Procedure

Metal concentrations were determined by inductively coupled plasma atomic emission spectrometry (ICP-MS) (a Plasma Quant MS Series (Jena, Germany) equipment located at Hatay Mustafa Kemal University. Operating conditions were given in Table 2. Calibration standards were prepared from a multi-element ICP Standard (Merck). The quality of data was checked against the analysis of standard reference material DORM-2 (National Research Council of Canada; dogfish muscle and hepatopancreas MA-A-2/TM Fish Flesh). The recovery values for Al, Cd, Co, Ni, Pb, Cr, Cu, Fe, Mn and Zn were measured as 99.98, 94.16, 96.57, 91.22, 97.12, 99.98, 91.94, 97.35, 89.83 and 96.59% respectively. Metal concentrations were calculated in micrograms per gram wet weight ($\mu\text{g g}^{-1}$ ww). Detection limit of Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb (in $\mu\text{g g}^{-1}$ ww) were 0.032, 0.009, 0.008, 2.151, 0.003, 0.008, 0.009, 0.603, 0.001 and 0.004, respectively.



Table 2. ICP-MS (Plasma Quant MS Series) operating conditions

RF power	1.20 kW
Plasma gas flow rate	9L min ⁻¹
Auxiliary gas flow rate	1.65L min ⁻¹
Carrier gas flow rate	1.1L min ⁻¹
Helium collision gas flow rate	mL min ⁻¹ (Not used)
Spray chamber T	2 °C
Sample depth	6 mm
Sample introduction flow rate	1 mL min ⁻¹
Nebulizer pump	0.1 rps
Extract lens	1.5 V

3.4. Determination of Malondialdehyde Concentration

Malondialdehyde concentration was carried out based on the double boiling method modified by Hammouda et al., [15]. In the first boiling process, proteins were precipitated using Trichloro Acetic Acid (TCA) to release the protein-bound MDA. In the second boiling process, a colored complex was formed by reacting total MDA with TCA in a hot and acidic environment. The resulting-colored complex was measured spectrophotometrically at 532nm wavelength. Results are given in nmol/mg protein.

3.5. Determination of Catalase Enzyme Activity

Catalase enzyme activity was determined according to the Aebi [16] method. The wavelength at which H₂O₂ gives maximum absorbance is 240nm (Schimadzu UV 1601, Japan). This method is based on the spectrophotometric measurement of the conversion of H₂O₂ added to the test medium of catalase to water and oxygen at 240nm. Catalase activity was calculated according to the changes in absorbance and the results were given in k/g protein. In the present study, k is the rate constant for the reaction defined by Aebi [16].

3.6. Statistical Analysis

One-way ANOVA and Duncan multiple comparison test were used to determine the significant difference among tissues and stations. Spearman correlation analysis was employed to investigate the relationship of metals and oxidative stress biomarkers. Lastly, principal component analysis was employed to understand the parameters which represent the variations metal concentrations and oxidative stress parameters. For that purpose, raw data including results of metal concentrations and oxidative stress parameters were processed after normalization for each tissue separately. All statistical calculations were done by PAST statistical software package [17] and Microsoft Excell. In all cases, p<0.05 were indicated the statistical significance.

3.7. Health Risk Assessment

The Target Hazard Quotient (THQ) is a recently employed tool for the assessment of potential health risks of individual heavy metals [13, 18 and 19]. The THQ≥1.0 refers that people may experience significant health risks from the intake of individual metals through fish consumption [20]. To determine the health risk of consumption of the studied species, THQ was calculated in accordance with the below formula [21].

$$THQ = [(E_F \times E_D \times F_{IR} \times C) / (R_{FD} \times W_{AB} \times T_A)] \times 10^{-3}$$

Where, EF is the exposure frequency: 365 days/year, ED is the exposure duration: the average lifetime is assumed as 70 years according to Bennett et al. [22]. FIR is the food ingestion rate is selected as 17.26g/day which is the average fisheries consumption of Turkish consumers, according to TUIK [23]. C is the determined metal concentration in edible tissue (µg g⁻¹). WAB is the average body weight:



72.8kg, TA is the average exposure time for non-carcinogens (365 days/year x ED, assuming 70 years in this study). Oral reference dose (R_{FD}) for Al, Cr, Co, Ni, Cd, Pb, Mn, Fe, Cu, Zn are taken as 1.00, 3x10⁻³, 3x10⁻⁴, 2x10⁻², 1x10⁻³, 4x10⁻³, 0.14, 0.7, 0.04, 0.3µg g⁻¹day⁻¹, respectively) [24]. The lifetime cancer risk was also investigated. The CR>10⁻⁵ indicates a high carcinogenic risk depending on the consumption of investigated species. CR was calculated in accordance with the below formula.

$$CR = [(E_F \times E_D \times F_{IR} \times C \times C_{SF}) / (W_{AB} \times T_A)] \times 10^{-3}$$

where C_{SF} is the cancer slope factor. C_{SF} value for Cr, Cd, Ni and Pb is taken as 0.5, 6.3, 1.7 and 8.5x10⁻³, respectively [25].

4. FINDINGS AND DISCUSSION

Metals found in the marine environments may accumulate in the organs which pose risk to the health of marine animals. In addition, toxicity risk increases depending on the consumption by upper trophic levels. For that reason, consumption of marine animals by humans endanger the human health [3]. Therefore, this study was undertaken to evaluate metal accumulation levels on the most commonly consumed marine fish species from Turkish coastal waters. According to TUIK, 12.319 tonnes of *T. trachurus* and 1.604 tonnes of *M. barbatus* were harvested in 2020 [23].

4.1. Metal Accumulation Levels in Tissues

In this study, metal accumulation levels in the muscle, gill and liver of *M. barbatus*, *B. boops*, *T. trachurus* from different parts of Turkish coastal waters were examined. Mean accumulation levels (in µg g⁻¹ ww) with standard errors (x±s) in the tissues of *M. barbatus*, *B. boops*, *T. trachurus* were given in Table 3, Table 4 and Table 5, respectively. Statistical analysis showed significant variations depending on the tissues (Table 3, Table 4 and Table 5).



Table 3. Metal concentration (in $\mu\text{g g}^{-1}$ ww) in the tissues of *Mullus Barbatulus* from Turkish coastal waters

Metals	Tissues	İskenderun	Antalya	Bodrum	Aydın	Çanakkale	Tekirdağ	Bandırma
Al	M	0.925±0.162a.b. x	1.385±0.346b.x	0.530±0.155a.c.x	0.325±0.212a.c. x	0.675±0.212a.c. x	0.860±0.056a.b. c.x	0.220±0.042c.x
	G	59.045±2.524a.y	21.780±1.428b.y	28.350±3.054b.y	67.065±3.627a.y	8.725±0.742c.y	11.270±1.131c.y	7.355±0.459c.y
	L	1.600±0.113a.x	0.905±0.106b.x	0.845±0.063b.x	0.935±0.091b.x	1.520±0.056a.x	0.235±0.049c.x	0.320±0.028c.x
Cr	M	0.140±0.042a.b. x	0.145±0.035a.b. x	0.205±0.035a.x	0.075±0.007a.b. x	0.440±0.028c.x	0.445±0.063c.x. y	0.075±0.007a.b. x
	G	3.590±0.113a.y	0.455±0.049c.y	1.010±0.070d.y	0.335±0.021b.c. y	0.395±0.021b.c. x	0.320±0.028b.c. x	0.140±0.014b.y. y
	L	0.090±0.028a.f. x	0.215±0.035b.x	0.380±0.014c.x	0.050±0.014a.x	0.785±0.021d.y	0.515±0.035e.y	0.150±0.014b.f. y
Co	M	0.015±0.007a.x	0.025±0.007a.x	0.015±0.006a.x	0.025±0.007a.x	0.025±0.006a.x	0.025±0.007a.x	0.025±0.006a.x
	G	0.240±0.028a.y	0.085±0.007b.c. x	0.055±0.007b.x	0.135±0.007c.x	0.045±0.007b.x	0.055±0.006b.x	0.055±0.007b.y
	L	0.340±0.028a.d. z	0.460±0.056a.c. d.y	0.650±0.021b.x	0.700±0.141c.y	0.410±0.042d.y	0.210±0.014b.d. y	0.235±0.007b.d. z
Ni	M	0.155±0.021a.c. x	0.040±0.014b.x	0.035±0.021b.x	0.025±0.007b.x	0.045±0.007b.x	0.230±0.042c.x	0.025±0.007b.x
	G	4.010±0.296a.y	0.290±0.014b.y	0.325±0.049b.y	0.410±0.042b.y	0.315±0.035b.y	0.165±0.007b.x	0.275±0.006b.y
	L	0.230±0.028a.x	0.120±0.028b.c. x	0.075±0.021c.x	0.120±0.028b.c. x	0.170±0.014a.b. z	0.155±0.021a.b. c.x	0.175±0.021a.b. z
Cd	M	0.005±0.001a.x	0.015±0.007a.x	0.010±0.001a.x	0.010±0.001a.x	0.020±0.001a.x	0.020±0.001a.x	0.010±0.001a.x
	G	0.010±0.0001a.x	0.025±0.006a.x	0.025±0.007a.x.y	0.015±0.006a.x	0.015±0.006a.x	0.020±0.001a.x	0.020±0.001a.x
	L	0.070±0.014a.y	0.080±0.014a.y	0.035±0.007a.y	0.045±0.003a.x	0.060±0.028a.x	0.045±0.007a.y	0.040±0.014a.x
Pb	M	0.380±0.070a.b. x	0.275±0.007a.x	0.250±0.042a.x	0.270±0.056a.x	0.565±0.176a.b. x	0.430±0.240a.b. x	0.155±0.021a.x
	G	1.505±0.091a.b. y	1.080±0.056a.f. y	23.845±0.332c.y	6.360±0.296d.y	3.105±0.148e.y	1.160±0.155a.f. x	0.730±0.014f.y. x
	L	0.910±0.127a.z	0.885±0.134a.y	1.500±0.311a.b.z	3.325±0.049c.z	5.150±0.791d.z	0.685±0.374a.x	0.765±0.134a.y. z
Mn	M	0.0205±0.063a.b. x	0.305±0.007b.c. x	0.075±0.007a.x	0.195±0.3007b.a x	0.125±0.035b.a x	0.460±0.084c.x	0.690±0.098d.x
	G	10.710±0.438a.y	3.340±0.226c.y	2.920±0.579c.y	15.030±0.608d.y	2.920±0.579c.y	4.325±0.445c.y	4.650±0.445b.c. y
	L	1.330±0.070a.d. z	0.945±0.162a.c. x	0.705±0.091a.c.x	0.285±0.061c.x	1.145±0.007a.x	2.035±0.049b.d. z	0.815±0.007a.c. x
Fe	M	6.005±0.700a.c. d.x	18.070±2.206b.x	7.375±0.516a.c.x	4.280±0.254a.d. x	8.500±0.155c.x	3.360±0.325d.e. x	0.755±0.021e.x
	G	440.610±0.905a. y	89.230±1.626c.y	103.235±3.061c.y	196.745±4.334d. y	53.695±7.940e. y	64.055±1.138e.y	63.440±3.054e. y
	L	134.205±2.637a. z	126.515±9.920a. z	71.035±9.043c.z	16.270±4.058d.x	122.715±0.275a. z	5.365±0.502d.x	13.265±1.887d. z
Cu	M	0.070±0.014a.x	0.670±0.014b.x	0.085±0.007a.x	0.075±0.007a.x	0.055±0.021a.x	0.085±0.007a.x	0.075±0.021a.x
	G	0.705±0.148a.c. e.y	0.600±0.028c.d. x	0.495±0.063a.c.d. x	0.495±0.021a.c. d.x	0.390±0.028b.d. y	0.905±0.021e.y	0.700±0.084c.e. y
	L	2.505±0.205a.z	2.795±0.106a.b. y	2.310±0.268a.y	2.565±0.318a.y	2.715±0.091a.z	4.715±0.247c.z	3.870±0.028d.z
Zn	M	4.455±0.487a.x x	4.015±0.091a.c. x	3.065±0.162b.c.d. x	2.700±0.098b.d. x	4.420±0.042a.x	3.560±0.438a.d. x	3.555±0.445a.d. x
	G	14.140±0.127a.e. y	14.940±0.028a.e. y	24.160±1.414b.y	18.175±0.261a.d. y	22.560±3.337b. c.y	7.130±0.820d.y	10.985±0.898d. e.y
	L	33.920±2.390a.b. z	25.335±0.926b.c. z	28.490±0.014a.b. c.z	26.580±0.848b.c. z	33.905±3.641a. b.y	36.595±1.067a.b. z	22.320±2.687c. z

Note: M=Muscle, G=Gill, L=Liver
Horizontally a, b indicates differences among stations (p<0.05), vertically letters x.y indicate differences among tissues (p<0.05)



Table 4. Metal concentration (in µg g⁻¹ ww) in the tissues of *Boops boops* from Turkish coastal waters

Metals	Tissues	İskenderun	Antalya	Bodrum	Aydın	Çanakkale	Tekirdağ	Bandırma
Al	M	0.850±0.042 _{a,x}	0.380±0.042b _{,x}	0.605±0.021a.b.x	0.225±0.035b.x	0.395±0.021b.x	0.795±0.233a.x	0.165±0.007b.x
	G	1.755±0.106 _{a,b,y}	1.075±0.007b _{,y}	1.490±0.141a.b.y	1.160±0.070a.b.y	1.515±0.289a.b.y	1.765±0.304a.b.y	1.940±0.113a.y
	L	1.070±0.084 _{a,c,x}	0.710±0.028a _{,b,z}	0.920±0.042a.b.x	0.535±0.077b.z	1.040±0.028a.x.y	1.435±0.077c.x.y	1.420±0.183a.c.y
Cr	M	0.310±0.042 _{a,x}	0.135±0.035b _{,x}	0.050±0.042b.x	0.070±0.014b.x	0.245±0.049a.x	0.345±0.021a.x	0.060±0.014b.x
	G	0.565±0.035 _{a,y}	0.205±0.007b _{,x}	0.310±0.042a.b.y	0.890±0.070c.y	0.580±0.014a.y	0.800±0.141c.x	0.510±0.127a.y
	L	0.665±0.035 _{a,y}	0.305±0.007b _{,c,y}	0.380±0.028c.y	0.100±0.014b.x	0.580±0.028a.c.y	0.540±0.183a.c.x	0.705±0.021a.y
Co	M	0.015±0.007 _{a,x}	0.015±0.006a _{,x}	0.015±0.007a.x	0.015±0.006a.x	0.015±0.007a.x	0.020±0.014a.x	0.015±0.006a.x
	G	0.035±0.007 _{a,b,x,y}	0.015±0.007a _{,x}	0.015±0.006a.x	0.0135±0.007a.b.x.y	0.035±0.006a.b.x	0.040±0.028a.b.x	0.035±0.006a.b.x
	L	0.055±0.007 _{a,y}	0.155±0.021a _{,b,y}	0.090±0.014a.y	0.055±0.007a.y	0.230±0.042a.b.y	0.275±0.063b.y	0.265±0.077b.y
Ni	M	0.200±0.042 _{a,x}	0.020±0.014b _{,x}	0.040±0.014b.c.x	0.020±0.014b.x	0.030±0.014b.x	0.045±0.021b.c.x	0.020±0.014b.x
	G	0.710±0.028 _{a,y}	0.235±0.021b _{,y}	0.275±0.035b.y	0.560±0.070a.y	0.360±0.127b.y	0.535±0.077a.y	0.370±0.028b.y
	L	0.235±0.049 _{a,x}	0.025±0.007b _{,x}	0.050±0.028b.x	0.050±0.014b.x	0.090±0.014b.x.y	0.090±0.014b.x	0.090±0.015b.x
Cd	M	0.010±0.001 _{a,x}	0.010±0.001a _{,x}	0.010±0.001a.x	0.010±0.001a.x	0.015±0.006a.x	0.025±0.021a.x	0.010±0.001a.x
	G	0.015±0.007 _{a,x}	0.015±0.006a _{,x}	0.015±0.007a.x	0.020±0.001a.x	0.025±0.007a.x	0.30±0.014a.x	0.030±0.001a.x
	L	0.135±0.007 _{a,c,y}	0.290±0.042c _{,d,y}	0.070±0.014a.y	0.145±0.007a.c.y	0.355±0.063d.y	0.070±0.014a.x	0.500±0.056b.d.y
Pb	M	0.415±0.063 _{a,x}	0.110±0.0042a _{,x}	1.755±0.443c.x	0.275±0.120a.x	0.370±0.098a.x	0.525±0.049a.x	0.145±0.021a.x
	G	2.010±0.268 _{a,b,y}	0.870±0.098a _{,y}	3.595±0.572b.x	1.345±0.261a.y	3.075±0.473b.y	2.265±0.275a.b.y	0.830±0.141a.y
	L	1.300±0.325 _{a,b,x,y}	0.550±0.084b _{,d,y}	3.000±0.353c.x	0.750±0.113a.b.d.x.y	1.440±0.254a.x	1.275±0.134a.b.z	0.685±0.148a.b.d.y
Mn	M	0.145±0.035 _{a,x}	0.165±0.049a _{,x}	0.110±0.014a.x	0.085±0.021a.x	0.385±0.091a.b.x	0.520±0.084b.x	0.400±0.028b.x
	G	4.175±0.077 _{a,b,y}	5.340±0.395b _{,y}	3.650±0.113a.c.y	12.675±0.544d.y	7.020±0.245e.y	13.125±0.176d.y	3.615±0.247a.c.y
	L	1.895±0.091 _{a,z}	0.965±0.021b _{,c,x}	0.545±0.077c.z	1.145±0.063b.x	0.750±0.084b.c.x	2.680±0.254d.z	0.440±0.282c.x
Fe	M	6.380±0.311 _{a,x}	5.675±0.558a _{,x}	4.785±0.431a.b.x	3.375±0.205b.d.x	3.685±0.388b.x	3.695±0.176b.x	2.090±0.296b.d.x
	G	75.310±4.72 _{3a,y}	175.740±18.059b,y	74.940±3.917a.y	91.490±1.173a.y	199.410±15.372b.y	44.075±1.689a.y	76.630±4.737a.y
	L	189.050±6.279a,z	262.965±8.209b,z	98.345±1.053c.z	130.985±4.886d.z	314.905±20.286e.z	85.105±3.245c.z	145.620±7.269d.z
Cu	M	0.030±0.014 _{a,x}	0.035±0.007a _{,x}	0.045±0.006a.x	0.045±0.007a.x	0.070±0.014a.x	0.045±0.007a.x	0.060±0.028a.x
	G	0.345±0.035 _{a,y}	0.700±0.084b _{,c,y}	0.300±0.028a.x	0.320±0.056a.x	0.850±0.042c.x	0.500±0.113a.b.x	0.865±0.077c.y
	L	2.035±0.077 _{a,z}	2.305±0.148a _{,b,z}	2.180±0.183a.y	1.865±0.162a.y	4.350±0.410c.y	2.520±0.367a.b.y	2.720±0.226a.b.z
Zn	M	4.675±0.007 _{a,x}	4.360±0.183a _{,b,x}	3.525±0.572b.c.x	2.955±0.148c.x	2.130±0.127c.x	5.765±0.148e.x	0.675±0.021f.x
	G	19.835±0.275 _{a,b,e,y}	18.220±0.636a,d,e,y	23.470±1.753b.y	21.745±1.633a.b.y	13.745±1.124d.y	22.185±1.279a.b.y	6.035±0.021f.y
	L	33.820±2.347 _{a,z}	21.945±1.251b,d,z	36.440±0.395a.z	33.750±2.630a.z	27.065±1.209d.z	39.395±0.855a.z	15.670±1.725b.z

Note: M=Muscle, G=Gill, L=Liver
Horizontally a,b indicates differences among stations (p<0.05), vertically letters x,y indicate differences among tissues (p<0.05)



Table 5. Metal concentration (in $\mu\text{g g}^{-1}$ ww) in the tissues of *Trachurus trachurus* from Turkish coastal waters

Metals	Tissues	Iskenderun	Antalya	Bodrum	Aydın	Çanakkale	Tekirdağ	Bandırma
Al	M	0.775±0.190a .x	2.310±0.367 b.x.y	0.445±0.12 0a.x	0.820±0.183 a.x	0.415±0.035a .x	0.405±0.077 a.x	0.300±0.014a .x
	G	18.485±0.586 a.y	2.770±0.197 b.x	1.000±0.09 8b.y	13.020±1.10 3c.y	1.955±0.049b .y	1.330±0.141 b.y	1.390±0.084b .x
	L	1.680±0.240a .x	1.360±0.296 a.y	0.385±0.16 2a.x	0.995±0.035 a.x	2.200±0.014a .z	2.250±0.169 a.z	41.625±3.401 c.y
Cr	M	0.205±0.035a .x	0.150±0.028 a.x	0.105±0.02 1a.x	0.435±0.063 b.x	0.360±0.042b .x	0.435±0.021 b.x	0.090±0.014a .x
	G	0.55±0.042a y	0.530±0.070 a.y	0.405±0.07 7a.y	0.510±0.070 a.x	0.545±0.106a .x.y	0.445±0.035 a.x	0.660±0.028a y
	L	0.485±0.063a .y	1.035±0.021 b.z	0.070±0.01 4c.x	0.495±0.007 a.x	0.835±0.063b .y	0.545±0.035 a.x	0.900±0.155b y
Co	M	0.025±0.007a .x	0.0150±0.00 7a.x	0.015±0.00 7a.x	0.0150±0.00 7a.x	0.015±0.007a .x	0.015±0.007 a.x	0.025±0.007a .x
	G	0.045±0.007a .x	0.045±0.006 a.y	0.025±0.00 6a.x	0.045±0.007 a.x	0.060±0.014a .x	0.035±0.007 a.x	0.065±0.007a .x
	L	0.290±0.028a .y	0.165±0.007 a.z	0.055±0.02 1b.x	0.335±0.077 c.y	0.280±0.028a .y	0.105±0.007 b.y	0.400±0.056a c.y
Ni	M	0.0175±0.035 a.x	0.025±0.007 b.x	0.015±0.00 7b.x	0.045±0.007 b.x	0.045±0.035b .x	0.015±0.007 b.x	0.015±0.007b .x
	G	0.575±0.007a y	0.375±0.021 b.y	0.325±0.03 5b.y	0.465±0.035 a.y	0.550±0.042a y	0.275±0.049 b.y	0.565±0.007a y
	L	0.275±0.077a .x	0.140±0.014 a.b.z	0.045±0.02 1b.x	0.260±0.028 a.z	0.085±0.007b .x	0.110±0.014 b.x	0.215±0.063a z
Cd	M	0.015±0.007a .x	0.015±0.006 a.x	0.010±0.00 1a.x	0.015±0.006 a.x	0.015±0.007a .x	0.015±0.06a .x	0.015±0.006a .x
	G	0.015±0.007a .x	0.035±0.007 a.x	0.015±0.00 7a.x	0.025±0.007 a.x	0.025±0.007a .x	0.030±0.001 a.x	0.035±0.007a .x
	L	0.175±0.049a y	0.325±0.035 b.y	0.100±0.02 8a.y	0.150±0.014 a.y	0.245±0.007b .y	0.150±0.014 a.y	0.450±0.042c y
Pb	M	0.360±0.014a .x	0.165±0.007 a.x	0.195±0.04 9a.x	0.405±0.063 a.x	0.525±0.077a .b.x	0.390±0.042 a.x	0.225±0.063a .x
	G	1.340±0.183a .b.y	0.760±0.042 b.y	1.010±0.15 5a.b.y	0.885±0.049 a.b.y	1.040±0.155a .b.x	1.040±0.155 a.b.y	0.835±0.007b y
	L	0.755±0.120a .b.x	0.955±0.106 a.y	0.205±0.07 7b.x	1.285±0.162 a.y	1.500±0.367c .x	2.555±0.049 d.z	2.175±0.021d z
Mn	M	0.150±0.014a .x	0.080±0.028 a.x	0.070±0.02 8a.x	0.155±0.063 a.x	0.055±0.021a .x	0.060±0.014 a.x	0.300±0.084b .x
	G	3.185±0.219a y	2.520±0.268 b.y	1.620±0.19 7b.y	2.445±0.049 b.y	2.020±0.240b .y	1.895±0.021 b.y	4.125±0.091d y
	L	2.105±0.148a z	0.420±0.056 b.x	0.685±0.06 3b.z	2.195±0.275 a.y	0.800±0.113b .z	0.705±0.077 b.z	2.170±0.212a z
Fe	M	6.985±0.374a .x	7.480±0.424 a.x	4.260±0.86 2a.b.x	10.020±0.76 3a.x	6.715±0.813a .x	8.635±0.586 a.x	1.655±0.063b .x
	G	69.880±1.470 a.y	75.025±1.67 5a.y	64.365±5.8 33a.y	106.500±15. 457b.y	72.650±1.880 a.y	96.795±8.12 4a.b.y	63.300±0.678 a.y
	L	198.450±6.54 7a.b.z	159.950±0.4 80a.z	108.455±9. 439d.z	480.150±21. 156e.z	192.360±5.81 2a.b.z	49.330±9.36 2c.f.z	136.625±8.73 2b.d.z
Cu	M	0.925±0.077a .x	0.200±0.028 b.x	0.535±0.16 2b.x	0.185±0.091 b.x	0.880±0.042a .x	0.780±0.042 a.x	0.305±0.021b .x
	G	1.535±0.473a .x	1.275±0.106 a.y	1.100±0.19 7a.x	1.105±0.134 a.x	1.290±0.028a .y	1.715±0.219 a.x.y	1.420±0.042a y
	L	4.835±0.700a c.y	2.825±0.120 b.d.z	3.740±0.25 4a.b.y	2.350±0.466 b.d.y	1.715±0.063d .z	2.505±0.530 b.d.y	2.555±0.417b d.z
Zn	M	3.590±0.226a .x	17.405±2.11 4b.x	3.745±0.61 5a.x	7.010±0.169 c.x	3.065±0.007a .x	8.950±0.240 c.x	3.995±0.120a .x
	G	16.735±0.516 a.y	23.740±0.43 8b.y	18.415±0.7 14a.y	15.860±0.55 1a.y	15.080±2.503 a.y	13.280±1.27 2a.x.y	18.300±1.074 a.y
	L	31.575±0.714 a.c.z	20.875±0.02 1b.x.y	20.660±0.0 70b.y	37.985±1.98 6c.z	19.955±0.289 b.y	17.650±1.79 6b.y	21.425±1.251 b.y

Note: M=Muscle, G=Gill, L=Liver
Horizontally a,b indicates differences among stations ($p<0.05$), vertically letters x.y indicate differences among tissues ($p<0.05$)

Aluminum (Al) concentration in the muscle of *M. barbatus*, *B. boops*, *T. trachurus* was varied between $0.19\text{--}1.63\mu\text{g g}^{-1}$, $0.16\text{--}0.96\mu\text{g g}^{-1}$, $0.29\text{--}0.48\mu\text{g g}^{-1}$, respectively. Turan et al., Fındık and Çiçek were reported higher Al accumulation in the muscle tissue of *M. barbatus* from the Mediterranean Sea and the Black Sea; whereas, Gündoğdu et al., reported similar accumulation levels from the Black Sea [26, 27 and 28]. Similar accumulation levels in the muscle of *B. boops* were reported from İzmir, Aegean Sea; on the other hand, higher concentrations were reported from Mersin, Mediterranean Sea [29 and 30]. Al accumulation in the muscle of *T. trachurus* estimated in this study was higher than the previous study conducted in Black Sea [28].

Minimum and maximum chromium (Cr) concentration in the muscle, liver and gill of studied species were found as follows: *M. barbatus*: $0.07\text{--}0.49\mu\text{g g}^{-1}$, $0.13\text{--}3.67\mu\text{g g}^{-1}$, $0.04\text{--}0.8\mu\text{g g}^{-1}$; for *B. boops*: $0.02\text{--}0.36\mu\text{g g}^{-1}$, $0.2\text{--}0.97\mu\text{g g}^{-1}$, $0.09\text{--}0.72\mu\text{g g}^{-1}$, for *T. trachurus* $0.08\text{--}0.48\mu\text{g g}^{-1}$



g^{-1} , $0.35-0.68\mu g g^{-1}$, $0.06-0.44\mu g g^{-1}$, respectively. Similar Cr accumulation levels in the muscle and liver tissue were reported in Albanian Sea, Aegean Sea, Black Sea, Mediterranean Sea, Algeria [31, 32, 33 and 34]. Cr concentration in the gill tissue was found to be lower than the Aegean Sea [33]. Cr concentration in the muscle tissue of *B. boops* were similar to studies reported from İzmir Bay, Aegean Sea [29], Antalya, northeastern Mediterranean [35]; lower than the studies conducted in Mersin, Northeastern Mediterranean Sea [30]; Egypt, Eastern Mediterranean Sea [36], and higher than the conducted in Nigeria [38]. Cr concentration in the liver and gill of this study were higher than the study conducted in Antalya, Northeastern Mediterranean [35]. Reported Cr concentration in the muscle gill and liver of *T. trachurus* was similar to study reported from the Black Sea [37]; however, concentration in the muscle tissue was higher than studies reported from Nigeria [38], Adriatic Sea [39].

Cobalt (Co) concentration in the muscle, gill and liver tissue of *M. barbatus* was varied from 0.01 to $0.03\mu g g^{-1}$, 0.04 to $0.26\mu g g^{-1}$ and 0.05 to $0.8\mu g g^{-1}$ which were coherent to report from Sicily channel [40]. Co concentration in the muscle, gill and liver tissue of *B. boops* was varied between $0.01-0.03\mu g g^{-1}$, $0.01-0.06\mu g g^{-1}$ and $0.05-0.32\mu g g^{-1}$ which was coherent to report from İzmir Bay, Aegean Sea [29]. Reported Co concentration in the muscle, gill and liver tissue of *T. trachurus* was varied between $0.01-0.03\mu g g^{-1}$, $0.02-0.07\mu g g^{-1}$ and $0.04-0.44\mu g g^{-1}$, respectively. Estimated concentration in the muscle and liver tissue of *T. trachurus* was similar to the studies conducted in the Black Sea [37 and 41].

Minimum and maximum nickel (Ni) concentration in the muscle, gill and liver of *M. barbatus*, *B. boops* and *T. trachurus* were found as $0.02-0.26\mu g g^{-1}$, $0.16-4.22\mu g g^{-1}$, $0.06-0.25\mu g g^{-1}$, $0.01-0.23\mu g g^{-1}$, $0.22-0.73\mu g g^{-1}$, $0.02-0.27\mu g g^{-1}$, $0.01-0.2\mu g g^{-1}$, $0.24-0.58\mu g g^{-1}$, $0.03-0.33\mu g g^{-1}$, respectively. Ni accumulation in the muscle tissue of *M. barbatus* was similar to studies conducted in the Mediterranean Sea [4, 11 and 26], Black Sea [26], Sicily channel [40], and lower than Black Sea (27). Ni concentration in the muscle tissue of *B. boops* was similar to those reported in İzmir, Aegean Sea [29], Mediterranean Sea [35]; on the other hand, concentration in the liver was lower than previous study from the Mediterranean Sea [35]. Ni concentration in the muscle of *T. trachurus* was coherent to previous studies from the Turkish coastline [37], Black Sea [28 and 41]; whereas, concentration in the liver tissue was lower than previous studies [35].

Minimum and maximum cadmium (Cd) concentration in the muscle, gill and liver were determined as $0.01-0.02\mu g g^{-1}$, $0.01-0.03\mu g g^{-1}$, $0.02-0.09\mu g g^{-1}$ for *M. barbatus*; $0.01-0.04\mu g g^{-1}$, $0.01-0.04\mu g g^{-1}$, $0.06-0.54\mu g g^{-1}$ for *B. boops*; $0.01-0.02\mu g g^{-1}$, $0.01-0.04\mu g g^{-1}$, $0.08-0.48\mu g g^{-1}$ for *T. trachurus*, respectively. Higher Cd concentrations in the muscle of *M. barbatus* was reported in the Albanian Sea [39], Mediterranean Sea [26], Black Sea [26 and 27], Aegean Sea [32], Algeria [34], lower concentrations were reported in the Mediterranean Sea [4, 11 and 42], Sicily channel [40], and similar results were reported from Mediterranean Sea [43], Aegean Sea [33]. Estimated Cd concentration in the muscle of *B. boops* were higher than those reported from Aegean Sea [29 and 44], Mediterranean Sea [35, 36 and 44], lower than those reported from Mersin, Mediterranean Sea [30], Tripoli Port, Libya [45], and similar to those reported from Atlantic Sea [46]. Cd concentration in the gill and liver tissue of *B. boops* was similar to a previous study from the Mediterranean Sea [35]. Cd concentration in the muscle of *T. trachurus* was similar to studies reported from Sinop, Black Sea [37], Ghana [47], higher than those reported from Adriatic Sea [39], Black Sea [28] and lower than those reported from Egypt [48]. Cd concentration in liver tissue of *T.*



trachurus was similar to previous studies from Egypt [48], Black Sea [37] and Adriatic Sea [39].

Minimum and maximum lead (Pb) concentration in the muscle, gill and liver of *M. barbatus* was varied between 0.01-0.02 $\mu\text{g g}^{-1}$, 0.01-0.03 $\mu\text{g g}^{-1}$, 0.02-0.09 $\mu\text{g g}^{-1}$, respectively. Lower accumulation levels in the muscle tissue of *M. barbatus* were reported from Albanian Sea [39], Mediterranean Sea [4, 26, 42, 43], Black Sea [26, 28 and 49], Aegean Sea [33], Algeria [34], Sicily channel [40], and similar accumulation levels were reported from Black Sea [27] and Mediterranean Sea [11]. Pb concentration in the liver and gill estimated in this study were lower than those reported from Aegean Sea [33] and higher than those reported from Algerian [34]. Pb concentration in the muscle, gill and liver of *B. boops* was varied between 0.08-2.07 $\mu\text{g g}^{-1}$, 0.73-4.00 $\mu\text{g g}^{-1}$, 0.49-3.25 $\mu\text{g g}^{-1}$, respectively. In literature similar accumulation levels in the muscle tissue were reported from the Mediterranean Sea [40 and 44]; while, lower levels in the muscle, gill and liver tissue were reported from Egypt [35 and 36], the Aegean Sea [29]. Pb concentration in the muscle, gill and liver of *T. trachurus* was varied between 0.16-0.58 $\mu\text{g g}^{-1}$, 0.73-1.47 $\mu\text{g g}^{-1}$, 0.15-2.59 $\mu\text{g g}^{-1}$, respectively. Previous studies reported comparable accumulation levels in the muscle and liver tissues of *T. trachurus* from the Mediterranean Sea [48], Turkish coastline [37], Ghana [47]. Lower accumulation in the muscle and liver tissue were reported in the Adriatic Sea [39], Black Sea [28]; whereas; higher accumulation levels in the gill and liver tissues were reported from Egypt, Mediterranean Sea [48].

Minimum and maximum manganese (Mn) concentration in the muscle, gill and liver of *M. barbatus* were estimated as 0.01-0.02 $\mu\text{g g}^{-1}$, 0.01-0.03 $\mu\text{g g}^{-1}$, 0.02-0.09 $\mu\text{g g}^{-1}$, respectively. Reported accumulation levels in the tissues of *M. barbatus* in this study were comparable to those reported from the Albanian Sea [39]; Mediterranean Sea [4, 11 and 26], Black Sea [26 and 27], Aegean Sea [33], Algeria [34]; whereas, higher accumulation in the muscle tissue was reported from Black Sea [49]. Manganese concentration in the muscle, gill and liver of *B. boops* was varied between 0.07-0.58 $\mu\text{g g}^{-1}$, 3.44-13.25 $\mu\text{g g}^{-1}$, 0.24-2.86 $\mu\text{g g}^{-1}$, respectively. Similar concentrations in the muscle tissue were reported from the Aegean Sea [29], the Mediterranean Sea [35]; while higher concentrations were reported from Mersin, Mediterranean Sea [30]. In addition, Tekin-Özan [35] reported lower accumulation levels in the gill and liver tissue from Mersin, Mediterranean Sea. Mn concentration in the muscle, gill and liver of *T. trachurus* was varied between 0.04-0.36 $\mu\text{g g}^{-1}$, 1.48-4.19 $\mu\text{g g}^{-1}$, 0.38-2.39 $\mu\text{g g}^{-1}$, respectively. Reported concentrations in the tissues of *T. trachurus* were similar to those reported from Nigeria [38], Turkish coastal waters [37], Ghana [47]; whereas, lower than those reported from the Black Sea [41].

Minimum and maximum Fe concentration in the muscle, gill and liver of *M. barbatus*, *B. boops* and *T. trachurus* were found as 0.74-19.63 $\mu\text{g g}^{-1}$, 48.08-441.25 $\mu\text{g g}^{-1}$, 5.01-136.07 $\mu\text{g g}^{-1}$, 1.88-6.60 $\mu\text{g g}^{-1}$, 42.88-210.88 $\mu\text{g g}^{-1}$, 82.81-329.25 $\mu\text{g g}^{-1}$, 1.61-10.56 $\mu\text{g g}^{-1}$, 60.24-117.43 $\mu\text{g g}^{-1}$, 42.71-495.11 $\mu\text{g g}^{-1}$, respectively. Fe accumulation levels in the tissues of *M. barbatus* was within a similar range of previous studies reported from the Mediterranean Sea, Black Sea, Aegean Sea [4, 11, 26, 27, 28, 33, 34, 49 and 50]. Fe accumulation in the tissues of *B. boops* was comparable with the studies conducted in the Mediterranean Sea, Aegean Sea [29, 30, 35 and 44]; whereas, lower accumulation levels were reported from Libya [45]. Fe concentration in the muscle of *T. trachurus* was lower than the studies reported in the Black Sea [28, 37 and 45]; however, concentration in the liver tissue was within the same variation range [37].

Minimum and maximum copper (Cu) concentration in the muscle, gill and liver of *M. barbatus* were varied between 0.04-0.68 $\mu\text{g g}^{-1}$, 0.37-0.92 $\mu\text{g g}^{-1}$



g^{-1} , 5.01–136.07 $\mu g g^{-1}$, respectively. Reported accumulation levels in the tissues of *M. barbatus* in this study were comparable to those reported from the Mediterranean Sea [11, 26, 40 and 43], Aegean Sea [32 and 40]; Black Sea [26, 28 and 49], Algeria [34]; whereas, higher accumulation levels were reported from Mediterranean Sea [4] and Black Sea [50]. Cu concentration in the muscle, gill and liver of *B. boops* was varied between 0.02–0.08 $\mu g g^{-1}$, 0.28–0.92 $\mu g g^{-1}$, 1.75–4.64 $\mu g g^{-1}$, respectively. Higher concentration levels in the tissues of *B. boops* were reported from Aegean Sea, Black Sea and Mediterranean Sea [29, 30, 35, 36, 44 and 45]. Cu concentration in the muscle, gill and liver of *T. trachurus* was varied between 0.12–0.98 $\mu g g^{-1}$, 0.96–1.87 $\mu g g^{-1}$, 1.67–5.33 $\mu g g^{-1}$, respectively. Reported concentrations in the tissues of *T. trachurus* were similar those reported from Nigeria [38], Egypt [48], Turkish coastal waters [37], Ghana [47]; whereas, higher concentrations in the muscle and liver tissue were reported from Egypt [46], Black Sea [41].

Minimum and maximum zinc (Zn) concentration in the muscle, gill and liver of *M. barbatus* were determined as 0.04–0.68 $\mu g g^{-1}$, 0.37–0.92 $\mu g g^{-1}$, 5.01–136.07 $\mu g g^{-1}$, respectively. Accumulation levels in the tissues were similar to the studies reported from Aegean Sea [32 and 33], Black Sea [26, 28 and 49] and Mediterranean Sea [26, 40 and 43], Algeria [34]; on the other hand, higher levels were reported from Mediterranean Sea [4, 11 and 50]. Zn concentration in the muscle, gill and liver of *B. boops* was varied between 0.67–5.87 $\mu g g^{-1}$, 6.02–24.71 $\mu g g^{-1}$, 14.45–40.00 $\mu g g^{-1}$, respectively. Zn accumulation levels in the tissues of *B. boops* were similar to the studies reported from the Aegean Sea [29 and 44], Mediterranean Sea [35, 36, 44 and 45]; on the other hand, higher levels were reported from Mersin, Mediterranean Sea [30]. Zn concentration in the muscle, gill and liver of *T. trachurus* was varied between 3.06–18.9 $\mu g g^{-1}$, 12.38–24.05 $\mu g g^{-1}$, 16.38–39.39 $\mu g g^{-1}$, respectively. Zn accumulation levels in the tissues of *T. trachurus* were similar to the studies reported from Black Sea [37 and 41] and Mediterranean Sea [48]; whereas, lower levels were reported from Black Sea [28], Ghana [47].

As it can be seen from the literature, metal accumulation in the tissues did not show clear picture even in the studies conducted in same region. This situation represents that metal accumulation strongly influenced by environmental factors. Metal accumulation levels in the fish impacted by the pollution level in the surrounding environment and represent the pollution status of sampling area [51]. Mutlu et al., [37] reported variable accumulation levels in muscle and liver tissues of *T. trachurus* from Turkish coastline. In addition, this heterogeneity observed in the metal accumulation levels might be related with the sampling time and season. In fact, season is the most well-known parameter which impact the accumulation rate in fish. For example, Uçar [30] found seasonal variation in the heavy metal accumulation levels in the muscle of *M. barbatus* from Mediterranean Sea. Similarly, Durmuş [41] reported seasonal variation in the metal concentration in the muscle tissue *T. trachurus* sampled from Black Sea. Furthermore, heavy metal accumulation was affected by gender [41], size of the examined species [31 and 32]. To sum up, all these environmental and biological factors create a heterogeneity in the reported accumulation levels.

4.2. Metal Accumulation Levels Between Species

In this study, metal accumulation levels were varied depending on the specie (Table 3 and Table 5). Variations in the metal accumulation levels may arise from the variations in reproductive cycle, growth cycle, metabolism, habitat, feeding strategies of species [52 and 54]. *M. barbatus* is demersal specie which usually found on gravel, sand and mud bottoms. It feeds on small benthic crustaceans, worms and mollusks. *B. boops* usually found in shelf and coastal pelagic waters on various



bottoms. It is omnivorous and usually feed on planktophagous and crustaceans. *T. trachurus* is pelagic-neritic specie that feeds on fish, crustaceans, and cephalopods [55]. Results obtained in this study showed that highest Co, Ni, Mn, Fe accumulation was observed in the muscle of demersal *M. barbatus*; whereas, highest Al, Cr, Cd, Cu, Zn accumulation were found in the muscle of pelagic *T. trachurus*. Concentration of metals were distributed to the water column depending on the redox potential of environment [49]. For that reason, species whose have close relationship with sediment accumulate higher amounts of metals in their tissues [30 and 43]. In this study, a clear difference could not be obtained, some metals were found to be more accumulative in pelagic fish species; while, some metals were more accumulative in demersal fish *B. boops*. This conflicting result was also observed in previous studies. For instance, similar to this study, Varol et al., [29] found the highest Al, As, Cd and Pb concentration in the flesh of demersal *M. barbatus*. On the other hand, Uçar [30] found higher accumulation levels in the *B. boops* which have close relationship with sediment.

4.3. Accumulation Levels Among Metal Types

Accumulation levels in the tissue was differed depend on type of the metal. Accumulation levels, depending on mean concentrations, in the tissues of studies species are as follows:

M. barbatus (muscle: Fe>Zn>Al>Pb>Mn>Cr>Cu>Ni>Co>Cd; gills: Fe>Al>Zn>Mn>Pb>Cr>Ni>Cu>Co>Cd; liver: Fe>Zn>Cu>Pb>Mn>Al>Co>Cr>Ni>Cd),
B. boops (muscle: Fe>Zn>Pb>Al>Mn>Cr>Ni>Cu>Co>Cd; gills: Fe>Zn>Mn>Pb>Al>Cu>Cr>Ni>Co>Cd; liver: Fe>Zn>Cu>Pb>Cd>Al>Cr>Cd>Co>Ni),
T. trachurus (muscle: Zn>Fe>Al>Cu>Pb>Cr>Mn>Ni>Co>Cd; gill: Fe>Zn>Al>Cu>Pb>Mn>Cr>Cd>Co>Ni). In this study, essential elements Fe Al, Zn were found as the most accumulated metal; whereas, Co, Cd, Ni were the least accumulated metals (Table 3, Table 4 and Table 5). Similar outcome was reported from Aegean Sea Black Sea, North eastern Mediterranean [33]. Higher accumulation of Fe and Zn in the tissues may be related with the higher availability of them in sea water; since fish have tendency the bioaccumulate metals in water column [38 and 46].

4.4. Accumulation Levels Between Tissues

Results showed variation in the metal accumulation levels between tissues that could be explained with the difference ratios of metallothioneins in different tissues [57] (Table 3 and Table 5). Muscle was found as the least accumulative tissue regardless of specie or metal type which is coherent to the previous studies [12 and 33]. The highest Al, Ni, Mn accumulation was observed in gill; whereas, the highest Co, Cd, Fe, Cu, Zn accumulation was found in liver. In literature, it has been reported that metals like Mn have tendency to accumulate in gills [58 and 59]. This is expected since gills are responsible for the uptake of resuspended and ionized metals from water column [32]. Higher accumulation of metals in the liver tissue is a well-known phenomenon and reported in many studies [32, 34, 35 and 48]. Metallothioneins found in tissues transport the metal ions into liver because, liver has vital role in synthesis of metal binding proteins and detoxification [60 and 61].

4.5. Variations in Oxidative Stress Biomarkers

Malondialdehyde (MDA) is the end product of lipid peroxidation and used as indicator for free radical production in stressed cells [62]. For that reason, MDA activity in the organism is considered as typical defense component against metal contamination. On the other hand, catalase enzyme activity initiates under oxidative stress resulting from pollutant-induced stress, so enhanced levels in the CAT activity may

represent formation of adaptation equilibrium [63]. As a result, MDA and CAT levels were used as sensitive indicators to monitor pollution status in marine environments [64 and 65]. Mean MDA and CAT activity of all studies fish was varied between 1.40-13.70nmol/mg, 2.05-10.10k/g protein in muscle and 3.90-88.95nmol/mg, 2.05-5.00-18.20k/g protein in liver, respectively. Estimated concentrations were similar to previous studies conducted in marine environment [64 and 67] and freshwater resources [68 and 69]. Previous studies conducted in the surface rivers clearly showed that as the metal concentrations in the ambient environment increases, MDA and CAT levels in the tissues were increases [63 and 70]. Similar conclusion was reported from laboratory studies [71]. This could be the explanation of the variation in the MDA and CAT levels among stations (Table 6).

Table 6. Oxidative stress parameters (MDA and CAT) of *M. babatus*, *B. boops* and *T. trachurus*

Specie	Station	CAT (k/g protein)		MDA (nmol mg ⁻¹)	
		Muscle	Liver	Muscle	Liver
<i>M. Barbatus</i>	İskenderun	6.20±0.1	9.10±0.1	3.33±0.03	18.10±13.77
	Antalya	5.90±0.1	11.70±0.1	2.51±0.04	10.83±0.03
	Tekirdağ	5.70±0.05	12.10±0.1	1.78±0.01	9.47±0.07
	Çanakkale	5.40±0.05	12.30±0.15	2.30±0.05	11.37±0.10
	Aydın	2.10±0.1	12.70±0.15	5.47±0.07	8.44±0.14
	Bandırma	6.10±0.1	6.10±0.1	3.13±0.10	62.96±0.06
<i>B. Boops</i>	Bodrum	5.80±0.05	10.90±0.2	2.92±0.04	6.73±0.03
	İskenderun	7.10±0.1	14.20±0.2	3.54±0.04	26.10±0.10
	Antalya	8.20±0.1	15.40±0.2	2.37±0.07	9.84±0.04
	Tekirdağ	4.10±0.05	8.90±0.2	12.41±0.05	55.97±0.07
	Çanakkale	8.10±0.1	13.80±0.1	8.37±0.07	43.04±0.04
	Aydın	3.70±0.15	14.30±0.1	13.70±0.10	47.77±0.06
<i>T. trachurus</i>	Bandırma	4.10±0.1	5.10±0.1	12.74±0.04	88.85±0.06
	Bodrum	8.30±0.15	13.30±0.2	2.25±0.05	50.69±0.09
	İskenderun	5.60±0.1	11.20±0.2	3.24±0.04	8.38±0.08
	Antalya	3.30±0.1	9.90±0.2	9.01±0.06	11.62±0.07
	Tekirdağ	7.90±0.1	17.40±0.4	2.45±0.05	4.00±0.10
	Çanakkale	4.90±0.1	18.20±0.2	3.58±0.08	4.43±0.07
	Aydın	9.90±0.1	16.90±0.3	1.45±0.05	7.84±0.04
	Bandırma	8.60±0.05	17.30±0.3	2.34±0.04	4.64±0.10
	Bodrum	10.10±0.1	16.40±0.3	1.47±0.07	5.97±0.07

Correlation analysis showed that there is a significant positive correlation between Fe, Cu, Cr and MDA levels and negative correlation between MDA and CAT in the muscle (Figure 2). Similarly, MDA levels were found to be positively correlated with Al, Cr, Cd and CAT levels were found to be negatively correlated with Cd, Al and MDA. Previous studies from marine environments also reported similar correlations [65, 66 and 68].

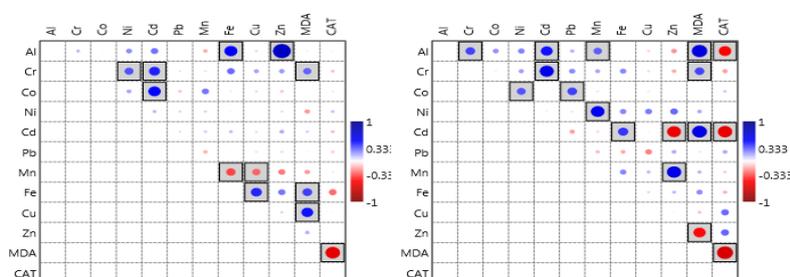


Figure 2. Heatmap of correlations between metal levels and oxidative stress parameters in muscle (on the left) and liver (on the right).

Scale color bar indicate correlation between -1 and +1. Square indicate the significant coefficients with 0.05 significance level (p<0.05)

PCA (Principal Component Analysis) was conducted to understand the parameters that describe the difference in metals and biostress parameters. In muscle, first two principal component explained 50% and 25% of variance, respectively. First component have positive strong loading of Fe, Zn, MDA and moderate negative loading of CAT. In liver, first principal component explained 94% of total variance which has high Fe and moderate MDA loading (Figure 3). Therefore, PCA analysis showed that MDA and CAT activity levels could effectively be used to address pollution differences between stations.

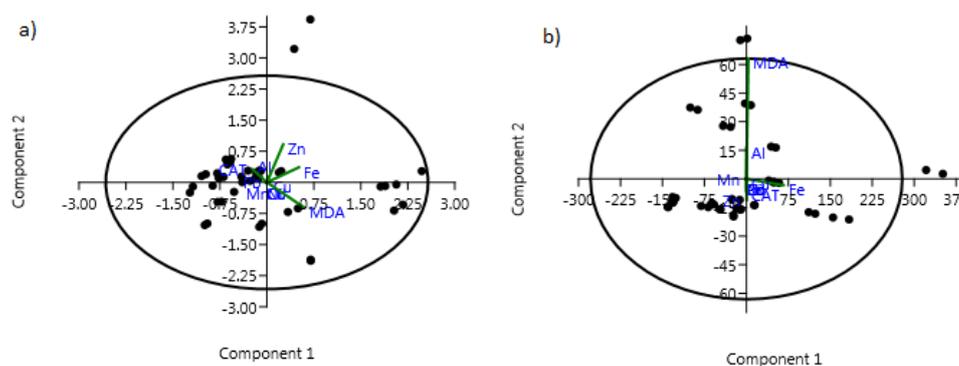


Figure 3. PCA diagram indicating the variation in (a) muscle, (b) liver of fish

Similar to the metal's levels, MDA and CAT activity levels were varied between tissues and higher levels were reported from liver tissue (Table 6). Presumably, this is the result of higher concentrations of metals in the liver tissue. Higher MDA and CAT levels in the liver were also reported from previous studies [9, 62 and 69]. Concentrations of biostress parameters were varied among species. MDA and CAT activity levels in the muscle and liver (based on mean measurements) were ranked as *T. trachurus*>*B. boops*>*M. barbatus* and *M. barbatus*>*T. trachurus*>*B. boops*, respectively. In literature, studies examining the biostress parameters of these species were limited. Therefore, concentrations in the accumulation levels and differences depending on habitat and feeding strategy needs to be further evaluated.

4.6. Health Risk Assessment

Even though some metals are essential for growth, overconsumption may be toxic and have harmful effects on reproductive system, nervous system, immune system [72 and 73]. Previous studies underline that metal toxicity may cause cancer development, cause cardio vascular diseases, Alzheimer, dementia and Parkinson's disease [12, 74 and 75]. For that reason, health risk assessment depends on the consumption of commonly consumed fish species from Turkish territorial waters (*M. barbatus*, *B. boops*, *T. trachurus*) were determined (Table 7 and Table 9).



Tablo 7. Results of health risk assessment for *M. barbatus*

		Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
İskenderun	THQ	2×10^{-5}	1×10^{-4}	1×10^{-3}	1×10^{-3}	4×10^{-5}	2×10^{-4}	3×10^{-5}	2×10^{-4}	2×10^{-3}	4×10^{-4}
	CR	-	7×10^{-7}	-	2×10^{-6}	-	-	-	6×10^{-6}	8×10^{-8}	-
Antalya	THQ	3×10^{-5}	4×10^{-4}	2×10^{-3}	1×10^{-3}	4×10^{-4}	6×10^{-4}	5×10^{-5}	5×10^{-5}	2×10^{-3}	3×10^{-4}
	CR	-	2×10^{-6}	-	2×10^{-6}	-	-	-	2×10^{-6}	6×10^{-8}	-
Bodrum	THQ	1×10^{-5}	2×10^{-4}	1×10^{-3}	2×10^{-3}	5×10^{-5}	2×10^{-4}	1×10^{-5}	4×10^{-5}	1×10^{-3}	2×10^{-4}
	CR	-	1×10^{-6}	-	2×10^{-6}	-	-	-	2×10^{-6}	5×10^{-8}	-
Aydın	THQ	8×10^{-6}	2×10^{-4}	2×10^{-3}	6×10^{-4}	4×10^{-5}	1×10^{-4}	3×10^{-5}	3×10^{-5}	2×10^{-3}	2×10^{-4}
	CR	-	1×10^{-6}	-	9×10^{-7}	-	-	-	1×10^{-6}	5×10^{-8}	-
Çanakkale	THQ	5×10^{-5}	5×10^{-4}	2×10^{-3}	3×10^{-3}	3×10^{-5}	3×10^{-4}	2×10^{-5}	5×10^{-5}	3×10^{-3}	3×10^{-4}
	CR	-	3×10^{-6}	-	5×10^{-6}	-	-	-	2×10^{-6}	1×10^{-7}	-
Tekirdağ	THQ	2×10^{-5}	5×10^{-4}	2×10^{-3}	4×10^{-3}	5×10^{-5}	1×10^{-4}	7×10^{-5}	3×10^{-4}	3×10^{-3}	3×10^{-4}
	CR	-	3×10^{-6}	-	5×10^{-6}	-	-	-	9×10^{-6}	9×10^{-8}	-
Bandırma	THQ	5×10^{-9}	2×10^{-7}	2×10^{-6}	6×10^{-7}	4×10^{-8}	3×10^{-8}	1×10^{-7}	3×10^{-8}	9×10^{-7}	3×10^{-7}
	CR	-	1×10^{-6}	-	9×10^{-7}	-	-	-	1×10^{-9}	3×10^{-8}	-

Tablo 8. Results of health risk assessment for *B boops*

		Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
İskenderun	THQ	2×10^{-5}	2×10^{-4}	1×10^{-3}	2×10^{-3}	2×10^{-5}	2×10^{-4}	2×10^{-5}	2×10^{-4}	2×10^{-3}	4×10^{-4}
	CR	-	1×10^{-6}	-	4×10^{-6}	-	-	-	8×10^{-6}	8×10^{-8}	-
Antalya	THQ	9×10^{-6}	2×10^{-4}	1×10^{-3}	1×10^{-3}	2×10^{-5}	2×10^{-4}	3×10^{-5}	2×10^{-5}	7×10^{-4}	3×10^{-4}
	CR	-	1×10^{-6}	-	2×10^{-6}	-	-	-	8×10^{-7}	2×10^{-8}	-
Bodrum	THQ	1×10^{-5}	2×10^{-4}	1×10^{-3}	4×10^{-4}	3×10^{-5}	2×10^{-4}	2×10^{-5}	5×10^{-5}	1×10^{-2}	3×10^{-4}
	CR	-	1×10^{-6}	-	6×10^{-7}	-	-	-	2×10^{-6}	4×10^{-7}	-
Aydın	THQ	5×10^{-6}	2×10^{-4}	1×10^{-3}	6×10^{-4}	3×10^{-5}	1×10^{-4}	1×10^{-5}	2×10^{-5}	2×10^{-3}	2×10^{-4}
	CR	-	1×10^{-6}	-	8×10^{-7}	-	-	-	8×10^{-7}	6×10^{-8}	-
Çanakkale	THQ	9×10^{-6}	4×10^{-4}	1×10^{-3}	2×10^{-3}	4×10^{-5}	1×10^{-4}	7×10^{-5}	4×10^{-5}	2×10^{-3}	2×10^{-4}
	CR	-	2×10^{-6}	-	3×10^{-6}	-	-	-	1×10^{-6}	7×10^{-8}	-
Tekirdağ	THQ	2×10^{-5}	6×10^{-4}	2×10^{-3}	3×10^{-3}	3×10^{-5}	1×10^{-4}	9×10^{-5}	5×10^{-5}	3×10^{-3}	5×10^{-4}
	CR	-	4×10^{-6}	-	4×10^{-6}	-	-	-	2×10^{-6}	1×10^{-7}	-
Bandırma	THQ	4×10^{-6}	2×10^{-4}	1×10^{-3}	5×10^{-4}	4×10^{-5}	7×10^{-5}	7×10^{-5}	2×10^{-5}	9×10^{-4}	5×10^{-5}
	CR	-	1×10^{-6}	-	7×10^{-7}	-	-	-	8×10^{-7}	3×10^{-8}	-

Tablo 9. Results of health risk assessment for *T. trachurus*

		Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
İskenderun	THQ	2×10^{-5}	4×10^{-4}	2×10^{-3}	2×10^{-3}	5×10^{-4}	2×10^{-4}	3×10^{-5}	2×10^{-4}	2×10^{-3}	3×10^{-4}
	CR	-	2×10^{-6}	-	2×10^{-6}	-	-	-	7×10^{-6}	7×10^{-8}	-
Antalya	THQ	5×10^{-5}	4×10^{-4}	1×10^{-3}	1×10^{-3}	1×10^{-4}	3×10^{-4}	1×10^{-5}	3×10^{-5}	9×10^{-4}	1×10^{-3}
	CR	-	2×10^{-6}	-	2×10^{-6}	-	-	-	1×10^{-6}	3×10^{-8}	-
Bodrum	THQ	1×10^{-5}	2×10^{-4}	1×10^{-3}	8×10^{-4}	3×10^{-4}	1×10^{-4}	2×10^{-5}	2×10^{-5}	1×10^{-3}	3×10^{-4}
	CR	-	1×10^{-6}	-	1×10^{-6}	-	-	-	6×10^{-7}	4×10^{-8}	-
Aydın	THQ	2×10^{-5}	4×10^{-4}	1×10^{-3}	3×10^{-3}	1×10^{-4}	3×10^{-4}	3×10^{-5}	5×10^{-5}	2×10^{-3}	5×10^{-4}
	CR	-	2×10^{-6}	-	5×10^{-6}	-	-	-	2×10^{-6}	8×10^{-8}	-
Çanakkale	THQ	1×10^{-5}	4×10^{-4}	1×10^{-3}	3×10^{-3}	5×10^{-4}	2×10^{-4}	9×10^{-6}	5×10^{-5}	3×10^{-3}	2×10^{-4}
	CR	-	2×10^{-6}	-	4×10^{-6}	-	-	-	2×10^{-6}	1×10^{-7}	-
Tekirdağ	THQ	9×10^{-6}	4×10^{-4}	1×10^{-3}	3×10^{-3}	5×10^{-4}	3×10^{-4}	1×10^{-5}	2×10^{-5}	2×10^{-3}	7×10^{-4}
	CR	-	2×10^{-6}	-	5×10^{-6}	-	-	-	6×10^{-7}	8×10^{-8}	-
Bandırma	THQ	7×10^{-6}	3×10^{-4}	2×10^{-3}	7×10^{-4}	2×10^{-4}	6×10^{-5}	5×10^{-5}	2×10^{-5}	1×10^{-3}	3×10^{-4}
	CR	-	2×10^{-6}	-	1×10^{-6}	-	-	-	6×10^{-7}	5×10^{-8}	-

In this study, target hazard quotient was found to be less than 1 which indicates safe consumption of these species from Turkish waters. In fact, estimated numbers were so small that, even consumption on daily basis does not pose a health problem. Similarly, lifetime cancer risk of Cr, Cd, Pb, Ni $<10^{-5}$ indicating no health risk depending on the consumption. Previous studies conducted in coastal waters of Turkey was also reported safe consumption [4, 11, 13, 51, 76 and 77]. On the other hand, Pazi et al. [78] reported that consumption of *M. barbatus*, *P. erythrinus* could be dangerous to human health due to high Hg content in Aliaga Bay; whereas, they were safe to be consumed from İzmir Bay. Kosker et al., [19] estimated that consumption of fish and crustacean in Mersin Bay is dangerous in terms of as intake; whereas, it is safe in terms of other metals in Mersin Bay.

5. CONCLUSION AND RECOMMENDATIONS

This study was conducted to insight some information on metal accumulation and related health risk assessment depending on consumption of *M. barbatus*, *B. boops*, *T. trachurus*. This study includes the first report regarding the oxidative stress parameters of studied fishes from Aegean, Marmara and Mediterranean Sea. Results revealed that heavy metal accumulation showed significant differences depend on heavy metal type and tissue. The highest Al, Ni, Mn accumulation was observed in gill; whereas, the highest Co, Cd, Fe, Cu, Zn accumulation was found in liver. In general, Fe and Zn were found to be most accumulative metals in the



tissues; on the other hand, Co and Cd were found to be least accumulative ones regardless of specie. Health risk assessment showed safe consumption of these species from Turkish coastal waters. Even though, results obtained in this study were consistent with previous studies, monitoring programs should be continued to ensure the quality of marine environments. In addition, further studies should focus on the metal toxicity response of these species and usage of biostress parameters as pollution indicator.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Serdar Doğan, Önder Duysak, Erkan Uğurlu and statistical analysis were conducted by Önder Duysak and Ece Kılıç. The first draft of the manuscript was written by Ece Kılıç and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY

The datasets generated during the current study are not publicly available due to but are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

The authors have no conflicts of interest to be disclosed.

FINANCIAL DISCLOSURE

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

DECLARATION OF ETHICAL STANDARDS

This is an observational study and dead specimens obtained from fisherman was analyzed. So, no ethical approval is required for this study.

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