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Evaluation of heavy metal accumulation and associated human health risks in three commercial marine fish species from the Aegean Sea, Türkiye

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ORIGINAL ARTICLE

Abstract

This study investigates the presence and concentrations of ten specific heavy metals in the muscle tissues and gonads of three commercially important marine fish species: Garfish (*Belone belone*), European barracuda (*Sphyraena sphyraena*), and Anglerfish (*Lophius piscatorius*) collected from the Aegean Sea in Türkiye. The results indicate variations in metal accumulation, both between genders and among species. The fish species tend to accumulate higher levels of heavy metals in their gonads than in muscle tissues ($p<0.05$). While Cr, Cd, and Pb were not detected, Zn, Cu, and Fe are the predominant metals in muscle tissues due to their essential biological roles. However, within toxic elements, Hg was predominant, and *L. piscatorius*, a benthic fish, exceeded its recommended safety limits. A health risk assessment suggests that except for *L. piscatorius*, consuming these fish species from the Aegean Sea is generally safe, with estimated weekly intake (EWI) values below permissible limits. Total Hazard Quotient (THQ) values for most metals are below 1, indicating no significant non-carcinogenic health risks for consumers. This study emphasizes the critical need for regularly monitoring heavy metal levels in marine fish from the Aegean Sea, Turkiye. While overall safety in consuming these fish is highlighted, exceptions exist, notably concerning Hg levels in *L. piscatorius*. Therefore, persistent monitoring of heavy metal concentrations, especially in *L. piscatorius*, is advised to ensure ongoing safety and mitigate potential risks of metal accumulation.

Keywords: Aegean Sea, gonad, heavy metal, human health**,** marine fish, muscle tissue

Introduction

In the last several decades, heavy metal pollution in water bodies (in aquatic systems) has been a significant and growing concern in many fast-growing cities worldwide. This is because heavy metals, when present in excessive amounts in the environment, can pose significant threats to water supplies and human health by consuming fish and other aquatic organisms (Akoto *et al.*, 2014). This problem results from various human activities and industrial processes that release heavy metals into the environment. As aquatic inhabitants, fish are highly susceptible to the harmful effects of heavy metals in aquatic environments. Because marine organisms can accumulate these contaminants from various environmental sources, including water, food, bottom sediment, and suspended particles in the water column (Ayas *et al.*, 2007; Bat *et al.*, 2020). However, many aquatic organisms can accumulate metals in concentrations much higher than those in the surrounding water or sediment (Usman and Mustapha, 2017). Heavy metals such as Mercury (Hg), Lead (Pb), Cadmium (Cd), and Arsenic (As) tend to accumulate in the muscle tissues and gonads of aquatic organisms, and they are generally not biodegradable, and they have long biological half-lives (Olgunoglu *et al*., 2015). The bioaccumulation of heavy metals in seafood can pose significant health risks to humans and other consumers when they consume contaminated seafood (Han *et al*., 2021).

According to the World Health Organization (WHO), managing the presence of heavy metals in food sources is crucial to ensure public safety (Olgunoglu *et al*., 2015; Olgunoglu, 2015). Hence, we must be sure that fish and seafood are not contaminated with heavy metals and do not exceed their acceptance limits is crucial for both human health and environmental sustainability (Sabbir *et al*., 2018). Several marine fish species serve as bioindicators to assess the quality of aquatic environments because they can accumulate significant amounts of certain metals in their various tissues (Farombi *et al*., 2007; Safahieh *et al*., 2011). Therefore, utilization of several fish species as bioindicators of heavy metal pollution in environmental monitoring studies has been widely recognized and emphasized in recent years by numerous investigators and researchers (Chen-Yi *et al*., 2001; Yılmaz, 2003; Dural *et al*., 2007; Yılmaz *et al*., 2007; Uysal *et al*., 2008; El-Moselhy *et al*., 2014; Khezri *et al*., 2014; Külcü *et al*., 2014; Oguz and Yeltekin, 2014; Gu *et al*., 2017; Anser and Benamal, 2018; Astani *et al*., 2018).

In the study, for the assessment of metal accumulation in fish, edible muscle tissue and gonads were chosen as the main organs since the metal content in muscle tissue indicates the prevailing metal levels in the surrounding waters. In contrast, the accumulation of metals in the gonads sheds light on the long-term retention of these elements within the fish (Ateş *et al*.,2015). Therefore, the main goal of this study is to investigate the presence and concentrations of ten metals including Aluminum (Al), Copper (Cu), Iron (Fe), Nickel (Ni), Chromium (Cr), Zinc (Zn), Manganese (Mn), Mercury (Hg), Cadmium (Cd), and Lead (Pb), in the muscle tissues and gonads of three fish species collected from the Aegean Sea in Türkiye. We assessed the heavy metal content in the muscle tissue of various fish species, which are important protein sources for human life, and examined its potential impact on public health.

Material and Methods

Collection and preparation of raw materials

The marine fish species examined in this research, which inhabit various parts of the water column and possess distinct dietary habits, were collected from the Aegean Sea in Türkiye in February 2023 (Figure 1). The Aegean Sea is situated between the western and southwestern shores of Türkiye and the eastern shores of Greece. It is an elongated embayment of the Mediterranean Sea, home to a series of islands known as the Aegean Islands (Makedonski *et al*., 2015).

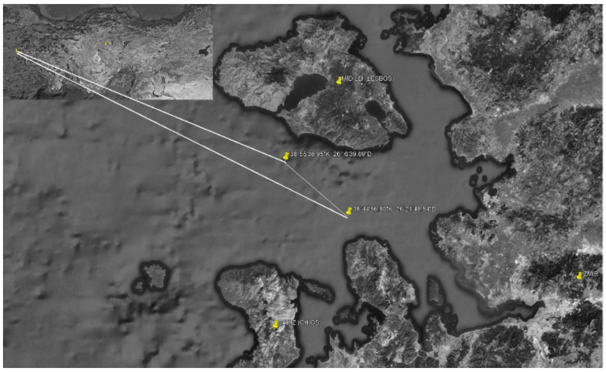

Figure 1. Sampling area. (38˚55´38.95´´ N-26˚6´39.69´´ E / 38˚44´56.80´´N-26˚21´48.54´´E).

The collected samples were immediately washed with clean seawater at the collection point to remove any external contaminants. To maintain the freshness of the samples and prevent spoilage during transportation to the laboratory, cleaned samples were placed in a container and preserved in crushed ice. In the laboratory, the samples were subject to measurements, which included determining the total size, weight, and gender of the fish. Based on the gender determination, various components, including edible tissues and gonads, were separated. After measurements and separation, the samples were placed in labeled polyethylene bags and stored at a temperature of -20ºC until they were processed for metal analysis. Fifteen specimens were collected from each fish species in the sampling area.

Determination of heavy metals

The muscle tissue and gonad samples were transported to the Accredited Industrial Services Laboratory of Türkiye in Izmir using dry ice to ensure low temperatures and preserve the sample integrity during transportation. For each fish species, 2 grams of edible muscle tissue and gonad samples were weighed and placed in a digestion vessel. To prepare the samples for analysis, 5 ml of concentrated (65%) nitric acid (HNO_3) and 2 ml of (30%) hydrogen peroxide (H_2O_2) were added to the vessel. The samples were digested in a microwave oven (NMKL, 1998). The determination of heavy metals was carried out using Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES-Perkin Elmer Optima 8000º) instrument. The metal concentration results were expressed in micrograms per gram (μg/g) of wet weight.

Assessment of health risk

The equation associated with public health risks is presented as follows:

Equation (1): Estimated daily intake (EDI) of heavy metals (Sadeghi *et al.*, 2020):

$$EDI = \frac{MC \times FDC}{BW}$$

MC (µg/g) is the concentration of heavy metals in the fish muscle; FDC is the average food daily consumption of fish muscle (g/person/day), which is 17.81g/person/day in Türkiye (Yalçın ve Çakmak, 2023) and BW is the body weight (average 70 kg for adults). EDI was expressed as µg/kg bw/day (Sadeghi *et al.*, 2020):

Equation (2): Estimated weekly intake (EWI) of heavy metals (Alipour *et al.*, 2015):

$$EWI = EDI \times 7 \text{ days}$$

Equation (3): Target hazard quotients (THQ) (Sadeghi *et al.*, 2020):

$$THQ = \frac{EF \times ED \times FIR \times C}{RfDs \times BW \times ATn} \times 10^{-3}$$

Table 1 provides the factors, units, and values in the target hazard quotients (THQ) formula.

Equation (4): Total Target Hazard Quotient (ΣTHQ) (Pokorska *et al.*, 2022):

$$\Sigma THQ = THQ(Zn) + THQ(Mn) + \cdots THQ(Hg)$$

Statistical analysis

The statistical analysis of the data was performed using SPSS 21.0 for Windows. One-way Analysis of Variance (ANOVA), Independent Samples t Test, and Post hoc test (Duncan) were employed to compare group means, with a significance level set at 0.05. The reported mean values represent the results of three experiments and are presented as X±SD.

Results and Discussion

Metal concentrations in the fish muscle and gonads

Table 2 shows the mean weights (g) and total length of female and male fish species obtained from the Aegean Sea in Türkiye.

Table 1. Parameters and values used in THQ analysis (Javed and Usmani, 2016; Sadeghi *et al.*, 2020; Çiftçi *et al.*, 2021; Pokorska *et al.*, 2022; Tecimen *et al.*, 2023).

Factor	Statement	Unit	Value
EF	Exposure frequency	Days per year	365
ED	Exposure duration	Years	70
FIR	Food ingestion rate	g/person/day	17.81
C	Metal concentration	µg/g	Present study
BW	Body weight	kg	70
ATn	Average exposure time for non-carcinogen effects	days per year × ED (365*70)	25550
RfDs	Oral reference dose	mg/kg/day	Pb=0.0035; Ni=0.02; Cd=0.001; Al=1; Cr=0.003; Cu=0.04; Fe=0.7; Mn=0.14; Zn=0.3; Hg=0.0001

Table 2. The mean weights (g) and total length of female and male fish species (mean±SD).

Species	Average Total Length (cm)	Weight (g)	Habitat	Feeding habits
B. belone	33.06± 2.63^{a} ♀	42.86±6.52^{a} ♀	Pelagic	Carnivorous
	32.53±1.94^{a} ♂	39.46±6.87^{a} ♂		
S. sphyraena	41.05± 2.01^{a} ♀	253.77± 10.85^{a} ♀	Pelagic	Carnivorous
	35.50±1.00^{a} ♂	200.53±5.96^{a} ♂		
L. piscatorius	62.66± 2.51^{a} ♀	2418± 105.15^{a} ♀	Benthic	Carnivorous
	39.23±3.60^{b} ♂	766.67±87.36^{b} ♂		

♀: Female; ♂: Male. Significant statistical differences are indicated by distinct letters (a, b) within the same column (p<0.05).

The total length and weight differences between female and male *L. piscatorius* were statistically significant (p <0.05). Conversely, no noticeable variations in total length and weight were identified between males and females of *B. belone* and *S. sphyraena* (p>0.05).

The elements were categorized into two groups based on their significance in biological systems and their potential roles in environmental pollution. The first group, which includes Cu, Zn, Fe, Mn, and Cr, consists of essential elements required for various biological functions in organisms and naturally present in animal tissues, playing vital roles in maintaining health. The second group, Al, Ni, Hg, Cd, and Pb, consists of non-essential elements that can have toxic effects on living organisms (Makedonski *et al*., 2015). The data provided in the present study is presented in Table 3.

As shown in Table 3, for ***B. belone,*** the levels of Cu were significantly higher in the gonads of both female (♀) and male (♂) fish, with values of 1.288±0.148 μg/g and 1.238±0.142 μg/g, respectively, compared to the muscle tissue where Cu concentrations were lower at 0.346±0.040 μg/g for females and 0.454±0.052 μg/g for males (p<0.05). Similar patterns were observed for Zn, which were significantly higher in the gonads (34.71±3.77 μg/g for females and 16.85±1.83 μg/g for males) than the muscle (7.071±0.768 μg/g for females and 9.731±1.057 μg/g for males) (p<0.05). Fe concentrations also exhibited the same trend, with significantly higher levels in the gonads (79.01±8.31 μg/g for females and 48.25±5.08 μg/g for males) compared to the muscle tissue (2.354±0.248 μg/g for females and 5.044±0.531 μg/g for males) (p<0.05). Mn concentrations were below the detection limit in the muscle tissue for both females and males but were detectable in the gonads, with values of 1.568±0.339 μg/g for females and 1.138±0.149 μg/g for males (p<0.05). Cr was also below the detection limit in the muscle tissue for both genders but was present in the gonads, with values of 0.232±0.030 μg/g for females and 0.156±0.020 μg/g for males (p<0.05). Al was detected in the muscle tissue of females at a concentration of 0.287±0.029 μg/g, while it was below the detection limit in males. Al exhibited higher levels in the gonads of females, measuring 2.248±0.229 μg/g, whereas the levels were below the detection limit (<0.250 μg/g) in male gonads. Notably, while the differences in Al concentrations between gonads and muscle tissue were not statistically significant (p>0.05), they were found to be statistically significant between the genders (p<0.05). Ni concentrations

Table 3. Concentrations of heavy metals in muscle tissue and gonad of *B. belone, S. sphyraena,* and *L. piscatorius* (μg g^{-1} wet weight).

Metals	*B. belone*		*S. sphyraena*		*L. piscatorius*	
	Muscle	*Gonad*	*Muscle*	*Gonad*	*Muscle*	*Gonad*
Essential elements						
Cu	0.346±0.040♀ 0.454±0.052*♂	1.288±0.148*♀ 1.238±0.142♂	0.309±0.036*♀ 0.263±0.030♂	1.040±0.120*♀ 0.915±0.105♂	0.158±0.018♀ 0.181±0.021*♂	2.073±0.353*♀ 1.840±0.212♂
Zn	7.071±0.768♀ 9.731±1.057*♂	34.71±3.77*♀ 16.85±1.83♂	3.297±0.358♀ 3.702±0.402*♂	36.873±0.746*♀ 25.77±10.40♂	3.999±0.434♀ 7.958±0.864*♂	11.75±1.28♀ 13.73±1.49*♂
Fe	2.354±0.248♀ 5.044±0.531*♂	79.01±8.31*♀ 48.25±5.08♂	1.13±0.090♀ 1.936±0.204*♂	23.356±0.353*♀ 17.54±2.90♂	1.025±0.250♀ 1.244±0.131*♂	7.447±0.783♀ 13.40±3.51*♂
Mn	nd♀ nd♂	1.568±0.339*♀ 1.138±0.149♂	nd♀ nd♂	0.351±0.046♀ 0.544±0.071*♂	0.182±0.024*♀ 0.106±0.014♂	0.368±0.048♀ 0.470±0.061*♂
Cr	nd♀ nd♂	0.232±0.030*♀ 0.156±0.020♂	nd♀ nd♂	nd♀ nd♂	nd♀ nd♂	nd♀ nd♂
Non-essential (Toxic) elements						
Al	0.287±0.029*♀ nd♂	2.248±0.229*♀ nd♂	nd♀ nd♂	0.310±0.032♀ 0.542±0.059*♂	nd♀ nd♂	1.132±0.115*♀ 0.753±0.077♂
Ni	0.126±0.019♀ 0.138±0.021*♂	0.183±0.028♀ 0.236±0.036*♂	nd♀ nd♂	0.236±0.036*♀ 0.111±0.017♂	nd♀ 0.147±0.022*♂	0.105±0.016♀ 0.289±0.044*♂
Hg	0.025±0.005*♀ 0.024±0.005♂	0.048±0.010*♀ 0.034±0.007♂	0.069±0.014♀ 0.088±0.018*♂	0.486±0.09*♀ 0.417±0.085♂	1.109±0.225*♀ 0.211±0.043♂	1.398±0.283*♀ 0.290±0.059♂
Cd	nd♀ nd♂	0.045±0.009*♀ 0.037±0.007♂	nd♀ nd♂	nd♀ nd♂	nd♀ nd 1♂	0.087±0.018*♀ 0.076±0.013♂
Pb	nd♀ nd♂	0.058±0.011*♀ 0.026±0.005♂	nd♀ nd♂	nd♀ nd♂	nd♀ nd♀	0.011±0.002*♀ 0.010±0.002♂

♀: Female; ♂: Male; nd: below detection limit; limits of detection of measurements are Al<0.250; Mn<0.1; Cr<0.1; Ni<0.1; Cd<0.01; Pb<0.01;
* indicates the higher one; The data are presented as the mean ± standard deviation of triplicate measurements.

in gonads were significantly higher than in muscle for both genders, with values of 0.183±0.028 μg/g for females and 0.236±0.036 μg/g for males ($p<0.05$). Hg levels were lower in the muscle than in the gonads for both genders. Importantly, these differences in Hg concentrations between gonads and muscle tissue were not statistically significant ($p>0.05$). Cd and Pb concentrations were below the detection limit in the muscle tissue for both females and males. However, they were detectable in the gonads, with Cd concentrations of 0.45±0.009 μg/g for females, 0.037±0.007 μg/g for males, and Pb concentrations of 0.058±0.011 μg/g for females and 0.026±0.005 μg/g for males. The differences in Cd and Pb concentrations between the gonads and muscle tissue were found to be statistically significant ($p<0.05$)

In the muscle tissue of both genders, the level of Zn was higher than other metal concentrations. The order of mean metal concentrations in the muscle of *B. belone* was as follows.

In females, Zn> Fe> Cu> Al> Ni> Hg

In males, Zn> Fe> Cu> Ni> Hg

Within the gonads, Fe exhibited the highest concentration, followed by others, with the order being Fe> Zn> Al>Mn> Cu> Cr>Ni>Pb> Hg> Cd for females.

In males, the order of concentration in gonadal tissues was Fe> Zn> Cu >Mn>Ni>Cr> Cd>Hg>Pb.

Except for Al, the variation in heavy metal concentrations between genders was not found to be statistically significant ($p>0.05$).

For *S. sphyraena*, Cu concentrations were found to be significantly higher in female gonads (1.040±0.120 μg/g) and male gonads (0.915±0.105 μg/g) compared to muscle tissues, where Cu concentrations were lower in females (0.309±0.036 μg/g) and males (0.263±0.030 μg/g) ($p<0.05$). Similarly, Zn concentrations were higher in female gonads (36.873±0.746 μg/g) and male gonads (25.77±10.40 μg/g) compared to muscle tissues ($p<0.05$). Fe concentrations also exhibited a similar trend, with significantly higher levels in female gonads (23.356±0.353 μg/g) and male gonads (17.54±2.90 μg/g) compared to muscle tissues ($p<0.05$). Mn concentrations were below the detection limit in the muscle tissues for both females and males, while they were detectable in the gonads, with values of 0.351±0.046 μg/g for females and 0.544±0.071 μg/g for males ($p<0.05$). Al was also below the detection limit in the muscle tissues for both sexes, while they were detectable in the gonads with values of 0.310±0.032 μg/g for females and 0.542±0.059 μg/g for males ($p<0.05$). In the gonads, Ni levels were higher than in muscle tissue ($p<0.05$). Hg levels were lower in the muscle tissues than in the gonads for both genders ($p<0.05$), with Hg concentrations of 0.088±0.018 μg/g for males and 0.069±0.014 μg/g for females in muscle tissues and 0.486±0.09 μg/g for females and 0.417±0.085 μg/g for males in gonads. Cr, Cd, and Pb concentrations were below the detection limit in the muscle tissues and gonads for both females and males.

The order of concentration in muscle tissues for females was Zn> Fe> Cu>Hg

For males, the order of concentration in muscle tissues is as follows: Zn> Fe> Cu> Hg

In females, the order of concentration in gonads from highest to lowest is as follows: Zn> Fe> Cu>Hg>Mn>Al> Ni

In males, Zn> Fe >Cu> Mn> Al> Hg> Ni

The variation in heavy metal concentrations between genders was not found to be statistically significant ($p>0.05$).

For *L. piscatorius*, Cu concentrations in the muscle were recorded at 0.158±0.018 μg/g for females and 0.181±0.021 μg/g for males. However, higher Cu concentrations were observed in the gonads, with values of 2.073±0.353 μg/g for females and 1.840±0.212 μg/g for males, with a statistically significant difference ($p<0.05$) compared to the muscle tissue. A similar pattern was also observed for Zn ($p<0.05$). Fe levels in the muscle were 1.025±0.250 μg/g for females and 1.244±0.131 μg/g for males. In contrast, the gonads exhibited higher Fe concentrations, with females at 7.447±0.783 μg/g and males at 13.40±3.51μg/g ($p<0.05$). Mn concentrations showed a similar pattern, with 0.182±0.024 μg/g in female muscle and 0.106±0.014 μg/g in male muscle, while in the gonads, females had much higher concentration with 0.368±0.048 μg/g of Mn, whereas males had 0.470±0.061 μg/g ($p<0.05$). Cr concentrations were below the detection limit (<0.1 μg/g) in both muscle and gonads for both genders. Al levels were also below the detection limit (<0.250 μg/g) in the muscle for both females and males. However, in the gonads, females had 1.132±0.115 μg/g of Al, while males had 0.753±0.077 μg/g($p<0.05$).

Ni concentrations were below the detection limit (<0.1 μg/g) in female muscle. Ni was found to be 0.147±0.022 μg/g in male muscle. In the gonads, males had higher concentrations, with levels at 0.289±0.044 μg/g, while females had lower concentrations at 0.105±0.016 μg/g. The levels of Hg in the muscle were 1.109±0.225 μg/g for females and 0.211±0.043 μg/g for males. In the gonads, Hg values were found to be higher than muscle, with females at

1.398±0.283 μg/g and males at 0.290±0.059 μg/g. Notably, the differences in Ni and Hg concentrations between the gonads and muscle tissue were insignificant ($p>0.05$). However, they were statistically significant between the genders ($p<0.05$). Cd concentrations were below the detection limit (<0.01 μg/g) in muscle tissue for both genders. However, it was observed to be 0.087±0.018 μg/g for females and 0.076±0.013 μg/g for males in the gonads. A similar trend was observed in Pb ($p<0.05$).

The order of heavy metal levels in the samples was as follows:

In the muscle tissue (Female): Zn>Hg>Fe>Mn>Cu
In the muscle tissue (Males): Zn>Fe>Hg>Cu>Ni>Mn
In gonad (Female): Zn>Fe>Cu>Hg>Al>Mn>Ni>Cd>Pb
In gonad (Male): Zn>Fe>Cu>Al>Mn>Hg>Ni>Cd>Pb

Except for Ni and Hg, the variation in heavy metal concentrations between genders was not found to be statistically significant ($p>0.05$).

As a result, when comparing all the species examined in this study, it was determined that heavy metal levels in the gonadal tissues were higher than those in the muscle ($p<0.05$). Gonads are considered target organs for metal accumulation due to their metabolic activity, which can result in the accumulation of metals, sometimes at high levels (Terra *et al*., 2008). Therefore, this difference was attributed to the physiological activities of the gonads, as muscles are not actively involved in metal accumulation. It has also been reported that the reason for the high metal accumulation in the gonads is that the fish's defense mechanism regulates metals and pollutants. In this process, some organic and mineral pollutants are transferred from the body to the eggs instead of being eliminated from the body (Astani *et al*., 2018). Several studies have reported similar findings, indicating that muscle is not an actively involved tissue in accumulating heavy metals (Olgunoglu *et al.,* 2015; Yılmaz, 2013). For instance, in a study, Wong *et al*., (2001) reported that the bioaccumulation of heavy metals in muscle tissue is lower than in gonads in marine fishes. Astani *et al*. (2018) reported in their study that the muscle, as the target organ, exhibited the lowest concentration of metals compared to gonads. In another study, it was reported that the concentrations of heavy metals in gonads (both testis and ovary) were higher than in the other organs, as reported by Ebrahimi and Taherianfar (2010). Our findings align with those reported by researchers.

Among the species within toxic elements, Hg was predominant, while Cr, Cd, and Pb were not detected in the muscle tissues. The concentrations in gonads of Fe, Mn, Cr, and Pb in *B. belone* were found to be statistically more significant ($p<0.05$). At the same time, Hg in benthic *L. piscatorius* was also significantly different in muscle and gonads compared to the species examined in the study. It was reported that Hg concentrations in fish can vary depending on the habitat due to habitat-specific differences in the biogeochemical processes related to the cycling and methylation of Hg and variations in the structure of the food web (Smith *et al*., 2016). Based on a study conducted by Storelli and Marcotrigiano *et al*. (2000), Lophiidae are positioned at the highest trophic level, leading to the potential for higher body mercury accumulation as a result of mercury biomagnification. It appears that our study's findings align with the researchers' interpretations.

It is a fact that heavy metal accumulation can vary among species and is influenced by various factors. Furthermore, it was also observed that, when comparing males and females, heavy metal levels mostly tend to be higher in the muscles of males. It was noted that the association between metal accumulation and gender could be ascribed to differences in metabolic activity between males and females. Typically, the sex that grew faster, often females, could be expected to have lower metal concentrations (Pourang *et al*.,2004; Yılmaz and Yılmaz, 2007; Olgunoglu *et al*., 2015). Hence, the average metal concentrations in males' tissues were higher than those in the tissue of females ($p>0.05$). In a study to determine the accumulation of heavy metals in little tunny (*Euthynnus alletteratus*) fish, the levels of some heavy metals in muscle were significantly higher in males than in females Ansel and Benamar (2018). Thus, it appears that the results obtained were consistent with previous studies.

Additionally, to provide insight into the pollution at the location where the fish are caught, recorded heavy metal levels in muscle ranged from 0.158 to 0.454 μg/g for Cu, 3.297 to 9.731 μg/g for Zn, 1.025 to 5.044 μg/g for Fe, <0.1 to 0.182 μg/g for Mn, <0.250 to 0.287 μg/g for Al, <0.1 to 0.147 μg/g for Ni, and 0.024 to 1.109 μg/g for Hg, regardless of fish species and sexes. Cr, Cd, and Pb were not detected.

In the gonads, heavy metal levels were as follows: Cu ranged from 0.915 to 2.073 μg/g, Zn from 11.75 to 36.873 μg/g, Fe from 7.447 to 79.01 μg/g, Mn from 0.368 to 1.568 μg/g, Cr from <0.1 to 0.232 μg/g, Al from <0.250 to 2.248 μg/g, Ni from 0.105 to 0.289 μg/g, Hg from 0.034 to 1.398 μg/g, Cd from <0.01 to 0.087 μg/g, and Pb from <0.01 to 0.058 μg/g.

Distribution patterns of metal concentration in both fish muscle samples and gonads follow a sequence, irrespective of fish species and sexes.

For muscle: $\text{Zn}_{(5.96)} > \text{Fe}_{(2.12)}) > \text{Cu}_{(0.29)} > \text{Hg}_{(0.25)} > \text{Ni}_{(0.07)>}$ $\text{Al}_{(0.05)} = \text{Mn}_{(0.05)}$

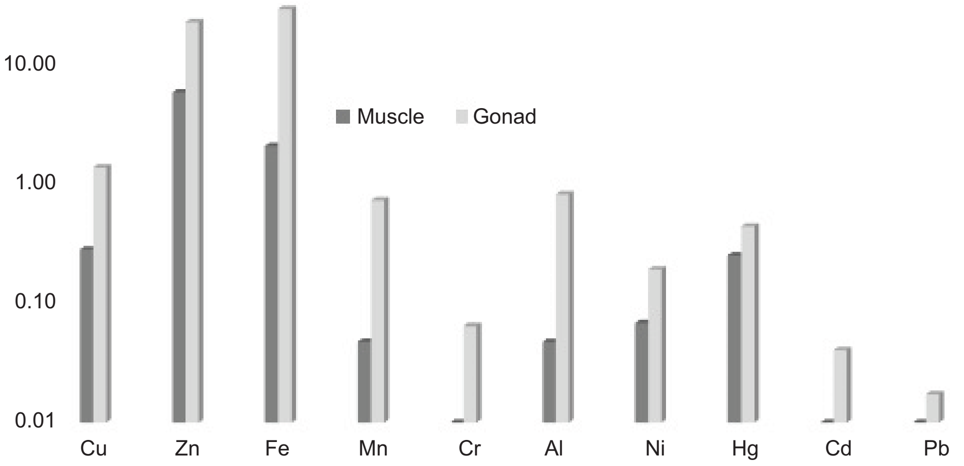

Figure 2. **The ranking of heavy metal levels from highest to lowest in fish.**

For gonads: Fe $_{(31.50)}$> Zn $_{(23.28)}$> Cu $_{(1.40)}$> Al $_{(0.83)}$> Mn $_{(0.74)}$> Hg $_{(0.45)}$> Ni $_{(0.19)}$> Cr $_{(0.06)}$> Cd $_{(0.04)}$> Pb $_{(0.02)}$

The graph of the ranking of heavy metal levels from highest to lowest is given in Figure 2.

Zn, as an essential element crucial for organisms' normal growth and metabolic processes, demonstrated the highest accumulation in the tissue samples compared to other metals. Similarly, iron (Fe) and copper (Cu) are essential trace elements for animal metabolism. Studies have reported that Zn, Fe, and Cu are the most abundant elements in tissues, surpassing the levels of other metals (Pourang *et al*., 2005; Krishna *et al*., 2014). Our results are consistent with the findings reported for Zn, Cu, and Fe.

In Table 4, heavy metal concentrations in selected fish species caught from different fishing locations in the Aegean Sea were summarized, demonstrating the variability of metal levels in organisms and their reflection of contamination levels in the sampling areas for comparison.

The comparison of mean metal concentrations in muscle tissues and gonads (Table 4) reveals variations in muscle tissue metal levels among different organisms. Notably, some studies have reported significantly higher concentrations of heavy metals, including Zn, Fe, Cr, Al, Mn, Pb, and Cd, compared to the levels found in our study for all species. On the other hand, Ni concentrations were compatible with the previous studies on some fish species (Erdem *et al*., 2021). In the case of Hg, a higher concentration was noted in *L. piscatorius*. Additionally, it has been observed that the concentration of Cu, particularly in the muscles of *B. belone*, is partially higher when compared to the species listed in Table 4. Tecimen *et al.* (2023) reported higher levels of Zn, Cu, and Pb in the muscle tissue of *S. sphyraena* compared to those found in our study for the same species. This situation is thought to arise from the differences in sampling areas and pollution levels. When the accumulation of Zn, Cu, Pb, and Cd in the gonads was compared with the values provided in Table 4, it was evident that the Zn, Cu, and Cd levels obtained in the study were mostly by previously reported values. However, the reported quantity of Pb appeared to be higher.

In summary, prior research has demonstrated that a multitude of factors, including species variations, seasonal changes, fish length and weight, diverse ecological requirements, metabolic processes, feeding patterns, water's physical and chemical attributes, and pollution levels, can collectively impact the accumulation of metals in fish tissues.

Health risk assessment

The permissible limits determined in the present study for edible fish muscles intended for human consumption may vary depending on the region and regulatory authority. However, while there are no specific maximum acceptable values for all elements, they were lower than the limits, including Cu, Zn, Fe, Mn, and Pb, set by national and international agencies (Table 5) such as FAO, WHO, and EC (Verep and Mutlu, 2022).

Sometimes, even though muscle tissue in marine species often has low metal concentrations, the risk it poses may vary depending on the quantity consumed (Kilercioglu *et al*., 2022). Therefore, EWI and THQ were calculated for each of the heavy metals in three female and male fish species to assess health risks associated with the common consumption of muscle tissue (Table 5). Also, Table 5

Table 4. Concentrations of heavy metal in specific fish species in various fishing grounds in the Aegean Sea (μg g^{-1}).

Metals	Species	Muscle	Gonad	Author
Zn	*Engraulis encrasicolus*	20.03±0.11 39.44±2.18/41.60±8.41		Erdem *et al*., 2021 Tecimen *et al*., 2023
	Sardinella aurita	26.06±6.05/32.30±1.42		Tecimen *et al*., 2023
	Sphyraena sphyraena	13.09±1.19/21.15±3.01		Tecimen *et al*., 2023
	Mugil cephalus	25.63±2.43/39.69±6.01		Tecimen *et al*., 2023
	Mullus barbatus	5±0.8–15.5±1.06 26.63±4.89/41.38±2.67	39±5.3/117.2±9.6	Zyadah and Chouikhi, 1999 Tecimen *et al*., 2023
	Merluccius merluccius	5.5±0.23/15± 2.05	38.5±5.3/182±12.6	Zyadah and Chouikhi, 1999
	Boops boops	4.5±1.03/30±4	32±4.5/118.4±14	Zyadah and Chouikhi, 1999
	Lithognathus mormyrus	5.01±0.15		Yabanlı *et al*., 2016
	Diplodus vulgaris	4.95±0.16		Yabanlı *et al*., 2016
	Pagellus erythrinus	5.04±0.27 18.94±2.55/32.64±3.99		Yabanlı *et al*., 2016 Tecimen *et al*., 2023.
	Sparus aurata	1.01±0.24		Döndü *et al*., 2023
	Pomatomus saltatrix	5.26±1.55		Türkmen *et al*., 2009
	Merlangius merlangus	12.8±2.02		Tepe *et al*., 2008
Fe	*Engraulis encrasicolus*	13.6±0.01		Erdem *et al*., 2021
	Sparus aurata	3.43±0.75		Döndü *et al*., 2023
	Pomatomus saltatrix	11.6±2.78		Türkmen *et al*., 2009
	Merlangius merlangus	21.9±3.26		Tepe *et al*., 2008
Al	*Engraulis encrasicolus*	1.44±0.04		Erdem *et al*., 2021
	Lithognathus mormyrus	0.41±0.11		Yabanlı *et al*., 2016
	Diplodus vulgaris	0.45±0.13		Yabanlı *et al*., 2016
	Pagellus erythrinus	0.45±0.12		Yabanlı *et al*., 2016
Cu	*Engraulis encrasicolus*	0.88±0.0 1.54±0.23/5.28±0.85		Erdem *et al*., 2021 Tecimen *et al*., 2023.
	Merluccius merluccius	0.04-0.4±0.15	0.4±0.06/2.2±0.45	Zyadah and Chouikhi, 1999
	Sphyraena sphyraena	0.80±0.66/ 5.82±0.58		Tecimen *et al*., 2023
	Sardinella aurita	1.26±0.22/3.16±0.53		Tecimen *et al*., 2023
	Mugil cephalus	0.94±0.08/3.44±1.40		Tecimen *et al*., 2023
	Mullus barbatus	0.13±0.04 0.03-0.35± 0.08 0.96±0.22/4.41±0.93	 1.15±0.21/3.45± 0.71	Yabanli and Alparslan, 2015 Zyadah and Chouikhi, 1999 Tecimen *et al*., 2023
	Boops boops	0.05/0.85±0.13	0.22±0.05/2.55±0.15	Zyadah and Chouikhi, 1999
	Mullus surmuletus	0.18±0.05 0.17±0.05		Yabanli and Alparslan, 2015
	Lithognathus mormyrus	0.17±0.05 0.17±0.05		Yabanli and Alparslan, 2015 Yabanlı *et al*., 2016
	Pagellus erythrinus	0.22±0.14 0.21±0.03 0.97±0.15/3.03±1.54		Yabanli and Alparslan, 2015 Yabanlı *et al*., 2016 Tecimen *et al*., 2023.
	Diplodus vulgaris	0.21±0.03 0.22±0.14		Yabanli and Alparslan, 2015 Yabanlı *et al*., 2016
	Pomatomus saltatrix	0.68±0.10		Türkmen *et al*., 2009
	Sparus aurata	1.31±2.30		Döndü *et al*., 2023
	Merlangius merlangus	3.97±0.68		Tepe *et al*., 2008
Mn	*Engraulis encrasicolus*	0.57±0.02		Erdem *et al*., 2021
	Merlangius merlangus	1.03±0.25		Tepe *et al*., 2008
	Pomatomus saltatrix	0.14±0.04		Türkmen *et al*., 2009
	Sparus aurata	1.80±1.12		Döndü *et al*., 2023

(*continues*)

Table 4. Continued.

Metals	Species	Muscle	Gonad	Author
Ni	*Engraulis encrasicolus*	0.14±0.22		Erdem *et al*., 2021
	Pomatomus saltatrix	0.80±0.18		Türkmen *et al*., 2009
	Merlangius merlangus	0.58±0.13		Tepe *et al*., 2008
Cr	*Engraulis encrasicolus*	0.03±0.00		Erdem *et al*., 2021
	Mullus barbatus	0.28±0.07		Yabanli and Alparslan, 2015
	Mullus surmuletus	0.27±0.10		Yabanli and Alparslan, 2015
	Lithognathus mormyrus	0.38±0.11 0.38±0.11		Yabanli and Alparslan, 2015 Yabanlı *et al*., 2016
	Pagellus erythrinus	0.31±0.14 0.39±0.07		Yabanli and Alparslan, 2015 Yabanlı *et al*., 2016
	Diplodus vulgaris	0.39±0.07 0.31±0.14		Yabanli and Alparslan, 2015 Yabanlı *et al*., 2016
	Pomatomus saltatrix	0.06±0.01		Türkmen *et al*., 2009
	Merlangius merlangus	0.27±0.11		Tepe *et al*., 2008
Pb	*Thunnus alalunga*	0.020-0.557		Stamatis *et al*., 2019
	Engraulis encrasicolus	0.70±0.37		Tecimen *et al*., 2023.
	Sardinella aurita	0.41±0.28/0.46±0.23		Tecimen *et al*., 2023
	Sphyraena sphyraena	0.21±0.10/0.98±0.54		Tecimen *et al*., 2023
	Mugil cephalus	0.28±0.10/1.30±0.49		Tecimen *et al*., 2023
	Mullus barbatus	0.10±0.02 0.07-0.85±0.14 0.21±0.10/ 0.78±0.19	0.55±0.16-0.80±0.22	Yabanli and Alparslan, 2015 Zyadah and Chouikhi, 1999 Tecimen *et al*., 2023
	Boops boops	0.55±0.09 /0.8±0.23	0.5±0.06/3.15±0.65	Zyadah and Chouikhi, 1999
	Mullus surmuletus	0.10±0.02		Yabanli and Alparslan, 2015
	Lithognathus mormyrus	0.10±0.02 0.11±0.03		Yabanli and Alparslan, 2015 Yabanlı *et al*., 2016
	Pagellus erythrinus	0.10±0.02 0.10±0.02 0.20±0.15/ 0.92±0.15		Yabanli and Alparslan, 2015 Yabanlı *et al*., 2016 Tecimen *et al*., 2023
	Diplodus vulgaris	0.10±0.02 0.12±0.05		Yabanli and Alparslan, 2015 Yabanlı *et al*., 2016
	Merlangius merlangus	1.00±0.16		Tepe *et al*., 2008
	Pomatomus saltatrix	0.24±0.11		Türkmen *et al*., 2009
Cd	*Thunnus alalunga*	0.021-0.669		Stamatis *et al*., 2019
	Mullus barbatus	0.03±0.01 0.04/0.25±0.05	0.03-0.65±0.1	Yabanli and Alparslan, 2015 Zyadah and Chouikhi, 1999
	Merluccius merluccius	0.04/0.15±0.05	0.04±0.01/ 0.25±0.08	Zyadah and Chouikhi, 1999
	Boops boops	0.04/0.15±0.04	0.04/0.75±0.15	Zyadah and Chouikhi, 1999
	Mullus surmuletus	0.03±0.01		Yabanli and Alparslan, 2015
	Lithognathus mormyrus	0.03±0.01 0.03±0.01		Yabanli and Alparslan, 2015 Yabanlı *et al*., 2016
	Pagellus erythrinus	0.03±0.01 0.03±0.01		Yabanli and Alparslan, 2015 Yabanlı *et al*., 2016
	Diplodus vulgaris	0.03±0.01 0.03±0.01		Yabanli and Alparslan, 2015 Yabanlı *et al*., 2016
	Pomatomus saltatrix	0.01±0.00		Türkmen *et al*., 2009
	Sparus aurata	0.01±0.01		Döndü *et al*., 2023
	Merlangius merlangus	0.05±0.01		Tepe *et al*., 2008

(*continues*)

Table 4. Continued.

Metals	Species	Muscle	Gonad	Author
Hg	*Mullus barbatus*	0.10±0.03		Yabanli and Alparslan, 2015
	Mullus surmuletus	0.09±0.03		Yabanli and Alparslan, 2015
	Lithognathus mormyrus	0.10±0.03 0.10±0.03		Yabanli and Alparslan, 2015 Yabanlı *et al*., 2016
	Pagellus erythrinus	0.09±0.03 0.09±0.03		Yabanli and Alparslan, 2015 Yabanlı *et al*., 2016
	Diplodus vulgaris	0.09±0.02 0.09±0.03		Yabanli and Alparslan, 2015 Yabanlı *et al*., 2016
	Thunnus alalunga	0.141-0.938		Stamatis et., 2019
	Sparus aurata	2.79±0.85		Döndü *et al*., 2023

compares the estimated EWI to the recommended values, such as PTWI. The PTWI value calculates the quantity of a contaminant that humans can ingest throughout their lifetime without significant risk. PTWI standards are determined by the Expert Committee on Food Additives (JECFA), a collaborative body of the Food and Agriculture Organization (FAO), and the World Health Organization (WHO) for the United Nations. The quantity of food consumed influences the PTWI, the duration of consumption, and the level of contamination in the food (Alipour *et al*., 2015).

Therefore, in the current study, PTWI values were employed as reference standards (safe levels) for heavy metals to compare them with EWI levels, and all samples exhibited EWI values lower than the corresponding $PTWI_{(k)}$ values. In general, there is no health-threatening concern due to the consumption of edible muscle of these three fish species from the Aegean Sea.

THQ is recognized as an indicator used to evaluate potential health risks to consumers. A THQ or ∑THQ below 1 indicates no adverse hazard for the exposed population. When THQ or ∑THQ equals 1, it suggests that the affected individuals may face noncarcinogenic health risks, with the likelihood of these risks increasing as the THQ value rises (Miri *et al*., 2017; Kilercioglu *et al*., 2022). In the present study, the sum of THQ values for all the related heavy metals was higher only in the muscle of male *L. piscatorius* than in females and other species, exceeding 1. Moreover, except for Hg in the muscle of male L. *piscatorius*, THQ values for all other metals were below 1, indicating an absence of health hazards for the population who consume B.belone S. Sphyraena from the Aegean Sea. These variations in THQ values have been attributed to the fact that *L. piscatorius* is a demersal fish species and disparities in average weight and lifespan.

Conclusions

This study evaluated heavy metal accumulation in the muscle tissues and gonad-selected fish species (*B. belone, S. sphyraena, , L. Piscatorius*) from the Aegean Sea to assess health risk potential. Results showed different accumulation patterns among the genders and between the species. Generally, the mean metal concentrations in the muscle of male fish were higher than in female fish species. When comparing muscle and gonads, it has been determined that gonads, especially in females, tend to accumulate heavy metals at higher concentrations than muscles ($p<0.05$). In the case of the edible part of the fish, primarily the muscle tissue, THQ and ∑THQ values were calculated, and the results did not exceed 1.00, except for *L. piscatorius*. Based on the results obtained, the primary concern for human health among the examined metals is the level of Hg in female *L. piscatorius* ($p<0.05$). In light of the potential for the accumulation of toxic levels of this metal, it is recommended to continue monitoring heavy metal concentrations in the benthic fish, *L. piscatorius*, caught in the Aegean Sea, Türkiye. Additionally, the following suggestions should be considered.

- Monitoring heavy metal levels in different seasons and regions as part of scientific research should be continuous
- It is recommended to conduct and monitor heavy metal studies in other aquatic species in the region.
- Studies on heavy metal monitoring should encompass water and sediment samples, as well as organisms in the Aegean Sea
- Efforts to reduce pollution from industrial and agricultural activities should be supported
- Caution should be exercised regarding the consumption of fish gonads due to potential heavy metal contamination
- Efforts should be made to raise awareness about potential risks associated with fish consumption among the

Table 5. **Estimated weekly intake (EWI), compared with provisional tolerable weekly intake (PTWI), target hazard quotient (THQ), and total THQ (∑THQ) of heavy metals in edible tissues of *B. belone S. sphyraena*, *L. piscatorius.***

	Cu	Zn	Fe	Mn	Cr	Al	Ni	Hg	Cd	Pb
PTWI	3500*	7000*	5600^{++}	2500**	700*	28.6 $^{++}$	35^{-}	4^{+}	–	25^{+}
$PTWI_{(k)}$	2.45E5	4.90E5	3.92E5	175E3	4.9E3	2000	2450	280	–	1750
PTMI	–	–	–	–	–	–	–	–	25***	–
EWI Bb♀	0.6162	12.593	4.1924	–	–	0.5111	0.2244	0.0445	–	–
EWI Bb♂	0.8085	17.330	8.9833	–	–	–	0.2457	0.0427	–	–
EWI Ss♀	0.5503	5.8719	2.0125	–	–	–	–	0.1228	–	–
EWI Ss♂	0.4684	6.5932	3.4480	–	—	–	–	0.1567	–	–
EWI Lp♀	0.2813	7.1222	1.8255	0.3241	–	–	–	1.9751	–	–
EWI Lp♂	0.3223	14.173	2.2155	0.1887	–	–	0.2618	0.3757	–	–
THQ Bb♀	2.20081E–03	5.99688E–03	8.55607E–04	–	–	7.30210E–05	1.60290E–03	6.36071E–02	–	–
THQ Bb♂	2.88776E–03	8.25281E–03	1.83334E–03	–	–	–	1.75556E–03	6.10629E–02	–	–
∑THQ Bb					7.43364E–02♀ 7.57923E–02♂					
THQ Ss♀	1.96546E–03	2.79617E–03	4,10720E–04	–	–	–	–	1.75556E–01	–	–
THQ Ss♂	1.67287E–03	3.13965E–03	7.03677E–04	–	–	–	–	2.23897E–01	–	–
∑THQ Ss					1.80728E–01♀ 2.29413E–01♂					
THQ Lp♀	1.00499E–03	3.39153E–03	3,72556E–04	3,30757E–04	–	–	–	2.82161E+00	–	–
THQ Lp♂	1.15129E–03	6.74914E–03	4.52156E–04	1.92639E–04	–	–	–	5.36844E–01	–	–
∑THQ Lp					2.82671E+00♀ 5.47260E–01♂					
PL[1]	<20	40	100	1.00						0.20–0.50

PTWI: Provisional Tolerable Weekly Intake (µg kg^{-1} body weight); $PTWI_{(k)}$: Provisional Tolerable Weekly Intake (µg kg^{-1} 70 kg body weight) PTMI: Provisional Tolerable Monthly Intake (µg kg^{-1} body weight); EWI: Estimated Weekly Intake (µg kg^{-1} body weight); THQ: Target Hazard Quotient (Bb: *B. belone*; Ss: *S. sphyraena*, Lp: *L. piscatorius*); PL: Permissible limits (µg g^{-1}) +: FAO/WHO, 2011; ++Çiftçi *et al*., 2021: *Mohamed *et al*., 2017: **Mohamed *et al*., 2019; ***EFSA, 2011; [1]Verep and Mutlu, 2022; $^{-}$Kilercioglu *et al*., 2022; ♀: Female; ♂: Male public, and specific recommendations should be provided, especially for at-risk groups

Conflict of Interest

The authors affirm that their work is unaffected by financial interests or personal relationships.

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