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## BIOACCUMULATION OF HEAVY METALS AND MINERAL CONTENT IN EUROPEAN FLOUNDER (*PLATICHTHYS FLESUS*) FILLETS AND GONADS IN THE SOUTHERN BLACK SEA

Seval DERNEKBAŞI<sup>\*1</sup>, Basim S. A. AL SULIVANY<sup>2</sup>, Ismihan KARAYÜCEL<sup>1</sup>, Fatemeh JAFARI<sup>3</sup>, Muhammad OWAIS<sup>4</sup>

<sup>1</sup>University of Sinop, Faculty of Fisheries, Department of Aquaculture, Akliman, 57000-Sinop, Türkiye

<sup>2</sup>Department of Biology, College of Science, University of Zakho, Zakho, 42002, Duhok, Iraq

<sup>3</sup>Artemia and Aquaculture Research Institute, Urmia University, Urmia, Iran

<sup>4</sup>Department of Zoology, Ghazi University, Dera Ghazi Khan, Punjab, Pakistan

Seval Dernekbaşı: [sevalyaman@hotmail.com](mailto:sevalyaman@hotmail.com), ORCID ID: <https://orcid.org/0000-0001-5735-2486>

Basim S. A. Al Sulivany: [basim.ahmed@uoz.edu.krd](mailto:basim.ahmed@uoz.edu.krd), ORCID ID: <https://orcid.org/0000-0002-0117-7022>

Ismihan Karayücel: [ismihank@hotmail.com](mailto:ismihank@hotmail.com), ORCID ID: <https://orcid.org/0000-0003-2520-7545>

Fatemeh Jafari: [f.jafari9254@iau.ir](mailto:f.jafari9254@iau.ir), <https://orcid.org/0000-0002-1023-5562>

Muhammad Owais: [owaisgulmuhammad@gmail.com](mailto:owaisgulmuhammad@gmail.com), <https://orcid.org/0009-0005-3966-4191>

\*Corresponding author: Seval DERNEKBAŞI, [sevalyaman@hotmail.com](mailto:sevalyaman@hotmail.com) (+90 542 290 23 25)

### Abstract

The research investigated the accumulation of essential and non-essential elements in the fillets and gonads of European flounder (*Platichthys flesus*) from the Southern Black Sea, specifically the Sinop coast of Türkiye. Twelve samples collected during January and February 2022 underwent ICP-MS analysis following microwave digestion. Findings showed distinct variations between tissues and months: gonadal sodium (Na) levels rose from  $708.34 \pm 0.001$  mg/kg in January to  $977.54 \pm 0.003$  mg/kg in February, while fillet Na increased from  $910.26 \pm 0.002$  mg/kg to  $1194.27 \pm 0.002$  mg/kg. Gonadal phosphorus (P) decreased from  $4291.53 \pm 0.001$  mg/kg to  $3902.91 \pm 0.002$  mg/kg, whereas fillet P increased from  $1896.78 \pm 0.001$  mg/kg to  $2054.80 \pm 0.001$  mg/kg. Concentrations of non-essential metals such as cadmium (Cd) (gonad: 0.012–0.002 mg/kg; fillet: <0.000–0.009 mg/kg) and lead (Pb) (gonad: 0.042–0.058 mg/kg; fillet: 0.022–0.031 mg/kg) were within regulatory limits, except for arsenic (As), which exceeded thresholds in fillets (5.927–5.773 mg/kg). Health risk assessments using Hazard Quotient (HQ) and Risk Index (RI) revealed potential non-carcinogenic risks for children (THQ >1) due to elevated As levels in fillets. Although most metals did not pose immediate health risks, the presence of arsenic raises concerns for vulnerable groups. The study emphasizes the

importance of ongoing monitoring of Black Sea ecosystems and implementing targeted measures to reduce pollution, ensuring the protection of marine biodiversity and human health.

**Keywords:** *Platichthys flesus*, flounder, metal contamination, mineral matter

## Introduction

Water pollution has intensified in recent decades due to the increasing use of heavy metals in industrial and technological applications (Ossai et al., 2020). The release of these metals into aquatic environments poses a significant threat to both aquatic organisms and humans who consume contaminated fish (Arisekar et al., 2020; Arisekar et al., 2022; Sabullah et al., 2015; Ulaganathan et al., 2022). Although heavy metals occur naturally in ecosystems, anthropogenic activities have led to elevated concentrations in water bodies, resulting in ecological imbalance and biological toxicity (Ahmed et al., 2023). Once introduced into aquatic systems, heavy metals can dissolve and accumulate in the tissues of aquatic organisms, particularly fish, and subsequently enter the human food chain (Jamil Emon et al., 2023). The toxicity of heavy metals depends on several factors, including species sensitivity, metal concentration, and exposure duration, and many metals exhibit carcinogenic, mutagenic, and teratogenic effects (Ngo et al., 2011). Metals such as mercury (Hg), cadmium (Cd), and lead (Pb) are considered particularly hazardous due to their persistence and bioaccumulative properties (Burger et al., 2002). Exposure to these contaminants can impair fish health by reducing reproductive performance, fertilization success, and hatching rates, resulting in growth retardation, reproductive disorders, and increased mortality (Jeziarska & Witeska, 2006). The European flounder (*Platichthys flesus*) is a demersal species that inhabits estuarine and benthic environments and is therefore highly exposed to sediment-associated contaminants (Bat and Arıcı, 2018). This species is closely associated with estuarine habitats during its juvenile and adult stages (Kirby et al., 2000) and lives in direct contact with sediments, which serve as major reservoirs for pollutants in aquatic environments (Koebler, 2004; Minier et al., 2000). Most studies using flounder as a bioindicator have focused on large individuals because smaller specimens provide limited tissue for chemical analyses (Kerambrun et al., 2013). While some trace metals are essential for biological functions, many become toxic at elevated concentrations. Metals such as arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), mercury (Hg), nickel (Ni), selenium (Se), and zinc (Zn) are known for their toxicological significance (Elbeshti et al., 2018). The accumulation of these metals in fish tissues not only affects fish physiology but also poses health risks to humans, particularly vulnerable populations such as pregnant women and children due to the neurotoxic effects of mercury (Chen & Dong, 2022). The Black Sea is the world's largest natural anoxic basin below 180 m depth and is a semi-enclosed sea with limited water exchange with the global oceans. It receives substantial freshwater input from major European rivers such as the Danube, Dniester, and Dnieper (Stancheva et al., 2013). For this reason, the Black Sea is considered one of the most polluted marine ecosystems, and increasing nutrient inputs have led to severe eutrophication. This environmental degradation has resulted in a significant decline in fishery resources and negatively affected tourism along the Bulgarian Black Sea coast. Numerous studies have reported heavy metal accumulation in various fish species from the Black Sea (Al Sayed et al., 1995; Türkmen et al., 2008; Makedonski et al., 2017). Despite the recognized health risks associated with consuming contaminated wild fish, these species remain widely consumed by local populations. Therefore, reducing heavy metal bioaccumulation in fish is essential to minimize potential risks to human health.

In this context, the present study aims to determine the concentrations of heavy metals in the fillet and gonad tissues of European flounder (*Platichthys flesus*) collected from the Southern Black Sea. The results will provide valuable information for environmental monitoring and help raise awareness about pollution-related risks and mitigation strategies.

## Material and Method

### *Study Area and Sample Collection*

The research was conducted in the Southern Black Sea, with a particular emphasis on the Sinop coast (41°59'89" N – 35°10'19" E), an area well-known for its intensive fishing activities and its importance in both aquaculture and fisheries. This region is distinguished by its abundant marine biodiversity (Figure 1). The European flounder (*Platichthys flesus*) was collected through sampling conducted using bottom trammel nets. The nets featured a mesh size of 160 mm for the main net and 40 mm for the inner tor mesh. Sampling was conducted during January and February, a winter period that has been reported in previous studies as corresponding to the reproductive season of *Platichthys flesus* in the Southern Black Sea region. This timing was selected to enable comparison of heavy metal accumulation between muscle and gonadal tissues during a period when gonadal development is expected to be at an advanced stage. The study was therefore designed as a targeted, cross-tissue comparison during a presumed reproductive phase rather than a fully verified reproductive staging analysis. A total of 12 samples (6 individuals per month) were collected for metal analysis. Immediately after capture, fish were placed in pre-cleaned polythene bags, sealed, labeled, and stored in ice boxes for transport to the laboratory at Sinop University.



**Figure 1.** Fish sampling area from Sinop coasts of the Black Sea, Türkiye (Google Earth)

### *Sample Preparation, Digestion, and Metal Extraction*

Upon arrival at the laboratory, the fish were measured for body length and weight, with the smaller individuals averaging  $27.17 \pm 0.25$  cm in length and  $226.05 \pm 0.42$  g in weight, while the larger ones measured  $35.12 \pm 0.24$  cm and weighed  $598.31 \pm 0.28$  g. To eliminate external contaminants, they were rinsed with deionized water. After recording the measurements, the fish were dissected to collect gonad and fillet tissues for subsequent analyses.

The collected tissues were dried at  $105^\circ\text{C}$  in an oven (CARBOLITE GERO 30–3000, UK) until constant weight was achieved. Microwave-assisted acid digestion was performed according to

AOAC method 999.10 with slight modifications. Briefly, 1.5 g of dried, homogenized tissue (APCO tissue homogenizer, USA) was accurately weighed and digested in Teflon vessels using a mixture of concentrated nitric acid (HNO<sub>3</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (7:1, v/v) in a microwave digestion system (Milestone SK10). The digestion program was carried out by heating samples to 200°C for 15 min and maintaining this temperature for an additional 15 min. After cooling, digested solutions were transferred to 50 mL polypropylene tubes and diluted with ultra-pure water. Elemental concentrations were determined using inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7700x, USA). Quality assurance and quality control (QA/QC) procedures included analysis of certified reference material (TORT-2, lobster hepatopancreas, NRC Canada), procedural blanks, and triplicate measurements. Calibration curves were prepared using multi-element standard solutions (Agilent). Analytical precision was within ±10%, and recoveries of certified reference materials ranged between 90–100%. Limits of detection (LOD) and quantification (LOQ) were calculated for each element and are provided in Table 1.

Total arsenic concentrations were determined in gonad and fillet tissues. The analytical procedure quantified total arsenic without speciation. Therefore, inorganic arsenic was not directly measured in this study. Since arsenic in marine organisms predominantly occurs in organic forms, particularly arsenobetaine, total arsenic values do not directly reflect the toxicologically relevant inorganic fraction (Neff, 1997; Taylor et al., 2018; Sadee et al., 2024). For this reason, no conversion factor was applied, and results are reported as total arsenic only.

### **Health Risk Assessment of Fish**

#### *Estimated Daily Intake Assessment*

The estimated daily intake (EDI) of metals was calculated based on the mean metal concentration in fish samples, the daily fish consumption rate, and the average body weight of consumers. The EDI was determined using the exposure assessment approach recommended by the United States Environmental Protection Agency (US EPA, 2011):

$$EDI = C_{\text{samples}} \times CR / Bwt$$

Where:

C<sub>samples</sub> is the mean metal concentration in *Platichthys flesus* samples (mg/kg)

CR is the daily fish consumption rate (kg/day)

Bwt is the mean body weight of the consumer (kg)

Daily fish consumption varies according to age and body weight. Therefore, the calculations were performed for two population groups (children and adults) using the average body weights reported by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR, 2000), which are 30 kg for children and 70 kg for adults.

The annual per capita fish consumption in Türkiye was reported as 7.3 kg in 2022 (Orajiaka-Uchegbu et al., 2020), and this value was converted to daily consumption rates for the EDI calculations.

#### *Non-Carcinogenic Risk Assessment: Target Hazard Quotient*

Non-carcinogenic risk was evaluated using the Hazard Quotient (HQ), calculated according to the US EPA Risk Assessment Guidance for Superfund (RAGS) framework (US EPA, 1989–2004):

$$HQ = EDI / RfD$$

Where,

RfD (reference dose) represents the maximum acceptable oral daily exposure level without adverse health effects (mg/kg/day), as provided by the US EPA Integrated Risk Information System (IRIS).

The Hazard Quotient (HQ) is used to evaluate potential non-carcinogenic risk from individual metals. The total hazard quotient (THQ), representing cumulative risk from multiple metals, was calculated as:

$$THQ: THQ = \sum HQ$$

A THQ value greater than 1 indicates potential health risk, while values below 1 suggest no significant risk, according to US EPA risk characterization principles.

### *Risk Index*

Carcinogenic risk was estimated using the US EPA cancer risk assessment framework. The Risk Index (RI), expressed as lifetime cancer risk, was calculated as:

$$RI = EDI \times SF$$

Where,

SF is the cancer slope factor (mg/kg/day), obtained from the US EPA Integrated Risk Information System (IRIS) database.

Slope factors were applied for Cd, Pb, and As according to US EPA toxicological reference values.

### *Statistical Analysis*

Descriptive statistics, including means and standard deviations, were calculated using Microsoft Excel (Microsoft 365, Microsoft Corporation, Redmond, WA, USA). Differences in heavy metal concentrations between sampling months (January and February) were evaluated using the non-parametric Mann–Whitney U test, with statistical significance set at  $p < 0.05$ . Results are expressed as mean  $\pm$  standard error (SE). Statistical analyses were conducted using Minitab 17 (Minitab Inc., State College, PA, USA). R software (version 4.4.1) was employed for multiple regression analyses and graphical visualization of the data using the ggplot2 package.

## **Results and Discussion**

The analysis of element concentrations in gonads and fillets of *Platichthys flesus* revealed significant variations between tissues and months, as illustrated in Table 1 and Figure 2. In gonads, the order of essential elements by concentration was  $P > K > Na > Mg > Ca > Zn > Fe > Se > Cu$ , while in fillets, it was  $K > P > Na > Mg > Ca > Zn > Fe > Se > Cu$ . These differences indicate tissue-specific metabolic demands and physiological roles of macro- and microelements.

Statistical analysis demonstrated notable monthly changes in several elements. Gonadal Na increased significantly from  $708.344 \pm 0.001$  mg/kg in January to  $977.543 \pm 0.003$  mg/kg in February ( $p < 0.001$ ), while fillet Na rose from  $910.261 \pm 0.002$  mg/kg to  $1194.265 \pm 0.002$  mg/kg ( $p < 0.01$ ). Conversely, gonadal P decreased from  $4291.530 \pm 0.001$  mg/kg in January to  $3902.909 \pm 0.002$  mg/kg in February ( $p < 0.05$ ), whereas fillet P increased from  $1896.775 \pm 0.001$  mg/kg to  $2054.795 \pm 0.001$  mg/kg ( $p < 0.05$ ). Aluminum (Al) in gonads exhibited a pronounced

increase from  $0.221\pm 0.029$  mg/kg in January to  $9.686\pm 0.001$  mg/kg in February ( $p<0.001$ ), suggesting possible temporal shifts in environmental exposure or sediment interaction.

Non-essential elements, particularly Cd (gonad:  $0.012\pm 0.000$  to  $0.002\pm 0.000$  mg/kg; fillet:  $<LOD$  to  $0.009\pm 0.000$  mg/kg) and Co (gonad:  $0.072\pm 0.004$  to  $0.029\pm 0.008$  mg/kg; fillet:  $<LOD$  to  $0.010\pm 0.01$  mg/kg), consistently showed the lowest concentrations ( $p<0.001$ ), consistent with previous reports indicating their presence at trace levels due to high toxicity even at low concentrations (Bat et al., 2024). Lead (Pb) exhibited higher accumulation in gonads ( $0.042\pm 0.001$  to  $0.058\pm 0.004$  mg/kg) than in fillets ( $0.022\pm 0.008$  to  $0.031\pm 0.005$  mg/kg,  $p<0.05$ ), highlighting tissue-specific retention patterns of certain toxic metals.

**Table 1.** The essential, non-essential and other elements in European flounder (*Platichthys flesus*) gonad and fillet by month (mg/kg)

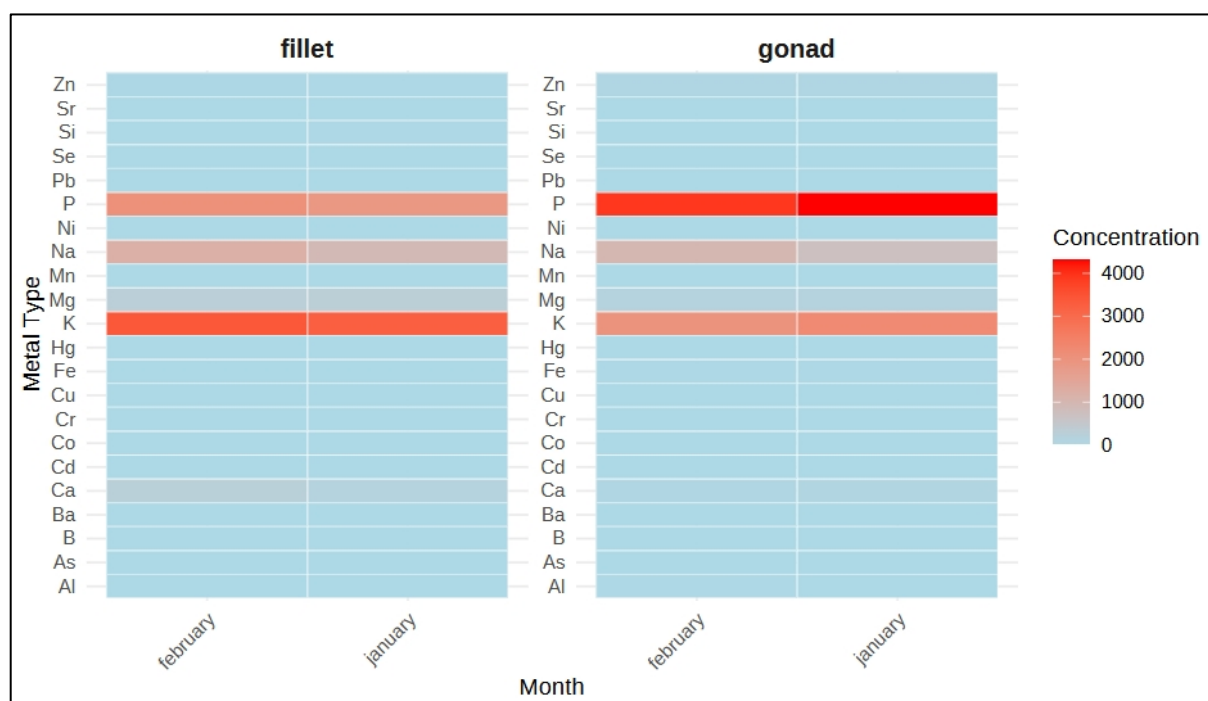
Elements	January		February	
	Gonad	Fillet	Gonad	Fillet
<b>Essential (mg/kg)</b>				
Calcium (Ca)	$68.749\pm 0.001$	$157.337\pm 0.002$	$59.677\pm 0.001$	$249.595\pm 0.002$
Iron (Fe)	$11.554\pm 0.001$	$4.161\pm 0.000$	$11.278\pm 0.001$	$6.942\pm 0.001$
Potassium (K)	$2223.604\pm 0.002$	$3236.444\pm 0.000$	$1991.977\pm 0.003$	$3399.882\pm 0.000$
Magnesium (Mg)	$143.201\pm 0.002$	$271.710\pm 0.001$	$143.016\pm 0.002$	$268.013\pm 0.001$
Sodium (Na)	$708.344\pm 0.001$	$910.261\pm 0.002$	$977.543\pm 0.003$	$1194.265\pm 0.002$
Phosphorus (P)	$4291.530\pm 0.001$	$1896.775\pm 0.001$	$3902.909\pm 0.002$	$2054.795\pm 0.001$
Manganese (Mn)	$0.990\pm 0.002$	$0.169\pm 0.011$	$1.832\pm 0.001$	$0.281\pm 0.001$
Zinc (Zn)	$46.827\pm 0.001$	$11.960\pm 0.001$	$46.793\pm 0.000$	$15.418\pm 0.001$
Copper (Cu)	$1.657\pm 0.001$	$0.391\pm 0.001$	$1.505\pm 0.001$	$1.353\pm 0.000$
<b>Non-Essential (mg/kg)</b>				
Arsenic (As)	$0.884\pm 0.001$	$5.927\pm 0.000$	$1.000\pm 0.002$	$5.773\pm 0.001$
Nickel (Ni)	$0.054\pm 0.003$	$0.382\pm 0.001$	$0.114\pm 0.003$	$0.753\pm 0.001$
Cadmium (Cd)	$0.012\pm 0.000$	$<LOD$	$0.002\pm 0.000$	$0.009\pm 0.000$
Lead (Pb)	$0.042\pm 0.001$	$0.022\pm 0.008$	$0.058\pm 0.004$	$0.031\pm 0.005$
Mercury (Hg)	$0.121\pm 0.004$	$0.241\pm 0.008$	$0.266\pm 0.017$	$0.195\pm 0.003$
Aluminum (Al)	$0.221\pm 0.029$	$2.031\pm 0.003$	$9.686\pm 0.001$	$1.263\pm 0.005$
<b>Other (mg/kg)</b>				
Cobalt (Co)	$0.072\pm 0.004$	$<LOD$	$0.029\pm 0.008$	$0.010\pm 0.011$
Barium (Ba)	$0.103\pm 0.003$	$0.070\pm 0.007$	$0.242\pm 0.003$	$0.142\pm 0.004$
Rubidium (Rb)	$0.724\pm 0.001$	$0.878\pm 0.002$	$0.541\pm 0.002$	$1.048\pm 0.002$
Strontium (Sr)	$0.480\pm 0.001$	$0.762\pm 0.002$	$0.475\pm 0.003$	$1.393\pm 0.002$
Chromium (Cr)	$0.065\pm 0.002$	$0.146\pm 0.005$	$0.070\pm 0.006$	$0.350\pm 0.001$

Selenium (Se)	1.915±0.003	0.625±0.004	1.487±0.004	0.845±0.001
Boron (B)	0.152±0.003	0.145±0.001	0.313±0.010	0.176±0.006
Lithium (Li)	0.043±0.001	0.028±0.007	0.051±0.007	0.034±0.011
Silicium (Si)	4.463±0.004	12.494±0.002	5.588±0.006	8.282±0.001

LOD: Below Limit of Detection

Figure 2 visually supports these findings, showing distinct distribution patterns between tissues. Essential elements such as K and P displayed relatively higher concentrations in metabolically active tissues, whereas toxic metals including Cd, Hg, and Pb remained consistently low throughout the sampling period. These findings are in agreement with earlier studies conducted in the Black Sea region, including those by Bat et al. (2024) who reported similarly low Cd and Pb concentrations in several marine fish species, confirming that heavy metal levels generally remained below regulatory thresholds.

Comparable results were also reported in a recent investigation of *Scorpaena porcus* by Bat and Öztekin (2025) where concentrations of Pb, Cd, Hg, Cu, and Zn were found to be below established regulatory limits. The authors further demonstrated that estimated daily intake (EDI) values indicated safe consumption levels, while hazard quotient (THQ) values remained below 1, suggesting negligible non-carcinogenic risks. Additionally, the carcinogenic risk index (CRI) for Pb remained within acceptable limits, supporting the general observation that fish species from the Black Sea region typically present low health risks in terms of heavy metal exposure.



**Figure 2.** Distribution of element concentrations in fillet and gonad of European flounder (*Platichthys flesus*) by months ( $\text{mg kg}^{-1}$ )

Fish are widely acknowledged as an excellent source of essential nutrients, including macronutrients, but certain elements classified as heavy metals can present substantial health risks when they accumulate in elevated amounts. Cadmium, for instance, is considered highly toxic even at minimal concentrations, causing both acute and chronic impacts on aquatic life and ecosystems (Elbeshti et al., 2018).

Previous investigations conducted along the Black Sea coast have demonstrated considerable variability in heavy metal concentrations among different fish species. For example, Bat et al. (2012) reported notable differences in the concentrations of Zn, Cu, Pb, and Cd among ten fish species collected from the Sinop coast. Despite this variability, the majority of measured values remained below the maximum allowable limits established by national and international authorities. In their study, Cd exhibited the lowest concentrations among the analyzed metals, followed by Pb, while Zn and Cu were generally detected at comparatively higher levels. These findings are consistent with the present study, where Cd and Pb were also detected at relatively low concentrations in both fillet and gonad tissues.

In this study, heavy metal concentrations were generally found to be within the maximum tolerable limits, with the exception of arsenic (As) (Table 2). Similar observations have been reported for other marine organisms from the Black Sea region. For instance, Bayraklı et al. (2024) demonstrated that Rapa whelk (*Rapana venosa*) can serve as a valuable dietary source of essential elements such as Cr, Cu, Fe, Mn, Mo, Se, and Zn. However, the same study indicated that concentrations of As, Cd, Ni, and Pb exceeded permissible limits, raising concerns regarding potential human health risks. A comparable risk-oriented perspective was presented by Bayraklı (2021), who investigated trace metal accumulation in the warty crab (*Eriphia verrucosa*). Although the measured metal concentrations were generally below the regulatory thresholds established by the Turkish Food Codex and the European Union, the target hazard quotient (THQ) values exceeded 1 for certain elements, particularly As, Cu, and Hg. These findings highlight that even when metal concentrations comply with legal limits, cumulative exposure and consumption frequency may still pose potential health risks. This observation supports the present findings, where arsenic was identified as the primary element contributing to elevated risk indices.

Similarly, several studies have emphasized that seafood can act both as an essential nutrient source and as a pathway for exposure to toxic elements. Yılmaz et al. (2017) and Ervik et al. (2018) reported that although many seafood species provide essential micronutrients, toxic metals such as As and Cd may occasionally exceed recommended safety thresholds. These findings demonstrate the dual role of seafood as both a source of essential micronutrients and a potential exposure pathway for toxic heavy metals, underscoring the importance of continuous monitoring and strict regulatory control to ensure food safety.

Trace element distribution patterns reported in earlier regional studies further support the observations obtained in the present research. For example, Yıldız et al. (2023) investigated trace element concentrations in whiting collected from Kastamonu, Sinop, and Samsun along the southern Black Sea coast. Their results demonstrated that element concentrations in muscle tissues followed the order Zn>Fe>Sr>As>Al>Se>B>Mn>Cu>Hg>Li>Ni>Ba>Pb>Cr>Cd, while roe samples showed a similar distribution pattern. Importantly, all measured values remained below the permissible limits established by the European Union. These findings are consistent with the present study, where Zn and Fe were among the most abundant elements detected in both fillet and gonad tissues.

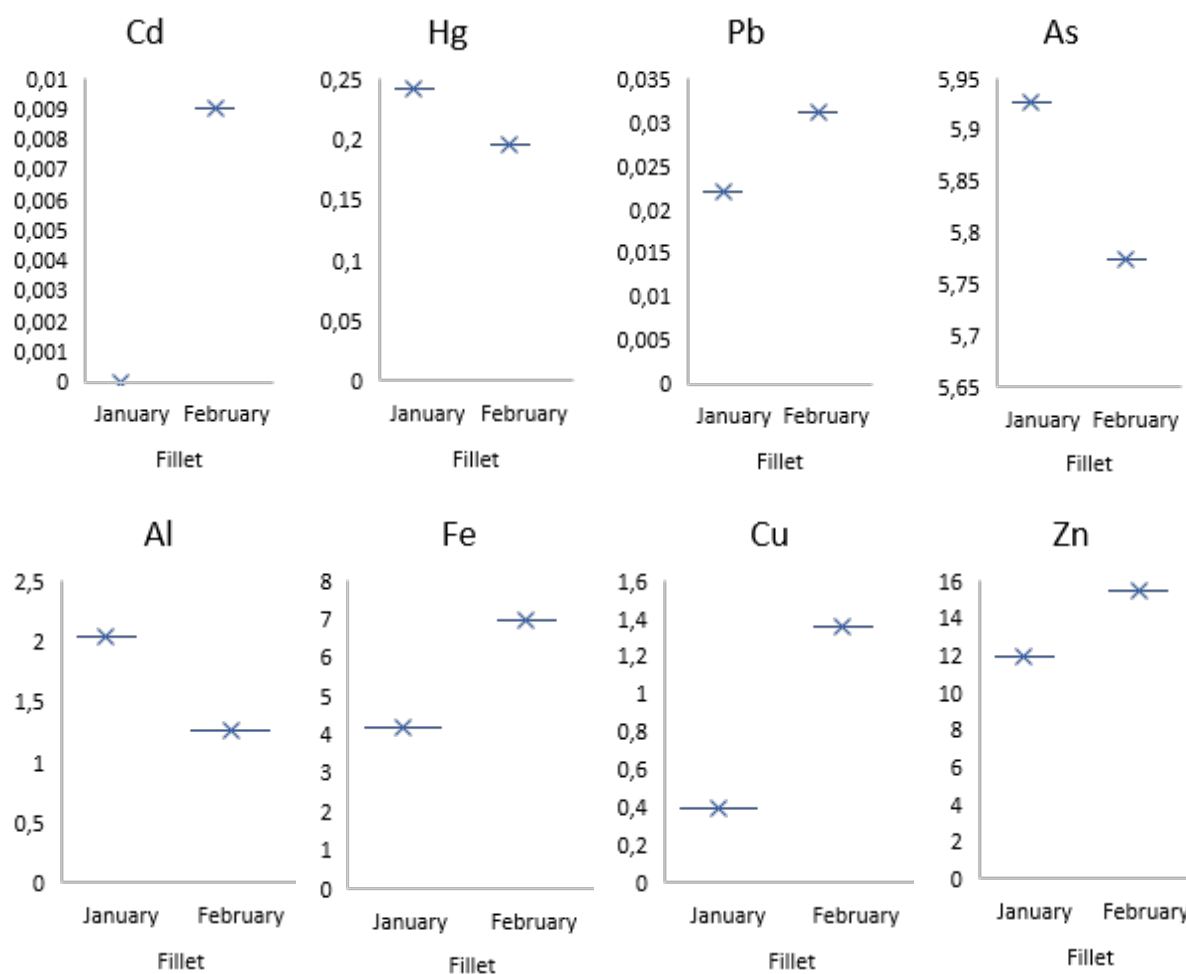
**Table 2.** Maximum Tolerable Values (mg/kg)

Heavy metals	Maximum Tolerable Value (mg/kg)	Source/Reference
Zinc (Zn)	50-100 (for all food products)	FAO/WHO (1989); Codex Alimentarius Commission (1995, updated)
Iron (Fe)	50-200 (for all food products)	FAO/WHO (1989)
Copper (Cu)	20 (for fish and seafood)	FAO/WHO (1989); Turkish Food Codex (2002)
Cadmium (Cd)	0.05-0.1 (for fish)	EU Commission Regulation (EC) No. 1881/2006 (as amended)
Mercury (Hg)	0.5 (for most fish species), 1.0 (predatory fish)	EU Commission Regulation (EC) No. 1881/2006 (as amended)
Lead (Pb)	0.3 (for fish)	EU Commission Regulation (EC) No. 1881/2006 (as amended)
Arsenic (As)	2 (for inorganic arsenic)	FAO/WHO Codex Alimentarius (Codex Stan 193-1995, updated)
Aluminum (Al)	7-10 (for some seafood)	FAO/WHO (JECFA, 2011)

The concentrations of heavy metals in fish fillets were analyzed, revealing that Cd, Pb, Fe, Cu, and Zn reached their highest levels in February, while Hg, As, and Al peaked in January (Figure 3). Despite these variations, all detected heavy metal concentrations remained within the maximum permissible limits. Additional regional comparisons further reinforce these observations. Studies focusing on commercially important marine species have reported similar accumulation patterns, where essential metals such as Zn and Fe were typically dominant, while toxic metals such as Cd and Pb were present at relatively low levels (Perumal et al., 2023). Such patterns indicate that trace metal accumulation in marine organisms is influenced by both environmental conditions and species-specific metabolic characteristics, as demonstrated by Jia et al. (2017), who reported significant interspecies differences related to habitat conditions and physiological properties. Furthermore, environmental parameters such as pollution sources and water chemistry have been identified as major factors affecting metal bioaccumulation in aquatic organisms (Naz et al., 2023).

Comparable consumption-based risk assessments were also conducted by Yıldız et al. (2023) who evaluated trace element concentrations and associated health risks in whiting collected from the southern Black Sea coast. Their study demonstrated that consumption of whiting meat and roe within recommended monthly portion limits did not pose significant health risks to consumers. The authors emphasized that adherence to recommended intake limits plays a critical role in minimizing long-term exposure to trace metals. Comparable consumption-based risk assessments were also conducted by Yıldız et al. (2023) who evaluated trace element concentrations and associated health risks in whiting collected from the southern Black Sea coast. Their study demonstrated that consumption of whiting meat and roe within recommended monthly portion limits did not pose significant health risks to consumers. The authors emphasized that adherence to recommended intake limits plays a critical role in minimizing long-term exposure to trace metals. Further supporting these observations, Okbah et al. (2018) reported that Fe and Zn were the most abundant metals in fish species from the Mediterranean Sea, whereas Cd and Pb concentrations remained consistently low. Similarly, Mutlu (2024)

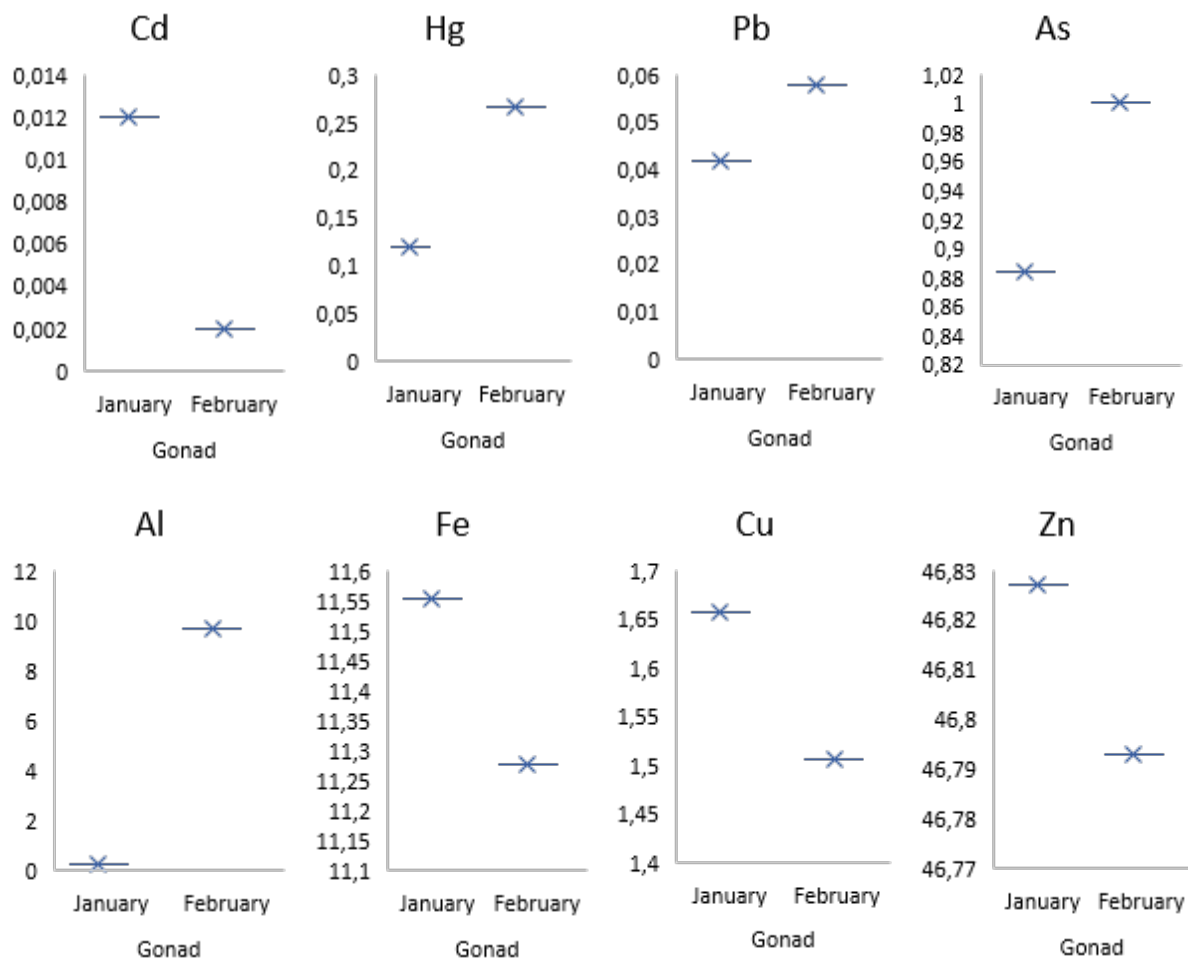
reported that arsenic levels in fish species from the Ionian Sea showed temporal variability but did not exceed regulatory thresholds. Bat et al. (2012) also concluded that toxic metals such as Cd and Pb remained below levels of concern in Black Sea fish, reinforcing the reliability of the present findings. Alkan et al. (2016) analyzed heavy metals in five commonly consumed fish species collected from the Bulgarian coast of the Black Sea and reported that Fe and Zn exhibited the highest accumulation levels, whereas Cd, Cu, Pb, and Hg did not pose significant health risks. These findings collectively demonstrate that trace metal distribution patterns in marine organisms exhibit consistent trends across different regions, although localized variations may occur due to environmental conditions.



**Figure 3.** The heavy elements levels (mg/kg) in the fillets of European flounder (*Platichthys flesus*) in different months.

Highest concentrations of heavy metals in gonads were observed for Cd, Fe, Cu, and Zn in January, while Hg, Pb, As, and Al peaked in February (Figure 4). Among the analyzed metals, arsenic (As) concentrations exceeded the ranges reported in the paper, whereas the levels of other metals remained within the recommended legal limits. These results are consistent with those reported by Alkan et al. (2016), who determined the concentrations of As, Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn in the muscle, gill, and gonad tissues of pelagic fish species (*Trachurus mediterraneus*, *Engraulis encrasicolus ponticus*, and *Sprattus sprattus*) of commercial and ecological importance in the Black Sea. They reported that Cd concentrations in muscle tissues exceeded the maximum permissible limits for human consumption.

Similar findings have also been reported by Shafeeka Parveen et al. (2024), who investigated heavy metal concentrations in commercially important pelagic fish species from the Black Sea and noted that Cd levels in muscle tissues exceeded maximum acceptable concentrations in some cases. Additionally, Hala and Bakiu (2024) reported that although most metals remained within safe limits in fish from the Adriatic Sea, As and Cd occasionally surpassed recommended thresholds, emphasizing the importance of continuous monitoring programs. Türkmen and Ögütçü (2020) also reported moderate concentrations of Zn and Cu in fish from the Turkish Black Sea coast, while toxic metals such as Cd and Pb remained generally within acceptable safety ranges.



**Figure 4.** The heavy elements levels (mg/kg) in the gonads of European flounder (*Platichthys flesus*) in different months.

Peycheva et al. (2017) In their study evaluating toxic metal pollutants in a total of 24 different flatfish species from the marine biosphere reserve, they reported the metal concentration order in flatfish as Zn>Cu>Pb>Cd. In the present study, the order of metal concentration in the fillets of fish was determined as Zn>Fe>As>Al>Cu>Hg>Pb>Cd, while in the gonads it was determined as Zn>Fe>Al> Cu>As>Hg>Pb>Cd. Madgett et al. (2021), determined the distribution of heavy metal concentrations in muscle tissues of sprat, goby (*Neogobius melanostomus*), Mediterranean horse mackerel and turbot (*Psetta maxima*) fish as Zn>As>Cu>Pb>Hg~Cr~Ni>Cd. They reported that although Zn showed the highest

accumulation level among the analyzed metals, the concentrations of the examined metals were close to or lower than the levels reported in the literature and the results for toxic and trace elements in the analyzed species were within acceptable limits for human consumption. From the results of the present study, the highest heavy metal concentration determined in gonads and fillets in January and February was determined as Zn (within the legal limits recommended in the literature).

Madgett et al, (2021) in their study examining the variability (inter- and intra-specific variation) in concentrations of three priority heavy metals (Hg, Cd and Pb) and six additional trace metals and metalloids (As, Ni, Se, Zn, Cu and Cr) in twenty-three species at four trophic levels from different parts of Scotland, Hg 20.10-341.0, Pb<13.6-19.80, Cd<4.76-9.460, As 5380-23200, Ni<13.00-27.50, Cr<30.6-112.0, Cu 122.0-1200, Se 210.0-1940, Zn 5070-11100 µg/kg ww were detected in flatfish muscles. Additionally, in all species studied, all detected and trophically adjusted Hg concentrations were reported to be above the EU Hg biota EQS. Bat et al. (2023) reported that the average concentrations of heavy metals (Cd, Hg, Pb, Cu, Zn, Fe) in both male and female turbot (*Scophthalmus maximus*) samples collected from the Black Sea coast were consistently below the safety limits set by national and international regulatory authorities. Tuzen (2009) reported that the toxic element contents in ten different fish samples collected from the Black Sea were 25-84 µg/kg for Hg, 0.11-0.32 µg/g for As, 0.28-0.87 µg/g for lead, 0.10-0.35 µg/g for Cd, 1.14-3.60 µg/g for Ni, 36.2-145 µg/g for Fe, 0.65-2.78 µg/g for Cu, 2.76-9.10 µg/g for Mn, 38.8-93.4 µg/g for Zn, and 0.63-1.74 µg/g for Cr and the Pb and Cd levels in the samples were above the legal limits recommended for human consumption.

In the current study, in order to assess the risks of Cd, Pb, As, Hg, Al, Fe, Cu and Zn elements on human health through consumption of different tissues and in different months in the European flounder, EDI, HQ and RI values were calculated (Table 3). EDI values showed that the intake rate for gonads and fillets was at safe levels in January and February. HQ values were determined at safe intake levels for all elements except As in both gonads and fillets. In January and February, THQ was calculated as <1 for adults in gonads, while THQ was >1 for children. For fillets, THQ was found as >1 in both adults and children, and it was determined that the safe intake level was exceeded. In the study conducted by Bošković et al. (2023), two fish species (*Mullus barbatus* and *Merluccius merluccius*) were examined. The study reported that THQ values for Hg in *M. barbatus* in Boka Kotorska Bay were above 1 and EDI values showed that the Hg uptake rate was not at a safe level. In contrast, the Hg uptake rate was reported to be at a safe level regarding THQ and EDI values for *M. merluccius*. For As it was reported that THQ and EDI values were high in both fish species and As uptake rate was not safe for human health.

Consistent with these findings, Bat et al. (2024) investigated heavy metal contamination in European anchovy (*Engraulis encrasicolus*) from the Black Sea and reported that concentrations of Pb, Cd, Hg, Cu, and Zn remained below established regulatory thresholds. Their results demonstrated that estimated daily intake values were within safe limits across all age groups and that THQ values remained below 1, indicating negligible non-carcinogenic risks. Furthermore, carcinogenic risk index values for Pb exposure remained within acceptable limits, reflecting minimal cancer risk.

**Table 3.** The estimated daily intake (EDI), hazard quotient (HQ), risk index (RI) of selected element of European flounder in the Black Sea

Months	Tissues	Elements	RfD	EDI		HQ		RI	
				Children	Adults	Children	Adults	Children	Adults
January	Gonad	Cd	0.001	8E-06	3.42E-06	0.008	0.003	1.27E-06	5.44E-07
		Hg	0.0003	8.07E-05	3.46E-05	0.269	0.115		
		Pb	0.002	2.8E-05	1.2E-05	0.014	0.006	3.29E-03	1.41E-03
		As	0.0003	5.89E-04	2.53E-04	1.964	0.842	3.93E-04	1.68E-04
		Al	1	1.47E-04	6.31E-05	1.47E-04	6.31E-05		
		Fe	0.7	0.008	0.003	0.011	0.005		
		Cu	0.04	0.001	4.7E-04	0.028	0.013		
	Zn	0.3	0.031	0.013	0.104	0.045			
	Fillets	Cd	0.001	0	0	0	0	0	0
		Hg	0.0003	1.61E-04	6.89E-05	0.536	0.229		
		Pb	0.002	1.47E-05	6.29E-06	0.007	0.003	1.73E-03	7.39E-04
		As	0.0003	0.004	0.002	13.171	5.645	2.63E-03	1.13E-03
		Al	1	0.001	5.8E-04	0.001	5.8E-04		
		Fe	0.7	0.003	0.001	0.004	0.002		
Cu		0.04	2.61E-04	1.12E-04	0.007	0.003			
Zn	0.3	0.008	0.003	0.027	0.011				
February	Gonad	Cd	0.001	1.33E-06	5.71E-07	0.001	0.001	2.12E-07	9.07E-08
		Hg	0.0003	1.77E-04	7.6E-05	0.591	0.253		
		Pb	0.002	3.87E-05	1.66E-05	0.019	0.008	4.55E-03	1.95E-03
		As	0.0003	6.67E-04	2.86E-04	2.222	0.952	4.44E-04	1.9E-04
		Al	1	0.007	0.003	0.007	0.003		
		Fe	0.7	0.008	0.003	0.011	0.005		
		Cu	0.04	0.001	4.3E-04	0.025	0.011		
	Zn	0.3	0.031	0.013	0.104	0.045			
	Fillets	Cd	0.001	6E-06	2.57E-06	0.006	0.003	9.52E-07	4.08E-07
		Hg	0.0003	1.3E-04	5.57E-05	0.433	0.186		
		Pb	0.002	2.07E-05	8.86E-06	0.010	0.004	2.43E-03	1.04E-03
		As	0.0003	0.004	0.002	12.828	5.498	2.57E-03	1.1E-03
		Al	1	8.42E-04	3.6E-04	8.42E-04	3.61E-04		
		Fe	0.7	0.005	0.002	0.007	0.003		
Cu		0.04	9.02E-04	3.87E-04	0.023	9.67E-03			
Zn	0.3	0.011	0.004	0.034	0.015				

RfD, reference doses (mg/kg/day) for metals

Yu et al. (2014) also reported that children are more vulnerable to contaminant exposure through fish consumption due to higher intake rates relative to body weight. Their study indicated that although overall non-carcinogenic risks were generally low, certain pollutants, particularly arsenic, could contribute to increased carcinogenic risk levels in both children and adults. These findings highlight the importance of considering age-related exposure differences in health risk assessments.

Overall, the findings of the present study demonstrate that most heavy metal concentrations in European flounder remained within acceptable safety limits, although arsenic showed

comparatively elevated levels and contributed most significantly to calculated risk indices. The absence of arsenic speciation in the present study represents an important limitation, as inorganic arsenic is the most toxic form. Nevertheless, previous studies have demonstrated that arsenic in marine organisms is predominantly present in organic forms, while inorganic arsenic generally constitutes only a small fraction of total arsenic (Yılmaz et al., 2017; Ervik et al., 2018). Therefore, the use of total arsenic concentrations may result in conservative or overestimated risk values.

Previous investigations have shown that environmental contamination and increasing anthropogenic activities may lead to elevated trace metal levels in marine ecosystems over time. Even in relatively low-industrialized coastal regions such as the southern Black Sea, contamination signals have been detected in marine organisms. These observations emphasize the necessity of continuous environmental monitoring and implementation of effective pollution control strategies to protect both ecosystem health and food safety.

### **Conclusion**

The study highlights the bioaccumulation of essential and non-essential elements in European flounder (*Platichthys flesus*) from the Southern Black Sea, revealing significant variations between fillets and gonads. While most heavy metals remained within safe limits, arsenic (As) exceeded permissible levels, posing health risks, particularly for children. The findings emphasize the need for continuous monitoring of seafood safety and stricter pollution control measures to protect both marine ecosystems and human health from the adverse effects of heavy metal contamination.

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### **Ethical Statement**

An "Ethics Committee Approval Certificate" is not required for this study.

### **Informed consent**

Not available.

### **Data availability**

Data will be made available on request.

### **Conflict of Interest**

The authors declare no competing interests.

### **Funding organizations**

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### **Declaration of AI USE**

The authors declare that no generative artificial intelligence tools were used in the design of the study, data analysis, or interpretation of the results; any language editing support did not influence the scientific content.



### Contributions of authors

Seval Dernekbaşı: Conceptualization, Data curation, Visualization, Formal analysis, Investigation, Writing original draft

Basim S. A. Al Sulivany: Formal analysis, Visualization, Methodology, Writing original draft, Software

Ismihan Karayücel: Formal analysis, Investigation, Writing, Review, Editing.

Fatemeh Jafari: Data curation, Formal analysis, Visualization, Writing

Muhammad Owais: Visualization, Writing, Review, Editing, Software

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