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Sex-Specific Bioaccumulation and Health Risk Assessment of Trace Elements in Alosa immaculata Bennett, 1835 from the Southern Black Sea

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Abstract

The Black Sea, a vital fishing area for Türkiye, is subject to severe anthropogenic pollution. The Pontic shad (*Alosa immaculata*), a prized anadromous fish rich in omega-3 fatty acids, is a dietary staple in the Sinop region, where the Inceburun coast is a proposed site for a nuclear power plant. This necessitates establishing robust baseline data on contaminant levels. This study aimed to determine the concentrations of 12 elements (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Hg, Pb) in the edible tissues of male and female Pontic shad from Inceburun of the south of Black Sea and to conduct a comprehensive human health risk assessment. Elemental concentrations were quantified using ICP-MS. Health risks were evaluated by calculating the Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), Hazard Index (HI), Carcinogenic Risk (RI), and Metal Pollution Index (MPI), and comparing results to international safety standards (EU, WHO, Codex Alimentarius). All elemental mean concentrations were well below regulatory limits. Significant sex-specific differences were identified: females exhibited higher levels of Cr, while males accumulated more Hg. The THQ for each element and the HI for the metal mixture were below 1 (HI_{female}=0.22, HI_{male}=0.15). The total carcinogenic risk indices (RI_{female}=3.0x10^{-5}, RI_{male}=1.8x10^{-5}) were within the acceptable range (<10^{-4}). The consumption of Pontic shad from the Sinop Inceburun coast is considered safe and poses no significant non-carcinogenic or carcinogenic health risks to consumers. However, the distinct accumulation patterns between sexes underscore the critical importance of gender-specific analysis in biomonitoring studies. This research provides vital baseline data for future environmental monitoring in this ecologically and economically significant region.

Keywords Pontic shad · potential toxic elements · sexual dimorphism · Inceburun

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Introduction

The Black Sea, a semi-enclosed marine ecosystem (Sorokin, 1983), holds paramount ecological and economic significance for the surrounding nations (Zaitsev & Mamaev, 1997), particularly Türkiye, where it represents the most productive fishing ground. It supports a major fraction of the country's commercial fisheries, providing a vital source of protein and livelihood for coastal communities. However, its unique hydrographic structure, characterized by limited water exchange with the Mediterranean Sea, makes it exceptionally vulnerable to anthropogenic pollution (Mee, 1992). Over decades, industrial discharge, agricultural runoff, and urbanization have led to the accumulation of various pollutants, including trace elements, posing a significant threat to marine biodiversity and human health (Bat et al., 2018).

Within this critical context, Inceburun, located on the Southern coast of the Black Sea, is a region of particular interest and growing importance. This area has been designated as a potential site for a future nuclear power plant, a project that brings with it profound environmental considerations and necessitates extensive baseline ecological studies. Establishing a comprehensive understanding of the existing pollution levels is imperative for future monitoring and assessing the environmental impact of such a large-scale industrial facility. This delineates the critical need for comprehensive environmental and health risk assessments, particularly concerning trace element contamination, in regions designated for significant industrial development like nuclear power plants (Tuncer et al., 2024). Such assessments are paramount for safeguarding public health and ecological integrity, informing policy decisions, and promoting sustainable development in areas potentially impacted by industrial emissions and effluents (Bat et al., 2023).

Among the valuable biological resources of the Black Sea, the Pontic shad (*Alosa immaculata*), known locally as 'tirsi', holds a special place. It is the largest species of the family Clupeidae in the Black Sea (Balık, 2019) and is an endemic fish species for the Black Sea (Turan et al., 2015) and it is available off the coasts of the southern Black Sea in Türkiye (Turan et al., 2007). Despite being a small bony fish, the Pontic shad is highly sought after for its high nutritional value, being exceptionally rich in health-beneficial omega-3 polyunsaturated fatty acids (Balçik Misir et al., 2016; Merdzhanova et al., 2021). It is a staple in the local diet, especially in regions like Sinop, where it is widely consumed and represents an important component of the culinary culture and food security. Its trophic position as zooplankton (mainly crustaceans, (*Crangon* sp., *Upogebia* sp., *Idothea* sp., gammarids) and small fish (*Engraulis* sp., *Clupeonella* sp., *Sprattus* sp.) (Froese & Pauly, 2025) and its key role in the marine food web also make it a valuable bioindicator species for monitoring ecosystem health and the bioavailability of contaminants (Bat, 2017; Bat & Arıcı 2018; Nachev et al., 2022).

The consumption of fish, while nutritionally beneficial, is also a primary pathway for human exposure to potentially toxic elements such as mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As). These elements can bioaccumulate in fish tissues and biomagnify through the food chains, leading to concentrations that may pose health risks to consumers. A critical, yet often overlooked, aspect of such bioaccumulation studies is the potential for variation between sexes. Physiological differences, such as reproductive strategies and metabolic rates, can lead to significant disparities in metal accumulation between male and female individuals, which in turn can affect risk assessment accuracy.

In 2024, the total amount of fish caught through fishing was 933,194 tons, of which 322,463 tons were marine fish (TurkStat, 2024). The catch of the Pontic shad in 2024 amounted to 1,946.2 tons (TurkStat, 2024). According to Sinop Provincial Directorate of Agriculture and Forestry, along the Sinop coasts, 24,620 kg of Pontic shad were caught in 2022 (Bat et al., 2024a). Therefore, the present study aims to (1) determine the concentrations of 12 elements (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Hg, Pb) in the edible tissues of male and female Pontic shad captured from the coastal waters of Sinop Inceburun using inductively coupled plasma mass spectrometry (ICP-MS); (2) conduct a comparative analysis of the accumulation levels between the two sexes; and (3) perform a human health risk assessment for consumers through the calculation of estimated daily/weekly intake and target hazard quotients. The findings of this research will provide crucial baseline data on elemental pollution in a key species from a strategically important location, thereby contributing to public health safety and fostering awareness on food safety issues.

Materials and Methods

Sampling procedure

The sampling site was located on the Inceburun coast, positioned at 42°05′51.29″N latitude and 34°56′40.18″E longitude. Inceburun represents the northernmost tip of Anatolia and lies on the northwestern edge of the Sinop Peninsula, projecting into the Black Sea. It is situated approximately 20 km from the provincial centre of Sinop (Figure 1). The construction of a nuclear power plant in this region underlines the environmental significance of the present investigation.

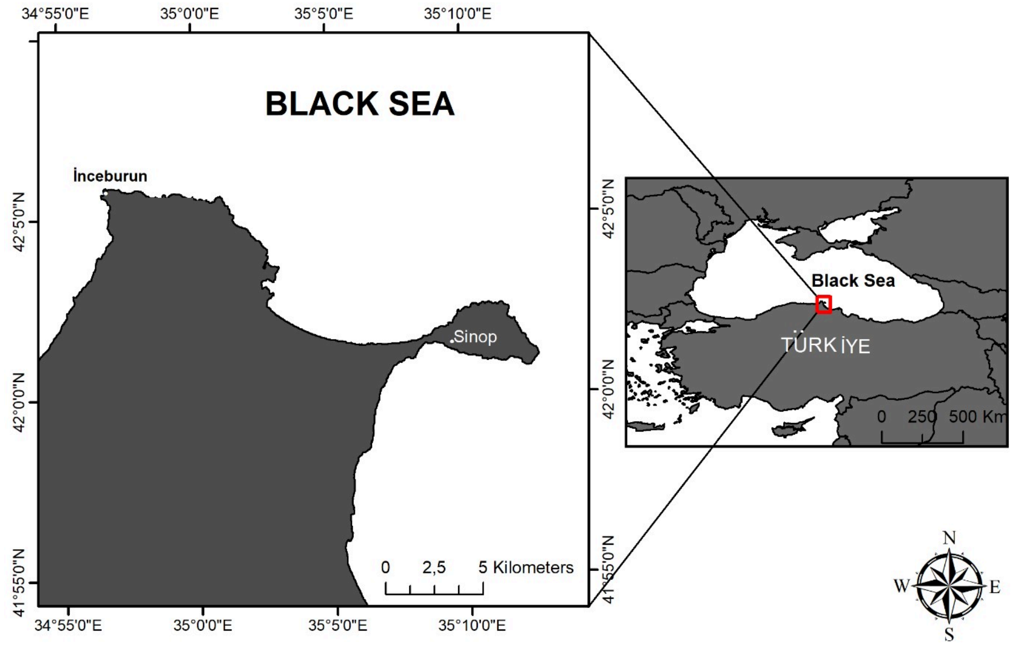

Figure 1. Sampling area (modified from Bat et al., 2022a)

A. immaculata specimens were collected throughout the 2023–2024 fishing seasons. Samples originated from commercial fishing activities carried out between September 1 and April 15, the legal period for industrial-scale fishing (using trawlers and purse seiners). After capture, the Pontic shad were preserved at 5 °C and transported on the same day to the Hydrobiology Laboratory of the Faculty of Fisheries, Sinop University. The sex of the specimens (female or male) was determined at the macroscopic level by examining the gonads, following the methods and descriptions outlined by King (2007), and Follesa & Carbonara (2019). Both male and female, twenty individuals (n=20, 10 for each sex) were randomly chosen. The samples were rinsed with tap water to remove external residues, followed by washing with double-distilled water. Subsequently, muscle tissues were dissected, and approximately 1.5 g of tissue was taken from each specimen for elemental analysis (Bat & Öztekin, 2025).

Element determination in fish

Elemental concentrations were determined in accordance with the U.S. EPA Method 200.3, which describes the preparation of biological samples for spectrochemical quantification of total recoverable elements (USEPA, 2024). Approximately 1.5 g of tissue was digested in Teflon vessels using a microwave digestion system (Milestone SK10). The digestion mixture consisted of concentrated nitric acid (HNO_3, 65% suprapur) and hydrogen peroxide (H_2O_2, 30% suprapur) at a ratio of 7:1, applied under a controlled temperature and pressure program (HPR-FO-67). The sealed vessels were heated at 200 °C for half an hour. After digestion, solutions were transferred into 50 mL polypropylene tubes and diluted to volume with ultrapure water. The certified reference material, Lobster TORT-2, was employed, and quality assurance and control were maintained through triplicate measurements (Bat & Öztekin, 2025). All elements in the certified reference material were confirmed to have an accuracy within 10% of their certified values. It falls within the expected or acceptable bounds for this material (Bat et al., 2022b,c). An Agilent 7700X Inductively Coupled Plasma Mass Spectrometer (ICP-MS) was employed for quantification. Calibration was performed using Agilent multi-element standards containing a 27-element mixture (8500-6940 2A and 8500-6940 Hg). An internal standard of 1 ppm (Agilent 5188-6525) was continuously monitored. All analyses were conducted in triplicate, and mean values were reported. Quality assurance and control procedures were applied, with limits of quantification (LOQ) set at three times the detection limit (LOD × 3) (Bat & Öztekin, 2025). Results were expressed in mg/kg wet weight (wet wt.). All analyses were carried out at Sinop University's Scientific and Technological Research Application and Research Centre (SUBİTAM) through service procurement.

Human health risk assessment

Estimation of Daily Intake (EDI)

The estimated daily intake of metals was calculated according to standard risk assessment models (RAIS, 2024; USEPA, 2024), using the equation (USEPA, 2000, 2001):

$$EDI = \frac{C \times FIR}{BW} \tag{1}$$

where *C* is the mean metal concentration in fish tissue (mg/kg wet weight), *FIR* is the daily fish ingestion rate (g/day), and *BW* is the average body weight. For this study, the per capita fish consumption rate was set at 19.72 g/day (Çöteli, 2024).

Target Hazard Quotient (THQ)

The non-carcinogenic risk from metal exposure was assessed using the target hazard quotient, calculated as follows (RAIS, 2024; USEPA, 2024):

$$THQ = \frac{E_{fr} \times ED \times FIR \times C}{RfD \times BW \times AT_n} \times 10^{-3} \tag{2}$$

where *Efr* = exposure frequency (365 days/year), *ED* = exposure duration (years), *FIR* = ingestion rate, *C* = mean element concentration, *RfD* = oral reference dose, *BW* = body weight (70 kg for adults), and *ATn* = averaging time for non-carcinogens (365 days/year × 70 years). A THQ value < 1 indicates no expected non-carcinogenic risk, while values > 1 suggest potential health concerns.

Hazard Index (HI)

The hazard index represents the combined risk from multiple elements and was obtained by summing the THQ values for all metals (USEPA, 2000, 2001):

$$HI = \sum_{i=1}^{n} THQ_i \tag{3}$$

An HI < 1 implies negligible risk, whereas HI > 1 signals possible health hazards associated with fish consumption.

Risk Index (RI)

Carcinogenic risk was evaluated for Cr, As, and Pb using their respective oral slope factors (0.16, 1.5, and 0.0085 mg/kg-day; RAIS, 2024; USEPA, 2024). The risk index was estimated using:

RI= EDI x SF

An RI < 10^{-6} is considered insignificant, values between 10^{-6} and 10^{-4} indicate tolerable risk, while RI > 10^{-4} reflects a significant carcinogenic threat.

Metal Pollution Index (MPI)

The MPI was employed as an integrated metric to assess overall metal contamination in fish, following Usero et al. (1997):

$MPI = (C_1 \times C_2 \times C_3 \ldots \times C_n)^{1/n}$

where *C* represents the mean concentration (mg/kg) of each metal and *n* is the number of metals analysed. This index provides a useful tool for monitoring toxic element accumulation in seafood and marine ecosystems.

Statistical Analysis

The data did not follow a normal distribution; therefore, non-parametric tests were applied. Differences in heavy metal concentrations between sexes were assessed using the Mann-Whitney U test. Pearson correlation analysis was employed to examine the relationships among length, weight, and element concentrations. A p-value of less than 0.05 was considered statistically significant.

Results and Discussion

In the regulation governing fisheries, no minimum catch size has been established for some commercially important fish species. One of these species is the Pontic shad. It has economic importance in the Black Sea, there are currently no fishing restrictions in place (Özdemir et al., 2018). In the present study, the mean length of the Pontic shad individuals was measured as 30.5 cm, while the mean weight was recorded as 295 g.

The analysis of 12 trace elements in the edible tissues of male and female Pontic shad revealed distinct accumulation patterns, with several elements showing notable differences between the sexes (Figure 2, Table 1). These variations can be attributed to differences in physiology, metabolism, and reproductive strategies. However, the statistical analyses showed no significant differences between the sexes ($p>0.05$) and all mean trace element concentrations were below international regulatory limits, indicating compliance with food safety standards.

Zn exhibited the most pronounced sexual dimorphism. Female individuals showed a wide range (8.56 - 18.44 mg/kg) but an overall lower average accumulation compared to males (11.72 - 18.44 mg/kg). Zn is a crucial micronutrient involved in numerous enzymatic processes and is vital for oogenesis (egg production) in females. However, it appears that the high metabolic demand for growth and maintenance in males, potentially related to different spawning behaviours or energy utilization, leads to a higher uptake or retention of Zn. Concentrations of Fe were highly variable in both

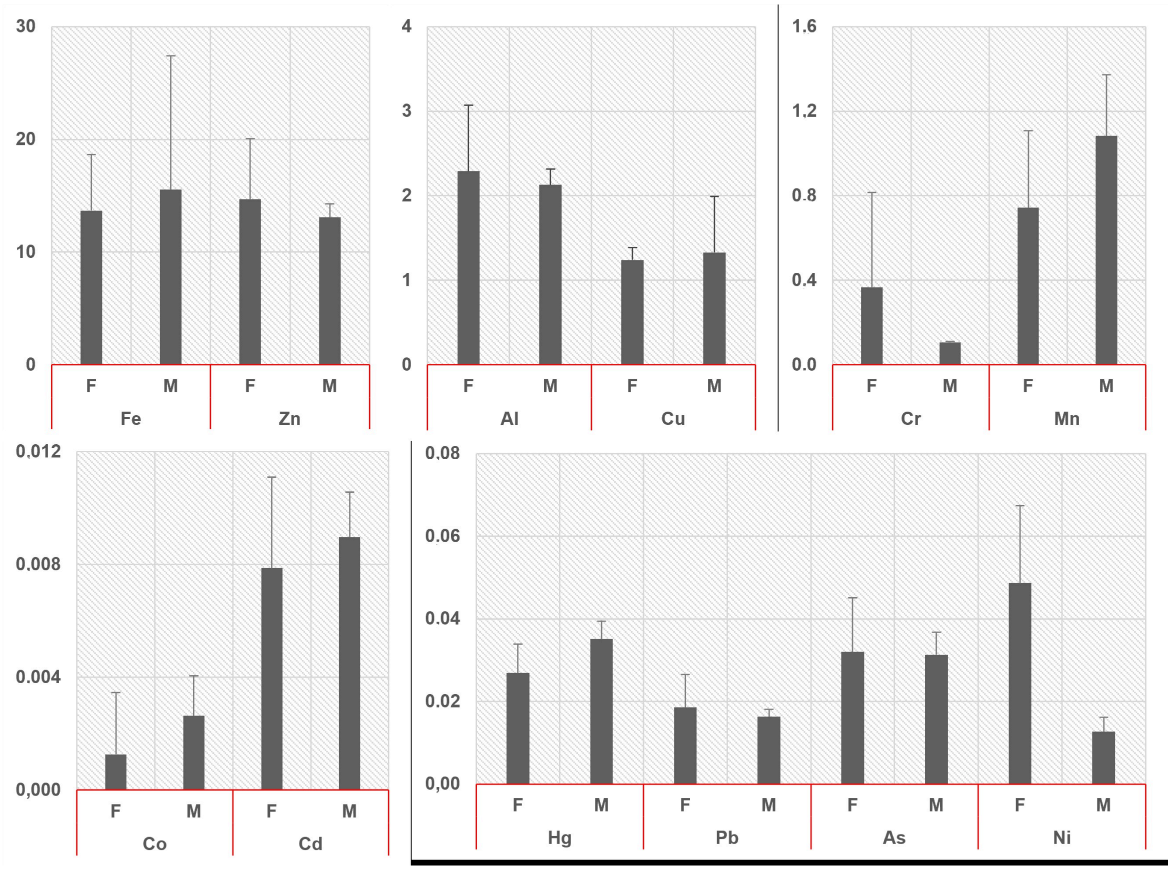

Figure 2. The means with standard deviations of trace elements in the edible tissues of female (F) and male (M) *Alosa immaculata* collected from Sinop coasts

groups. One male individual showed an exceptionally high value (29.05 mg/kg), which skews the average. Excluding this outlier, the levels between sexes are comparable. Fe, like Zn, is an essential element for oxygen transport and metabolic processes. The high variability is likely influenced by the diet of individual fish rather than a consistent sex-specific trend. Cu and Mn showed relatively consistent levels across all individuals with no clear sex-based pattern. Cu values clustered around 1.0-1.3 mg/kg, and Mn ranged from 0.33-1.34 mg/kg. This suggests that the physiological regulation of these essential elements is similar for both male and female Pontic shads. Concentration of Al was consistently higher in female Pontic shads (1.63 - 3.15 mg/kg) compared to males (1.99 - 2.34 mg/kg). Al is often associated with sediment resuspension and anthropogenic runoff. The reason for its higher accumulation in females is unclear but could be linked to differences in feeding habits or gill function. Cr and Ni demonstrated significantly higher concentrations in female individuals. For Cr, females showed levels up to 0.88 mg/kg, while the maximum in males was 0.11 mg/kg. Similarly, Ni was higher in females (up to 0.069 mg/kg vs. 0.016 mg/kg in males). Bat et al. (2024b) found that surface sediment Cr amounts exceeded average shale values, while Ni amounts were lower. These findings are supported by high total organic carbon contents in the surface sediments. The metal content found in the Black Sea sediments is strongly dictated by the presence of suspended particles in the water column, especially following periods of intense precipitation or deluge events. Furthermore, agricultural runoff carried by terrestrial waterways, such as major rivers, constitutes a substantial pathway for metal delivery into the marine environment (Bat et al., 2024b). These elements are industrial pollutants, and their elevated levels in females may indicate a reduced ability to excrete them or a higher rate of uptake. The results for highly toxic elements Pb and Cd are mixed but show a trend. Male Pontic shads generally accumulated higher levels of both Pb (0.0144 - 0.0181 mg/kg) and Cd (0.0079 - 0.0108 mg/kg) compared to females (Pb: 0.0117 - 0.0273 mg/kg; Cd: 0.0058 - 0.0116 mg/kg). However, the ranges overlap, indicating that while the trend points towards higher male accumulation, it is not an absolute rule and may be influenced by individual exposure history. A clear and consistent trend is observed for Hg, with male Pontic shads (0.0301 - 0.0380 mg/kg) accumulating higher concentrations than females (0.019 - 0.0322 mg/kg). Hg is a potent neurotoxin that bioaccumulates over time. Higher levels in males could be related to a higher metabolic rate, leading to greater food consumption and thus higher Hg intake, or to the offloading of Hg from

Table 1. Mean element concentrations (mg/kg wet wt.) in A. immaculata Inceburun coasts of Sinop province in the Black Sea, EDI, EWI, THQ, HI, RI and MPI values.

Metals	Female					Male				
	Mean	EDI	EWI	THQ	RI	Mean	EDI	EWI	THQ	RI
Al	2.29	6.5×10^{-4}	4.5×10^{-3}	6.4×10^{-4}		2.13	6.0×10^{-4}	4.2×10^{-3}	6.0×10^{-4}	
Cr	0.37	1.0×10^{-4}	7.2×10^{-4}	1.1×10^{-1}	1.6×10^{-5}	0.11	3.0×10^{-5}	2.1×10^{-4}	3.3×10^{-2}	4.8×10^{-6}
Mn	0.74	2.1×10^{-4}	1.5×10^{-3}	1.5×10^{-3}		1.08	3.1×10^{-4}	2.1×10^{-3}	2.2×10^{-3}	
Fe	13.68	3.8×10^{-3}	2.7×10^{-2}	5.5×10^{-3}		15.54	4.4×10^{-3}	3.1×10^{-2}	6.2×10^{-3}	
Co	0.0013	3.5×10^{-7}	2.5×10^{-6}	1.2×10^{-3}		0.0026	7.4×10^{-7}	5.2×10^{-6}	2.5×10^{-3}	
Ni	0.049	1.4×10^{-5}	9.6×10^{-5}	6.9×10^{-4}		0.013	4.0×10^{-6}	2.5×10^{-5}	1.8×10^{-4}	
Cu	1.24	3.0×10^{-4}	2.4×10^{-3}	8.7×10^{-3}		1.33	4.0×10^{-4}	2.6×10^{-3}	9.4×10^{-3}	
Zn	14.69	4.1×10^{-3}	2.9×10^{-2}	1.4×10^{-2}		13.07	4.0×10^{-3}	2.6×10^{-2}	1.2×10^{-2}	
As	0.032	9.0×10^{-6}	6.3×10^{-5}	3.0×10^{-2}	1.3×10^{-5}	0.031	8.8×10^{-6}	6.2×10^{-5}	2.9×10^{-2}	1.3×10^{-5}
Cd	0.008	2.2×10^{-6}	1.5×10^{-5}	2.2×10^{-2}		0.009	2.5×10^{-6}	1.8×10^{-5}	2.5×10^{-2}	
Hg	0.027	7.5×10^{-6}	5.3×10^{-5}	2.5×10^{-2}		0.035	9.8×10^{-6}	6.9×10^{-5}	3.3×10^{-2}	
Pb	0.019	5.2×10^{-6}	3.7×10^{-5}	1.7×10^{-3}	4.4×10^{-8}	0.016	4.6×10^{-6}	3.2×10^{-5}	1.5×10^{-2}	3.9×10^{-8}
			HI=	2.2×10^{-1}				HI=	1.5×10^{-1}	
				Total RI=	3.0×10^{-5}				Total RI=	1.8×10^{-5}
MPI=			1.8×10^{-1}					1.6×10^{-1}		

females into their eggs during spawning. Concentrations of As were relatively low and stable across all samples, with no significant difference between the sexes. The primary form of As in marine organisms is often arsenobetaine, an organic compound considered non-toxic to humans. Levels of Co were near the detection limit for most individuals. The values were generally higher in males, but the extremely low concentrations make it difficult to draw a definitive conclusion regarding sex-specific accumulation. The results of this study, when contextualized with similar investigations into heavy metal bioaccumulation in marine biota, further emphasize the imperative for rigorous environmental monitoring, especially in coastal areas susceptible to industrial discharges (Bat et al., 2009). This is particularly relevant for proposed nuclear power plant sites, where the long-term ecological impact of operational discharges and potential accidental releases necessitates a proactive risk assessment framework (Karahan et al., 2020). Such frameworks are essential for identifying potential pathways of human exposure and for estimating the risks associated with consuming contaminated seafood, a common dietary component in many coastal.

The data suggests that sex is a significant factor in the bioaccumulation of certain trace elements in Pontic shad. Female individuals tended to accumulate higher levels of Al, Cr, and Ni. In contrast, male Pontic shads show a strong tendency for higher accumulation of Zn and Hg, with a weaker trend observed for Pb and Cd. These findings underscore the importance of considering sexual dimorphism in bio-monitoring studies. Using a mixed-sex sample pool without distinction could mask important trends and lead to an inaccurate assessment of both environmental contamination and human health risks. The physiological mechanisms behind these differences such as the transfer of elements to gonads during reproduction in females or differences in metabolic rates between sexes, warrant further investigation. To further explore the potential influence of biometric parameters on trace element accumulation, the relationships between metal concentrations and fish length and weight were examined. When assessing the relationship between metal concentrations and biometric parameters (length and weight), no statistically significant correlations were detected ($p>0.05$). The Pontic shad is a larger anadromous clupeid, migrating between the sea and freshwater to spawn, and likely feeding on smaller fish and zooplankton (higher trophic level) (Froese & Pauly, 2025). This places the Pontic shad higher in the food web, which has major implications for the bioaccumulation of certain elements.

Risk Assessment

The mean concentrations of all elements in both female and male fish were found to be significantly below the maximum permitted levels set by the international Codex Alimentarius. This is the primary and most reassuring finding. The European Union does not set a limit for essential elements. 100 mg/kg wet weight for Fe (FAO, 1983), 50 mg/kg wet weight for Zn and 30 mg/kg wet weight for Cu (MAFF, 1995), 0.3 mg/kg wet weight for Pb, 0.5 mg/kg wet weight for Hg, and 0.05 mg/kg wet weight for Cd (Commission Regulation, 2006) are accepted as permissible values. It indicates that, on an individual element

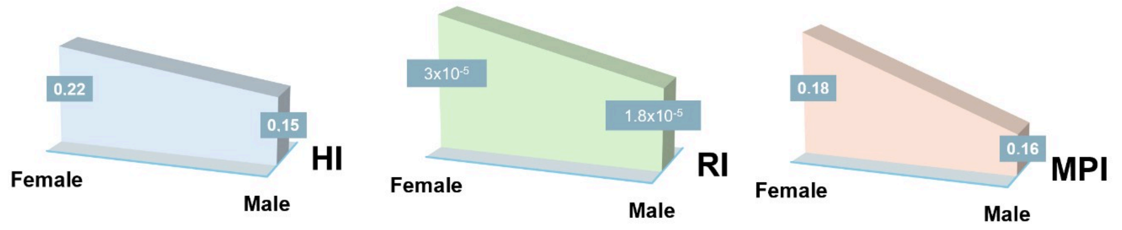

Figure 3. The means with standard deviations of trace elements in the edible tissues of female (F) and male (M) *Alosa immaculata* collected from Sinop coasts

basis, the consumption of this specific fish from this location does not pose an immediate or direct threat from any single metal.

Non-Carcinogenic Health Risks (THQ and HI)

The THQ values for all individual elements were less than 1 for both sexes. A THQ < 1 indicates that the estimated level of exposure is unlikely to cause non-carcinogenic adverse health effects over a lifetime. Notably, the highest THQ values were observed for Cr in females (0.11), followed by As, Cd, and Hg in both sexes (all in the range of 0.022 - 0.033). While all are below the hazard threshold, these elements are the primary contributors to the total risk and warrant continued monitoring. The HI, which represents the cumulative non-carcinogenic risk from all elements, was 0.22 for females and 0.15 for males (Figure 2). Both values are below 1, suggesting that the combined effect of these metals through consumption of Pontic shad is unlikely to pose a non-carcinogenic health risk to the general population. The relatively higher THQ for females is directly driven by their significantly higher accumulation of Cr.

Carcinogenic Health Risks (RI)

The carcinogenic risk was calculated for the known or probable carcinogens: Cr, As, and Pb. The Risk Index (RI) values for all these elements were several orders of magnitude below the USEPA's negligible risk level of $1x10^{-6}$ and the acceptable range of $1x10^{-6}$ to $1x10^{-4}$ (Figure 3). The total RI was $3.0x10^{-5}$ for females and $1.8x10^{-5}$ for males. These values fall within the "acceptable" or "essentially negligible" risk range according to most international guidelines. This indicates that the lifetime cancer risk from exposure to these carcinogenic metals through consumption of this fish is very low.

Metal Pollution Index (MPI) and Sex Differences

The MPI provides a simple overall measure of contamination. The values were low and similar for both sexes (0.18 for females, 0.16 for males), confirming the overall low contamination load (Figure 2). However, the breakdown reveals critical sex-specific differences: Female Pontic shad presented a higher overall non-carcinogenic risk (HI) due to elevated levels of Cr. Male Pontic shad, while having a lower overall THQI, consumers who preferentially consume male fish.

Comparisons of different studies conducted on *Alosa* species

The element concentrations in muscles of *Alosa* species from the Black Sea coasts were compared with other studies (Table 2). Looking at the values obtained from the current study, compared to other studies, Al, Ni, As, Cd, Hg and Pb were found to be low, Cr, Fe, Cu, Zn were found to be similar or slightly high, and Mn was found to be high. Apart from our study, only Visnjic-Jeftic et al. (2010) have measured metal levels in both male and female individuals of the Pontic shad. They found a significant difference in trace element concentrations in muscle and liver between males and females but did not find such a difference in gills. While Mg concentrations in muscle tissue were higher in males, As and Mg concentrations in liver tissue were higher in females. Cd, B, Ba, Fe, Zn, and Li concentrations in liver tissue were also recorded as higher in males. Visnjic-Jeftic et al. (2010) found that Cd and As concentrations in the muscle tissue of Pontic shad exceeded the maximum acceptable concentrations for human consumption and therefore emphasised the need to take measures to prevent metal pollution in the future.

Table 2. Comparison of the amounts of trace elements (mg/kg) in the edible tissues of different Alosa species from the Black Sea coasts

Metals	Location		Mean	wet/dry weight	References
Al	Danube River	Female	7.910±11.845	dry wt.	Visnjic-Jeftic et al., 2010
		Male	7.489±7.849	dry wt.	Visnjic-Jeftic et al., 2010
	Black Sea-Sinop	Female	2.29±0.7	wet wt.	Present study
		Male	2.13±0.18	wet wt.	Present study
Cr	Black Sea-Romania		0.11	wet wt.	Jitar et al., 2015
	Black Sea-Bulgaria		0.05±0.01	wet wt.	Stancheva et al., 2014
	Black Sea-Samsun		0.11±0.01	wet wt.	Türkmen & Dura, 2016
	Black Sea-Trabzon		0.29±0.04	wet wt.	Türkmen & Akaydin, 2017
	Black Sea-Rize		0.27±0.07	wet wt.	Türkmen & Akaydin, 2017
	Black Sea-Sinop	Female	0.37±0.45	wet wt.	Present study
		Male	0.11±0.006	wet wt.	Present study
Mn	Black Sea-Bulgaria		0.11±0.01	wet wt.	Stancheva et al., 2014
	Black Sea-Samsun		0.17±0.02	wet wt.	Türkmen & Dura, 2016
	Black Sea-Trabzon		0.19±0.04	wet wt.	Türkmen & Akaydin, 2017
	Black Sea-Rize		0.25±0.04	wet wt.	Türkmen & Akaydin, 2017
	Black Sea-Sinop	Female	0.74±0.36	wet wt.	Present study
		Male	1.08±0.29	wet wt.	Present study
Fe	Danube River	Female	39.845±35.86	dry wt.	Visnjic-Jeftic et al., 2010
		Male	41.185±47.29	dry wt.	Visnjic-Jeftic et al., 2010
	Black Sea-Samsun		16.6±9.29	wet wt.	Türkmen & Dura, 2016
	Black Sea-Trabzon		38.6±4.39	wet wt.	Türkmen & Akaydin, 2017
	Black Sea-Rize		35.0±4.11	wet wt.	Türkmen & Akaydin, 2017
	Danube River		6.00±1.00	wet wt.	Simionov et al., 2021
	Black Sea-Romania		10.50±1.60	wet wt.	Simionov et al., 2021
	Black Sea-Sinop	Female	13.68±5	wet wt.	Present study
		Male	15.54±11	wet wt.	Present study
Co	Black Sea-Samsun		0.03±0.00	wet wt.	Türkmen & Dura, 2016
	Black Sea-Trabzon		0.03±0.00	wet wt.	Türkmen & Akaydin, 2017
	Black Sea-Rize		0.02±0.00	wet wt.	Türkmen & Akaydin, 2017
	Black Sea-Sinop	Female	0.0013±0.002	wet wt.	Present study
		Male	0.0026±0.001	wet wt.	Present Study
Ni	Black Sea-Romania		0.06	wet wt.	Jitar et al., 2015
	Black Sea-Bulgaria		0.07±0.01	wet wt.	Stancheva et al., 2014
	Black Sea-Samsun		3.74±0.08	wet wt.	Türkmen & Dura, 2016
	Black Sea-Trabzon		1.57±0.01	wet wt.	Türkmen & Akaydin, 2017
	Black Sea-Rize		1.70±0.09	wet wt.	Türkmen & Akaydin, 2017
	Black Sea-Sinop	Female	0.049±0.018	wet wt.	Present study
		Male	0.013±0.004	wet wt.	Present study
Cu	Danube River	Famale	4.045±2.498	dry wt.	Visnjic-Jeftic et al., 2010
	Danube River	Male	4.123±2.392	dry wt.	Visnjic-Jeftic et al., 2010
	Danube River		5.34±3.5	dry wt.	Ioniţă et al., 2014
	Black Sea-Romania		2.25±0.81	wet wt.	Jitar et al., 2015
	Black Sea-Bulgaria		0.45±0.03	wet wt.	Stancheva et al., 2014
	Danube River		0.40±0.10	wet wt.	Simionov et al., 2021

Metals	Location		Mean	wet/dry weight	References
	Black Sea-Samsun		1.28±0.15	wet wt.	Türkmen & Dura, 2016
	Black Sea-Trabzon		3.00±0.86	wet wt.	Türkmen & Akaydin, 2017
	Black Sea-Rize		1.74±0.16	wet wt.	Türkmen & Akaydin, 2017
	Black Sea		2.62±0.58	dry wt.	Nisbet et al., 2010
	Black Sea-Türkiye		1.21	wet wt.	Bat et al., 2017
	Black Sea-Sinop	Female	1.24±0.15	wet wt.	Present study
		Male	1.33±0.66	wet wt.	Present study
Zn	Danube River	Female	68.224±76.03	dry wt.	Visnjic-Jeftic et al., 2010
	Danube River	Male	62.554±31.03	dry wt.	Visnjic-Jeftic et al., 2010
	Danube River		44.55±2.6	dry wt.	Ioniţă et al., 2014
	Bulgaria		9±1	wet wt.	Stancheva et al., 2014
	Danube River		3.60±0.30	wet wt.	Simionov et al., 2021
	Black Sea-Romania		4.00±0.40	wet wt.	Simionov et al., 2021
	Black Sea-Samsun		3.62±0.15	wet wt.	Türkmen & Dura, 2016
	Black Sea- Trabzon		3.65±0.55	wet wt.	Türkmen & Akaydin, 2017
	Black Sea-Rize		4.28±0.7	wet wt.	Türkmen & Akaydin, 2017
	Black Sea-Türkiye		8	wet wt.	Bat et.al., 2017
	Black Sea-Sinop	Female	14.69±5.3	wet wt.	Present study
		Male	13.07±1.2	wet wt.	Present study
As	Danube River	Female	7.861±2.191	dry wt.	Visnjic-Jeftic et al., 2010
	Danube River	Male	7.499±2.885	dry wt.	Visnjic-Jeftic et al., 2010
	Black Sea-Bulgaria		0.38 0.02	wet wt.	Stancheva et al., 2014
	Black Sea		30.87±7.11	dry wt.	Nisbet et al., 2010
	Black Sea-Sinop	Female	0.032±0.01	wet wt.	Present study
		Male	0.031±0.005	wet wt.	Present study
Cd	Danube River	Female	0.453±0.202	dry wt.	Visnjic-Jeftic et al., 2010
	Danube River	Male	0.399±0.139	dry wt.	Visnjic-Jeftic et al., 2010
	Danube River		0.091±1.5	dry wt.	Ioniţă et al., 2014
	Black Sea-Romania		0.02±0.003	wet wt.	Jitar et al., 2015
	Black Sea-Bulgaria		0.007±0.001	wet wt.	Stancheva et al., 2014
	Black Sea-Samsun		0.12±0.03	wet wt.	Türkmen & Dura, 2016
	Black Sea-Romania		0.02±0.001	wet wt.	Simionov et al., 2021
	Danube River		0.02±0.001	wet wt.	Simionov et al., 2021
	Black Sea-Trabzon		0.18±0.06	wet wt.	Türkmen & Akaydin, 2017
	Black Sea-Rize		0.22±0.10	wet wt.	Türkmen & Akaydin, 2017
	Black Sea		0.022±0.002	dry wt.	Nisbet et al., 2010
	Black Sea-Türkiye		< 0.02	wet wt.	Bat et al., 2017
	Black Sea-Sinop		0.071±0.035	dry wt.	Ergönül and Altındağ, 2014
	Black Sea-Sinop	Female	0.008±0.003	wet wt.	Present study
		Male	0.009±0.002	wet wt.	Present study
Hg	Black Sea-Bulgaria		0.08±0.01	wet wt.	Stancheva et al., 2014
	Black Sea-Türkiye		< 0.05	wet wt.	Bat et al., 2017
	Black Sea-Sinop	Female	0.027±0.007	wet wt.	Present study
		Male	0.035±0.004	wet wt.	Present study
	Black Sea-Romania		0.20±0.09	wet wt.	Jitar et al., 2015

Metals	Location		Mean	wet/dry weight	References
Pb	Danube River		0.65±1.4	dry wt.	Ioniţă et al., 2014
	Black Sea-Bulgaria		0.05±0.01	wet wt.	Stancheva et al., 2014
	Danube River		0.01±0.004	wet wt.	Simionov et al., 2021
	Black Sea-Romania		0.02±0.002	wet wt.	Simionov et al., 2021
	Black Sea-Samsun		0.98±0.21	wet wt.	Türkmen & Dura, 2016
	Black Sea-Trabzon		0.85±0.22	wet wt.	Türkmen & Akaydin, 2017
	Black Sea-Rize		2.32±0.76	wet wt.	Türkmen & Akaydin, 2017
	Black Sea		0.86±0.16	dry wt.	Nisbet et al., 2010
	Black Sea-Türkiye		< 0.05	wet wt.	Bat et al., 2017
	Black Sea-Sinop		0.68±0.31	dry wt.	Ergönül and Altındağ, 2014
	Black Sea-Sinop	Female	0.019±0.008	wet wt.	Present study
		Male	0.016±0.002	wet wt.	Present study

Conclusion

The significant differences in metal accumulation between the sexes of Pontic shad from the Sinop Inceburun region underscore the need for a gender-based approach in human health risk assessment and environmental biomonitoring. The assessment is based on EDI, THQ, HI, RI, and MPI. This comprehensive risk assessment demonstrates that the consumption of *A. immaculata* caught from the Inceburun coast of Sinop is safe for human health regarding the 12 analysed trace elements. All individual metal concentrations comply with international food safety standards. The calculated indices THQ, HI, and RI, all consistently indicate that the non-carcinogenic and carcinogenic health risks for the average consumer are negligible. A health risk analysis based on a mixed-sex sample would underestimate the risk for consumers who primarily eat male and overestimate it for those eating female *A. immaculata*.

As a migratory species that connects freshwater and marine ecosystems, *A. immaculata* is a valuable bioindicator for monitoring metal pollution across a broad environmental gradient. The differences in metal accumulation between sexes must be accounted for in such monitoring programs. These findings highlight the necessity of sex-specific analysis in future studies on anadromous and predatory fish species to ensure accurate environmental monitoring and public health risk evaluation. We strongly advocate for the inclusion of gender-specific analysis in future biomonitoring studies to ensure accurate risk evaluation. This study provides an essential baseline of elemental pollution levels in a key fishery species from a region slated for significant industrial development (the nuclear power plant). These results are invaluable for future monitoring programs, allowing scientists to accurately detect any potential changes in pollution status attributable to new anthropogenic activities.

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