

HEAVY METALS IN SOME FISH SPECIES FROM BLACK SEA: A HEALTH RISKS ASSESSMENT

Violina ANGELOVA

Agricultural University - Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: vileriz@yahoo.com

Abstract

Consumption of fish contaminated with heavy metals poses a significant risk to human health, particularly with prolonged intake. This study investigates the concentrations of heavy metals in fish caught from the Black Sea and evaluates the associated health risks. The content of toxic metals and trace elements was determined in the muscle tissue of 15 different Black Sea fish species. The highest concentrations of specific metals were observed in the following species Pb in piked dogfish; Cd in European sprat; Zn in European sprat and Mediterranean horse mackerel; Cu in leaping mullet; Fe in European sprat and Atlantic bonito; Mn and Al in European sprat; Cr in Atlantic bonito; Ni in Atlantic bonito and bluefish; As in round goby; and Hg in bluefish and piked dogfish. In general, metal concentrations in muscle tissue were below the maximum permissible limits established by EU Commission Regulation, except for cadmium in Mediterranean horse mackerel. A human health risk assessment was conducted using target hazard quotients (THQ) and the hazard index (HI). The HI values were below 1 for all species, except for mercury in piked dogfish, indicating no significant health risk from consumption of these fish under typical dietary exposure.

Key words: fish, food safety, risk assessment, toxic metals, ICP-OES.

INTRODUCTION

Seafood, particularly fish, is an essential source of nutrients that are the foundation of a balanced diet. It provides high-quality proteins, lipids, essential nutrients and fat-soluble vitamins. For this reason, the World Health Organization recommends fish as an important component of a healthy diet. In recent years, fish consumption has risen significantly (Gu et al., 2017). According to the National Statistical Institute, in 2023, Bulgarian households consumed an average of 6.2 kg of fish per capita.

Contaminants can accumulate in fish and eventually enter the human body through the food chain (Turan et al., 2009; Popescu et al., 2019; Ture et al., 2021). The higher a fish is in the trophic chain, the greater its concentration of toxic substances. Although muscle tissue generally contains lower metal loads, it remains the primary focus of research for assessing human health risks related to fish consumption (Sirin et al., 2024). This raises an important question how safe is fish as a food source, and what the benefit-risk balance of seafood consumption is. In recent years, researchers have increasingly focused on evaluating the

risks associated with fish consumption (Barone et al., 2015; Traina et al., 2019).

To ensure seafood safety, the European Communities (EC) has established regulatory limits for chemical contaminants in seafood intended for human consumption (Regulation (EC) 1881/2006; 2023/915). Additionally, global food safety agencies, including JECFA and EFSA, have set health-based guidelines for acceptable exposure to toxicants (e.g., tolerable intake reference doses and health risk factors) for various contaminants (Copat et al., 2013). Moreover, the Marine Strategy Framework Directive (2008/56/EC) provides ecological descriptors to assess the sustainable use and management of marine environments. The ninth descriptor, Contaminants in fish and other seafood, aims to determine the levels of contamination of edible parts of fish, crustaceans, molluscs etc., caught or collected from nature for human consumption for which maximum levels have been established at European, regional or national level.

Monitoring and analysing heavy metal concentrations in marine fish, an essential link in the food chain, is crucial to preventing potential adverse health effects, as contamination is a continuous process.

Numerous studies have examined heavy metal levels in the muscle and other tissues of fish caught in the Black Sea and other marine environments (Turkmen et al., 2007; Tepe et al., 2008; Turan et al., 2009; Stancheva et al., 2013a; 2013b; Stancheva et al., 2014; Peycheva et al., 2017; Peycheva et al., 2019; Bat et al., 2022).

The objective of this study is to determine the levels of toxic metals and trace elements in the muscle tissue of 15 fish species from the southern Black Sea coast of Bulgaria. These species include round goby, European sprat, whiting, Mediterranean horse mackerel, Pontic shad, annular seabream, leaping mullet, flathead grey mullet, bluefish juvenile, bluefish, turbot, red mullet, garfish, Atlantic bonito, and piked dogfish. The study also aims to assess the public health risks associated with fish consumption in this region.

MATERIALS AND METHODS

Tissue sampling

This study examined the following fish species from the Black Sea: *Neogobius melanostomus*, *European sprattus*, *Merlangius merlangus*, *Trachurus mediterraneus*, *Alosa immaculata*, *Diplodus annularis*, *Liza saliens*, *Mugil cephalus*, *Pomatomus saltatrix* juvenile, *Pomatomus saltatrix*, *Scophthalmus maximus*, *Mullus barbatus*, *Belone belone*, *Sarda sarda*, and *Squalus acanthias*.

Fish samples were collected from commercial catches between September and November in 2022, 2023, and 2024 at two locations along the southern Bulgarian coast of the Black Sea: Nessebar and Burgas. Specimens were transported to the Agricultural University-Plovdiv laboratory in an ice-cooled container. Upon arrival, samples were either analysed immediately or stored at -20°C to maintain their integrity until analysis. In the laboratory, fish were rinsed with distilled water and dried using filter paper. Dissections were performed using a stainless steel knife. For smaller species (European sprat, round goby, Mediterranean horse mackerel, whiting, and Pontic shad), the entire edible portion of each individual was combined to form a composite sample. Larger species were processed by removing the head, tail, and viscera before filleting. The filleted

tissue was minced and homogenized for further analysis.

Determination of metal content in fish

The fish samples were prepared for toxic metal and trace element analysis using the microwave mineralization method with a MARS 6 microwave system (CEM Corporation, USA). Element concentrations were determined using an ICP-OES spectrometer (Prodigy Model 7, Teledyne Leeman Labs, USA). Mercury content was analyzed separately using a mercury analyzer MA 3000.

All analyses were performed in triplicate, and results were expressed as mean values. Data processing was conducted using SPSS 26 statistical software.

Risk assessment

Metal Pollution Index (MPI)

MPI is a reliable metric for monitoring heavy metal contamination in food and aquatic ecosystems. In this study, the MPI values were calculated using the following equation:

$$\text{MPI (mg/kg)} = (\text{Cf1} \times \text{Cf2} \times \dots \times \text{Cfn})^{1/n} \quad (1)$$

where: Cf represents the average concentration of each metal (mg/kg) in fish muscle, and n represents the number of metals considered.

Estimated daily intake (EDI)

The Estimated Daily Intake (EDI) was calculated to assess human health risks associated with metal intake from fish consumption:

$$\text{EDI} = \frac{\text{C} \cdot \text{IR} \cdot \text{EF} \cdot \text{ED} \cdot 10^{-3}}{\text{BW} \cdot \text{AvT}} \quad (2)$$

where: C - Metal concentration in fish (mg/kg); IR - Ingestion rate of fish (0.017 kg/day); EF - Exposure frequency (365 days/year); ED - Exposure duration (70 years); BW - Average body weight of an adult (70 kg); AvT - Average exposure time for non-carcinogenic effects (365 days/year × ED)

Target hazard quotient (THQ) – Non-carcinogenic risk

THQ estimates the non-carcinogenic health risk associated with metal exposure through fish consumption. It was calculated using the formula:

$$\text{THQ} = \frac{\text{EDI}}{\text{RfD}} \quad (3)$$

where: EDI - Estimated Daily Intake; RfD - Reference dose for each metal (mg/kg/day), as defined by U.S. EPA (2014): Al:1000; As:0.3 Cd:1; Cr:3; Cu:40; Fe:700; Hg:0.1; Mn:140; Ni:20; Pb:3.5; Zn:300.

A THQ < 1.0 suggests no significant health risks, whereas a THQ \geq 1.0 indicates a potential for adverse health effects due to metal exposure.

Hazard Index (HI)

HI represents the cumulative risk from multiple metals and is calculated as the sum of individual THQ values:

$$TTHQ = \sum THQi \quad (4)$$

where THQi is the target hazard quotient for each metal. According to U.S. EPA (2024), an HI > 1.0 indicates potential health concerns due to combined metal exposure.

RESULTS AND DISCUSSIONS

Assessing heavy metal concentrations in marine fish is vital for preventing potential adverse health effects. To evaluate the safety of marine fish flesh, the measured concentrations of metals were compared with the maximum permissible levels established by Bulgarian and European legislation. The concentrations of toxic metals and trace elements detected in the 15 fish species examined are presented in Figure 1.

Lead (Pb): Pb is a highly toxic metal known to suppress immune responses, cause neurological disorders, and severely impact the nervous system. The Pb content in the analysed fish samples ranged from 0.003 mg/kg in turbot to 0.144 mg/kg in piked dogfish. Pb was undetectable in European sprat, round goby, whiting, Pontic shad, flathead grey mullet, red mullet, garfish, and Atlantic bonito (Figure 1). All measured Pb levels were significantly below the maximum permissible concentration (0.3 mg/kg) set by Commission Regulation (EU) 2023/915 for Pb in marine food.

Compared to previous studies on the Bulgarian Black Sea coast, Pb levels in fish appear to have declined over the years. Earlier research reported Pb concentrations between 0.05 mg/kg and 0.160 mg/kg in frequently consumed species such as turbot, Mediterranean horse

mackerel, flathead grey mullet, bluefish, round goby, and Pontic shad (Stancheva et al., 2013a; 2013b; Stancheva et al., 2014; Peycheva et al., 2019).

Higher Pb concentrations were also recorded in fish from the Turkish Black Sea coast, ranging from 0.04 mg/kg to 1.07 mg/kg (Turkmen et al., 2007; Turkmen et al., 2008; Mutlu, 2021; Bat et al., 2022; Mutlu, 2024; Sirin et al., 2024).

Cadmium (Cd): Cd is a highly toxic metal that can enter the human body through food consumption, leading to renal dysfunction, skeletal damage, and reproductive issues. According to Commission Regulation (EU) 2023/915, the maximum permissible concentration for Cd in fish ranges from 0.05 to 0.25 mg/kg, depending on species.

In this study, Cd concentrations ranged from 0.0078 mg/kg (Atlantic bonito) to 0.075 mg/kg (European sprat) (Figure 1). While the highest Cd level (0.075 mg/kg) was detected in European sprat, it remained below the regulatory threshold of 0.1 mg/kg. However, a slight exceedance was observed in Mediterranean horse mackerel (0.058 mg/kg) compared to the permissible limit of 0.05 mg/kg.

Cd concentrations in fish from the Bulgarian Black Sea coast have remained relatively stable over time. Previous studies reported Cd levels ranging from 0.005 mg/kg to 0.048 mg/kg in commonly consumed species such as turbot, Mediterranean horse mackerel, flathead grey mullet, bluefish, round goby, and Pontic shad (Stancheva et al., 2013a, 2013b; Stancheva et al., 2014; Makedonski et al., 2017; Peycheva et al., 2017; Peycheva et al., 2019).

Mercury (Hg): Hg is one of the most toxic heavy metals, causing neurological disorders, depression, irritability, tremors, and degenerative diseases affecting the brain, liver, and kidneys. Long-term Hg exposure is linked to impaired fetal development, reproductive disorders, and coma.

In this study, Hg levels varied across fish species, with the lowest concentration detected in garfish (6.00 μ g/kg) and the highest in piked dogfish (914.0 μ g/kg). Other species with notable Hg concentrations included round goby (39.45 μ g/kg), turbot (76.90 μ g/kg), red mullet (32.48 μ g/kg), Pontic shad (27.06 μ g/kg),

flathead grey mullet (26.23 $\mu\text{g}/\text{kg}$), and bluefish (143.9 $\mu\text{g}/\text{kg}$).

According to Commission Regulation (EU) 2023/915, the maximum permissible Hg concentration in fish ranges from 0.3 to 1.0 mg/kg , depending on the species. All analyzed fish samples in this study remained below these regulatory thresholds. Hg levels in the analyzed fish species were within the permissible limits, except for piked dogfish, where the Hg

concentration was close to the threshold value of 1 mg/kg . Previous research by Kirov et al. (2022) reported higher Hg levels in piked dogfish (1.39 mg/kg). Earlier studies also found Hg concentrations ranging from 0.05 mg/kg to 0.20 mg/kg (Stancheva et al., 2013a, 2013b; Stancheva et al., 2004; Makedonski et al., 2017; Peycheva et al., 2017; Manev et al., 2022).

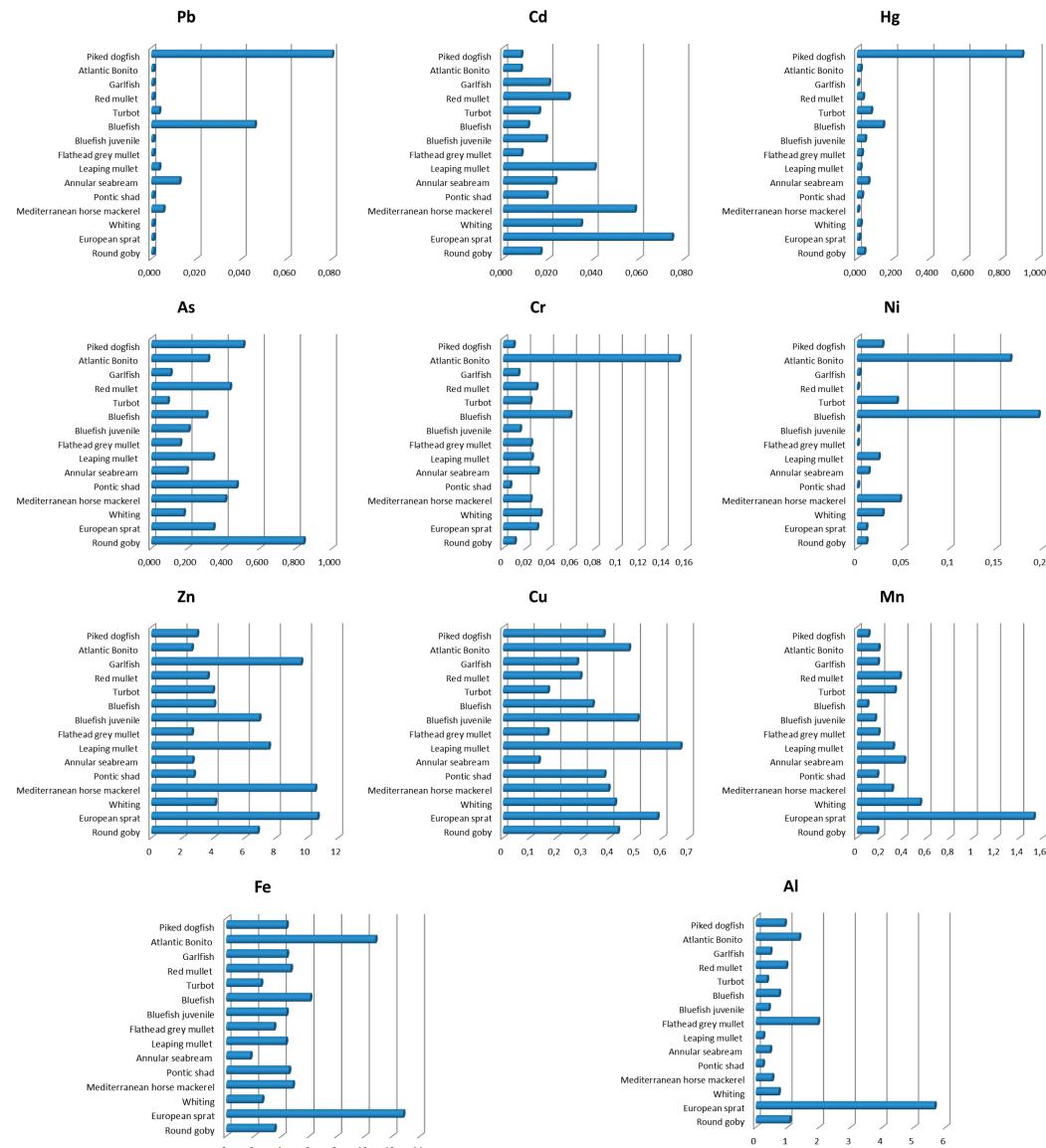


Figure 1. Toxic and essential element content (mg/kg) of the fish species sampled during the autumn season (September-November) from the Black Sea

Arsenic (As): As is a widespread metalloid present in rocks, soil, water, and air. The general population is primarily exposed to As through food and drinking water. The most toxic forms of arsenic are inorganic As(III) and As(V), but fish typically contain organic arsenic, which is non-toxic. Chronic exposure to arsenic can lead to cardiovascular, nervous system, respiratory, and renal disorders, as well as liver and prostate cancer.

In this study, As ranged from 0.09 mg/kg in turbot to 0.84 mg/kg in round goby (Figure 1). These values remain below the permissible limit of 5.00 mg/kg fresh weight (Bulgarian Ministry of Health, 2004). While fish As is mostly non-toxic, monitoring is recommended for round goby and European sprat, which showed relatively higher As levels.

Previous studies found As levels in fish samples ranging from 0.35 mg/kg to 1.42 mg/kg (Stancheva et al., 2013a, 2013b, 2014; Peycheva et al., 2017, 2019; Manev et al., 2022).

Chromium (Cr): Cr is an essential element involved in enzyme synthesis, fat metabolism, and carbohydrate metabolism. In the analyzed fish, Cr concentrations ranged from 0.01 mg/kg in round goby to 0.153 mg/kg in Atlantic bonito (Figure 1).

Previous research reported Cr levels ranging from 0.020 mg/kg to 0.07 mg/kg (Stancheva et al., 2014; Peycheva et al., 2017, 2019). Studies in other regions found: 0.10–1.60 mg/kg in Turkish seas (Tepe et al., 2008); 0.06–0.84 mg/kg in Black Sea coastal waters (Topcuoglu et al., 2002; up to 1.69 mg/kg in Iskenderun Bay, Mediterranean Sea (Turkmen et al., 2007); 0.23–0.31 mg/kg in the Eastern Black Sea (Mutlu, 2021)

Nickel (Ni): Ni is essential for growth and reproduction but can be carcinogenic at high levels. The Ni content in the analyzed fish ranged from 0.01 mg/kg in round goby and European sprat to 0.196 mg/kg in bluefish. In Pontic shad, flathead grey mullet, and red mullet, Ni was below the detection limits of the method used (Figure 1).

All Ni concentrations were below the maximum permissible limit of 0.5 mg/kg (Regulation 31, 2004). Previous studies reported Ni levels between 0.009 mg/kg in bluefish and flathead grey mullet to

0.07 mg/kg in Pontic shad (Stancheva et al., 2003a).

Metal concentrations in fish depend on habitat, diet, and feeding behavior. Topping (1973) suggested that plankton-feeding fish accumulate higher concentrations of heavy metals than demersal species.

European sprat, a zooplankton-eating fish, showed elevated levels of Cd, Zn, Cu, Fe, Mn, and As, indicating its potential as an ecological indicator for coastal waters.

However, Bat et al. (2022) reported that demersal species accumulate higher heavy metal concentrations than pelagic fish. In this study, Hg concentrations were higher in demersal species like turbot and round goby than in pelagic fish.

Turbot, a demersal species, accumulated As at higher levels but contained less Hg. Research suggests that turbot muscle tissue has a strong affinity for Hg, making it a bioindicator for Hg contamination in the marine ecosystem (Polak-Juszczak, 2012).

Predatory fish, such as bluefish and piked dogfish, had the highest Hg concentrations due to bioaccumulation and biomagnification in the food chain (Stergiou and Karpouzi, 2002).

Trace element content

Zinc (Zn): The maximum permissible Zn content in fish is 50 mg/kg (Bulgarian legislation, 2004) and 30 mg/kg (FAO, 1983). The Zn content in this study ranged from 2.59 mg/kg in flathead grey mullet to 10.67 mg/kg in European sprat (Figure 1), remaining well below the legal limits.

Zn levels in flathead grey mullet ranging from 5.2 mg/kg to 11.0 mg/kg in European sprat (Stancheva et al., 2003b); 3.9 mg/kg in bluefish to 12.7 mg/kg in European sprat (Peycheva et al., 2017); Zn levels between 5.2 mg/kg in flathead grey mullet to 10.0 mg/kg in bluefish and Atlantic bonito (Makedonski et al., 2017); 3.15–22.9 mg/kg in Turkish seas (Turkmen et al., 2005; Tepe et al., 2008); 23.71–31.34 mg/kg in the Middle Black Sea (Tuzen, 2009); 4.53–26.47 mg/kg in the Eastern Black Sea (Mutlu, 2021, 2024)

Copper (Cu): Cu is an essential element but is toxic at excessive levels. The Cu content in the analyzed fish ranged from 0.17 mg/kg in turbot and bluefish to 0.67 mg/kg in leaping mullet

(Figure 1), remaining below the permissible limit of 10 mg/kg (Bulgarian Ministry of Health, 2004).

Past research found Cu levels ranging from 0.218 mg/kg to 12.7 mg/kg in common Black Sea fish (Stancheva et al., 2004; Makedonski et al., 2017; Peycheva et al., 2017, 2019). Other studies reported: 1.01-4.54 mg/kg in Black Sea coastal waters (Turkmen et al., 2008); 0.65 mg/kg in *M. horse mackerel* (Tuzen, 2009); 0.33-1.49 mg/kg in anchovy and whiting (Mutlu, 2024).

Manganese (Mn): Mn is an essential but low-toxicity element. The Mn content in this study ranged from 0.088 mg/kg in bluefish to 1.52 mg/kg in European sprat (Figure 1). Previous research found Mn levels between 0.06 mg/kg and 0.256 mg/kg in commonly consumed Black Sea fish (Stancheva et al., 2004; Peycheva et al., 2019).

Iron (Fe): Fe is a constituent of haemoglobin, ensuring oxygen transport and cell retention. Iron is involved in the synthesis of hormones and neurotransmitters. According to Bulgarian and European legislation, there are no set maximum levels for Fe in fish and fish products. Still, the American Academy of Sciences sets the total amount of Fe in canned fish not to exceed 30 mg/kg (U.S. EPA, 2011). Levels ranged from 2.59 mg/kg in whiting to 12.8 mg/kg in European sprat (Figure 1), with past studies reporting Fe contents between 1.47 mg/kg and 9.0 mg/kg (Stancheva et al., 2004; Peycheva et al., 2019). Mutlu (2024) found an average Fe content of 15.08 mg/kg in red mullet, 21.09 mg/kg in anchovy, 11.96 mg/kg in whiting, and 11.25 mg/kg in *M. horse mackerel* in the Black Sea.

Aluminum (Al): The highest Al concentration was found in Black Sea round goby (5.65 mg/kg), while the lowest was in Pontic shad (0.22 mg/kg). Higher Al values (9.85-95.31 mg/kg) were previously reported in Black Sea fish (Turan et al., 2009).

Risk Assessment

Fish consumption is widely recommended due to its high nutritional value; however, it may pose health risks due to the accumulation of toxic substances in fish muscle. Therefore, assessing the levels of metals in fish and

comparing them to regulatory limits is essential for evaluating potential health risks.

The concentrations of toxic metals and essential elements in the muscles of various fish species were compared with permissible values established by Regulation No. 31 (Bulgarian Ministry of Health, 2004) and Commission Regulation 2023/915 (EU Commission Regulation, 2023). The findings indicate that all metal concentrations were within the permissible limits, except for a slight exceedance of Cd in *M. horse mackerel*.

To further assess contamination levels, the MPI was calculated based on the average concentrations of detected elements in fish muscle. An MPI value >1 indicates contamination, whereas an MPI value <1 suggests no significant contamination. In this study, MPI values were well below 1 for all fish species analyzed, confirming minimal contamination risk.

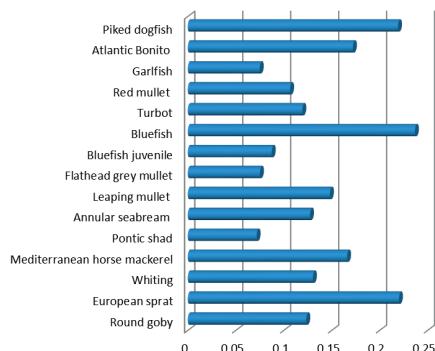


Figure 2. MPI values in muscle tissue of the fish species sampled during the autumn season (September–November) from the Black sea

The highest MPI values were observed in the following species: bluefish (0.237); European sprat (0.220); piked dogfish (0.219); Atlantic bonito (0.172); *M. horse mackerel* (0.166); leaping mullet (0.148); annular seabream (0.127); whiting (0.131); round goby (0.124); turbot (0.119); red mullet (0.107) (Figure 2).

As all MPI values remained below 1, the results suggest that consuming fish from the Bulgarian part of the Black Sea does not pose a significant risk of toxic metal exposure. However, continued monitoring is recommended to ensure long-term safety,

particularly for species with relatively higher metal accumulation.

The expected daily intake (EDI) and target hazard quotient (THQ) values were calculated based on the mean concentrations of metals in the studied fish species (Tables 1 and 2, Figure 3). The EDI values for both toxic and trace elements were below the limits recommended by the Food and Nutrition Board (2004). Comparisons were made with the tolerable daily intake (TDI) values established by EFSA (2010), which are as follows (in mg/g): As:2.14; Cd:0.8; Cr:300; Cu:500; Fe:800; Mn:140; Ni:12; Pb:1.5; and Zn:300 mg/g. The results indicate that the EDI values for these metals remain below the recommended TDI thresholds. These findings align with previous studies conducted in the eastern Black Sea (Bat et al., 2022), the Black Sea (Kalipci et al., 2022), and the eastern Black Sea (Mutlu et al., 2021).

Table 1. Estimated daily intake (EDI) from fish consumption by adults (mg/kg/day)

Specification	Pb	Cd	Cr	Ni	As	Hg
Round goby	0.0002	0.0040	0.0024	0.0024	0.2047	0.0096
European sprat	0.0002	0.0181	0.0072	0.0024	0.0834	0.0028
Whiting	0.0002	0.0083	0.0079	0.0067	0.0434	0.0048
M. horse mackerel	0.0013	0.0141	0.0058	0.0112	0.0992	0.0015
Pontic shad	0.0002	0.0047	0.0015	0.0002	0.1146	0.0066
Annular seabream	0.0030	0.0056	0.0073	0.0030	0.0476	0.0151
Leaping mullet	0.0008	0.0098	0.0061	0.0056	0.0828	0.0045
Gray mullet	0.0002	0.0020	0.0059	0.0002	0.0386	0.0064
Bluefish juvenile	0.0002	0.0046	0.0035	0.0002	0.0501	0.0106
Bluefish	0.0111	0.0027	0.0142	0.0476	0.0738	0.0350
Turbot	0.0008	0.0038	0.0058	0.0104	0.0219	0.0187
Red mullet	0.0002	0.0070	0.0070	0.0002	0.1055	0.0079
Garlfish	0.0002	0.0049	0.0032	0.0006	0.0257	0.0015
Atlantic Bonito	0.0002	0.0019	0.0372	0.0402	0.0762	0.0047
Piked dogfish	0.0350	0.0019	0.0022	0.0066	0.1235	0.2221
TDI	1.5	0.8	300	12	2.14	-

THQ values were calculated to assess the non-carcinogenic risk associated with long-term exposure to metals through fish consumption. According to U.S. EPA (2024), the acceptable THQ value should be equal to or less than 1.

The THQ values for tested metals in the studied fish species are presented in Figure 3.

The results show that the THQ values for all metals in the studied fish species were below 1, except for mercury (Hg). The THQ values for As, Cd, Pb, Ni, and Cr in all studied fish species remained below 1, suggesting no significant health risk from consuming these fish.

THQ_{Hg} values ranged from 0.02 to 2.22. In particular, the health risk is mainly due to exposure through the consumption of piked dogfish (2.22). Consumption of fish caught from the Black Sea other than piked dogfish is unlikely to have adverse health effects. These results indicate that metal intake poses a non-carcinogenic health risk to consumers.

Table 2. Estimated daily intake (EDI) from fish consumption by adults (mg/kg/day)

Specification	Zn	Cu	Fe	Mn	Al
Round goby	0.0002	0.0040	0.0024	0.0024	0.2047
European sprat	0.0002	0.0181	0.0072	0.0024	0.0834
Whiting	0.0002	0.0083	0.0079	0.0067	0.0434
M. horse mackerel	0.0013	0.0141	0.0058	0.0112	0.0992
Pontic shad	0.0002	0.0047	0.0015	0.0002	0.1146
Annular seabream	0.0030	0.0056	0.0073	0.0030	0.0476
Leaping mullet	0.0008	0.0098	0.0061	0.0056	0.0828
Gray mullet	0.0002	0.0020	0.0059	0.0002	0.0386
Bluefish juvenile	0.0002	0.0046	0.0035	0.0002	0.0501
Bluefish	0.0111	0.0027	0.0142	0.0476	0.0738
Turbot	0.0008	0.0038	0.0058	0.0104	0.0219
Red mullet	0.0002	0.0070	0.0070	0.0002	0.1055
Garlfish	0.0002	0.0049	0.0032	0.0006	0.0257
Atlantic Bonito	0.0002	0.0019	0.0372	0.0402	0.0762
Piked dogfish	0.0350	0.0019	0.0022	0.0066	0.1235
TDI	300	500	800	140	-

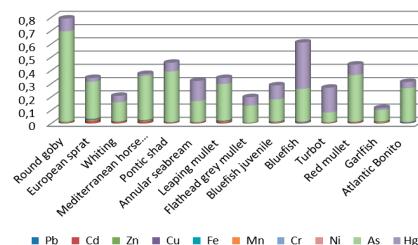


Figure 3. Target hazard quotients (THQ) of the fish species sampled during the autumn season (September–November) from the Black sea

The hazard index (HI), the cumulative value of THQ, was calculated for all metals studied. When the HI is less than 1, it indicates safety, meaning that fish consumption benefits health and that consumers are in a safe zone. On the other hand, when HI exceeds 1, it indicates potential hazards and risks to public health, which means that consuming fish may have adverse effects.

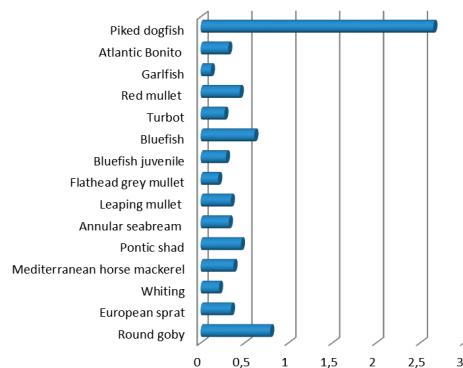


Figure 4. The hazard index (HI) of the fish species sampled during the autumn season (September–November) from the Black Sea

The results for HI do not exceed the value of one, which indicates that the consumption of these fish from the Bulgarian part of the Black Sea does not lead to excessive exposure to the action of these toxic metals (Figure 4).

Among the studied fish species, piked dogfish has the highest HI value (2.652). High values are obtained in round goby (0.792), bluefish (0.612), Pontic shad (0.459) and red mullet (0.445). These HI values are due to high THQHg and THQAs.

CONCLUSIONS

Based on the results of the levels of different metals (Al, As, Cd, Cu, Cr, Fe, Mn, Ni, Pb, Hg, and Zn) in the muscles of 15 fish (round goby, European sprat, whiting, M. horse mackerel, Pontic shad, annular seabream, leaping mullet, flathead grey mullet, bluefish juvenile, bluefish, turbot, red mullet, garlfish, Atlantic bonito, piked dogfish) caught in the Black Sea, it was found that the content of toxic elements in fish is lower compared to previous studies in the same region.

The highest concentration was observed for Pb in piked dogfish, Cd in European sprat, Zn in European sprat and M. horse mackerel, Cu in leaping mullet, Fe in European sprat and Atlantic bonito, Mn and Al in European sprat, Cr in Atlantic bonito, Ni in Atlantic bonito and bluefish, As in round goby, Hg in bluefish and piked dogfish.

However, the levels are generally below the maximum allowable concentration (MAC) according to Commission regulation 2023/915 of April 23, 2023 and the Bulgarian Ministry of Health, except for Cd in M. horse mackerel.

The risk assessment based on the THQ shows that the levels of metals in the studied fish species are below the recommended limits (< 1), except for piked dogfish. The THQ values indicate that long-term exposure to metals through consumption of the fish species examined in the study is unlikely to cause severe damage to the health of an adult.

The hazard index HI was < 1 for the fish studied (except for piked dogfish), indicating no potential health risk to human health. However, continuous monitoring of toxic metal content in fish is essential to maintain food safety and reduce potential health risks associated with long-term exposure.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Center for Scientific Research, Technology Transfer and Intellectual Property Protection at the Agricultural University - Plovdiv and was financed from Project 04-22.

REFERENCES

- Barone, G., Storelli, A., Garofalo, R., Busco, V. P., Quaglia, N. C., Centrone, G., & Storelli, M. M. (2015). Assessment of mercury and cadmium via seafood consumption in Italy: estimated dietary intake (EWI) and target hazard quotient (THQ). *Food Additives & Contaminants: Part A*, 32(8), 1277-1286. DOI: 10.1080/19440049.2015.1055594.
- Bat, L., Sahin, F., Oztekin, A., & Arici, E. (2022). Toxic metals in seven commercial fish from the southern Black Sea: toxic risk assessment of eleven-year data between 2009 and 2019. *Biological Trace Element Research*, 200, 832-843.
- Bulgarian Ministry of Health (2004). Ordinance No. 31 of July 29, 2004 on the maximum permissible amounts of pollutants in foods. (Promulgated: State Gazette No. 88 of October 8, 2004).

Copat, C., Arena, G., Fiore, M., Ledda, C., Fallico, R., & Sciacca, S. (2013). Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: consumption advisories. *Food and Chemical Toxicology*, 53, 33-37.

EFSA (2010). European Food Safety Authority, Scientific opinion on lead in food, *EFSA Journal*, 8 (4), 1570. <https://doi.org/10.2903/j.efsa.2010.1570>.

EU Commission Regulation (2006). *EU Commission Regulation Commission Regulation (EC) no. 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs*. Retrieved September 15, 2022, from <https://www.legislation.gov.uk/eur/2006/1881>.

EU Commission Regulation (2023). EU Commission Regulation 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. *Official Journal of the European Union*, L 119, 103-157.

FAO/WHO Expert Committee on Food Additives (1983). *Evaluation of certain food additives and contaminants: twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives*. World Health Organization. Retrieved September 22, 2024, from <https://apps.who.int/iris/handle/10665/39165>.

Food and Nutritional Board (2004). *Dietary reference intakes [DRIs]*. In Recommended Intake for Individuals. Washington, DC, USA.

Gu, G., Lin, Q., Huang, H. H., Wang, L. G., Ning, J. J., & Du, F. Y. (2017). Heavy metals in fish tissues/stomach contents in four marine wild commercially valuable fish species from the western continental shelf of South China Sea. *Marine Pollution Bulletin*, 114(2), 1125-1129.

Kalipci, E., Cuce, H., Ustaoglu, F., Dereli, M. A., & Turkmen, M. (2023). Toxicological health risk analysis of hazardous trace elements accumulation in the edible fish species of the Black Sea in Turkiye using multivariate statistical and spatial assessment. *Environmental Toxicology and Pharmacology*, 97, 104028. <https://doi.org/10.1016/j.etap.2022.104028>.

Kirov, V., Neshovska, H., & Manev, I. (2022). Heavy metal levels in meat of spiny dogfish (*Squalus Acanthias*) from Bulgarian Black Sea. *Tradition and Modernity in Veterinary Medicine*, 7(1(12)), 77-79.

Makedonski, L., Peycheva, K., & Stancheva, M. (2017). Determination of heavy metals in selected black sea fish species. *Food Control*, 72, 313-318.

Manev, I., Kirov, V., & Neshovska, H. (2022). Heavy metal concentrations in turbot (*Scophthalmus maximus*) from Bulgarian Black Sea coast. *Tradition and Modernity in Veterinary Medicine*, 7, 80-85.

Muthu, T. (2021). Heavy metal concentrations in the edible tissues of some commercial fish caught along the Eastern Black Sea coast of Turkey and the health risk assessment. *Spectroscopy Letters*, 54(6), 437-445.

Muthu, T. (2024). Distribution of toxic and trace metals in fish from the Black Sea: Implications for human health risks. *Emerging Contaminants*, 10(2), 100295. <https://doi.org/10.1016/j.emcon.2023.100295>.

Peycheva, K., Stancheva, M., Georgieva, S., & Makedonski, L. (2016). Heavy metals in water, sediments and marine fish from Bulgarian Black Sea. *Materials of XXVI International Coastal Conference "Managing risks to coastal regions and communities in a changing world"*, 1-1. DOI: 10.31519/conferencearticle_5b1b93d4d78bb6.88545 986. Retrieved October 10, 2024, from https://academuspub.com/en/nauka/conference_article/1990/view

Peycheva, K., Panayotova, V., Merdhanova, A., & Stancheva, R. (2019). Estimation of THQ and potential health risk for metals by consumption of some black sea marine fish and mussels in Bulgaria. *Bulgarian Chemical Communications*, 51(Special Issue D), 241-246.

Popescu, A., Vlase, G., Nicolae, C. G., Paun, C., & Nenciu, M. I. (2019). Fish meat contamination with heavy metals - a real concern for the food consumption. *Scientific Papers. Series D. Animal Science*, LXII(2), 254-258.

Polak-Juszczak, L. (2012). Bioaccumulation of mercury in the trophic chain of flatfish from the Baltic Sea. *Chemosphere*, 89(5), 585-591.

Sirin, M., Bayrak, E. Y., & Baltas, H. (2024). Human health risk assessment of heavy metals accumulation in different genders and tissues of whiting fish (*Merlangius merlangus euxinus* Nordmann, 1840) from Rize, Turkey. *Journal of Food Composition and Analysis*, 127, 105971.

Stancheva, M., Merdhanova, A., Petrova, E., & Petrova, D. (2013a). Heavy metals and proximate composition of Black Sea sprat (*European sprattus*) and goby (*Neogobius melanostomus*). *Bulgarian Journal of Agricultural Science*, 19(S1), 35-41.

Stancheva, M., Makedonski, L., & Petrova, E. (2013b). Determination of heavy metals (Pb, Cd, As and Hg) in Black Sea grey mullet (*Mugil cephalus*). *Bulgarian Journal of Agricultural Science*, 19(S1), 30-34.

Stancheva, M., Makedonski, L., & Peycheva, K. (2014). Determination of heavy metal concentrations of most consumed fish species from Bulgarian Black Sea coast. *Bulgarian Chemical Communications*, 46(1), 195 - 203.

Stergiou, K. I., & Karpouzi, V. S. (2002). Feeding habits and trophic levels of Mediterranean fish. *Reviews in Fish Biology and Fisheries*, 11(3), 217-254.

Tepe, Y., Turkmen, M., & Turkmen, A. (2008). Assessment of heavy metals in two commercial fish species of four Turkish seas. *Environmental Monitoring and Assessment*, 146, 277-284.

Topcuoglu, S., Kirbas, C., & Gungor, N. (2002). Heavy metals in organisms and sediments from Turkish coast of the Black Sea, 1997-1998. *Environment International*, 27, 521-526.

Topping, G. (1973). Heavy metals in fish from Scottish waters. *Aquaculture*, 1, 373-377.

Traina, A., Bono, G., Bonsignore, M., Falco, F., Giuga, M., Quinci, E. M., Vitale, S., & Sprovieri, M. (2019). Heavy metals concentrations in some commercially key species from Sicilian coasts (Mediterranean Sea): potential human health risk estimation, *Ecotoxicology and Environmental Safety*, 168, 466-478.

Turan, C., Dural, M., Oksuz, A., & Oztürk, B. (2009). Levels of heavy metals in some commercial fish species captured from the Black sea and Mediterranean coast of Turkey. *Bulletin of Environmental Contamination and Toxicology*, 82, 601-604.

Ture, M., Kilic, M. B., & Altinok, I. (2021). Relationship between heavy metal accumulation in fish muscle and heavy metal resistance genes in bacteria isolated from fish. *Biological Trace Element Research*, 199(4), 1595-1603.

Turkmen, M., Turkmen, A., & Tepe, Y. (2007). Metal contaminations in five fish species from Black, Marmara, Aegean and Mediterranean seas, Turkey. *Journal of the Chilean Chemical Society*, 52, 1314-1318.

Turkmen, M., Turkmen, A., Tepe, Y., Ates, A., & Gokkus, K. (2008). Determination of metal contaminations in sea foods from Marmara, Aegean and Mediterranean seas: twelve fish species. *Food Chemistry*, 108(2), 794-800.

Turkmen, A., Tepe, Y., Turkmen, M., & Mutlu, E. (2009). Heavy metal contaminants in tissues of the Garfish, *Belone belone* L., 1761, and the Bluefish, *Pomatomus saltatrix* L., 1766, from Turkish waters. *Bulletin of Environmental Contamination and Toxicology*, 82, 70-74.

Tuzen, M. (2009). Toxic and essential trace elemental contents in fish species from the Black Sea, Turkey. *Food and Chemical Toxicology*, 47, 1785-1790.

U.S. EPA (United States Environmental Protection Agency) (2011). *Exposure Factors Handbook 2011 Edition (Final report) (EPA/600/R-09/052F)*. Washington, DC, US: U.S. Environmental Protection Agency Publishing House. Retrieved October 20, 2024 from <https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252>.

U.S. EPA (United States Environmental Protection Agency) (2014). Risk-Based Concentrations Table. Retrieved October 3, 2024 from <http://www.epa.gov/reg3hwmd/risk/human/index.htm>.

U.S. EPA (United States Environmental Protection Agency) (2024, November 25). Regional Screening Levels (RSLs) - Generic Tables. Retrieved from <https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables>.