

RISK ASSESSMENT OF ESSENTIAL AND TOXIC ELEMENTS IN SOME FISH SPECIES FROM THE BLACK SEA, BULGARIA

Violina R. Angelova*, Velika N. Kuneva

Agricultural University-Plovdiv, 12 Mendeleev str., Plovdiv, 4000, Bulgaria

Abstract

This study aimed to determine the concentrations of toxic and essential elements in the muscle tissue of 11 common fish species (Black Sea Goby, Sprat, Whiting, M. horse mackerel, Shad, Grey mullet, Bluefish, Turbot, Red mullet, Garlfish, Atlantic Bonito) from the southern Black Sea coast of Bulgaria were determined by Inductively-coupled plasma optic emission spectrometry (ICP-OES). The concentration ranges of toxic metals and essential elements in all samples, based on the overall average, were as follows (mg/kg wet weight): Pb: 0.001- 0.045 mg/kg, Cd: 0.008 – 0.07 mg/kg, Zn: 2.58-10.67 mg/kg, Cu: 0.17-0.58 mg/kg, Fe: 2.5-12.8 mg/kg, Mn: 0.088- 1.52 mg/kg, Cr: 0.006-0.15 mg/kg, Ni: 0.001-0.195 mg/kg, As:0.09-0.84 mg/kg, Hg:0.006-0.14 mg/kg. The metal concentrations in the muscle were below the maximum permissible levels set by international organizations for seafood, except Cd for Sprat and M. horse mackerel. The human risk was assessed by calculating the target hazard quotients (THQ) and hazard index (HI). The target hazard quotient for individual elements and hazard index was lower than 1, indicating no potential risk to humans from fish consumption.

Keywords: Black Sea, fish, hazard index, target hazard quotient, health risk, metals

1. INTRODUCTION

The Black Sea is an inland sea between southeastern Europe and Asia Minor, with an estimated area of 423 000 km² and a volume of 547 000 km³. Its average depth is about 1 240 m, reaching 2 212 m at its deepest. The total length of its coastline is about 4 340 km. B. Danube, Dnieper, Dniester, as well as 14 Bulgarian rivers.

Pollution of coastal waters can occur as a result of pollution from natural sources (such as rock weathering, volcanic eruptions, and soil erosion) and anthropogenic sources (industrial activities, industrial waste discharge, agricultural runoff, and sewage discharge (Domingo et al., 2007). The increasing concentration of nutrients in recent years has led to a higher degree of eutrophication of the Black Sea. Once in the aquatic environment, metals are redistributed in the water column, deposited or accumulated in sediments, and consumed by biota. Contaminants can accumulate in fish and eventually reach humans through the food chain (Turan et al., 2009; Ture et al., 2021)

Metals accumulate in fish by respiration, adsorption from water through the gills or skin, and ingestion (Zhou et al., 2001; Kwaansa-Ansah et al., 2019). The amount and distribution of metals vary considerably among tissues depending on the route of exposure, trophic level, type and properties of metals, and their concentrations in water and food. Once in fish, they are stored in tissues such as muscle, liver, and gills and, when consumed, enter humans through the food chain (Durmus, 2019). Fish are good indicators for long-term monitoring of metal accumulation in the marine environment (Turkmen et al., 2007). Liver and gills are considered good indicators of metal contamination in aquatic ecosystems (Anandkumar et al., 2019). While metal concentrations in gills and skin reflect levels in the surrounding water, the liver is vital for detoxifying and storing metals in fish (Yilmaz, 2003; Aydın & Tokalioglu, 2015). Although muscles generally have less metal load, they are the main object of investigation to assess the human health risks associated with fish consumption (Sirin et al. 2024). Consumption of metal-contaminated fish can lead to a range of adverse health effects, including neurological damage, teratogenic effects, impaired reproductive capacity, hypertension, kidney damage, and cancer (Djedjibegovic et al., 2020). Monitoring and determining the concentration of heavy metals in marine fish, which serve as an essential food source in the food chain, is crucial to prevent potential adverse effects on human health, as contamination is a continuous process.

Numerous studies in the literature examine heavy metal levels in muscle and other tissues of fish caught off the coast of the Black Sea and other marine environments (Andres et al., 2000; Tuzen, 2003; Turkmen et al., 2005; Turkmen and Ciminli, 2007; Turkmen et al., 2007; Tepe et al., 2008; Turan et al., 2009; Stancheva et al., 2013a, b); Stancheva et al., 2014; Bat et al., 2015; Makedonski et al., 2017; Peycheva et al., 2017; Peycheva et al., 2019; Baki and Baki, 2022).

The objective of this study was to determine the levels of metals (Pb, Cd, Hg, As, Cr, Ni, Zn, Cu, Fe, and Mn) in the muscles of black sea goby, Sprat, Whiting, M. horse mackerel, shad, grey mullet, bluefish, turbot, red mullet, garfish, and Atlantic bonito from the southern Black Sea coast of Bulgaria and to assess the public health risk associated with fish consumption,

2. MATERIALS AND METHODS

2.1. Materials

The fish used in this study are black sea goby, sprat, Whiting, M. horse mackerel, shad, grey mullet, bluefish, turbot, red mullet, garfish, and Atlantic bonito and the samples were collected from September to November in 2022 and 2023 from commercial catches at two locations in the Black Sea along the southern Bulgarian coast, Nessebar and Burgas. The sampled specimens were transported to the Agricultural University-Plovdiv laboratory in an ice cooler bag. The fish were analyzed immediately or stored without compromising their integrity in a freezer at -20°C until analysis. Fish were washed with distilled water and dried with filter paper in the laboratory. Fish were dissected with a stainless-steel knife. For small species (Sprat, tadpole, M. horse mackerel, Whiting, shad), the entire edible portion of each individual was included to prepare the mean sample. The giant fish were filleted after removing the head, tail, and guts. The filleted tissue was minced and homogenized.

2.2. Methods

2.2.1. Determination of the content of heavy metals in the fish

The microwave mineralization method (MARS 6 microwave system, CEM Corporation, USA) was used to prepare the fish for toxic metals and essential element analysis. A 1 g sample was weighed to the nearest 0.001 g in a Teflon container, and 10 ml of HNO₃ (65 %) was added; the containers were closed and placed in the microwave system (MARS 6, CEM Corporation, USA). The mineralization program includes three steps: (1) 20 minutes at 200°C, increasing the pressure to 800 PSI, (2) 15 minutes holding at 200°C with 800 PSI pressure, and (3) 30 minutes for cooling. After cooling, the samples are quantitatively transferred to a 25 cm³ volumetric flask. A blank is included in each series of mineralized copper samples to assess the purity of the reagents and glassware used. Elements were determined using an ICP-OES spectrometer (Prodigy model 7, Teledyne Leeman Labs, USA). The mercury content was determined using a mercury analyzer MA 3000.

Analyses were performed in triplicate, and all data were expressed as mean values. Data processing was performed using the statistical program SPSS 26.

2.2.2. Assessment of metal levels in fish species (MPI metal pollution index)

The Metal Pollution Index (MPI) is a mathematical model representing all metals' combined value. It is a reliable and accurate index for monitoring heavy metal contamination in food and aquatic ecosystems. In this study, the MPI values were calculated using Eq. by taking the average of the values of metals in the muscle of the fish:

$$\text{MPI (mg/kg)} = (\text{Cf}_1 \times \text{Cf}_2 \times \dots \times \text{Cf}_n)^{1/n}$$

Cf: Average metal concentrations in the fish muscle (mg/kg).

2.2.3. Evaluation of the safety of Black Sea fish as food (Target hazard quotient (THQ) and Hazard Index (HI))

Each chemical element exhibits different toxicity depending on its concentration (dose). The U.S. Environmental Protection Agency (U.S. EPA, 2011) offers various approaches to assess toxicity, with

indices being the most commonly used. The noncarcinogenic risk quotient (THQ) compares the ingested amount of a contaminant with a standard reference dose. When the THQ is less than unity, it means that the level of exposure is lower than the reference dose, and the daily exposure by consuming this product does not hurt human health over a person's lifetime in a given human population. The formula by which the THQ coefficient is calculated is as follows:

$$THQ = \frac{MC \cdot IR \cdot EF \cdot ED \cdot 10^{-3}}{RfD \cdot BWa \cdot ATn}$$

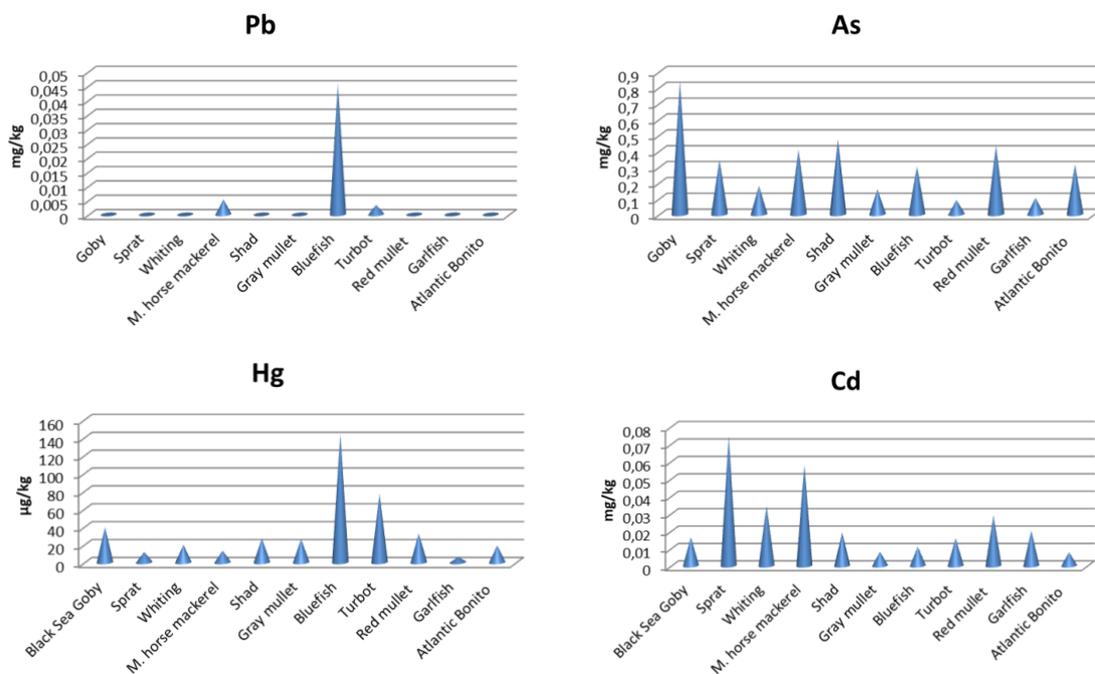
where THQ - noncarcinogenic risk quotient; MC - concentration of the analyzed chemical element in fish sample (mg/kg) IR - daily fish intake per year (13.7 g/day); EF - exposure frequency (350 day/year); ED - total exposure duration in an adult individual (30 years); RfD - reference dose (As - 3×10^{-4} ; Cu - 0.04; Cr (total) - 3×10^{-3} ; Hg - 3×10^{-4} ; Fe 9×10^{-3} ; Ni - 0.02; Zn - 0.3; Cd - 13×10^{-3} ; Pb - 4×10^{-3} Mn - 0.14 $\mu\text{g/g day}$) (U.S. EPA, 2011), BWa - the average weight of an adults (females - 65 kg and males - 79 kg), ATn - average exposure time for non-carcinogens ($365 \times ED = 10950 \text{ day/year}$).

The Hazard Index or HI can be represented as the sum of the different Target hazard quotient (THQ) (U.S. EPA, 2011) and is calculated using the formula:

$$HI = THQPb + THQcd + THQHg + THQAs + THQzn + THQcu + THQfe + THQMn + THQni + THQcr$$

3. RESULTS

The results obtained for the toxic metals and essential elements content of the 11 fish studied (Black Sea goby, Sprat, Whiting, M. horse mackerel, shad, grey mullet, bluefish, turbot, red mullet, garfish, Atlantic Bonito) are presented in Fig. 1.



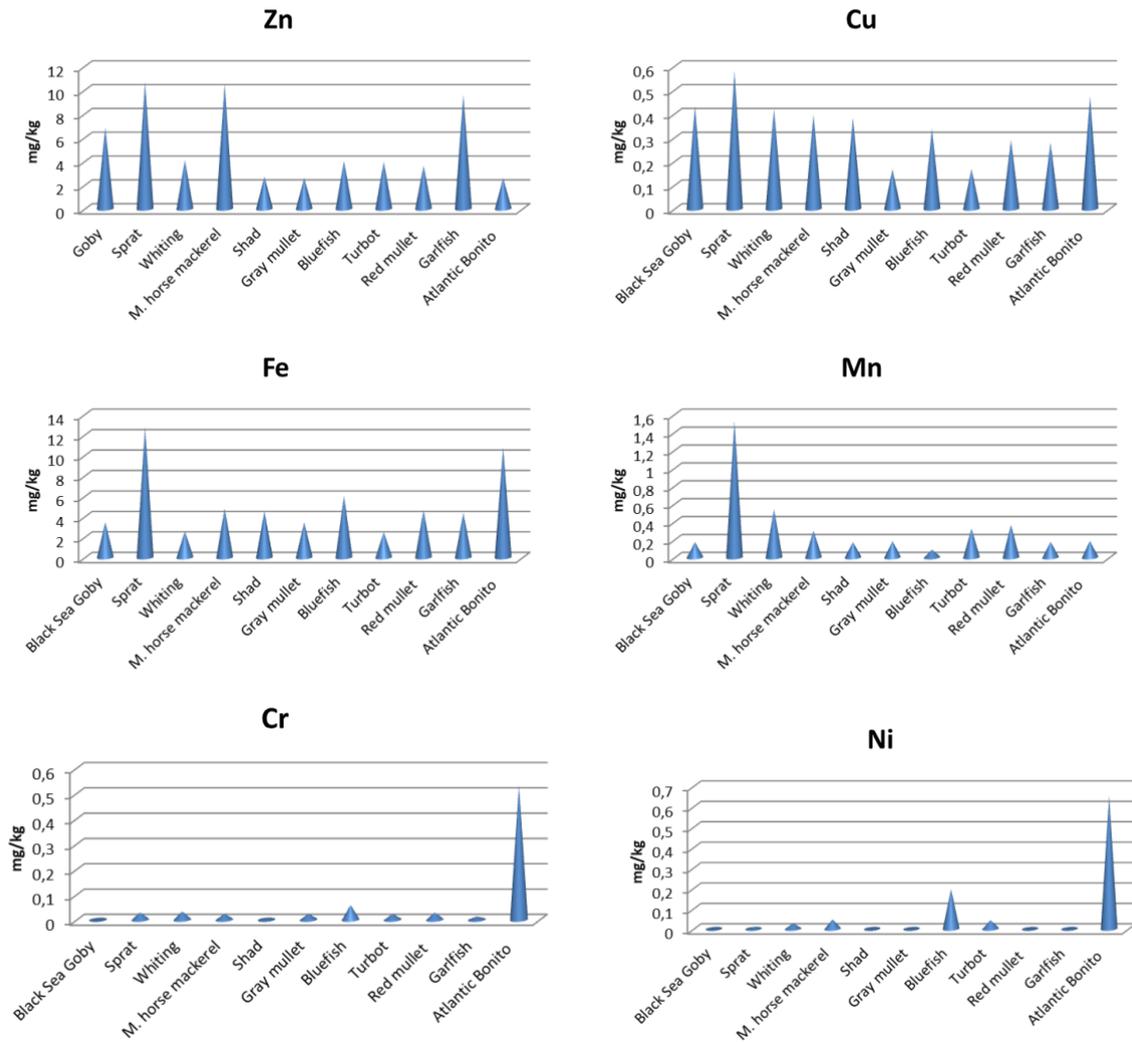


Fig. 1. Toxic and essential element content of the fish species sampled during the autumn season (September-November) from from the southern Black Sea coast of Bulgaria

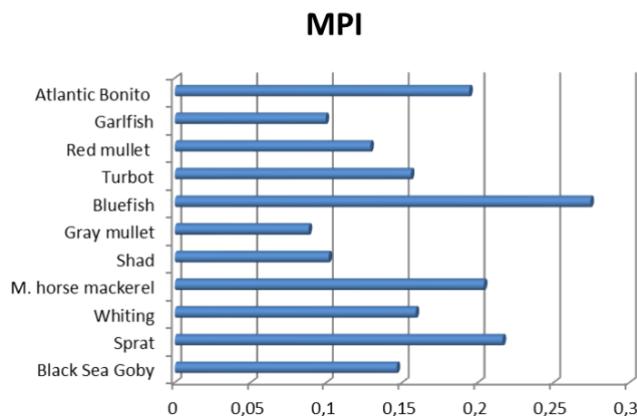


Fig. 2. MPI values in muscle tissue of the fish species sampled during the autumn season (September-November) from the southern Black Sea coast of Bulgaria

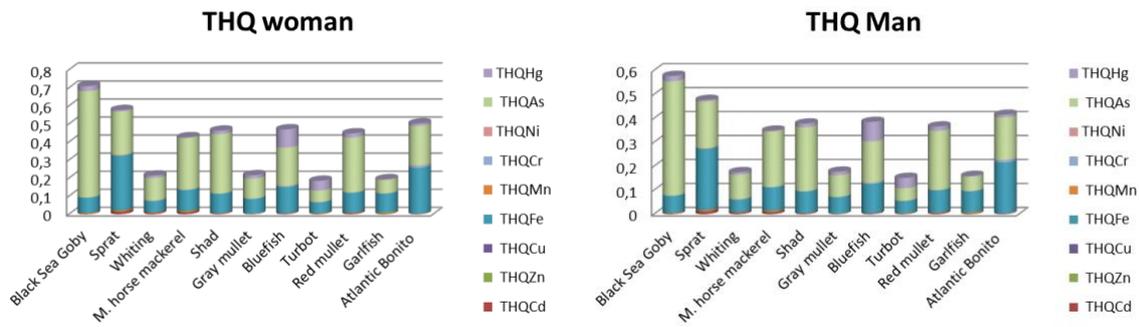


Fig. 3. Target hazard quotients (THQ) of the fish species sampled during the autumn season (September-November) from the southern Black Sea coast of Bulgaria

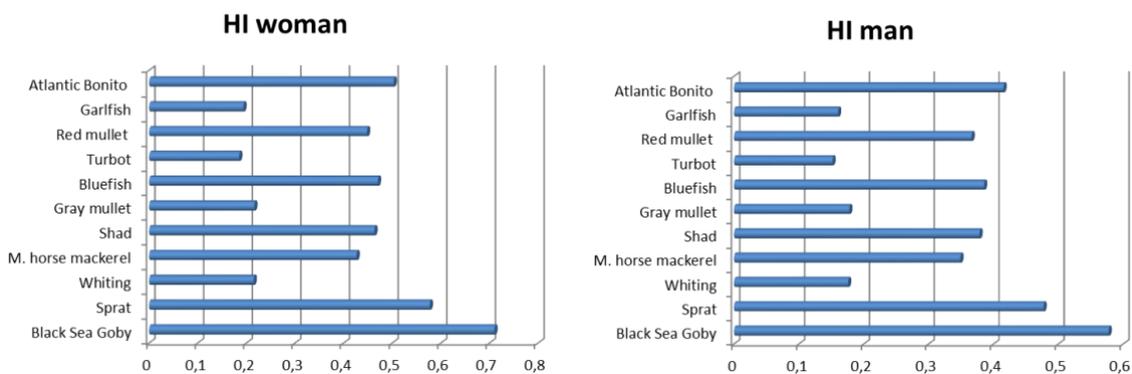


Fig. 4. Hazard index (HI) of the fish species sampled during the autumn season (September-November) from the southern Black Sea coast of Bulgaria

Calculated THQ values for toxic metals and essential elements for males and females are presented in Figure 3. The THQ values for the 11 marine fish species studied are below the U.S.EPA permissible unit standard (U.S.EPA, 2011) for all Black Sea fish.

The calculated values for HI (sum of THQ coefficients) for males and females are presented in Fig. 4.

4. DISCUSSION

4.1. Heavy metal content

Lead is a highly toxic metal that lowers the immune response, leads to brain disorders, and strongly affects the nervous system. According to Regulation No 31 from July 29, 2004 (Bulgarian Ministry of Health, 2004), on maximum levels of contaminants in foodstuffs, the standard for Pb in fish is 0.2 mg/kg fresh product, and according to Commission regulation No 1881/2006 of December 19, 2006 (EU Commission Regulation, 2006), a maximum level of Pb in fish of 0.3 mg/kg.

The Pb content of the samples analyzed ranged from turbot (0.003 mg/kg) and M. horse mackerel (0.005 mg/kg) to bluefish (0.046 mg/kg), while no Pb was detected in Sprat, Goby, Whiting, shad, grey mullet, red mullet, garfish, and Atlantic bonito (Fig.1). The values recorded in all the fish examined were significantly lower than the maximum permissible concentration for Pb in marine food.

A comparison of obtained results with previous studies on the Bulgarian Black Sea coast shows that the content of the toxic metal Pb decreases over the years in fish. It was found the content of Pb reached 0.023 mg/kg in goby, 0.08 mg/kg in sprat, 0.05 - 0.07 mg/kg in grey mullet (Stancheva et al.,

2013a, b), varies from 0.03 mg/kg in bluefish to 0.08 mg/kg in sprat (Stancheva et al., 2014; Makedonski et al., 2017) and to 0.160 mg/kg in garfish (Peycheva et al., 2019).

Higher values for Pb were also found for Pb in different fish species from the Turkish Black Sea coast (0.28 mg/kg for turbot, 0.04 - 0.099 for red mullet, 0.13-1.02 mg/kg for Whiting, 0.11-1.07 mg/kg for M. horse mackerel, 0.72 mg/kg for grey mullet, 0.19 to 0.81 mg/kg for garfish, 0.28 mg/kg for sprat and 0.09 to 0.87 mg/kg for bluefish (Bat et al., 2012; Tuzen, 2003; Uluozlu et al., 2007; Turkmen et al., 2007; Turkmen et al., 2008; Mutlu, 2021; Mutlu, 2024; Sirin et al., 2024)

Cadmium is a toxic metal that enters humans when contaminated foods are consumed, causing Cd poisoning (Arulkumar et al., 2017) and can cause renal dysfunction, skeletal damage, and reproductive deficiency. According to Commission regulation No 1881/2006 of December 19, 2006 (EU Commission Regulation, 2006), the maximum permissible concentration for Cd in fish is 0.05 - 0.1 mg/kg fresh weight, depending on the type of fish. The amount of Cd in the analyzed samples ranged from 0.0078 mg/kg w.w in Atlantic bonito to 0.058 mg/kg in M. horse mackerel, and 0.075 mg/kg in Sprat (Fig. 1). Only M. horse mackerel and sprat showed higher Cd concentration higher than the maximum permissible value of 0.05 mg/kg. Bat et al. (2012) found high levels of Cd (0.05 mg/kg) for Sprat from the Sinop area (Black Sea coast of Turkey), similar to results in this study.

A comparison of obtained results with previous studies on the Bulgarian Black Sea coast shows that the content of the toxic metal Cd does not change significantly over the years in fish. It was found the content of Cd reached 0.005 mg/kg in goby, 0.006 mg/kg in sprat, and 0.012 - 0.024 mg/kg in grey mullet (Stancheva et al., 2013a,b), varying from 0.005 mg/kg in sprat to 0.012 mg/kg in grey mullet (Stancheva et al., 2014), differs from 0.08 mg/kg in bluefish and M. horse mackerel to 0.015 mg/kg in Atlantic bonito (Makedonski et al., 2017), varies from 0.004 mg/kg in goby and M. horse mackerel to 0.005 mg/kg in sprat and turbot (Peycheva et al., 2017) and to 0.048 mg/kg in garfish (Peycheva et al., 2019).

Cd content in fish from the Turkish Black Sea coast varies from 0.004-0.21 mg/kg in Whiting, 0.011 - 0.32 mg/kg in M. horse mackerel, 0.012 mg/kg in grey mullet, and 0.01-0.17 mg/kg in red mullet, 0.01 to 0.07 mg/kg in garfish and 0.01 to 0.38 mg/kg in bluefish (Tuzen, 2003; Turkmen et al., 2008; Turkmen et al., 2009a,b; Mutlu, 2024; Almafrachi et al., 2024).

Hg is one of the most toxic elements and leads to depression, irritability, and tremors. As it accumulates in the body, degenerative diseases of the brain, liver, and kidneys progressively develop, and it can lead to impaired fetal development, reproductive problems, and coma. The lowest Hg content was found in garfish samples (6.00 µg/kg) and the highest in bluefish (143.9 µg/kg). Hg content was also found in Goby (39.45 µg/kg), turbot (76.90 µg/kg), red mullet (32.48 µg/kg), shad (27.06 µg/kg), and grey mullet (26.23 µg/kg). In all samples analyzed, the Hg content was lower than the maximum level of Hg for fish according to Commission regulation No 1881/2006 of December 19, 2006 (EU Commission Regulation, 2006) - 1.0 mg/kg.

The results of Stancheva et al. (2013a,b) showed higher Hg levels in sprat (0.12 mg/kg) compared to goby (0.05 mg/kg) and grey mullet (0.05-0.08 mg/kg). Hg contents ranged from 0.05 mg/kg in grey mullet to 0.16 mg/kg in M. horse mackerel (Stancheva et al., 2004), ranged from 0.05 mg/kg in grey mullet to 0.13 mg/kg in Atlantic bonito (Makedonski et al., 2017), varies from 0.05 mg/kg in goby to 0.1 mg/kg in sprat (Peycheva et al., 2017), to 0.096 mg/kg in garfish (Peycheva et al., 2019) , and to 0.15-0.20 mg/kg in turbot (Manev et al., 2022).

Bat et al. (2015) and Nisbet et al. (2009) reported that Hg was not detected in the muscle tissue of fish from the Turkish Black Sea coast. However, according to Tuzen (2009), Hg content in some Black Sea fish species ranged from 0.025 to 0.084 mg/kg fresh weight. Hg values of 0.11 mg/kg for red mullet, 0.078 mg/kg for M. horse mackerel, 0.055 mg/kg for anchovy, and 0.084 mg/kg for Whiting have been found (Tuzen, 2009; Alkan et al., 2016). According to Mutlu (2024), the mean value of Hg in Black Sea fish was 0.03 mg/kg, the maximum concentration of Hg was found in Whiting (0.34 mg/kg), and the minimum concentration was found in M. horse mackerel (0.05 mg/kg).

As is a widespread metalloid found in rocks, soil, water, and air. The general population's exposure to As is mainly through food and drinking water intake. The most toxic forms of As are the inorganic compounds of As(III) and (V), but the risk of consuming significant amounts of inorganic As from fish is generally low because fish usually contain As in an organic form that is not toxic. Chronic arsenic exposure can cause disorders in the cardiovascular, nervous, respiratory, and renal systems, liver and prostate cancer, and hyperpigmentation. The As content of the fish analyzed ranged from 0.09 mg/kg in turbot to 0.84 mg/kg for goby (Fig. 1). The As concentrations in the fish species studied did not exceed the specified standard of 5.00 mg/kg fresh weight (Bulgarian Ministry of Health, 2004). Although fish As is found mainly in organic form, which is not toxic, it is necessary to monitor the levels of this element in species such as goby and spat.

As contents reached 0.73 mg/kg in sprat, 0.66 mg/kg in goby, 0.90-1.10 in grey mullet (Stancheva et al., 2013a,b), ranged from 0.35 mg/kg in shad to 1.1 mg/kg in grey mullet (Stancheva et al., 2014), ranged from 1.42 mg/kg in mackerel to 3.99 mg/kg in turbot (Peycheva et al., 2017), to 0.128 mg/kg in garfish (Peicheva et al., 2019), to 0.86-1.49 mg/kg in turbot (Manev et al., 2022), ranged from 0.73 mg/kg in M. horse mackerel to 1.1 mg/kg in grey mullet (Makedonski et al., 2022).

Alkan et al. (2016) reported an As level of 2.58 mg/kg in M. horse mackerel and 1.85 mg/kg in anchovy. The average As level in fish species from the Turkish Black Sea coast was 1.94 mg/kg, with the highest value measured in anchovy (5.44 mg/kg) and the lowest in Whiting (0.54 mg/kg) (Mutlu, 2024), while Almafrachi et al. (2024) reported As levels from 0.850 mg/kg in M. horse mackerel to 1.078 mg/kg in grey mullet.

Cr is an element involved in synthesizing enzymes, fats, and cholesterol and metabolizing proteins and carbohydrates. The Cr in the analyzed samples ranged from 0.01 mg/kg in goby to 0.153 mg/kg in Atlantic Bonito (Fig.1). Stancheva et al. (2014) found Cr content between 0.03 and 0.07 mg/kg in the five most consumed marine fishes from the Bulgarian Black Sea Coast (turbot, M. horse mackerel, grey mullet, bluefish, and shad). Peycheva et al. (2017) and Peycheva et al. (2019) found that Cr ranged from 0.020 mg/kg in turbot to 0.050 mg/kg in goby and 0.048 mg/kg in garfish 0.048.

Studies have shown that Cr levels range from 0.10 to 1.60 mg/kg for fish from the Turkish seas (Tepe et al., 2008), 0.06-0.84 mg/kg for fish from coastal waters of the Black Sea (Topcuoglu et al., 2002), and reach up to 1.69 mg/kg for fish species from Iskenderun Bay, Mediterranean Sea, Turkey (Turkmen et al., 2005). According to Tuzen (2009), Cr ranged from 0.86 to 1.74 mg/kg. Mendil et al. (2010) reported similar Cr values (0.63-1.74 mg/kg). In another study conducted in the Eastern Black Sea region, Cr levels decreased and ranged between 0.23 and 0.31 mg/kg (Mutlu, 2021). According to Turkmen et al. (2009), Cr content ranged from 0.05 to 0.42 mg/kg in garfish and from 0.06 to 1.87 mg/kg in bluefish, respectively. Mutlu (2024) found that Cr content was highest in Whiting (1.55 mg/kg) and lowest in red mullet (0.12 mg/kg). Almafrachi et al. (2024) found 0.16 mg/kg in M. horse mackerel to 0.185 mg/kg in grey mullet.

While Ni is essential for average growth and reproduction in animals and humans, excessive Ni consumption can have carcinogenic effects. The Ni content of the fish studied ranged from 0.01 mg/kg in goby and Sprat to 0.165 mg/kg in Atlantic bonito and 0.196 mg/kg in bluefish. In shad, grey mullet, and red mullet, the Ni content was below the detection limits of the method used (Fig.1). The Ni content in the fish species studied was below the maximum permissible concentration for Ni in fish (0.5 mg/kg according to Regulation 31, 2004). According to Stancheva et al. (2003a), Ni content ranged from 0.009 mg/kg for bluefish and grey mullet to 0.07 mg/kg for shad.

Uluozlu et al. (2007) determined values for total Ni concentration ranging from 1.92 mg/kg for Whiting to 5.68 mg/kg for grey mullet from the Aegean Sea, Turkey. Turkmen et al. (2009) detected values from 0.06 to 0.92 mg/kg for garfish and from 0.44 to 4.70 mg/kg for bluefish, respectively. According to Mutlu (2024), Ni contents ranged from 0.05 mg/kg in red mullet to 0.54 mg/kg in Whiting, while Almafrachi et al. (2024) determined Ni values from 0.382 mg/kg in grey mullet to 1.066 mg/kg in M. horse mackerel.

Differences in metal concentrations have been found to be related to the habitat and diet of demersal, benthic, and pelagic fish species. Metal concentrations in fish tissues are known to be related to habitat

contamination status. Topping (1973) suggested that fish feeding mainly on plankton contain much higher concentrations of some heavy metals than demersal fish. The results of present study indicate elevated levels of Cd, Zn, Cu, Fe Mn, and As in sprat. Sprat migrate between the open sea and coastal areas. Sprat is a zooplankton-eating fish and can be used to monitor the ecological conditions of coastal waters.

However, according to Bat et al. (2012), demersal species typically accumulate higher concentrations of heavy metals than pelagic fish. In the present study, Hg content in turbot and goby was higher than in pelagic fish feeding on plankton and zooplankton. The skate is a demersal fish and accumulates As to the greatest extent and less Hg. It has been established that turbot muscle tissue has a high affinity for Hg. Therefore, turbot is an essential and suitable bioindicator for determining Hg levels in the marine ecosystem. Bioaccumulation and biomagnification factors indicate that flatfishes accumulate Hg from water, sediment, and food (Polak-Juszczak, 2012).

Predatory fish also accumulate high levels of Hg due to their higher trophic levels (Stergiou & Karpouzi, 2002), given the high bioaccumulation and bioaugmentation in the marine food chain. The results of this study show that Hg reaches the highest concentrations in predatory fish (bluefish).

4.2. Essentials element content

Micronutrient levels are known to vary in fish depending on various factors such as its habitat, feeding behavior and migration even within the same area (Romeoa et al., 1999; Andres et al., 2000; Anan et al., 2005).

Zinc is involved in immune responses, taste perception, wound healing, and fetal development during pregnancy and interacts with sex and thyroid hormones. Zinc deficiency leads to impaired immune function, growth, acne, and skin problems. The permissible maximum Zn content in fish and fish products is 50 mg/kg fresh weight according to the Bulgarian legislation (Bulgarian Ministry of Health, 2004) and 30 mg/kg (FAO, 1983). The Zn content ranged from 2.59 mg/kg in grey mullet to 10.51 mg/kg in *M. horse mackerel* (Fig.1). The zinc content was much lower than the established legislative standards in all samples analyzed.

Stancheva et al. (2003b) found the lowest Zn content in grey mullet (5.2 mg/kg) and the highest in sprat (11.0 mg/kg). Zn ranged from 3.9 mg/kg in bluefish to 12.7 mg/kg in Sprat (Peycheva et al., 2017), reached 5.365 mg/kg in garfish (Peycheva et al., 2019), ranged from 5.2 mg/kg in grey mullet to 10.0 mg/kg in bluefish and Atlantic bonito (Makedonski et al., 2017).

Zn contents in fish from the Turkish seas were in the range of 9.5-22.9 mg/kg (Tuzen 2003), 3.20-8.03 mg/kg (Turkmen et al. 2005) and 3.15-12.9 mg/kg (Tepe et al., 2008), 23.71 to 31.34 mg/kg in the Middle Black Sea (Nissbet et al, 2009), and from 38.8 to 75.5 mg/kg in the Black Sea (Tuzen, 2009), Mutlu (2021) reported that Zn concentrations of fish from the Eastern Black Sea ranged from 4.53 to 11.60 mg/kg in red mullet, from 5.72 to 32.37 mg/kg in *M. horse mackerel*, from 12.89 to 25.75 for anchovy, from 4.76 to 8.65 mg/kg for Whiting, 8.08 to 15.0 mg/kg for garfish, and 3.85 to 15.9 mg/kg for bluefish. Mutlu (2024) found the highest Zn content in *M. horse mackerel* (26.47 mg/kg) and the lowest in medjid (4.97 mg/kg).

Cu is essential for health, but excessive intake can cause toxic effects on fish, invertebrates, and amphibians. The Cu content ranged from 0.17 mg/kg in turbot and bluefish to 0.58 mg/kg in sprat (Fig.1). The Cu content was much lower than the established legislative standards in all samples analyzed (10 mg/kg in fish, Bulgarian Ministry of Health, 2004). Other authors found similar results. Cu contents ranged from 0.34 mg/kg in grey mullet to 1.4 mg/kg in sprat (Stancheva et al., 2004; Makedonski et al., 2017), from 3.9 mg/kg in goby to 12.7 mg/kg in sprat (Peycheva et al., 2017), to 0.218 mg/kg in garfish (Peycheva et al., 2019).

According to Turkmen et al. (2008), the Cu content ranged from 0.32 to 6.48 mg/kg in seafood from the Turkish part of the Sea of Marmara, Aegean and Mediterranean, from 1.01 to 4.54 mg/kg for fish from the coastal waters of the Black Sea (Topcuoglu et al., 2002), from 0.74 to 2.24 mg/kg for fish from the Gulf of Iskenderun (Turkmen et al. 2006) and from 0.15 to 5.06 mg/kg for fish from the Turkish seas (Tepe et al., 2008). Tuzen (2009) measured the lowest Cu value of 0.65 mg/kg for *M.*

horse mackerel caught in the Turkish part of the Black Sea. Turkmen et al. (2009) found that Cu content ranged from 0.21 to 5.89 mg/kg for garfish and from 0.52 to 2.97 mg/kg for bluefish, while Alkan et al. (2016) found 1.02 mg/kg for Whiting and 1.12 mg/kg for *M. horse mackerel*. According to Mutlu (2024), the Cu content ranged from 0.33 to 1.49 mg/kg, with the highest value of Cu found in anchovy and the lowest value in Whiting.

Mn is one of the least toxic heavy metals and an essential element for animals and humans. Manganese is essential for carbohydrate, amino acid, and cholesterol metabolism, connective tissue formation, bone formation, blood clotting, and energy production in the body. Manganese is also essential for the function of the nervous and immune systems.

The Mn content varies from 0.088 mg/kg in bluefish to 1.52 mg/kg in sprat (Fig.1). Other authors have obtained similar results. Mn contents ranged from 0.06 mg/kg in *M. horse mackerel* to 0.17 mg/kg in grey mullet (Stancheva et al., 2004), to 0.256 mg/kg in garfish (Peycheva et al., 2019).

The Mn content in fish from the Turkish seas varies between 0.69 and 3.56 mg/kg (Topcuoglu et al., 2002), 0.08-1.12 mg/kg (Tepe et al., 2008) and 0.10-0.99 mg/kg (Turkmen et al. 2008). The lowest concentration of Mn (0.12 mg/g) was measured in Whiting, and the highest concentration of Mn (2.01 mg/g) in *M. horse mackerel* (Mutlu, 2024). According to Turkmen et al. (2009a), the Mn content ranged from 0.25 to 1.33 mg/kg for garfish and from 0.14 to 0.85 mg/kg for bluefish, respectively.

Iron is a constituent of hemoglobin, ensuring oxygen transport and retention in cells. 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). The Fe content varies from 2.59 mg/kg in Whiting to 12.8 mg/kg in sprat. Other authors obtained similar results. Fe contents vary from 2.2 mg/kg for grey mullet to 9.0 mg/kg for sprat and shad (Stancheva et al., 2014) to 1.469 mg/kg for garfish (Peycheva et al., 2019).

Fe levels for fish ranged from 9.52 to 32.4 mg/kg (Tuzen, 2003), from 30 to 60 mg/kg (Topcuoglu et al., 2002), from 7.46 to 40.1 mg/kg (Turkmen et al. 2008) and from 4.49 to 145 mg/kg (Tuzen, 2009). 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. The highest concentration of Fe was observed in anchovy (37.45 mg/kg) and the lowest level in Whiting (5.11 mg/kg) (Mutlu, 2024). According to Turkmen et al. (2009a) Fe ranged from 9.99 to 43.3 mg/kg for garfish and from 11.6 to 41.2 mg/kg for bluefish.

4.3. Cluster analysis

Hierarchical cluster analysis was performed. A dendrogram was constructed to represent the formed clusters graphically. The dotted horizontal line on the dendrogram shows the rescaled distance at which the clusters were formed.

The cluster analysis results are presented in Fig. 5 by means of a dendrogram. The first cluster consists of two subclusters that are more homogeneous. The first sub-cluster unites the shad (sample 5), red mullet (sample 9), and Whiting (sample 3) similar in indicators: Pb, Cu, and Ni, with the smallest Euclidean distance between them. The second subcluster combines grey mullet (sample 6) and turbot (sample 8), similar in Cd, Cr, and Cu. The second central cluster includes *M. horse mackerel* (sample 4) and garfish (sample 10). They are most similar in Pb, Fe, Cr, and Hg. The most distant variants are Black Sea Goby (sample 1) and 2 Sprat (sample 2), which are distinguished by a significant difference in Fe, Zn, As, and Mn contents, with intergroup distance.

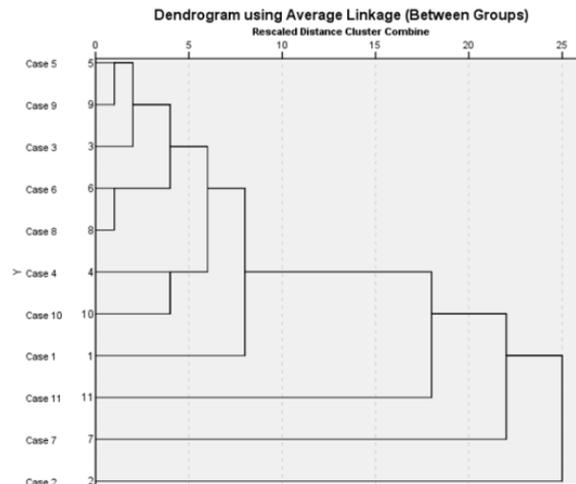


Fig. 5. Dendrogram of the hierarchical cluster analysis from the fish species sampled during the autumn season (September-November) from the southern Black Sea coast of Bulgaria

4.4. Risk Assessment

Although fish consumption is recommended for human health due to its high nutritional value, it may affect consumers' health due to the accumulation of toxic substances in the muscle of the fish. Therefore, it is essential to compare the content of metals in fish muscles with the permissible values in fish defined in regulatory documents. When comparing the content of toxic metals and essential elements in the muscles of different fish species with the permissible values, it was found that the limit values prescribed by Regulation No 31 from July 29, 2004 (Bulgarian Ministry of Health, 2004), and Commission regulation No 1881/2006 of December 19, 2006 (EU Commission Regulation, 2006) were not exceeded. An excess was observed only for Cd in sprat and M. horse mackerel.

A metal pollution index (MPI) was calculated based on the average value of the elements found in the fish muscles. If the MPI value is > 1 , it indicates that there is contamination; if it is < 1 , it indicates that there is no contamination. In this study, MPI values were much lower than 1 in all fish. The highest MPI value was found in bluefish (0.27), followed by sprat (0.22), M. horse mackerel (0.20), Atlantic bonito (0.19), Whiting (0.159), turbot (0.156), Black Sea Goby (0.146), red mullet (0.129), and shad (0.102) (Fig. 2).

THQ values were calculated to estimate the noncarcinogenic risk associated with long-term exposure to metals through fish consumption. The acceptable value for THQ is equal to or less than 1, according to the U.S. EPA (2011). The target hazard quotient (THQ) values were calculated based on the average values of Pb, Cd, Hg, As, Cr, Ni, Zn, Cu, Fe, and Mn in the studied fish, which are presented in Fig. 3. The THQ values of all metals in the studied fish species were below 1. These results indicate that metal intake poses a noncarcinogenic health risk to consumers.

The hazard index (HI), the cumulative value of THQ, was calculated for all metals studied. When the hazard index (HI) is less than 1, it indicates safety, which means that the consumption of fish is beneficial to 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. The calculated values for HI are presented in Fig. 4. 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. The absolute values of HI for women are higher than those of men. Among the studied fish species, Black Sea Goby has the highest HI value (0.71) due to high THQAs. High values in Sprat (0.58), Shad (0.46), M. horse mackerel (0.43) are due to THQ_{Cd}, THQ_{Fe} and THQ_{As}, Bluefish (0.47) - THQ_{Fe}, THQ_{As}, THQ_{Hg}, THQ_{Ni} and THQ_{Cr}, on Atlantic Bonito (0.50) - THQ_{As}, THQ_{Fe}, THQ_{Cr} and THQ_{Ni} and on Red mullet (0.45) - on THQ_{As} and THQ_{Fe}.

5. CONCLUSIONS

Based on the results of the levels of different metals (Pb, As, Cr, Ni, Zn, Cu, Fe, and Mn) in the muscles of 11 fish (black sea goby, sprat, Whiting, M. horse mackerel, shad, grey mullet, bluefish, turbot, red mullet, garfish, and Atlantic bonito) 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 bluefish, Cd and Zn - in sprat and M. horse mackerel, Cu – in Sprat, Atlantic bonito, goby and Whiting, Fe – in Sprat and Atlantic bonito, Mn in sprat, Cr – in Atlantic bonito, Ni- in Atlantic bonito and bluefish, As - in goby, Hg – in bluefish. However, the levels are generally below the maximum allowable concentration (MAC) according to Regulation No 1881/2006 of December 19, 2006 (EU Commission Regulation, 2006) and the Bulgarian Ministry of Health (2004), except for Cd in Sprat and M. horse mackerel.

The risk assessment based on the target hazard quotient (THQ) shows that the levels of metals in the studied fish species are below the recommended limits (< 1). 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, 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.

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