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Original scientific paper

Accumulation of metals in hake (*Merluccius merluccius*) from the Montenegrin coast

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Abstract: This study presents the results of the content of metals (Cr, Ni, Cu, Zn, Fe and Mn) in hake (*Merluccius merluccius*) from the Montenegrin coast, as well as the risk assessment and hazard index for human health caused by the consumption of hake. The examined hake samples were taken from the open sea on the Montenegrin coast in the period from spring 2019 to autumn 2020. During the entire research period, the mean concentrations of the examined metals in hake muscle tissue moved in the following decreasing order: Zn > Fe > Mn > Cu > Ni > Cr. The results of this research indicate that the concentrations of the tested metals in the hake samples were within or below the permitted limit values. Values of risk assessment and hazard index for all tested metals in hake muscle tissue were below unity, so the hazard index was also low. There are no health risks from the intake of Cr, Ni, Mn, Fe, Cu and Zn by consuming hake. However, further monitoring and analysis of the presence of non-essential metals in fish are recommended, as well as assessing the risks and dangers to human health caused by the consumption of fish.

Keywords: Hake, Metals, Risk assessment, Montenegrin coast

1. Introduction

Based on their biological role, metals can be divided into essential (with known biological function), such as Cu, Cr, Fe, Mn, Zn, and non-essential (with unknown biological function), such as Pb, Cd, Hg, As [1]. Essential metals regulate a number of physiological mechanisms crucial to the functioning and development of organs in humans, so their deficiency can lead to various diseases in humans [2]. Concentrations in which metal ions can be considered dangerous vary, so some of the essential metals in higher concentrations than the prescribed values are toxic [3].

In the marine environment, metals are naturally present in lower concentrations [4]. However, various anthropogenic activities lead to an increase in the available concentrations of metals in the environment and in marine ecosystems. Metals are considered one of the main polluting substances of anthropogenic origin in the marine environment [5]. The presence of metals in marine ecosystems above the maximum allowed concentration (MAC) directly threatens the life of plants, animals and even humans [6]. Metals, due to their toxicity and ability to accumulate in living organisms, represent a potential danger for the living world, both for plants and animals, and for humans [6, 7].

Fish, like other marine organisms, can contain various polluting substances as a result of seawater pollution. Fish is a valuable source of protein in the human diet. To ensure that its consumption does not pose a health risk, a continuous monitoring for harmful substances in fish is essential [6, 8]. Fish are considered important bioindicators in aquatic ecosystems for pollution assessment [9-11], where they can accumulate organic and inorganic pollutants and are good indicators of long-term effects [12]. Fish accumulate metals through food, water and sediment [13]. Apart from habitat, nutrition is an equally important and decisive factor for the accumulation of metals and therefore for the presence of metals in fish tissues [6]. Metals accumulated in fish can impair the beneficial nutritional value of fish, and through the food chain reach humans, which can lead to health risks. Due to all of the above, determining the concentration of metals in marine organisms and assessing the potential impact on human health is a very important aspect [14-16].

In this research, hake (*Merluccius merluccius*), which represents a benthopelagic bottom species in the Montenegrin sea, was selected as a bioindicator of metal pollution. This species has already been used as ecotoxicological bioindicators in the Mediterranean Sea due to its commercial value, ecological implications and different feeding habits [6, 17], which enables comparison with literature data from the region and beyond. The research represents a part of the unpublished results and research of the doctoral dissertation [6].

2. Materials and methods

The Adriatic Sea is susceptible to pollution as a relatively small and closed water area and is greatly influenced by various anthropogenic factors and activities that take place immediately along the coast, as well as at sea [6].

Fish samples were collected during the spring and fall of 2019 and 2020. Fig. 1 shows the fish sampling area. Fish sampling was done by trawling, i.e. bottom demersal nets, mesh size 40 mm (square shape), length about 50 m [6]. After sampling, 10 fish with similar parameters (length, weight) were selected.

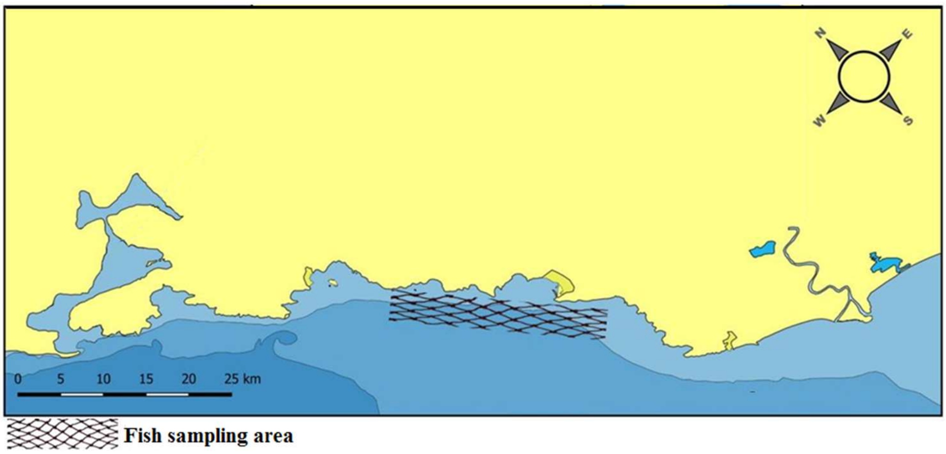

Fig. 1 – *Map of the fish sampling area* [6].

The preparation of the samples, as well as the analysis of metals in hake muscle tissue, was performed in the laboratory of the Centre for Ecotoxicological Research (CETI) in Podgorica according to the standard methods MEST EN 14084:2009 and according to the laboratory of the IAEA agency (International Atomic Energy Agency) and Marine Ecosystem Laboratory from Monaco [6]. In this study, hake muscle tissue was analysed for the presence of metals. Homogenized fish muscle tissues were digested in a microwave digestion device (Speedwave Xpert, Berghof). Concentrations of Fe, Mn, Zn, Cu, Cr, Ni in hake muscle tissue were determined using Inductively coupled plasma – optical emission spectrometry ICP-OES. Along with each batch of fish samples, the concentration was determined in certified reference materials (IAEA 407, 436) [6].

2.1. Hazard quotient risk (THQ) and hazard index (HI)

The risk to human health caused by the introduction of metals through the consumption of hake on the Montenegrin coast was assessed using the Target hazard quotient (THQ) [6]. The THQ represents the relationship between the Reference Dose (RfD) and the measured concentration, depending on the length and time frequency of exposure, amount of intake and body weight [18]. It is calculated using the equation [19]:

$$\mathrm{THQ} = \frac{EF \cdot ED \cdot MS \cdot C}{RfDo \cdot BW \cdot AT} \cdot 10^{-3} \tag{1}$$

EF – frequency of exposure (365 days per year);

ED – duration of exposure, average human lifespan (70 years);

MS – fish portion size (standard fish consumption rate is 17.5 g/day for the general population) [20];

C – concentration of the tested element in fish (mg/kg wet sample);

RfDo - Reference dose (RfD) is an estimate of the daily exposure of the human population to a certain agent without a significant risk of adverse effects during the lifetime (USEPA, 1993). Oral reference doses (RfDo) of metals (mg/kg·day): Cr 0.003; Ni 0.02; Mn 0.14; Zn 0.3; Fe 0.7; Cu 0.04 [20, 21].

BW – body mass of an adult (70 kg);

AT – average exposure time (EF · ED).

Hazard index (HI) was used to assess the risk to human health, by means of which the joint impact of a number of elements is evaluated (Bošković, 2022). The hazard index is calculated by summing the THQ values for all individual elements [19]:

$$HI = THQ_1 + THQ_2 + THQ_3 + \ldots\ldots\ldots\ldots + THQ_n \qquad (2)$$

where THQ1 is the risk coefficient of an individual element, and n is the number of examined elements. When the value of HI < 1, there is no apparent risk from the substance during lifetime exposure, however if the value of HI > 1, there may be a hazard or adverse effect on human health [18].

3. Results and discussion

The concentrations of the tested metals in hake muscle tissue during the entire research period are presented in Fig. 2. During the entire research period (2019-2020), the mean concentrations of the tested metals in the muscle tissue of the hake moved in the following decreasing sequence: Zn > Fe > Mn > Cu > Ni > Cr.

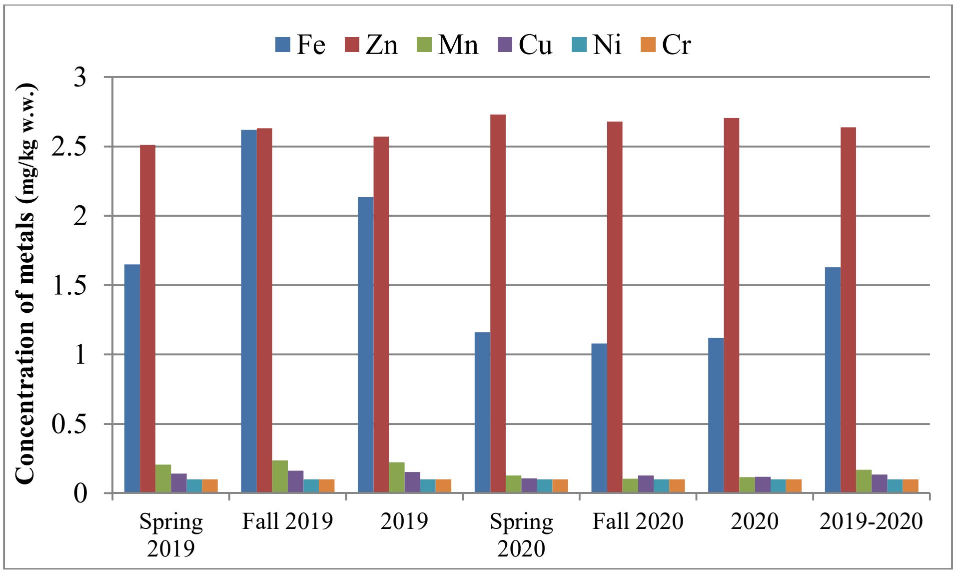

Fig. 2 – *Concentrations of metals in hake muscle tissue sampled in 2019 and 2020 (mg/kg wet weight - w.w.)* [6].

During 2019, slightly higher Fe concentrations were recorded in hake samples compared to 2020, while the sampling season did not have a significant impact on the Fe concentration in hake muscle tissue, Fig. 2. Fe is an essential trace element and one of the most widespread metallic elements in the Earth's crust, so its presence in the analysed fish from the Montenegrin coast is not surprising [22]. The range of Fe concentrations in hake muscle tissue during this study was within the range of values found in hake from the Mediterranean coast of Turkey and higher than the values recorded on the Adriatic coast of Montenegro [23, 24]. On the other hand, Fe concentrations in hake muscle tissue from the Mediterranean and Tyrrhenian seas were significantly higher than the values recorded in this study [25, 26].

Higher concentrations of Zn in hake samples were recorded during the 2020 sampling year, compared to 2019. Although Zn is an essential metal, necessary for the growth and development of living organisms, naturally present in the environment, mining and processing of ores, burning of coal and waste, disposal of waste, wastewater and the use of fertilizers and pesticides containing Zn are some of the anthropogenic sources of Zn in the environment [27]. The concentration of Zn in the hake muscle tissue during the entire research period did not vary significantly in the hake samples, Fig. 2. There were no significant differences in the Zn concentrations in the hake muscle tissue depending on the sampling season. In hake muscle tissue from the Montenegrin coast, Zn concentrations were lower than the values obtained in the Adriatic, Mediterranean and Tyrrhenian seas [25, 26, 28]. Also, the recorded values of Zn in hake were close to the values found in hake from the Adriatic and Mediterranean coasts and higher than the values recorded in the Ionian Sea [17, 23, 24].

In hake samples, there was no noticeable difference in Mn concentration depending on the sampling area. Concentrations of Mn in hake muscle tissue varied slightly depending on the season and the year of sampling. As Mn is an essential element, its concentrations in the analysed fish were expected, Fig. 2. The concentrations of Mn in hake muscle tissue were similar than the literature data recorded in the Adriatic, Mediterranean and Tyrrhenian seas and within the range of values recorded in the Adriatic Sea [23,24,26,28]. Lower Mn concentrations were recorded on the Adriatic and Ionian coasts than in hake muscle tissue in this study [17, 24].

It can be said that the concentration of Cu in hake muscle tissue during the entire study was quite uniform, without significant differences depending on the season and year of the study, Fig. 2. The concentrations of Cu in hake muscle tissue were lower than the literature data recorded in the Adriatic, Mediterranean and Tyrrhenian seas and within the range of values recorded in the Adriatic Sea [23, 24, 26, 28].

The concentrations of Cr and Ni in hake muscle tissue during the entire study were below the detection limits, Fig. 2. Ni concentrations in hake muscle tissue from the Montenegrin littoral were lower or within the range of values found in hake from the coast of the Adriatic, Ionian and Tyrrhenian seas [17, 24, 26]. In the Adriatic Sea in Montenegro, the measured concentrations of Cr in hake muscle tissue were very low, below the detection limits, as well as the values obtained in this study, while in the Ionian Sea, higher concentrations of Cr were measured in hake, compared to the values of the research in this study [17, 24].

In general, the examined metals in hake muscle tissue were mostly in the range or lower than the values recorded in hake muscle tissue from the Adriatic, Mediterranean, Ionian and Tyrrhenian seas.

Based on the above, it can be concluded that the mean concentrations of Fe, and Mn in hake muscle tissue were higher during the 2019 sampling year, while the mean concentration of Zn was higher during the year 2020. Also, during 2019, higher Cu concentrations were recorded in hake muscle tissue. The higher concentrations of examined metals in 2019 compared to 2020, the year of fish sampling, can be explained by the greater influence of anthropogenic factors such as the greater number of vessels, exhaust gases and the greater impact of tourism [29].

Differences in the concentrations of the tested metals in fish samples depending on the sampling season (spring-fall) were observed for Fe, whose concentrations were higher during the autumn sampling period, and from Zn and Mn, whose concentrations were higher during the spring sampling period of the analysed fish. At higher temperatures, metabolic activity increases, which can contribute to a higher level of metal accumulation in fish, and at lower temperatures, fish store energy reserves to a greater extent, before the period of reproduction [29, 30]. Also, the seasonal variability of the concentration of metals suggests that environmental factors such as the seasonal cycle of element absorption/dissolution in specific areas, local physicochemical parameters such as temperature, salinity and the nature of sediments can influence the bioaccumulation of metals by marine organisms [29, 31, 32].

Threshold values are not prescribed for all metals examined in this research, but only for Cu and Zn. Concentrations of Cu and Zn in hake muscle tissue from the Montenegrin coast were lower than the values prescribed by the FAO [33].

3.1. Risk assessment

The calculated values of the target hazard quotient risk (THQ) for the tested metals in hake, as well as the hazard index (HI), which assesses the joint impact of all tested elements, are given in Table 1. To calculate the THQ value, the mean concentrations of the tested elements during the two-year test period (2019/2020) were used.

Table 1 - *Target hazard quotient (THQ) and hazard index (HI) for the tested metals in hake for the general population, during the two-year study period [6]*

Metals	**THQ**
Cr	0.0083
Cu	0.0008
Fe	0.0006
Mn	0.0003
Ni	0.0013
Zn	0.0022
HI	0.0135

THQ values for all examined metals in hake muscle tissue were below unity, so the hazard index was also low (HI < 1). The results indicate that there are no health risks from the intake of Cr, Ni, Mn, Fe, Cu and Zn by consuming hake from the Montenegrin coast (THQ < 1). However, further monitoring and analysis of the presence of non-essential metals in fish are recommended, as is the assessment of risks and dangers to human health caused by the consumption of fish.

4. Conclusion

The results of this study showed that the concentrations of Cr, Ni, Cu, Zn, Fe and Mn in hake from the Montenegrin coast were within or below the permitted limit values and mostly within the range or lower than the values recorded in hake muscle tissue from the Adriatic, Mediterranean, Ionian and Tyrrhenian Seas. During the entire study period, the mean concentrations of the tested metals in hake muscle tissue followed a decreasing order: Zn > Fe > Mn > Cu > Ni > Cr. The risk assessment and hazard index values for all tested metals in hake muscle tissue were below unity, therefore the hazard index was low, indicating that there are no health risks from the intake of Cr, Ni, Mn, Fe, Cu and Zn through the consumption of hake. However, further monitoring and analysis of the presence of non-essential metals in fish, as well as the assessment of the risks and hazards to human health caused by fish consumption, are recommended.

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