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Arsenic (As), copper (Cu), zinc (Zn) and selenium (Se) in northwest Croatian seafood: A health risks assessment

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ABSTRACT

This study focuses on the health risk assessment of arsenic (As), copper (Cu), zinc (Zn), and selenium (Se) concentrations in seafood species commonly consumed in the northwestern region of Croatia. By measuring the concentrations of these elements coupled with data on seafood consumption, the health risks were evaluated using Target Hazard Quotients and Hazard Indexes. The results indicate a slightly increased health risks linked to seafood consumption for As, Cu and Zn in some of the tested seafood species. The findings of this study highlight the critical need for stronger food quality control measures, especially targeting certain types of seafood.

1. Introduction

Seafood consumption is increasingly being recognised as a source of essential nutrients, protein and omega-3 fatty acids contributing to a balanced and healthy human diet [1]. It is traditionally consumed in various forms including fish, shellfish, molluscs, and crustaceans. Nonetheless, due to increasing mainly land-based pollution loads to the marine environment, concerns have been raised about the potential health hazards associated with seafood intake [2–6].

Of particular interest as pollutants are various metals including, among others, arsenic (As), copper (Cu), zinc (Zn), and selenium (Se), which can bioaccumulate in aquatic organisms, including fish and shellfish. Heavy and trace metals in the marine environment can originate from natural sources, or from industrial activities, agricultural runoff, and atmospheric deposition. Once they have entered the aquatic ecosystem, these substances are absorbed by marine organisms and propagate through the marine food web having the potential to cause adverse health effects during human consumption [7–10].

Arsenic, a naturally occurring element, can occur in the environment in both organic and inorganic forms. It is highly toxic in its inorganic form, and as such has been associated with several adverse health effects, including skin lesions, cardiovascular effects, and various types of cancer [11–13]. It is mainly introduced into the marine environment by

weathering of geological formations and can thus contaminate water bodies including marine life.

Copper, an essential micronutrient, can also be harmful if humans are exposed to it in excessive amounts. While it is needed for various physiological processes, high levels of copper have been linked to liver and kidney damage, gastrointestinal disorders, and neurotoxic effects [14–17]. Sources of copper pollution in water bodies include industrial effluents, agricultural runoff, and corrosion of copper-containing infrastructure.

Zinc, another essential micronutrient, is critical for numerous biological functions including enzymatic activity, gene expression, and immune system function. However, excessive zinc intake leads to gastrointestinal disorders and affects copper and iron metabolism [18–20]. Sources of zinc contamination in seafood include industrial discharges, wastewater discharges and agricultural activities.

Selenium, an essential trace element, is required for proper thyroid function and antioxidant defence. It enters the marine environment mainly through weathering of rocks and can accumulate in marine organisms. Excessive selenium intake leads to selenosis, which causes hair and nail loss, skin lesions, and neurologic sequelae [21–25].

Given the potential health risks associated with the consumption of seafood contaminated with As, Cu, Zn, and Se, it is critical to quantify the extent of the risks posed by these elements in seafood. To our

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knowledge the literature does not abound with data on the levels and potential toxicities of these elements in seafood. In addition, the health risk associated with these elements have not been studied so far for the region of Croatia.

Thus, the main aim of this study was to assess the health risk posed by exposure to As, Cu, Zn, and Se through the consumption of seafood in the region of northwestern Croatia.

The decision to study the levels and potential toxic effects of As, Cu, Zn, and Se in seafood was based on several critical factors and concerns related to public health and environmental safety.

The unique properties of each heavy of the metal studied, their different toxicological profiles, including the various routes of exposure can lead to a spectrum of health effects ranging from acute toxicity to chronic disease with prolonged exposure. As already stated, arsenic, is known to be a potent carcinogen [26–28], while copper in excessive amounts can lead to adverse gastrointestinal effects and liver damage [29,30]. Zinc, too, despite its important role as a micronutrient, can pose health risks if accumulated above permissible limits [31,32]. Selenium, on the other hand, while important for human health in appropriate amounts, can be toxic in excessive concentrations [33].

Taking into consideration the above, the goal of this study was to provide a comprehensive insight into the current levels of As, Cu, Zn, and Se in seafood in the northwestern part of Croatia in order to obtain a more nuanced understanding of the potential health effects of these metals.

By measuring the concentration of these elements in marine

organisms, coupled with data on seafood consumption, we have investigated the extent of human exposure to these elements and provided a comprehensive understanding of the potential health risk associated with the presence of these elements in seafood.

In addition to contributing to the existing knowledge base, the results of this study will be useful for informing policy makers, health professionals, and consumers on health risks pertinent to seafood consumption, and will provide valuable insights for developing appropriate risk mitigation strategies.

2. Material and methods

2.1. Collection of samples

The samples were collected from the region of Primorje and Gorski Kotar, situated in the north-western area of the Republic of Croatia, encompassing the Kvarner Bay, the northern Croatian littoral, and the inland territory of Gorski Kotar (Fig. 1).

This location has been chosen since this specific area within the Republic of Croatia provides unique insights into marine and terrestrial ecosystems and is an ideal representation of the various environmental conditions and anthropogenic activities prevalent in the region. In addition, the region is as a major hub for commercial fishing which underscores the need to assess the potential risks associated with metal contamination of seafood.

The population in the region of Primorje and Gorski Kotar

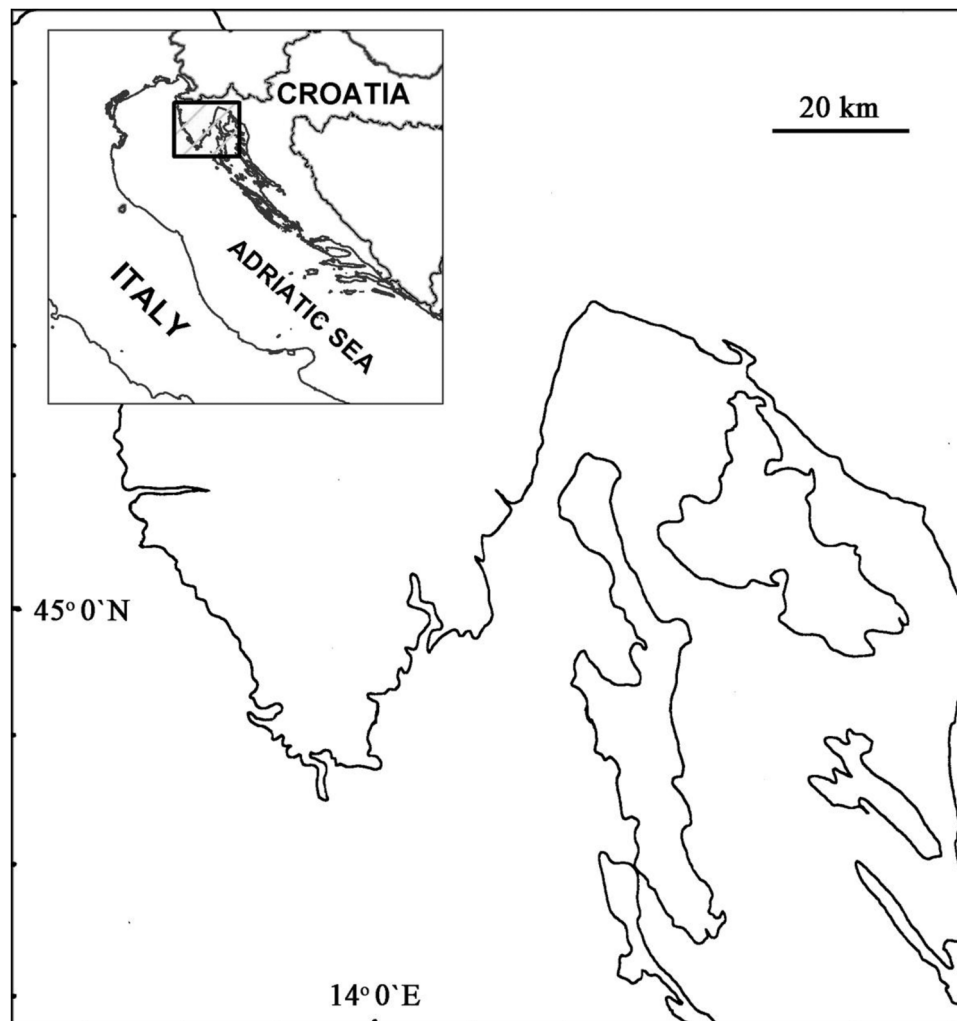


Fig. 1. Location of the Primorje and Gorski Kotar County.

predominantly relies on fresh seafood purchased from local markets. Throughout an 8-week sampling campaign from February 2022 to April 2022, samples of the gilthead seabream (*Sparus aurata*, L), European hake (*Merluccius merluccius*, Linnaeus 1758), sardines (*Sardinia pilchardus*, Walbaum, 1792), tuna fish (*Thunnus thynnus*, Linnaeus 1758), and the Patagonian squid (*Loligo gahi*, Orbigny 1835), were obtained from the local market. These are the fish species which, according to our previous research, are the ones most often consumed in the region [34]. The number of samples per species are shown in Table 2. From the samples obtained, 12 composite samples for each fish species have been prepared and analysed in triplicate. The number of samples of seafood were approximated based on other studies which analysed the presence of metals in various types of seafood [35–38].

Sardines and European hake were harvested from the Mediterranean Sea, more specifically from Croatian waters. Gilthead seabream was sourced from local fish farms. Tuna fish originated from aquaculture facilities located in Italy, while the Patagonian squid was caught in the Atlantic Ocean.

Following collection, the samples were held on ice, transported to the laboratory and stored at $-20\text{ }^{\circ}\text{C}$ until further processing. To facilitate the analyses, composite samples were prepared from sampled individuals. Prior to conducting the analyses, the samples underwent lyophilization using the LIO-5 PLT apparatus and pulverised using a cryogenic mill (MM 500 control, Retsch).

2.2. Analyses of heavy metals

Approximately 0.5 g of each sample was digested using the Anton Paar Multiwave 3000 microwave system (Anton Paar GmbH, Graz, Austria) equipped with pressurised vessels. The samples were digested with 5 mL of 65% nitric acid (Suprapur, Merck, Germany), over a 20 min operation cycle at $200\text{ }^{\circ}\text{C}$. After cooling, the digested samples were transferred to 25 mL volumetric flasks (Siemens Water Technologies Corp, Warrendale, PA, USA) and ultrapure water was added to the mark. The concentrations of As, Cu, Se and Zn were determined by inductively coupled mass spectrometer (ICP MS) equipped with S10 autosampler (PerkinElmer Instruments, Waltham, MA, USA).

2.3. Health risk assessment

The health risk assessment has been performed using the Target Hazard Quotients (THQ) and the Hazard Indexes (HI).

2.3.1. Target hazard quotient (THQ)

The THQ has been calculated according to the following equation:

$$THQ = \frac{EF * ED * FIR * C}{RfD * BW * TA} * 10^{-3} \quad (1)$$

where:

THQ – Target Hazard Quotient.

EF - exposure frequency (365 days year⁻¹).

ED - exposure duration equivalent to the average human lifetime (70 years).

FIR - fish and seafood ingestion rate (g day⁻¹).

C - metal concentration in fish tissue (mg kg⁻¹).

RfD - the oral reference dose for contaminant (mg kg⁻¹ day⁻¹).

BW - the average body weight (70 kg for adults).

TA - the exposure time for non-carcinogens (365 days year⁻¹ ED).

2.3.2. Hazard index (HI)

The HI has been calculated according to the following equation:

$$HI = \sum THQ \quad (2)$$

where:

HI – Hazard Index.

THQ – Target hazard Quotients for each heavy metal.

The RfD (reference dose) used to estimate the THQs is intended to provide a threshold below which an exposure over a human lifetime does not cause appreciable risks to human health. It is obtained by multiplying the no-observed-adverse-effect level (NOAELs) by an uncertainty (safety) factor. A10-fold uncertainty factor is used to account for differences in sensitivity among individuals in the population, another 10- fold factor is added when the NOAEL is based on animal data extrapolated to humans, and another 10-fold factor is used when the available data on toxic effects on humans and/or animals are very limited.

The RfD for the selected heavy metals were obtained by quiring the US EPA Integrated Risk information system (IRIS) database [39] coupled with data published in Taylor et in 2023 [40].

The RfD for As, Cu, Zn and Se are given in Table 1.

Of note is that when estimating the concentration of As, the concentration has been multiplied by 0,1 in order to account only for the inorganic As (inorganic As is approximately 10% of the total As), since organic As is not generally considered toxic.

2.4. Quality assurance

Multielement tuning solution (NexION Setup Solution, PerkinElmer Instruments, Waltham, MA, USA) was used for performance check and tuning, covering a wide range of masses of elements. The concentrations of metals were determined using an external standard method, with multielement standard solution (PerkinElmer Instruments, Waltham, MA, USA) used to prepare calibration curves. The calibration curves with $R^2 > 0,999$ were accepted for concentration calculations. For each experiment, a run included a blank, certified reference material (CRM) and samples, which were analysed in triplicate, to eliminate any batch-specific errors. Analytical blanks were prepared and ran in the same way as the samples. The method was validated using the IAEA-436 reference material (fish tissue), (International Atomic Energy Agency, Austria). Mean recoveries were: 91% for As, 99% for Cu, 88% for Se, and 82% for Zn.

2.5. Statistical analyses

The distribution of data sets has been tested with the Anderson-Darling test ($\alpha = 0,05$). Since the distribution of data was not normal differences between groups were tested using the non-parametric Kruskal-Wallis test followed by the Dunn's post-hoc test. All the analyses have been performed using GraphPad Prism version 9.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

3. Results and discussion

3.1. Concentration of metals in seafood

Descriptive statistics of heavy metal concentration in seafood are provided in Table 2.

In Fig. 2 box plots of the tested elements in different seafood species is shown.

As it can be seen from Fig. 2 the highest concentration, in general, has been observed for Zn and Cu. The concentration of the tested elements was in the following order: Zn > Cu > As > Se. The Kruskal-Wallis test revealed that there are statistically significant differences

Table 1

Reference doses (ingestion) for As, Cu, Zn and Se. The units are given in mg/kg d. w.*day.

As	Cu	Zn	Se
0,0003	0,04	0,3	0,005

Table 2

Median value including Min and Max values (in parenthesis) of the tested elements in seafood. p values are given for the Kruskal - Wallis analysis performed on the data. The table also includes the number of samples for each species.

	Patagonian squid	Tuna	Sardines	European hake	Gilthead seabream	p
n of samples	123	24	397	49	24	
As (mg/kg w.w.)	0,22 (0,08–0,26)	0,10 (0,02–0,24)	0,47 (0,38–0,24)	1,54 (0,42–2,56)	0,14 (0,07–0,34)	< 0,0001
Cu (mg/kg w.w.)	15,62 (11–20,36)	0,12 (0,09–0,78)	0,89 (0,64–1,22)	0,04 (0,01–0,18)	0,33 (0,21–0,56)	< 0,0001
Zn (mg/kg w.w.)	11,29 (6,49–33,06)	1,75 (1,29–3,53)	18,74 (14,98–25,72)	1,87 (1,20–2,61)	4,92 (4,05–12,85)	< 0,0001
Se (mg/kg w.w.)	0,60 (0,44–0,84)	0,70 (0,47–1,00)	0,62 (0,38–0,93)	0,27 (0,19–0,38)	0,24 (0,14–0,34)	< 0,0001

in the concentration of the analysed metals between species (Table 2). The Dunn's post hoc test revealed that there were statistically significant differences in the concentration of arsenic (As) between all species except between the Patagonian squid and European hake, tuna fish and the gilthead seabream and sardines and the European Hake. Regarding copper (Cu), there were statically significant differences between all species except between tuna fish and the European Hake. Differences in Zn concentration were statistically significant between all species except between tuna fish and European Hake. Finally, regarding selenium (Se), statistically significant differences have not been observed between the Patagonian squid and tuna fish, the Patagonian squid, and sardines, tuna fish and sardines as well as the European Hake and the Gilthead seabream. Statistical difference of the tested metals between the seafood species analysed in this study are shown in Fig. 2.

Regarding variations of heavy metal concentrations between species, selenium (Se) had the smallest variation whereas copper (Cu) had the highest variation, mainly due to the fact that Cu concentration in the Patagonian squid were comparatively high, which is in accordance with other published studies [41].

The observed high copper content in Patagonian squid, especially those from the Atlantic Ocean, could be attributed to both environmental and physiological factors. There are several plausible reasons which could contribute to the elevated Cu levels in this species. First, the dietary habits and preferences of the Patagonian squid could play a critical role. Given their position in the food chain and feeding habits, the Patagonian squid can consume prey organisms and/or aquatic plants that naturally have higher copper levels, since it is known that Cu has a tendency to bioaccumulate in tissues over time [42,43]. Consequently, transfer of Cu along the trophic chain from prey to predator may contribute significantly to the observed phenomenon. In addition, the geographic location and habitat of the Atlantic Ocean, where the Patagonian squid was fished, could expose the species to elevated Cu concentrations from natural or anthropogenic sources. Pollution from industrial activities, coastal runoff, or shipping could result in the introduction of copper into marine ecosystems. Consequently, the Patagonian squid could take up and accumulate higher levels of copper from their environment, resulting in the observed elevated copper levels in their tissues. The physiological characteristics and unique metabolic processes of Patagonian squid could also influence its ability to accumulate Cu [44]. Given these factors, further comprehensive studies involving ecological, environmental, and physiological aspects could be needed to elucidate the precise mechanisms underlying elevated copper levels in Patagonian squid from the Atlantic Ocean.

Regarding other metals analysed in this study, the obtained concentrations are similar to the concentrations published in other studies which analysed the presence of these metals in seafood [45–49].

Finally, it has to be stated that in Croatia, the Maximum Residual Levels (MRL) of metals in food, particularly seafood are regulated by the Rule on Maximum Permissible Levels of Certain Contaminants in Food (Croatian Official Gazette 154/2008). It has to be pointed out that in this research the MRL of the tested elements have not been exceed; however it has to be mentioned that scholarly research frequently highlights instances where the concentration of heavy metals in seafood are above the legal limits [50–53].

3.2. Characterisation of health risks

The Target Hazard Quotient (THQ) is a metric traditionally used to estimate the potential health risk associated with exposure to a specific contaminant in a particular food item. If the THQ value exceeds 1, it suggests an increased risk of adverse health effects and a potential health concern due to the consumption of that particular food item. On the other hand, to address the overall potential health risk associated with exposure to multiple contaminants, the Hazard Index (HI) has been calculated for the studied contaminants and seafood species.

The THQ and HI are provided in Table 3.

The target hazard quotient (THQ) values determined in our study indicate that the potential health risks associated with the consumption of the seafood species studied are relatively low in most cases, with THQ values mostly below 1. However, the findings indicate potential concerns for As in European hake, Cu in sardines, and Zn in both Patagonian squid and sardines, suggesting a slightly elevated risk for adverse health effects.

The elevated THQ levels for As in European hake may be attributed to bioaccumulation of this heavy metal in this species over time considering the general persistence of arsenic in the environment, its presence in aquatic ecosystems, and the dietary habits of the European hake.

Similarly, elevated THQ values for Cu in sardines suggest possible accumulation of this metal in the tissues of these fish. Sardines, which are often found in large schools and frequently consumed as a component of various diets, could be exposed to higher levels of copper in their natural habitat. In addition, the observed elevated THQ levels for zinc (Zn) in both Patagonian squid and sardines may be indicative of the propensity of these species to accumulate zinc. Factors such as dietary habits, habitat, and biological processes could contribute to the bioaccumulation of zinc in these species, increasing the potential health risk associated with their consumption.

Since the HI is the sum of the THQs, the HI for As, Cu and Zn were also above 1 indicating a heightened risk of adverse health effects due to the consumption the studied seafood. Regarding the THQ and HI the obtained results were in the order of magnitude reported by other studies [54–57]. Overall, the results of this assessment indicate that there is a slight potential health risk associated with the consumption of the tested seafood species due to elevated levels of As, Cu and Zn. To reach more robust conclusions, it would be necessary to perform a more intensive sampling campaign over several seasons and years, especially for the species of concern such as the European hake, the Patagonian squid and sardines. Further investigations into the concentration of As, Cu and Zn in these fish species are needed in order to obtain more granular data and develop adequate risk mitigation strategies.

4. Conclusions

The results of this study point out that there are potential health risks related to seafood consumption in north-western Croatia due to slightly elevated concentration of As, Cu and Zn. To mitigate the potential health risk associated with seafood consumption more stringent and frequent sampling protocols and tighter control from regulatory bodies are needed.

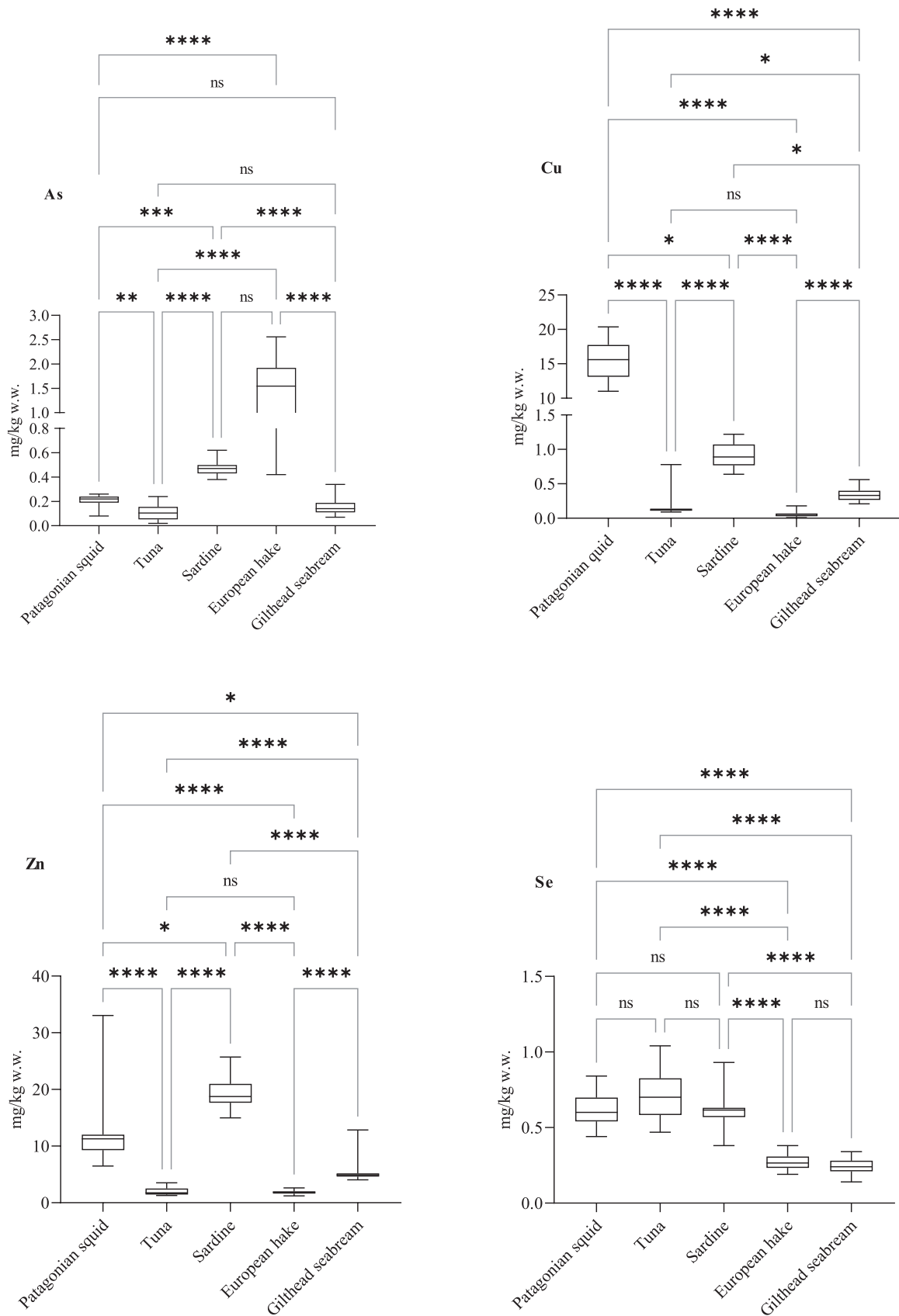


Fig. 2. Boxplot of the tested elements for each seafood species. Pairwise comparisons for each element between species are also indicated (* $p \leq 0,05$; **** $p \leq 0,0001$).

Table 3

THQ and HI of the tested elements in the selected seafood species.

	THQ (As)	THQ (Cu)	THQ (Zn)	THQ (Se)
European Hake	1,15	0	0	0,01
Gilthead seabream	0,04	0,82	0,35	0,01
Patagonian squid	0,09	0,17	1,44	0,05
Sardine	0,09	1,7	1,02	0,02
Tuna fish	0,02	0,27	0,11	0,03
HI	1,39	2,96	2,92	0,13

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Ethics approval

This is an observational study performed on fish which were not alive and were purchased from local markets, thus no ethic approval was needed.

CRedit authorship contribution statement

Luka Traven conceptualized the study, obtained funding, analysed the data and drafted the paper. Sandra Marinac Pupavac and Paula Žurga performed the analyses and contributed to data analysis. Marin Glad and Željko Linšak, Sandra Pavličić Žeželj, Dijana Tomić Linšak and Arijana Cenov contributed to data analyses and finalising the draft of the manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Luka Traven reports financial support was provided by the University of Rijeka.

Data availability

Data will be made available on request.

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