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Assessment of the Quality and Human Health Risk due to Heavy Metals Concentration in Marine Biota Species

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ABSTRACT

Marine ecosystems have been heavily contaminated in recent years by discharges from agricultural, domestic, chemical waste spills, ship emissions, etc. Among various pollutants, metals are considered the most serious ones, because they are not biodegradable and can show toxic effects on living organisms if exceeding certain concentration levels. In the present study, 24 biota species were randomly selected at the Vlora region, Albania and were analyzed for the content of some heavy metals in the edible part of their body. Except for Cd, the concentrations of heavy metals in the edible part of fish species did not exceed the permissible limits proposed by FAO (1983) and WHO (1996) and are suitable for human consumption. The estimated daily intake (EDI) of the selected species was lower than the daily reference intake (DRI) while low values of target hazard quotient (THQ < 1) had shown that the consumption of mussels containing heavy metals would not cause significant health risks to humans. Finally, studies of metal concentrations in coastal areas are relevant and useful for monitoring the health of environmental compartments, for the maintenance of biodiversity, and for assuring the quality of life, mainly for humans.

Keywords: heavy metals, marine biota, permissible limits, AAS/AET.

INTRODUCTION

In freshwater and marine environments, heavy metals are naturally occurring substances that are normally present in very low concentrations. They are recognized as major causes of contamination, because they induce substantial alterations in the chemistry of saltwater and infiltrate the marine environment through a variety of natural and man-made ways [1, 2]. In many of these natural systems, metal ion concentrations have unavoidably increased due to human activities. The increase in metal load in these waters is a result of various factors, such as main drainage, offshore oil and gas exploration, industrial (pesticides, paints, lather, textiles, fertilizers, pharmaceuticals), domestic effluents, agricultural runoff, acid rain, and others. Eventually, this increased metal load is incorporated into aquatic sediment [3, 4]. While some metals are merely helpful, some are essential to life, and some are exceedingly dangerous. Certain metals are required at low concentrations but are dangerous at greater concentrations, hence the threshold at which they can be considered crucial varies [5, 6]. To ascertain whether a trace heavy metal is necessary for the regular, healthy growth of plants and/or animals, the different standards are applied: the element has a direct impact on the organism and is engaged in its metabolism. It also cannot be entirely substituted by another element; without an appropriate supply of the element, the organism cannot grow or complete its life cycle [3].

Cr, Co, Cu, Fe, Mn, Mo, Se, Sn, V, As, and Zn are all necessary for animals. While several of these components are required at extremely low concentrations, their practical influence on deficiencies is minimal [7].

Marine species' heavy metal intake is influenced by various factors, including biological, environmental, and physicochemical factors [8, 9]. Generally speaking, the soft tissues of marine animals contain many times more metals than the surrounding saltwater [10, 11]. An organism can serve as a biological indicator of metal pollution, because its metal content is often proportionate to that of saltwater [11, 12]. The most well-known factor affecting an organism's metal concentration is its size. Another factor influencing metal toxicity to aquatic biota is salinity [13]. It has been discovered that most metals, including Cr, Cu, Hg, Ni, and Zn, become more harmful when salinity decreases [13, 14].

In aquatic systems, the most dangerous heavy metals include lead, copper, zinc, cadmium, and mercury. At higher concentrations, many metals are toxic to living things, while others (like zinc and copper) are necessary for metabolism at lower concentrations [15, 16]. Cadmium and lead have no biological function. Metals can negatively affect the abundance, diversity, and health of marine biota by being absorbed through the food chain or through saltwater. To determine the degree of harm to the marine biota, the dangerous effects of these metals on several types of marine species, including fish, zooplankton, and phytoplankton, must be measured. This will assist in carrying out prompt actions to stop metal contamination in the marine ecosystem [15, 16, 17].

Poisons that pose major risks to human health when consumed are typically made of heavy metals [14, 18, 19]. One of the main sources of readily digested protein that is high in fats, and contain macro and trace elements, important amino acids, and fat-soluble vitamins is fish. Valuable long-chain polyunsaturated omega-3 fatty acids can be found in abundance in fish [20, 21]. Fish tissues can, however, also acquire heavy metals, trace elements, pesticide residues, and persistent organic pollutants, such as polychlorinated biphenyls (PCBs) [20, 21]. The usual hierarchy of metal toxicity to human health is Hg > As > Pb > Cd > Ni > Zn > Cr > Se [10, 22, 23]. In some situations, eating seafood (fish, crab, shrimp, etc.) polluted with high concentrations of trace elements (including Hg, As, Pb, Cr, and Cd) can induce cancer and have an adverse effect on several human organs; in other cases, it operates similarly to fish [22, 23]. Others would entail maintaining the activity of Se-dependent enzymes, or selenium-enzymes, which, under some circumstances, may replace the requirement for mercury and methylmercury (CH_3Hg) [21]. Numerous factors, such as location, favored habitats, physical traits of the species, length of exposure to metal pollution, and so on, influence metal deposition in marine biota [24, 25]. Human electron transfer chains involve a large number of proteins that contain Fe and Cu. Among these proteins are the respiratory chain found in the inner membrane of the mitochondria, which is made up of cytochromes, Fe-S proteins, and the terminal component, Cu-Fe-dependent cytochrome c oxidase [24, 25]. Fe is also involved in oxygen activation (oxidases, hydroxylases), detoxification (Cu-Zn superoxide dismutase), as well as oxygen transport and storage proteins such myoglobin and haemoglobin.

Pb seems to target the proteins that typically bind zinc and calcium [25, 26]. Lead targets multiple proteins, such as δ-aminolaevulinate synthase (ALAD), the second enzyme in the route leading to hemoglobin formation, and synaptotagmin, a calcium sensor in neurotransmission. Since its ionic radius is similar to that of Ca^{2+}, Cd^{2+}, a soft Lewis acid that prefers readily oxidized soft ligands, especially sulfur, can exchange with Ca^{2+} in calcium-binding proteins, but it can also displace Zn^{2+} from proteins where sulfur dominates the Zn coordination environment [24–27].

There is disagreement on the biological advantages of chromium (III). The National Institutes of Health classifies chromium as a trace element because of its involvement in insulin action, a hormone that controls the metabolism and storage of proteins, fats, and carbohydrates. Chromium's necessity is questioned because the mechanism underlying its actions in the body is still unknown [25].

With a 380 km coastline, 284 km of which run along the Adriatic Sea to the north and the remaining 96 km facing the Ionian Sea, Albania is considered to have an abundance of water resources [28]. Although lagoon and inland fisheries are also significant, the marine capture fishery is the most significant part of the fishing industry.

The amount of catch produced in 2014 was 5,730 tons, of which 68% came from coastal fisheries and the remaining 38% from inland fisheries. The average amount of fish and seafood consumed by households nationwide has varied recently; according to FAO estimates, this amounts to 8.65 kg/capita, or roughly 166 g per week [28, 29]. Albania's coastal city of Vlora is well-known for its advanced human civilization. Because of the natural beauty, there has been a significant increase in tourism, which has led to the building of numerous hotels, restaurants, and beaches [29]. The city's population grows fivefold in the summer because of its large capacity to host a large number of visitors, both domestic and from foreign countries. In the present study, the concentration levels of heavy metals were determined in 24 marine biota species collected at the Vlora region aiming to estimate the quality of sea food which is being consumed by population.

MATERIALS AND METHOD

Study area

Samples of marine biota were randomly acquired from local fishermen across the coastal waters of Vlora bay region in Albania. The Bay of Vlora is situated along the Albanian Adriatic Sea Coast, on the Mediterranean Sea in southern Europe. It opens to the sea in the northwest and is largely surrounded by the lagoon of Narta in the north, the city of Vlorë in the northeast, the mountains of the Ceraunians in the east and southeast, and the peninsula of Karaburun in the southwest and west. The artisanal fishery covers all forms of fishing activity using fixed and selective gear, such as hooks, fixed nets, trammel nets, and gill nets. About 30% of the fishing vessels are located in Vlora bay, ranking the second city after Durres. As a member of GFCM, Albania is subject of catch limitations: from 2019, fishing vessels should not exceed 180 fishing days per year, with a maximum of 144 fishing days targeting sardine and 144 fishing days targeting anchovy [29]. In recent years, the national average household consumption of fish and seafood has fluctuated; FAO puts the estimate at 8.65 kg in 2020 (Fig. 1).

Figure 1. Map of sampling area

Sample collection and analysis

In total, 24 specimens were collected from September 2022 to June 2023. Fresh fish samples were obtained randomly from the fisherman of the area. Selected samples were packed in polyethylene ziplock bags, labeled, and transferred to the laboratory in cooler boxes. Upon their arrival, the samples were washed with distilled water, drained and representative parts of the body were stored in plastic bags at -40 °C freezer until the day of analysis. About 1.0 g of wet tissues of each biota sample was ground by a mixer, weighted in Teflon tubes and treated with 7 mL of nitric acid, HNO_3 (65%). First, the samples were left for 24 h at room temperature in closed Teflon tubes and then the temperature was raised to 150 °C for 3 hours. After 3 hours, the lids were opened, the temperature was raised to 200 °C and the samples were allowed to evaporate until they reached the consistency of wet salt. Then, 2 ml of HCl (37%) was added aiming to remove the NO_2 vapor and samples were left to cool at room temperature and were diluted to 50 ml with deionized water. Two replicates were prepared for each sample along with two blanks which also were prepared in a similar way as the biota samples. Determination of the concentration of metals in solutions was carried out by atomic absorption spectroscopy with electrothermal atomization, GFAAS, using an ANALYTIK JENA novAA 400 instrument [30].

Statistical analysis and quality control

The calibration curves were linear within the range of heavy metal contents (regression coefficients $R^2 \geq 0.999$). The detection limits (LOD) of the GFAAS technique were 0.005 mg/kg for Cu, 0.07 mg/kg for Fe, 0.002 mg/kg for Mn, 0.003 mg/kg for Zn, 0.005 mg/kg for Cd; 0.001 mg/kg for Pb; 0.03 mg/kg for Ni; 0.015 mg/kg for Cr; 0.05 mg/kg for Al. The lyophilized certified material (IAEA 407 fish homogenate, provided by IAEA Environment Laboratories) was also analyzed along with the considered samples for the content of metals. The recovery rates ranged between 92–108.0%. Statistical treatment of the obtained results was carried out by using MINITAB 22 statistical software. Cluster analysis was used to evaluate the similarities between metals and samples. In cases of the results under the detection limit of the method, values were replaced with half of the LOD value.

The contamination degree of biota samples with heavy metals was evaluated by comparing obtained results with the values recommended by WHO/FAO, 1997, European Commission, 2006 and the predicted non-effect concentration (PNEC) values, etc. [18, 31, 32, 33].

Bio-concentration factors

When exposed to high quantities, the metals present in seawater can accumulate to dangerous levels in marine biota. Bioconcentration refers to direct transfers of the chemical from the surrounding environmental medium into the animal – it does not account for the uptake by ingestion [11, 13, 34]. For a fish, bioconcentration of a substance in the water includes direct uptake from water through its gills. Some chemical pollutants can bioaccumulate in fatty tissues or bind to muscle tissue of fish and shellfish [11, 13]. Even very low concentrations of these pollutants in the water or sediment can result in fish or shellfish tissue concentrations high enough to pose health risks to consumers. A bioconcentration factor (BCF) can be measured but must be evaluated under controlled situations to avoid indirect uptake through the food chain, since it is the ratio of chemical concentration in the animal to chemical concentration in the water only [35, 36]. Generally, fish can accumulate toxic elements from the contaminated water, ingestion of suspended solids from water, ingestion of food material, adsorption through tissue or skin, and the lipophilic tissues like gills. Some of these elements, like Cu, Fe, Co and Zn are important for fish growth and metabolism but can be toxic when their concentrations increase and exceed the toxicity threshold [25]. However, non-essential elements, such as Cd, As, Hg and Pb are not only poisonous to aquatic organisms but also being linked to human health problem even at low concentration [25]. However, many factors may impact metal uptake and accumulation like sex, age, size, reproductive cycle, swimming pattern, feeding behavior, and geographical location. Besides, different affinity of metals to fish tissues, varying uptake, deposition and excretion rates cause the difference of bioaccumulation in the fish body. The bio-concentration factor (BCF) was calculated in the present study to understand the accumulation levels of each metal using the Equation below [1,10, 37]:

$$BCF = \frac{Cm}{Csw} \quad (1)$$

where: C_m is the metal concentration in marine organism (mg/kg dry weight) and C_{sw} is the same metal concentration in the seawater (mg/L).

Estimated daily intake

The estimated daily intake, estimated daily intake (EDI) depends on the metal content in species, fish consumption and body weight. According to FAO (2020) the estimated consumption of sea food per capita in Albania was 8.68 kg/year, giving about 23.8 g/day. Estimated daily intake, EDI, was calculated as:

$$EDI = \frac{C \times Cons.}{Bw} \quad (2)$$

where: *C* is metal concentration (mg/kg) in biota species; Cons. Is the average daily consumption of sea food; *Bw* is the average body weight of adult people in Albania. Obtained values were compared with dietary reference intake values (DRI) for essential and nontoxic elements and with risk reference values, for toxic elements [38, 39, 40].

Health hazard assessment

The non-carcinogenic effect, expressed as target hazard quotient (THQ) and defined as the ratio of the potential exposure to a substance and the level at which no adverse effects are expected, was determined following the Equation:

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

$$Intake = \frac{C_m \times IR \times EF \times ED}{BW \times AT} \quad (4)$$

where: C_m is the metals concentration in biota sample, (mg/kg ww); *IR* is the ingestion rate (taken as 0.0278 kg wet wt./day for Albanian [29]; *EF* is the exposure frequency (taken as 365 days·y^{-1}); *ED* is the exposure duration (assumed as 30 years); BW is the body weight (taken as 70 kg); *AT* is the averaging time (period over which exposure is averaged in days and assumed as 10950 days). The reference dose values (*RfD*) for metals were: Pb (0.0035), Mn (0.14), Zn (0.3), Cu (0.04), Cd (0.001), Cr (0.003), Ni (0.02), Fe (0.7), Zn (0.3) mg·kg^{-1}·day^{-1} [17].

A THQ value below 1 indicates no adverse effect for human health; if THQ is greater than 1, then adverse health effects are possible. Moreover, considering that exposure to more pollutants may cause cumulative and/or interactive risk effects, based on United States Environmental Protection Agency (USEPA) suggestions [35], the combined hazard quotient (CHQ) was calculated according to the Equation:

$$CHQ = \sum_{k=1}^{n} THQ \quad (5)$$

where: *n* = 1, 2,…, n is the individual HQ for the studied inorganic elements.

RESULTS AND DISCUSSION

Concentration of metals and daily intake assessment

Table 1 shows results regarding the content of heavy metals in marine biota species, expressed in mg/kg ww. The obtained results revealed that concentration of studied metals followed the order: Fe > Zn > Al > Cu > Mn > Ni > Cr > Cd > Pb. Descriptive statistics (Table 2) showed that Fe varied between 1.92–176 mg/kgww while Pb from < 0.001 to 0.026 mg/kgww.

The highest content of Fe was found in the liver and muscle of *Anguilla anguilla* species, 176 and 133 mg/kg respectively, not exceeding the values recommended by FAO/WHO and PNEC value. These values correspond to 4.2 and 3.2 mg/day Fe (daily intake) if a quantity of 23.8 g of eel is consumed, giving about 23.2 and 17.6% of the dietary reference intake for Fe (18 mg/day), respectively. DI for the remaining species ranged from 0.3–7.0% of the DI.

The highest content of Cu was found in *Sepia officinalis* species, 6.37 mg/kg respectively, not exceeding the values recommended by FAO/WHO as well as PNEC value. This value corresponds to 150 μg/day Cu, giving about 16.7% of the recommended DRI for Cu (900 μg/day). DI for the remained species ranged from 1.1–5.6% of the DRI.

The highest content of Cd was found in *Squilla mantis* and *Sepia officinalis* species, being 0.73 and 0.69 mg/kg ww, respectively, exceeding the values recommended by FAO/WHO as well as the PNEC value. Given the fact that Cd is not considered an essential element, the risk reference value was used in this evaluation. Concentration of Cd corresponds respectively to 0.017 and 0.016 mg/day, if a quantity of 23.8 g of species is consumed while the RRV for Cd is 0.07 mg/day, giving about 24% and 23% of the RRV, respectively.

The highest content of Pb was found in *Scomber scombrus* species, 0.026 mg/kgww, not exceeding the values recommended by FAO/WHO (1 mg/kg) as well as the PNEC value, (0.1 mg/kg). Given the fact that Pb is not considered

Table 1. Results of heavy metals in biota samples and descriptive statistics of results (mg·kg^{-1}, w.w)

Nr.	Species	Fe	Cu	Cd	Pb	Ni	Cr	Mn	Zn	Al
1	*Merluccius merluccius*	1.92^{a}	0.32^{a}	0.074^{a}	0.006^{a}	0.299^{d}	0.325^{c}	0.42^{a}	5.02^{a}	1.45^{a}
2	*Sphyraena sphyraena*	4.52^{b}	0.22^{a}	0.058^{a}	0.015^{b}	0.205^{c}	0.278^{c}	0.11^{a}	9.05^{b}	0.48^{a}
3	*Trigla lyra*	4.86^{b}	0.44^{a}	0.068^{a}	0.015^{b}	0.222^{c}	0.290^{c}	0.76^{b}	6.17^{a}	0.90^{a}
4	*Lithognatus mormyrus*	7.02^{b}	0.36^{a}	0.071^{a}	0.015^{b}	0.378^{d}	0.360^{c}	0.94^{b}	7.39^{a}	5.57^{b}
5	*Mugil cephalus*	4.39^{b}	0.41^{a}	0.060^{a}	0.006^{a}	0.229^{c}	0.261^{c}	0.38^{a}	5.04^{a}	1.09^{a}
6	*Sparus aurata*	6.61^{b}	0.31^{a}	0.063^{a}	0.017^{b}	0.356^{d}	0.359^{c}	0.85^{b}	7.97^{a}	2.90^{b}
7	*Dentex dentex*	6.05^{b}	0.21^{a}	0.088^{a}	0.017^{b}	0.301^{d}	0.408^{c}	0.77^{b}	5.30^{a}	3.52^{b}
8	*Solea vulgaris*	5.92^{b}	0.81^{b}	0.105^{a}	0.007^{a}	0.291^{d}	0.366^{c}	0.99^{b}	4.46^{a}	2.96^{b}
9	*Octopus vulgaris*	9.28^{b}	1.28^{b}	0.110^{a}	<0.001	0.719^{e}	0.278^{c}	0.56^{a}	5.83^{a}	5.43^{b}
10	*Diplodus vulgaris*	53.7^{c}	0.43^{a}	0.117^{a}	0.004^{a}	0.260^{d}	0.410^{c}	1.11^{b}	8.89^{b}	2.12^{b}
11	*Dicentrarchus labrax*	7.27^{b}	0.40^{a}	0.097^{a}	0.002^{a}	0.280^{d}	0.489^{c}	1.03^{b}	7.42^{b}	1.83^{b}
12	*Mullus surmuletus*	2.65^{a}	0.27^{a}	0.095^{a}	0.022^{b}	0.124^{b}	0.047^{a}	0.18^{a}	4.63^{a}	0.44^{a}
13	*Pagellus erythrinus*	3.25^{a}	0.31^{a}	0.119^{a}	<0.001	0.195^{c}	0.098^{a}	0.37^{a}	3.97^{a}	0.65^{a}
14	*Sepia officinalis*	2.02^{a}	6.37^{c}	0.693^{c}	<0.001	0.117^{b}	0.048^{a}	0.25^{a}	5.20^{a}	0.38^{a}
15	*Parapenaeus longirostris*	7.07^{b}	1.76^{b}	0.158^{a}	<0.001	0.133^{b}	0.213^{b}	0.90^{b}	5.21^{a}	22.5^{c}
16	*Squilla mantis*	3.25^{a}	2.27^{b}	0.727^{c}	<0.001	0.292^{d}	0.052^{a}	0.53^{a}	5.70^{a}	0.81^{a}
17	*Mytilus galloprovincialis*	6.64^{b}	0.52^{a}	0.219^{a}	<0.001	0.287^{d}	0.144^{b}	2.53^{c}	6.11^{a}	5.96^{b}
18	*Anguilla anguilla*	133^{c}	0.80^{b}	0.064^{a}	0.003^{a}	0.059^{a}	0.052^{a}	0.34^{a}	6.87^{a}	0.53^{a}
19	*Anguilla anguilla liver*	176^{c}	0.27^{a}	0.112^{a}	0.013^{b}	0.107^{a}	0.035^{a}	0.59^{a}	9.47^{b}	0.58^{a}
20	*Loligo vulgaris*	2.26^{a}	1.24^{b}	0.232^{b}	0.015^{b}	0.129^{b}	0.037^{a}	0.44^{a}	6.85^{a}	0.43^{a}
21	*Sardina pilchardus*	6.96^{b}	0.51^{b}	0.170^{b}	0.008^{a}	0.177^{c}	0.035^{a}	0.45^{a}	7.07^{a}	0.58^{a}
22	*Conger conger*	3.94^{b}	0.31^{a}	0.103^{a}	0.005^{a}	0.104^{a}	0.052^{a}	0.18^{a}	6.51^{a}	0.62^{a}
23	*Scomber scombrus*	4.18^{b}	0.36^{a}	0.133^{a}	0.026^{b}	0.184^{c}	0.035^{a}	0.22^{a}	5.23^{a}	1.13^{a}
24	*Sardinella aurita*	5.15^{b}	0.75^{a}	0.158^{b}	0.010^{a}	0.190^{c}	0.053^{a}	0.50^{a}	5.59^{a}	0.97^{a}
FAO/WHO		434.8	20	0.5	1.0	30	4.4	12	50	64.5
PNEC		1040	6.99	0.16	0.11	23.7	2.35	143.8	na	na
Dietary reference intake, DRI* (mg/day) Risk reference value, RRV** (mg/d).		18*	1.0*	0.07**	0.42**	1.4	0.025*	1.8*	8*	9.7*

DRI is the daily intake level of element to sufficiently meet the nutrient requirements of nearly (97–98%) of the healthy population. RRV value represents. *Values for adults; **Values for adults.

a, b, c, d, e – significant differences between the same element in different species ($p < 0.05$). The same letter indicates the absence of significant differences ($p > 0.05$).

an essential element, the risk reference value is used in this evaluation. Concentration of Pb corresponds to 0.62 μg/day Pb while the RRV for Pb is 0.42 mg/day, giving about 1.4×10^{-4} % of the RRV.

The highest content of Ni was found in *Octopus vulgaris* species, 0.719 mg/kg, not exceeding the values recommended by FAO/WHO as well as the PNEC value. Given the fact that Ni is not considered an essential element, the risk reference value is used in this evaluation. The concentration of Ni corresponds to 0.017 mg/day Ni, while US EPA recommends a 1.4 mg/day as a Risk Reference Value for Ni (for an average of body weight of 70 kg). On the basis of the obtained results, this value gives 1.2% of the RRV. The highest content of Cr was found in *Dicentrarchus labrax* species, 0.489 mg/kg, not exceeding the values recommended by FAO/WHO as well as the PNEC value. Given the fact that only Cr(III) is considered an essential element, the DRI value is used in this evaluation. Concentration of Cr corresponds to 11.6 μg/day while US EPA recommends a maximum quantity of 35 μg/day, (for an average of body weight of 70 kg). On the basis of the obtained results, this value gives about 33.2% of the maximum allowed value of DRI. The highest content of Mn was found in *Mytilus galloprovincialis* species, 2.53 mg/kg respectively, not exceeding the values recommended by FAO/WHO as well as the

Table 2. Descriptive statistics of metals concentration (mg/kg)

Variable	N	Mean	StDev	Minimum	Median	Maximum
Fe	24	19.5	43.3	1.92	5.53	176
Cu	24	0.872	1.281	0.210	0.424	6.37
Cd	24	0.162	0.175	0.0576	0.108	0.727
Pb	24	0.011	0.007	0.001	0.011	0.026
Ni	24	0.235	0.134	0.0588	0.2137	0.7191
Cr	24	0.197	0.154	0.0349	0.1785	0.4890
Mn	24	0.641	0.499	0.110	0.516	2.528
Zn	24	6.29	1.50	3.97	5.97	9.47
Al	24	1.83	1.73	0.378	1.03	5.96

PNEC value. This value corresponds to 0.055 mg/day Mn, giving about 2.39% of the recommended dietary reference intake for Mn (2300 μg/day). DRI for the remaining species ranged from 0.17–1.08%.

The highest content of Zn was found in *Anguilla anguilla* liver, *Sphyraena sphyraena* and *Diplodus vulgaris*, with 9.47; 9.05 and 8.90 mg/kg respectively, not exceeding the values recommended by FAO/WHO as well as the PNEC value. This value corresponds to 0.225; 0.215 and 0.212 mg/day Zn, respectively, giving about 2.05% of the recommended dietary reference intake for Zn (11000 μg/day). The percentage of DRI for the remaining species ranged from 1.0–1.7%. The highest content of Al was found in *Mytilus galloprovincialis* species, 5.96 mg/kg respectively, not exceeding the values recommended by FAO/WHO. This value corresponds to 0.142 mg/day Al, giving about 1.44% of the dietary reference intake for Al (9870 μg/day). DRI for the remaining species ranged from 0.11–0.85%.

Bioconcentration factors of metals in biota species

BCFs were calculated as the ratio of metal concentration in biota species, (mg/kg) to average metal concentration in sea water. The obtained results are presented in Table 3 and in the graph of Figure 2.

The results showed that mean BCFs of metals in selected species followed the order: Fe > Cu > Mn > Cd > Zn > Al > Cr > Ni > Pb which is different compared to the order of metals concentration in biota samples. Actually, Fe and Cu are essential elements being also naturally present in an organism's muscle. Species like *Anguilla Anguilla* and *Sepia officinalis* showed high values of bioaccumulation factors for Fe and Cu, respectively while *Mytilus galloprovincialis* exhibited the highest BCF for Mn. In fact, aquatic species appeared to have specific affinity for the bioaccumulation of substances from aquatic environment, depending on their characteristics, metabolism and habitat conditions.

Evaluation of health risk, THQ and CHQ

Total hazard quotient values, THQ, were calculated as a cumulative effect of metals concentration (except Al, for which there are not RfD values) in marine biota species. Results are presented in Table 4 and in Figure 4. Among the analyzed metals, Cd was characterized by higher values of THQ in species Sepia officinalis and Squilla mantis, (respectively THQ = 0.236 and 0.247). As it can be seen, the combined total hazard quotient, CHQ, in selected species has resulted CTHQ < 1, assuming no health risk hazard to human health.

Cluster analysis and principal component analysis

Cluster analysis was conducted to evaluate similarities between samples and metals. According to the obtained results, it was concluded that metals were grouped in four main clusters:

- Cluster I – Cu and Cd; Even though Cu is usually found naturally in living organisms, the presence of Cd in the same group also assumes anthropogenic origin of the elements.
- Cluster II – Ni, Cr, Al and Mn. In cluster II there are metals having mainly anthropogenic origin, deriving from human activities;
- Cluster III – elements found naturally in living organisms like Fe and Zn;
- Cluster IV – Pb. This element is clustered in a separate group mainly because it was found in very low concentration in selected species, in some cases below the detection limit of the method (Fig. 5).

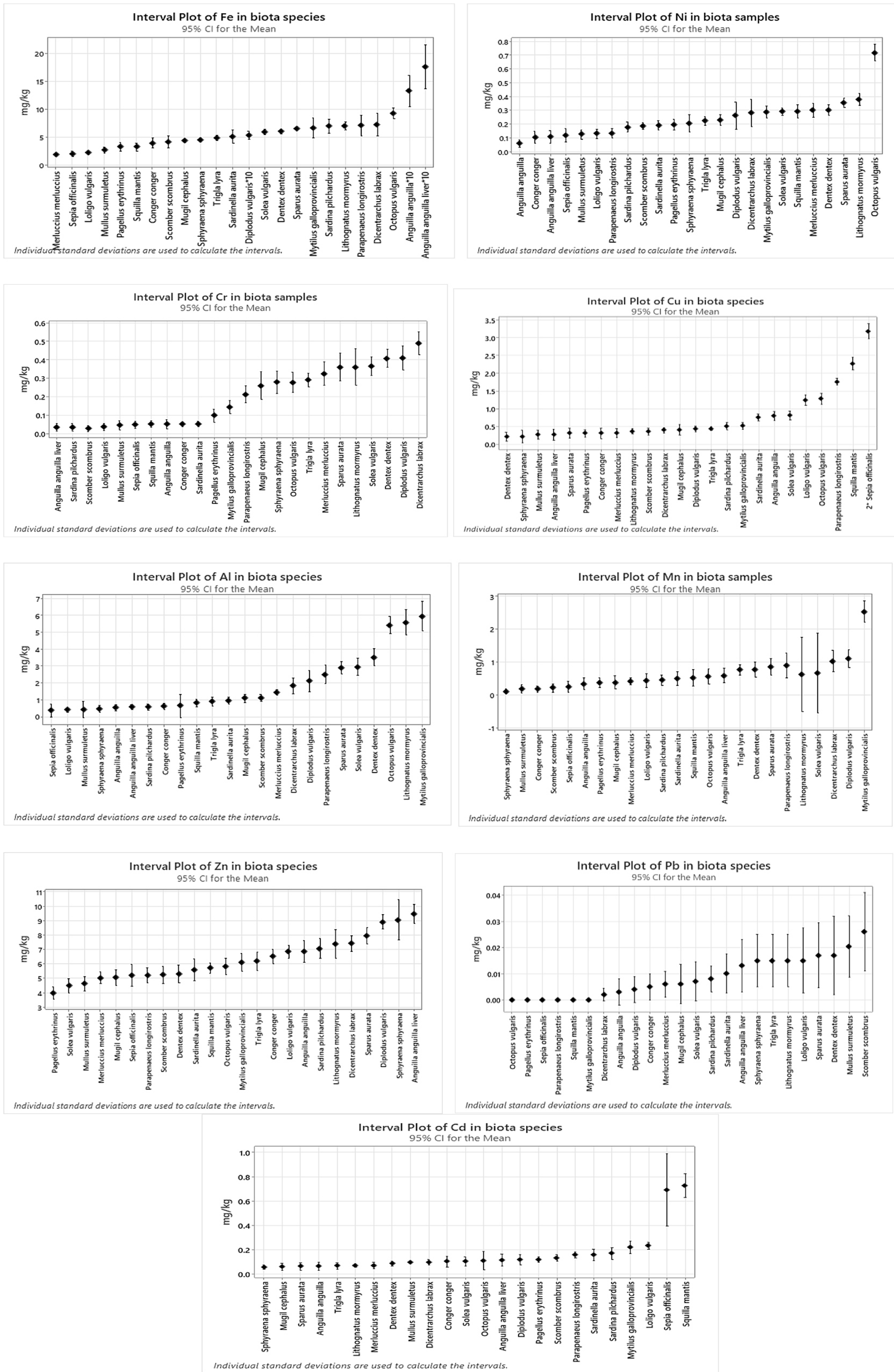

Figure 2. Interval plots of heavy metals concentration in biota samples

Table 3. Bio-concentration factors of metals

Species	Fe	Cu	Cd	Pb	Ni	Cr	Mn	Zn	Al
Merluccius merluccius	960	1285	670	184	535	1082	2100	1024	725
Sphyraena sphyraena	2259	868	524	501	367	927	550	1848	240
Trigla lyra	2432	1780	622	511	396	967	3800	1259	450
Lithognatus mormyrus	3510	1449	646	513	675	1199	4700	1509	2785
Mugil cephalus	2194	1658	542	205	409	870	1900	1029	545
Sparus aurata	3306	1225	576	572	635	1197	4250	1627	1450
Dentex dentex	3023	839	800	573	537	1359	3850	1081	1760
Solea vulgaris	2960	3250	953	246	519	1220	4950	910	1480
Octopus vulgaris	4642	5113	1003	33	1284	926	2800	1189	2715
Diplodus vulgaris	**26857**	1731	1061	150	465	1367	5550	1815	1060
Dicentrarchus labrax	3635	1611	886	68	501	1630	5125	1515	915
Mullus surmuletus	1325	1062	866	729	221	157	915	944	219
Pagellus erythrinus	1623	1234	1083	17	348	328	1833	810	327
Sepia officinalis	1012	**25485**	**6303**	17	209	160	1231	1061	189
Parapenaeus longirostris	3534	7045	1438	17	237	709	4503	1063	**11244**
Squilla mantis	1623	9064	**6607**	17	521	172	2657	1163	405
Mytilus galloprovincialis	3322	2075	1987	17	513	482	**12641**	1247	2980
Anguilla anguilla	**66289**	3188	578	110	105	172	1725	1402	267
Anguilla anguilla liver	**88202**	1084	1014	442	191	116	2941	1932	288
Loligo vulgaris	1130	4948	2112	511	229	125	2198	1398	213
Sardina pilchardus	3481	2044	1550	276	316	118	2269	1443	290
Conger conger	1969	1240	936	162	186	173	876	1329	312
Scomber scombrus	2088	1426	1211	854	328	117	1110	1066	567
Sardinella aurita	2573	2982	1436	350	339	177	2500	1140	487

Regarding selected samples, four main clusters were evident, respectively:

- Cluster I – most of the samples – similar concentration of metals.
- Cluster II– Sepia officinalis – because of the high concentration of Cd found in this species.
- Cluster III – Diplodus vulgaris; Anguilla anguilla; anguilla anguilla liver – exhibiting high concentration of Fe (Fig. 6)

PCA is one of the multivariate methods that use eigenvalue analysis of the correlation matrix

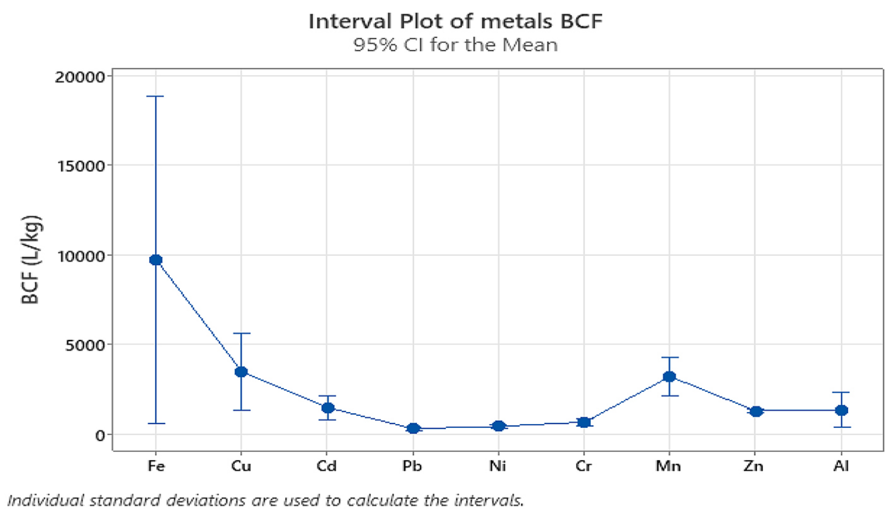

Figure 3. Variation of BCF values for metals

Table 4. THQ and CHQ results

Species	Fe	Cu	Cd	Pb	Ni	Cr	Mn	Zn	CHQ
Merluccius merluccius	0.001	0.003	0.025	0.001	0.005	0.037	0.001	0.006	0.078
Sphyraena sphyraena	0.002	0.002	0.020	0.001	0.003	0.032	0.000	0.010	0.071
Trigla lyra	0.002	0.004	0.023	0.001	0.004	0.033	0.002	0.007	0.076
Lithognatus mormyrus	0.003	0.003	0.024	0.001	0.006	0.041	0.002	0.008	0.090
Mugil cephalus	0.002	0.003	0.020	0.001	0.004	0.030	0.001	0.006	0.067
Sparus aurata	0.003	0.003	0.021	0.001	0.006	0.041	0.002	0.009	0.087
Dentex dentex	0.003	0.002	0.030	0.001	0.005	0.046	0.002	0.006	0.095
Solea vulgaris	0.003	0.007	0.036	0.001	0.005	0.041	0.002	0.005	0.100
Octopus vulgaris	0.005	0.011	0.037	0.000	0.012	0.032	0.001	0.007	0.105
Diplodus vulgaris	0.026	0.004	0.040	0.000	0.004	0.046	0.003	0.010	0.134
Dicentrarchus labrax	0.004	0.003	0.033	0.000	0.005	0.055	0.003	0.008	0.111
Mullus surmuletus	0.001	0.002	0.032	0.002	0.002	0.005	0.000	0.005	0.051
Pagellus erythrinus	0.002	0.003	0.040	0.000	0.003	0.011	0.001	0.004	0.065
Sepia officinalis	0.001	0.054	0.236	0.000	0.002	0.005	0.001	0.006	<u>0.305</u>
Parapenaeus longirostris	0.003	0.015	0.054	0.000	0.002	0.024	0.002	0.006	0.107
Squilla mantis	0.002	0.019	0.247	0.000	0.005	0.006	0.001	0.006	<u>0.287</u>
Mytilus galloprovincialis	0.003	0.004	0.074	0.000	0.005	0.016	0.006	0.007	0.116
Anguilla anguilla	0.065	0.007	0.022	0.000	0.001	0.006	0.001	0.008	0.109
Anguilla anguilla liver	0.085	0.002	0.038	0.001	0.002	0.004	0.001	0.011	0.145
Loligo vulgaris	0.001	0.011	0.079	0.001	0.002	0.004	0.001	0.008	0.107
Sardina pilchardus	0.003	0.004	0.058	0.001	0.003	0.004	0.001	0.008	0.082
Conger conger	0.002	0.003	0.035	0.000	0.002	0.006	0.000	0.007	0.055
Scomber scombrus	0.002	0.003	0.045	0.002	0.003	0.004	0.001	0.006	0.066
Sardinella aurita	0.003	0.006	0.054	0.001	0.003	0.006	0.001	0.006	0.080

Table 5. Unrotated factor loadings and communalities

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9
Fe	-0.182	-0.498	**0.723**	0.065	-0.083	-0.317	0.269	0.111	-0.000
Cu	-0.506	**0.716**	0.188	-0.281	0.076	0.042	0.262	-0.186	-0.079
Cd	-0.526	**0.714**	0.208	-0.210	0.236	0.037	-0.081	0.236	0.086
Pb	-0.009	-0.607	-0.478	-0.234	0.560	-0.023	0.172	0.058	-0.024
Ni	**0.740**	0.348	-0.108	-0.346	-0.086	-0.399	-0.123	0.089	-0.098
Cr	**0.787**	0.010	-0.052	-0.294	-0.289	0.361	0.257	0.106	0.035
Mn	**0.647**	0.274	**0.409**	0.398	0.332	0.234	-0.007	0.072	-0.101
Zn	0.154	-0.514	**0.627**	-0.455	0.123	0.168	-0.240	-0.103	0.005
Al	**0.849**	0.327	0.108	0.067	0.248	-0.216	0.084	-0.153	0.136
Variance	2.8944	2.2068	1.4159	0.7584	0.6604	0.5234	0.3228	0.1643	0.0536
% Var	0.322	0.245	0.157	0.084	0.073	0.058	0.036	0.018	0.006

to estimate the correlation structure of multidimensional data set variables. Every variable has a loading that indicates how much it adds to the significant variation in the data and helps to understand the relationships between the variables. Higher number PC components that only partially account for variance are practically ignored. In this study, a PCA bi-plot was used to assess the relationship between metals in the muscles of organisms (Fig. 2). While the first three factors account for almost 85% of the overall variance, the first two PCA components only explain 56.7% of the entire variation. Ni, Cr, Mn, and Al dominated PC1, with loadings of 0.74, 0.79, and 0.65.

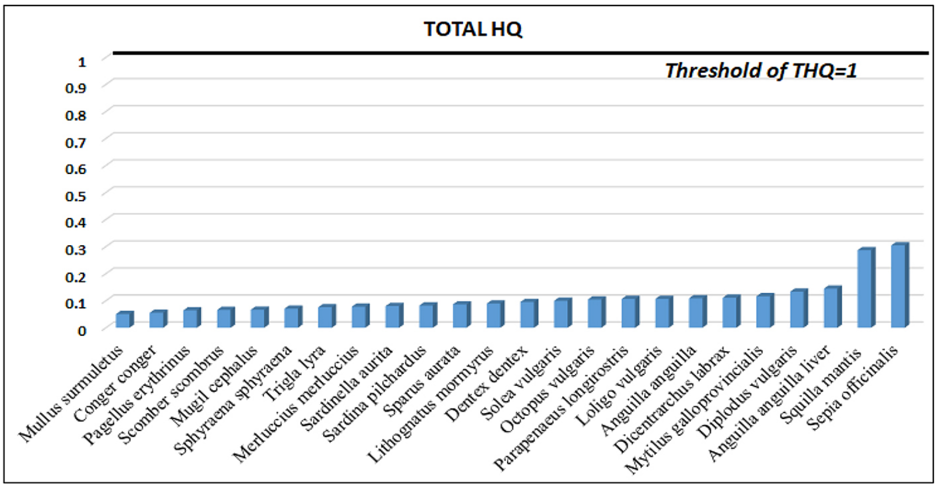

Figure 4. THQ values in selected species

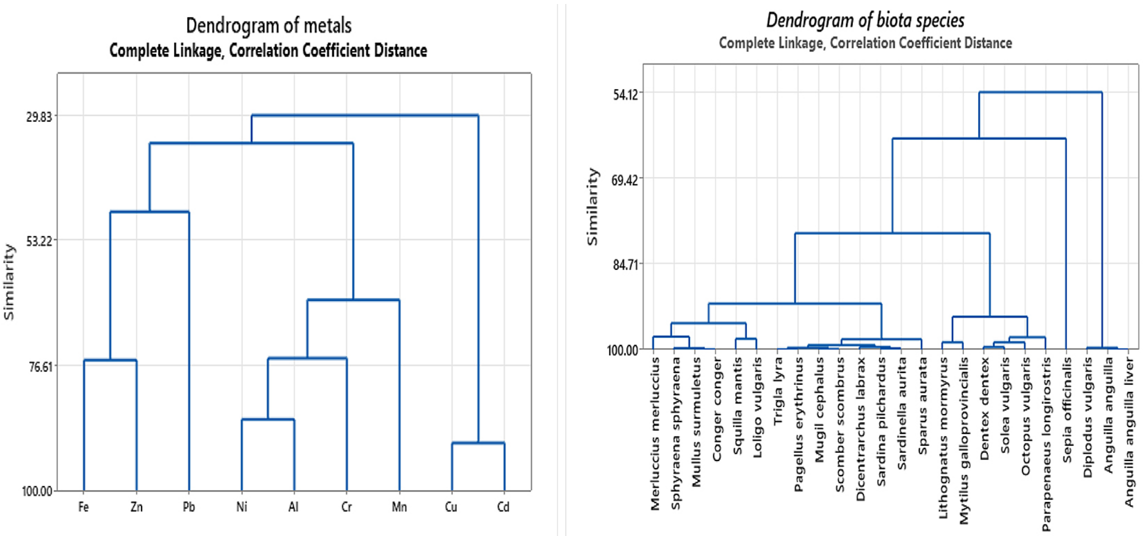

Figure 5. Cluster analysis of metals and biota samples

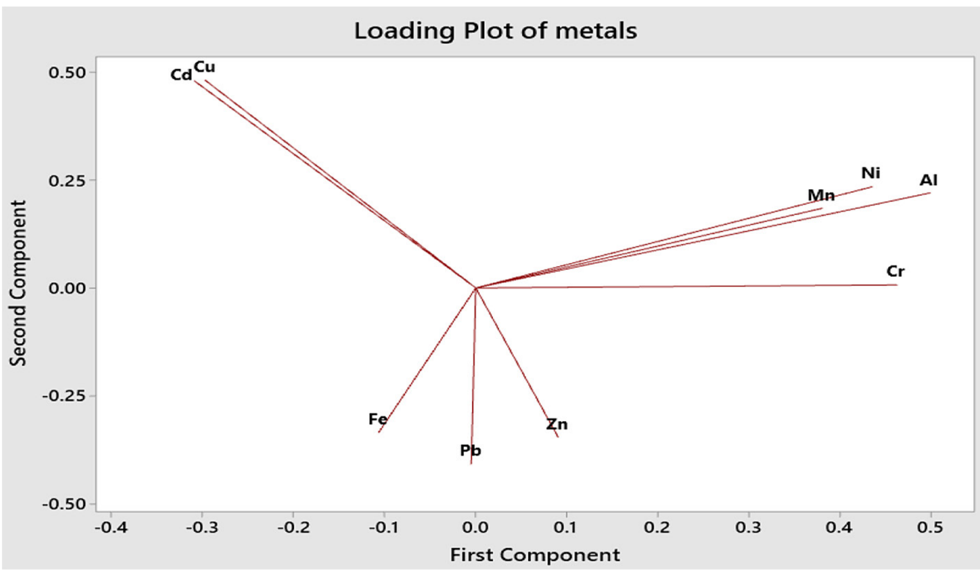

Figure 6. Principal component analysis

On the other hand, PC2 showed a strong correlation with both Cd and Cu (loading 0.71 and 0.71, respectively). Furthermore, it was discovered that Zn and Fe have representation in PC3 (loading 0.63 and 0.72, respectively). Since the amounts of Cu and Cd exceed or are near the legal limits in certain situations, it may be concluded from the analysis of the PCA data that they originated from comparable sources, namely anthropogenic origins. Although Fe and Zn are elements that occur naturally in living things, partial Mn contribution in PC3 (loading 0.49), which is also assumed to have an artificial origin, has demonstrated parallels. The elements that account for most of the variance (PC1), Cr, Ni, Mn, and Al, are thought to have comparable origins in biota species and are primarily acquired by anthropogenic inputs.

CONCLUSIONS

In the present study, the quality of marine biota species being mostly consumed by humans was assessed by determining the concentration of some heavy metals and their possible toxicity.

The obtained results revealed that Cd was the only element exceeding the recommended value according to FAO/WHO in species *Squilla mantis* and *Sepia officinalis*, accounting for about 24% of the Risk Reference Value. The concentration of the remaining elements was below the maximum allowed values. Daily intake of essential elements, including Fe, Cu, Zn and Cr resulted in 23%; 16.7%; 2.05% and 32% of the Daily Recommended Intake, respectively.

Daily intake of toxic elements like Cd, Pb, Ni, Mn and Al resulted in 24%; 0.0001%, 1.2%; 2.39 and 1.44% of the Risk Reference Value, respectively. Aquatic species appeared to have specific affinity for the bioaccumulation of substances from aquatic environment, depending on their characteristics, metabolism and habitat conditions. Some elements exhibited high accumulation degree like Fe, Cu, Mn and Cd, considering the different order in BCF values compared to metals concentration. Cluster analysis and PCA revealed that even though some elements are naturally found in living organisms, contribution of anthropogenic sources can increase metal concentrations in biota species. Low values of target hazard quotient (THQ < 1) had shown that the consumption of mussels containing heavy metals would not cause significant health risks to humans.

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