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Human exposure to heavy metals and possible public health risks via consumption of mussels *M. galloprovincialis* from the Albanian sea cost

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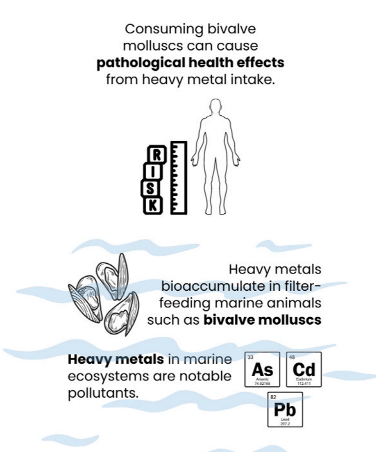
G R A P H I C A L A B S T R A C T



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A B S T R A C T

Heavy metals in the marine environment are significant contaminants that readily bioaccumulate in the tissues of aquatic organisms, particularly in filter-feeding animals such as bivalve molluscs. Human exposure to elevated concentrations of heavy metals, including essential elements such as Fe, Cu, and Zn, through the consumption of seafood can lead to various pathological effects. Research has demonstrated that among bivalve molluscs, mussels are the most effective indicators for monitoring marine pollution. Consequently, this study focused on the species *Mytilus galloprovincialis* to evaluate the levels of Al, As, Cd, Cr, Cu, Fe, Mg, Ni, Pb, and Zn in the two primary harvesting areas of Albania and to assess the associated human health risks from mussel consumption. The results revealed a concerning situation, particularly for Pb and Cd, with average concentrations of 2.15 μg/g and 4.14 μg/g, respectively, significantly exceeding the limits established by Regulation (EC) No. 915/2023. The levels of the other investigated elements also raised concerns, as only half of them were within the dietary intake values recommended by scientific authorities for weekly consumption of 250 g of mussels.

1. Introduction

Heavy metals (HMs) are defined as metallic elements with a density greater than water (Fergusson, 1990). They represent significant contaminants in marine environments, readily assimilated and bioaccumulated in hydrobionts' tissues (Jitar et al., 2015), resulting in concentrations several orders of magnitude higher than those in the surrounding water (Casas et al., 2008). Aquatic filter-feeding organisms, particularly bivalve molluscs, exhibit significant metal absorption due to their nutritional acquisition through water filtration. The process involves metal uptake by the apical membrane, subsequent transfer to the circulatory system, intracellular movement, interaction with ligands, and efflux across the basal membrane (Deb and Fukushima, 1999). Certain metals such as Cd, Hg, and Pb are considered non-essential for bivalve molluscs, posing harmful effects even at low concentrations (Sanz-Prada et al., 2022), while essential metals like Fe, Cu, and Zn can induce toxicity at elevated levels (Besada et al., 2014; Esposito et al., 2021; Türkmen et al., 2008).

The deposition and subsequent accumulation of heavy metals in aquatic environments can pose hazards to human health (Anandkumar et al., 2019; Patchaiyappan et al., 2023; Prabakaran et al., 2024). Prolonged exposure to metals or elevated concentrations thereof may result in detrimental impacts encompassing dermatological ailments, acute and chronic intoxications, neural, haematological, and gastrointestinal dysfunctions, respiratory impairments, as well as manifestations of mutagenicity and carcinogenicity (Chiesa et al., 2018; Martin and Griswold, 2009). To safeguard public health, the European Commission (EC) has established Maximum Levels (MLs) for Cd (1 mg/kg) and Pb (1.5 mg/kg) in bivalve molluscs, and for Hg (0.5 mg/kg) in seafood, as stipulated by Regulation (EC) No. 1881/2006, now repealed by Regulation (EC) No. 915/2023, which maintains the same limits.

Among bivalve molluscs, mussels are primary indicators of marine environment contamination (Besada et al., 2014; Çevik et al., 2008; Cunha et al., 2017; Sanz-Prada et al., 2022; Li et al., 2019). Extensive studies on metal bioaccumulation by mussels have established their efficacy as bioindicators (Rainbow and Phillips, 1993), particularly the species *Mytilus galloprovincialis*, which is renowned for its capacity to filter large volumes of water, adaptability to varying environmental conditions, and immobility (Belivermiş et al., 2016). Additionally, mussels are recognised as valuable dietary sources rich in omega-3 fatty acids and proteins, contributing to improved human health (Carboni et al., 2019). The European Food Safety Authority (EFSA) recommends a daily intake of 250 mg for combined marine-based long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (EFSA, 2010). Mussels also provide high-quality protein with an amino acid score comparable to eggs (Venugopal and Gopakumar, 2017), along with essential nutrients like iron and vitamin B12.

Recent statistical data reveals a notable increase in annual world production of bivalve molluscs, marking an increment of over 100% from 8.2 to 17.5 million tons during the period spanning 1995 to 2018. Within Europe, Spain leads as the foremost producer, yielding 287,000 tons, succeeded by France and Italy with 145 and 93 thousand tons, respectively (EUMOFA, 2022). The dominant species under cultivation in the European Union is the Mediterranean mussel (*Mytilus galloprovincialis*), contributing to 61% of the total production (EUMOFA, 2022).

In Albania, mussel production is concentrated in Butrinti Lagoon and Shengjin, contributing to the local economy. However, rapid developments in tourism, agriculture, and industrial activities in Albania have raised concerns about increased heavy metal levels in marine environments. Given that heavy metals are non-biodegradable and can accumulate to toxic levels, monitoring their concentrations in bivalve molluscs is crucial (Jović and Stanković, 2014). Scientific research and governmental monitoring programs play pivotal roles in assessing coastal pollution levels of heavy metals and trace elements.

Despite these efforts, there is limited current knowledge about the status of the Albanian coastline and its marine organisms, with outdated scientific literature and inaccessible monitoring reports. Therefore, expanding research in this area is essential to provide comprehensive data to stakeholders, including consumers, particularly in light of Albania's status as an EU candidate country since June 2014.

Consequently, the primary aims of this study were: (1) to evaluate the levels of Al, As, Cd, Cr, Cu, Fe, Mg, Ni, Pb, and Zn in mussels collected from major production sites in Albania, providing an updated assessment of heavy metal contamination in the region; and (2) to assess the potential health risks posed by mussel consumption through the estimation of target hazard quotient (THQ) and hazard index (HI) values and by comparing weekly metal exposures with Provisional Tolerable Weekly Intakes (PTWI) established by Joint FAO/WHO Expert Committee on Food Additives (JECFA).

2. Experimental section

2.1. Study area

The primary source of mussels (*Mytilus galloprovincialis*) collection originates from the Butrinti Lagoon, recognised as an optimal production site due to its suitable levels of salinity and temperature. Butrinti Lagoon spans an area of 16.3 km^2 with an average depth of 14 m and a maximum depth of 21 m (Cullaj et al., 2005). It is located near the city of Saranda in the southern part of the Ionian Sea (39.47° N, 20.1° E), opposite Corfu Island. The secondary source of mussel collection occurs in the Mediterranean Sea, specifically in the city of Shengjin, located 7 km west of Lezha (41.4849° N and 19.3538° E), (Fig. 1).

2.2. Sample collection

During the period of June to September 2023, a total of 67 batches of mussels were collected from the Butrinti Lagoon, and 44 batches were obtained from Shengjin, resulting in a combined total of 111 samples with an average weight of 45.9 g (MIN:26 g; MAX:80.5 g). The sampling protocol involved the collection of fresh mussels (not subjected to depuration treatments) at distances ranging from 0.5 to 1 km from the shoreline, encompassing various depth intervals: 1, 2, 2.5, 3.5, 5, and 6.5 m for the Butrinti Lagoon, and 1, 2, 2.5, and 5 m for the Shengjin region. Upon collection, all specimens were promptly rinsed and washed with seawater sourced from their respective collection sites to eliminate any particles and sediments. Subsequently, the mussels were immediately transported to the Element Analysis Laboratory of İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Department of Biophysics, under refrigerated conditions in a cold box maintained at 3 ± 1 °C. Upon arrival, all samples were stored at −80 °C until the time of analysis.

2.3. Apparatus

The concentrations of Al, total As (As_{tot}), Cd, Cr, Cu, Fe, Mg, Ni, Pb, and Zn in mussel tissues underwent analysis using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), (Thermo iCAP 6000 series), at the Element Analysis Laboratory of İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Department of Biophysics (Istanbul, Turkey). For inorganic arsenic (As_{in}), this study assumed that the typical proportion of As_{in} in mussels is 10% of the total arsenic content, as reported in previous studies (Sloth and Julshamn, 2008). A summary of the instrument parameters is provided in Table 1. Tissue samples underwent a three-step processing procedure in accordance with wet digestion protocols for element measurements: initially, each tissue sample was weighed, followed by digestion with a combination of nitric acid (2 mL) and perchloric acid (1 mL) in a furnace. After cooling to room temperature, the samples were rinsed with distilled water up to a total volume of 10 mL (Gunay Ucmak et al., 2023; Marenzoni et al., 2021). The performance of *ICP-OES* method was verified based on the obtained limits of detection (LODs) and quantification (LOQs), extraction recoveries, relative standard deviations (RSD), and linear ranges, (Table S1).

Element concentrations were measured at various wavelengths, as summarised in Table 2, and the results were expressed in μg/g.

2.4. Calibration standards

The calibration standards were prepared by diluting monoelemental stock standard solutions (1000 μg/mL) of Al, As, Cd, Cr, Cu, Fe, Mg, Ni, Pb and Zn. Internal quality controls for each analyte were obtained from Chem-Lab NV (Belgium) in the form of inorganic solutions traceable to NIST-certified reference materials. Tissue samples from all groups were analysed and compared. Each analysis was conducted in triplicate, and the levels of elements were averaged. All element levels were expressed as micrograms per gram of tissue (μg/g).

Table 1
ICP-OES instrumentation parameters.

Parameters	Assigned value
Nebulizer	V-Groove
Plasma Argon flow rate	15 L/min
Argon carrier flow rate	0.5 L/min
Nebulizer argon flow rate	0.7 L/min
Sample flow rate	1.51 L/min
The speed of peristaltic pump	100 rpm
RF Power	1.3 kW

Table 2
Wavelengths used for analysed elements with ICP-OES.

Elements	Wavelenghts (nm)
Aluminium (Al)	167.079
Arsenic (As)	189.042
Cadmium (Cd)	228.802
Chromium (Cr)	267.716
Copper (Cu)	324.754
Iron (Fe)	259.940
Magnesium (Mg)	202.582
Nickel (Ni)	341.476
Lead (Pb)	220.353
Zinc (Zn)	213.856

2.5. Estimated daily and weekly intake (EDI and EWI)

Provisional tolerable weekly intake (PTWI) is the accepted weekly intake of trace and toxic metals from seafood samples, set by the joint FAO/WHO expert committee (JECFA, 2003) to ensure no adverse health effects. PTWI values represent the permissible safe levels for metals in seafood samples. To make a comparison, the estimated daily intake (EDI) and then the estimated weekly intake (EWI) were calculated using Eqs. (1) and (2), respectively. If the EWI is lower than the PTWI, consuming fishery products poses an insignificant health risk (Police et al., 2021).

$$EDI\left(\frac{mg}{kg - day}\right) = \frac{mMC\ (mg/kgww)x\ Dfc}{BW\ (kg)} \tag{1}$$

$$EWI\left(\frac{mg}{kg - week}\right) = \frac{mMC\ (mg/kgww)x\ Wfc}{BW\ (kg)} \tag{2}$$

Where *mMC* is the mean metal concentration, *Wfc* is the average daily (Eq. (1) and weekly (Eq. (2)) consumption of sea food per capita in

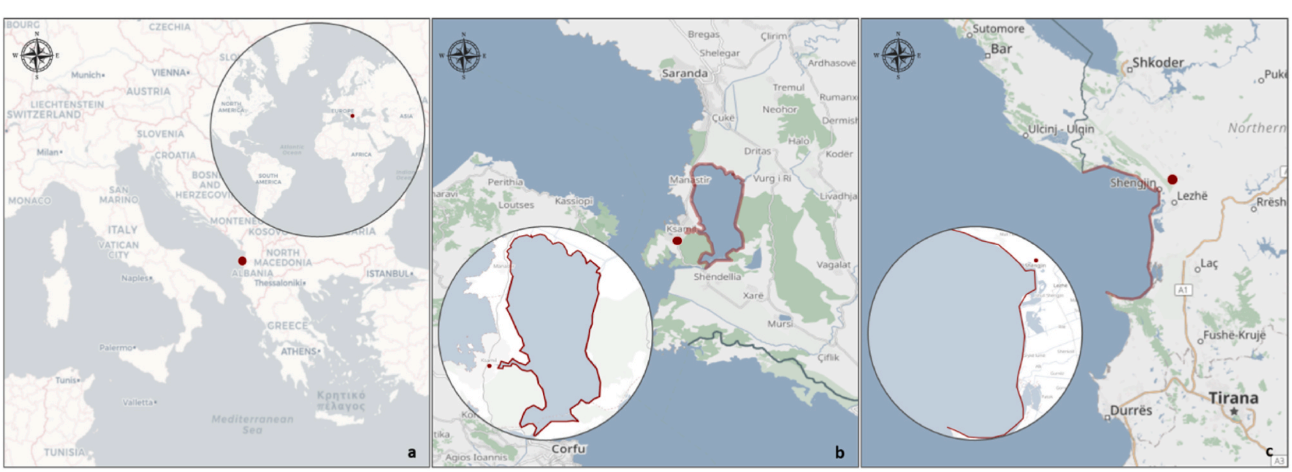

Fig. 1. Study area map, a) geographical location of the country Albania; b) sample collection location: Butrinti Lagoon and b) Sample collection location: Shengjin.

Albania (Haka et al., 2024); *BW* is the average body weight for an adult, assumed to be 70 kg.

2.6. Health hazard assessment: target hazard quotient (THQ) and hazard index (HI)

The Target Hazard Quotient (THQ) is a parameter used in health risk assessments to evaluate the intake of non-carcinogenic contaminants. It was calculated using Eq. (3), while the intake of these contaminants was determined using Eq. (4). Regarding the hazard index (HI) was expressed as the sum of THQs for a series of chemicals of interest (Police et al., 2021). A THQ value below 1 indicates no adverse effects on human health, whereas a THQ value greater than 1 suggests that adverse health effects are possible.

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

$$Intake\ \left(mg\ kg^{-1} day^{-1}\right) = \frac{mMCxIRxEFxED}{BWxAT} \quad (4)$$

Where the *mMC* is the mean metal concentration (mg/kg ww), *IR* refers to the ingestion rate, which is assumed to be 0.0278 kg of wet weight per day for the Albanian population, *IR* refers to the ingestion rate, which is assumed to be 0.0278 kg of wet weight per day for the Albanian population, according to Haka et al. (2024). *EF* stands for the exposure frequency, taken as 365 days per year. *ED* denotes the exposure duration, which is assumed to be 65 years, according to Police et al. (2021). *BW* is the body weight, set at 70 kg. *AT* is the averaging time, which is the period over which exposure is averaged, assumed to be 23, 725 days (365 days·year^{-1} × *ED*). *Rfd* is the heavy metal oral intake reference dose (mg kg^{-1} day^{-1}). *Rfd* for Al, As, Cd, Cr, Cu, Fe, Ni, Pb and Zn is 1.0, 0.0003, 0.001, 0.003, 0.04, 0.7, 0.02, Pb 0.004 and 0.3 mg/kg·day^{-1}, respectively (Burger et al., 2015; Haka et al., 2024; Police et al., 2021).

2.7. Statistical analysis

Descriptive statistics was used to present data as means, standard deviations (SD), medians, minimum and maximum, first (Q1) and third (Q3) quartile, absolute and relative frequencies. The effect of the site (2 levels) and sampling depth (2 levels: ≤2 m and >2 m), as well as their interactions, were assessed using generalised linear models with Tweedie (1.5) distribution. The associations between elements were evaluated using Pearson's correlation coefficient (r). The correlation was considered poor if r< |0.3|, medium if |0.3| ≤ r < |0.5|, and large if r ≥ |0.5| (Field et al., 2009). Statistical analyses were performed with SPSS 25.0 (SPSS Inc., Chicago, IL, USA), and statistical significance occurred when $p < 0.05$.

3. Results and discussion

The coastal Adriatic region represents a significant potential natural resource due to the presence of eight lagoon ecosystems, most of which are subject to intensive fishery and/or shellfish exploitation (Peja et al., 1996). Historically, it has been regarded as a heavily polluted sea because of its morphology and dynamics (Ianni et al., 2000). In this context, Table 3 presents descriptive statistics alongside the outcomes of inferential models of heavy metal contamination in mussels collected along the Albanian coast. Mussels sampled from Shengjin exhibited higher levels of Al, As, Cr, Fe, Mg, Pb, and Zn compared to those from Butrint ($p < 0.001$; Fig. 2). Additionally, concentrations of Cu, Fe, and Zn in mussels collected at both sites showed variations based on depth (interaction effect: $p < 0.05$; Table 3). Moreover, mussels from both locations highlighted Cadmium and Lead concentrations surpassing the maximum limits established by the Reg. (EC) No. 915/2023 for these metals in bivalve molluscs. Specifically, all samples exceeded the maximum Cd limit of 1 mg/kg, while non-compliant Lead concentrations (≥1.5 mg/kg) were observed in 88.63% of mussels from Shengjin and 19.40% of those from Butrint. In this regard, data from the literature indicate considerable variability in the contamination levels of heavy metals in the marine ecosystem along coastal areas (Corsi et al., 2011; Tursi et al., 2011). This variability suggests that the concentrations of elements are affected by multiple factors (Lazo et al., 2018; Sanz-Prada et al., 2022), although human activities play a crucial role. Cullaj et al. (2005) stated that in Albania, many aquatic ecosystems are compromised, and the state of water pollution poses a real risk to human health and the economy. Additionally, a recent input of Cr, Ni, Cu, and Mn was observed as a result of mining and industrial activities in selected areas such as Durres, Drin Bay, and Vlora Bay (Rivaro et al., 2011). Subsequent research confirmed this situation, showing rather high levels of metals in mussels at both sites. In fact, the values were well above those reported in other studies for seafood collected in the Adriatic Sea. As shown in Table 4, a retrospective survey conducted by Tavoloni et al. (2021) on *M. galloprovincialis* collected along the Marchigiana coast (Italy) as part of food safety monitoring plans from 2008 to 2018 found that the average concentrations of Cd, Pb, As, Cr, and Ni were several times lower than in our study. Similarly, lower values than ours were found in the study by Jović and Stanković (2014), which analysed the concentrations of Fe, Cu, Zn, Ni, Cd, and Pb in wild and farmed mussels from seven sites in Boka Kotorska Bay (Montenegro), (Table 4). The same evidence also emerges when analysing data from Albania's National Monitoring Programme on heavy metal concentrations in mussel samples taken in Durres and Vlora (Albania) during the period 2001–2005 (Cullaj et al., 2005). The study by Chiesa et al. (2018), which analysed mussels and clams from five different FAO areas (Chile; Thailand and Vietnam; New Zealand; northern Spain, northern France, and Portugal; and the Mediterranean Sea), also confirmed this situation. The levels of Pb, Cd, Ni, As, and Cr reported in their study were lower

Table 3
Descriptive statistics and results of GLM. Concentrations (μg/g), expressed as means, standard deviation (SD), median, first (Q1) and third (Q3) quartile of the analysed metals in mussel samples (n = 111).

Metal	Descriptive statistics							Test of model effects (p value)		
	Mean	SD	Minimum	Maximum	Median	Q1	Q3	Site	Depth	Site x depth
Al	365.23	455.80	17.52	2678.62	201.38	65.27	480.62	<0.001	0.054	0.123
As_{tot}	84.45	53.01	22.65	216.10	59.44	43.14	124.98	<0.001	0.272	0.932
As_{in}	8.44	5.30	2.26	21.61	5.94	4.31	12.49			
Cd	4.14	2.81	1.14	21.61	3.63	2.37	4.97	0.628	0.662	0.051
Cr	5.78	4.32	1.55	31.09	4.38	3.13	6.63	<0.001	0.929	0.443
Cu	20.95	11.16	2.38	61.33	18.85	13.66	27.16	0.203	0.017	0.010
Fe	1145.56	1502.24	160.02	9856.58	511.25	347.80	1344.93	<0.001	0.319	0.024
Mg	15526.46	7128.35	6621.24	50646.62	13763.91	10685.01	17860.14	<0.001	0.762	0.509
Ni	20.21	16.34	1.74	69.17	14.51	6.71	32.97	0.158	0.852	0.947
Pb	2.15	2.36	0.04	21.11	1.56	0.93	2.93	<0.001	0.455	0.423
Zn	284.14	195.18	79.31	1329.87	222.94	169.81	313.87	<0.001	0.013	<0.001

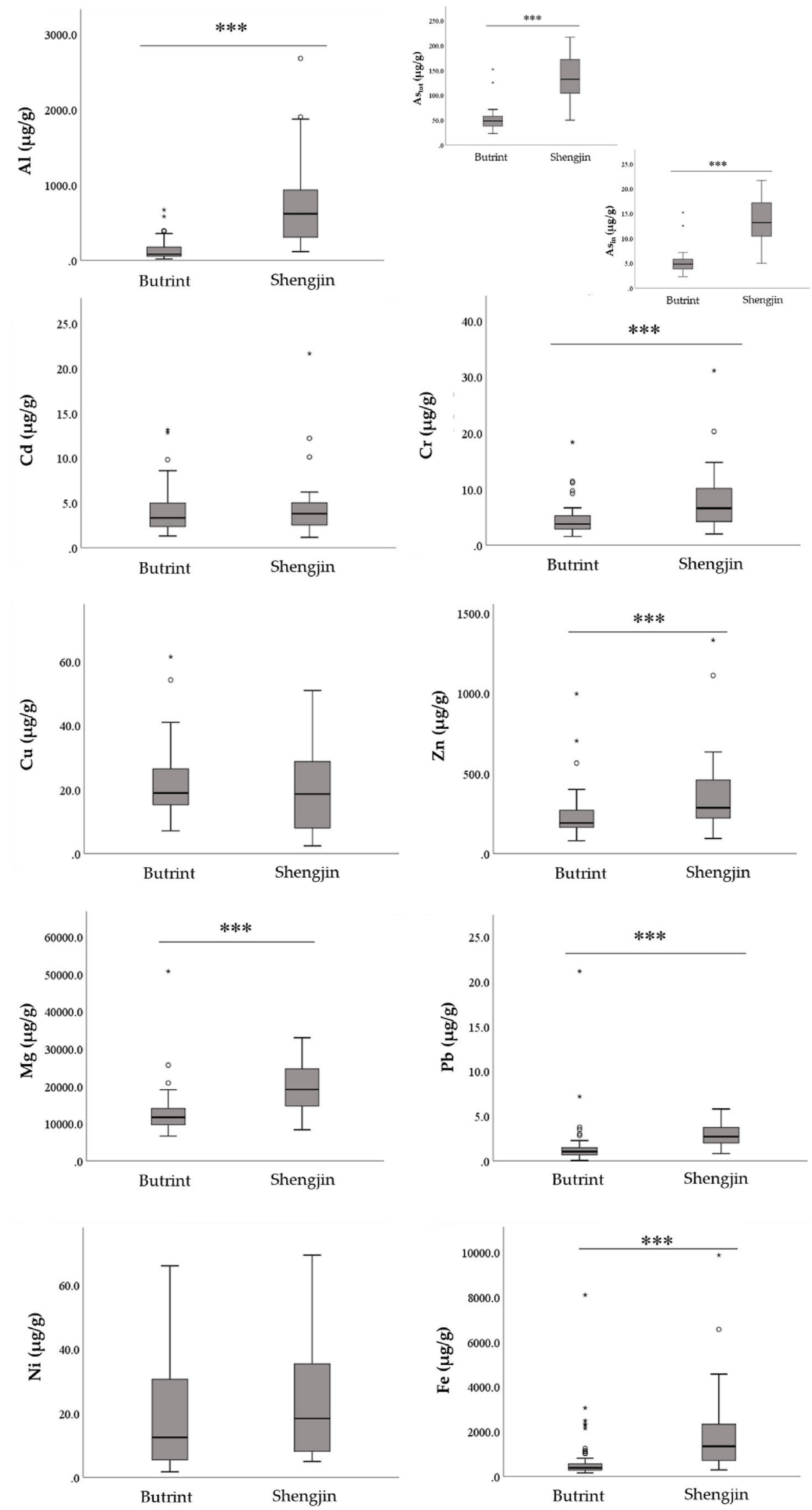

Fig. 2. Marginal means of the concentrations of metals in mussel samples according to the sampling site (Butrint: n = 67; Shengjin: n = 44) and results of the pairwise comparisons. ***P < 0.001.

Table 4
Comparison of average metal concentrations (μg/g) in mussels with those reported in other.

Al	As_{tot}	As_{in}	Cd	Cr	Cu	Fe	Mg	Ni	Pb	Zn	
724.72[a]	136.80[a]	13.68[a]	4.34[a]	7.91[a]	19.77[a]	1827.16[a]	19956.53[a]	22.67[a]	2.86[a]	360.05[a]	Present study value
129.15[b]	50.06[b]	5.00[b]	4.01[b]	4.38[b]	21.72[b]	697.94[b]	12617.17[b]	18.60[b]	1.62[b]	234.28[b]	
	3.50		0.15	0.27				0.48	0.19		Tavoloni et al. (2021)
			0.30		0.92	0.39		0.77	0.65	19.17	Jović and Stanković (2014)
			0.47[e]	5.81[e]	11.73[e]	771.55[e]		4.20[e]	3.41[e]	145.63[e]	Cullaj et al. (2006)
	5.04		0.29	0.051				0.96	0.23		Chiesa et al. (2018)
341.34[c]	1.41[c]		0.08[c]	0.26[c]	2.32[c]	212.03[c]	1150.79[c]	0.32[c]	0.14[c]	24.21[c]	Meloni et al. (2022)
96.01[d]	1.05[d]		0.04[d]	0.12[d]	3.08[d]	96.57[d]	1038.14[d]	0.11[d]	0.05[d]	21.69[d]	

[a] Shengjin site (n = 44).
[b] Butrint site (n = 67).
[c] Harvested in February.
[d] Harvested in May.
[e] Dry weight.

than those found in the present study (Table 4). In the recent study by Meloni et al. (2022), which assessed the concentrations of 24 elements in Mediterranean mussels (Calich Lagoon, Sardinia, Italy), the values were all lower than ours, the concentration of Ni in mussels harvested in May 2019 was found to deviate the most significantly from our results.

In more detail on our results, a different degree of contamination between the two areas under study also emerges. In fact, as many as seven out of ten investigated elements had higher average concentrations in the batches from the region of Shengjin compared to those from Butrint lagoon; only Cd, Cu, and Ni recorded similar concentrations between the two sites (Fig. 1). Particularly affected by the sampling site were the contents of Al, As, and Fe, with concentration differences of 5.7, 2.7, and 2.6 times higher in batches from the Shengjin region, respectively. This means that in this sampling area, higher quantities of heavy metals are present in the water column on average, regardless of the natural or anthropogenic origin of the elements (Sanz-Prada et al., 2022; Cullaj et al., 2006; Besada et al., 2011; Lazo et al., 2018). In fact, metals are normally present in the marine environment depending on the geology of the terrain. However, local emission sources stemming from anthropogenic activities can exponentially increase their concentration. In Albania, activities in ex-industrial sites of the copper, chromium, iron-nickel, and oil industries have produced 70 million tons of industrial waste, impacting the surrounding environment and posing adverse effects on natural resources, followed by potential health risks for people continuously exposed to this pollution. This critical situation is mostly connected with the direct discharge of untreated wastewaters originating from industry, agriculture, and domestic effluents into rivers, which serve as a route for these toxic elements to be discharged directly into the sea. Industrial pollution sources include makers of cement, leather, ceramics, and textiles, as well as mines, smelters, oil and gas producers, and wood processing facilities (UNEP, 2000). The areas of the sea most affected by human activities are precisely the coastal areas where bivalve molluscs are bred and harvested, particularly in stretches where watercourses flow. During the past decades in Albania, mining, enrichment, and metallurgy industries have produced high quantities of solid or liquid waste, often dumped on riverbanks or directly into rivers (Cullaj et al., 2005). Several studies have focused on the levels of heavy metals and trace elements in different rivers in Albania (Corsi et al., 2011; Haka et al., 2024; Rivaro et al., 2004; Tursi et al., 2011). In almost all the studies, high levels of heavy metals and trace elements have been noticed. In a research by Abazi and Astrit (2008), the amount of Cu, Cr, Pb, and Ni was especially high and at toxic levels in the Fan river, a consequence of mine and industrial discharges. Due to the geology of the zone, large areas of central Albania are covered by serpentine soils, which are highly enriched in heavy metals such as Cr, Cu, Ni, Fe, and Zn (Selenica et al., 2011). In another study by Abazi et al. (2013), high concentrations of Cu, Pb, and Cr were found in the waters of the Mati and Gjanica rivers due to geological and geochemical structures in the Northcentral area and south-central part of Albania, as well as anthropogenic activity. In a study by Accornero et al. (2004) on the determination of metal pollution in surface marine waters and nearshore areas in Durres, Albania, hot spots of land-based heavy metal pollution were identified. Highly populated areas were also adjacent to the Seman and Drin rivers, which exhibited the highest concentrations of trace metals (Accornero et al., 2004). Therefore, the situation of Albanian watercourses may have implications for the heavy metal content recorded in the present study, also considering that the Drin, Mati, and Fan rivers flow into the Lezha region, where the highest metal levels were found. However, specific investigations on the cause/effect relationship are needed to establish the contribution of Albanian rivers to the amount of metals in mussels collected along the coast. From a public health standpoint, the most alarming results concern the levels of Pb and Cd, for which the European Union, by the Regulation (EC) No. 915/2023, has set maximum limits (MLs) of 1.5 mg/kg and 1 mg/kg, respectively. Additionally, the levels of As, Cr, Ni, and Al are of concern, for which scientific authorities have defined other parameters.

The calculated EWI values in this study, as presented in Table 5, were found to be well above the PTWI values, indicating a health risk associated with the ingestion of the studied mussels.

In this regard, concerning As, EFSA, based on epidemiological studies on exposure to this metal through water and food, suggested a range rather than a single reference point. This range includes the benchmark dose lower confidence limit for a 0.1% increased incidence ($BMDL_{01}$) of skin lesions and cancer of the lung, skin, and bladder, ranging between 0.3 and 8 μg/kg of body weight/day (EFSA, 2009a, 2009b). For Cr, in 2014, EFSA suggested a Tolerable Daily Intake (TDI) of 300 μg/kg of body weight/day (EFSA, 2014). For Ni, in 2020, EFSA recommended a TDI of 13 μg/kg of body weight/day, obtained from studies on the dose-response curve of the incidence of litters with a post-implantation loss in rats (EFSA, 2020). Finally, for Al, EFSA (2008) recommended a PTWI of 1 mg/kg of body weight, which corresponds to 70 mg of Al in a week for an adult weighing 70 kg.

46.85% of the mussel batches had Pb concentrations above 1.5 mg/kg, with an average of 2.15 mg/kg and a maximum value of 21.11 mg/kg detected at Butrint (Table 3). This site, however, was less polluted with Pb, as the non-compliant samples were 19.40%, compared to 88.63% in Shengjin. Only 9 batches of Butrint were negative, while for Shengjin no batch was negative. Pb is a harmful metal, considered a probable carcinogen by the International Agency for Research on Cancer (IARC), which has no biological function (Stankovic and Jovic, 2012). When introduced into the body, it causes damage to the liver, kidneys, bones, and brain, particularly in children, where it leads to learning deficits and reduced IQ. For these reasons, the EFSA defined a dietary intake value of 0.63 and 1.50 μg/kg of body weight/day for nephrotoxic and cardiovascular effects in adults, respectively (EFSA, 2010). Considering these thresholds and the concentrations found in the mussels analysed in the current study, it emerges that weekly consumption of 108.0 g of mussels harvested in Shengjin is sufficient to reach the

Table 5
Results of estimated daily and weekly intake, and comparison with PTWI.

Metals	PTWIa (mg/week/kg BW)	PTWIa (mg/week/70 kg BW)	Shengjin site		Butrint site	
			EWIb (mg/week/70 kg BW)	EDIc (mg/day/70 kg BW)	EWIb (mg/week/70 kg BW)	EDIc (mg/day/70 kg BW)
Al	1	70	1723.384	246.198	307.119	43.874
As	0.015	1.050	32.531[a]	4.647[a]	11.904[a]	1.701[a]
Cd	0.007	0.490	10.321	1.474	9.536	1.362
Cr	0.023	1.631	18.810	2.687	10.416	1.488
Cu	3.5	245	47.013	6.716	51.650	7.379
Fe	5.6	392	4344.986	620.712	1659.701	237.100
Mg			47456.628	6779.518	30003.630	4286.233
Ni	0.035	2.450	53.909	7.701	44.231	6.319
Pb	0.025	1.750	6.801	0.972	3.852	0.550
Zn	7	490	856.199	122.314	557.118	79.588

a Provisional tolerable weekly intake.
b Estimated weekly intake.
c Estimated weekly intake.
[a] As_{in}.

reference dietary intake values indicated by EFSA (2010) for nephrotoxic (PTWI: 0.309 mg/person/week) effects (PTWI: 0.735 mg/person/week) (Table 4). This means that even an average consumption (125 g/week) of mussels collected in Shengjin exposes the consumer to lead levels exceeding 100% of PTWI for chronic kidney disease (Table 6). The situation in Butrint is better, where only high consumers of mussels exceed 100% of PTWI for nephrotoxic effect (Table 6). In the study by Jović and Stanković (2014), who evaluated human exposure to trace metals via consumption of mussels from 5 countries of the Adriatic coastal area, the amount of Pb ingested through the consumption of mussels by high-level mussel consumers never reached 100% of the permissible weekly doses indicated. Therefore, it is our opinion that the situation found is rather worrying for any level of consumer, especially for those consuming high amounts of mussels in the Shengjin area, which in the long term may have nephrotoxic effects.

For Cd, the situation is even more serious, with 100% non-compliant batches (>1 mg/kg) for both sites and a maximum level of 21.61 mg/kg recorded in Shengjin (Table 3). With these concentrations, PTWI of 7 μg/kg b. w. set by EFSA (2009b), corresponding to 0.49 mg of Cd in one week for an adult weighing 70 kg, is reached with a meal of 112.9 g of mussels from Shengjin and 122.2 g of mussels from Butrint (Table 6). Therefore, at both sites, even average mussel consumers are always exposed to weekly Cd concentrations above 100% of the PTWI (Table 6).

In other areas of the Adriatic coast, the highest recorded weekly Cd exposure was 44.18% of PTWI and was only reached by high mussel consumers in Croatia, where the highest Cd levels (0.61 mg/kg) were found among the 5 Adriatic coast countries investigated (Jović and Stanković, 2014).

Several authors reported that increased concentrations of cadmium in sediments observed in different areas of the Adriatic Sea were related to an increasing percentage of carbonate fraction (Cosma et al., 1979; Martinčić et al., 1990). High Cd concentrations were also found in Drin Bay sediments, rich in organogenic material (Rivaro et al., 2004).

Certainly, the most critical situation concerns As_{tot}, for which in Butrint, the PTWI is reached with a weekly consumption of 78.3 g of mussels, while in Shengjin, it is as low as 28.7 g. This means a very high degree of exposure for mussel consumers in these areas, particularly the high-end ones, who, with 250 g/week of mussels harvested in Shengjin, reach 871.1% of the PTWI. However, when considering only As_{in}, the values decrease by an order of magnitude, and the PTWI is never exceeded (Table 6). Regarding Cr, although the concentrations in mussels detected in this study were several times higher than those reported in other research (Chiesa et al., 2018; Meloni et al., 2022; Tavoloni et al., 2021; Cullaj et al., 2006), the average values for both sites were lower than 21 mg/day Cr intake for an adult person of 70 kg, calculated from the TDI value of 300 μg Cr/kg of body weight/day (EFSA, 2014). Ni was also detected at relatively high average concentrations at both sites; however, it did not expose mussel consumers to intake levels exceeding 100% of the 0.91 mg/day Ni intake for an adult weighing 70 kg, calculated from the TDI value of 13 μg Ni/kg of body weight/day (EFSA, 2020) (Table 6) and thus to potential toxic effects on their health. Al was the element with the greatest difference in concentration between the two sites, with mussels from Shengjin being 5.7 times more contaminated than those from Butrint. Consequently, even with an average consumption of mussels from Shengjin, the PTWI established by EFSA (2008) is exceeded by more than 1.3 times. Aluminium occurs naturally in the environment but also as a consequence of anthropogenic activities (EFSA, 2008). However, considering the differences between the two sites, it is our opinion that human activities are mainly responsible for the high Al concentrations in Shengjin mussels.

Among metals considered to be non-harmful, Fe recorded values several times higher than those reported in the literature (Arici et al., 2018; Bajc and Kirbiš, 2019; Jović and Stanković, 2014; Meloni et al., 2022; Cullaj et al., 2006), (Table 4). Fe is an essential element for the human body, but in excessive doses, it becomes detrimental to health (Jović and Stanković, 2014). For this reason, in 1983, JECFA established a provisional maximum tolerable daily intake (PMTDI) of 0.8 mg/kg of body weight, which corresponds to 392 mg/week for an adult weighing 70 kg (JECFA, 1983). Considering this value, high mussel consumers in the Shengjin area are exposed to doses of Fe that exceed the estimated PTWI (Table 4).

Cu and Zn also had rather high concentrations (Table 4), and in some cases, several times higher than those reported in other studies (Arici et al., 2018; Bajc and Kirbiš, 2019; Besada et al., 2011; Jović and Stanković, 2014; Meloni et al., 2022; Cullaj et al., 2006). However, for mussel consumers, exposure to these two metals remains below the PMTDI of 0.5 and 1 mg/kg b. w./day for Cu and Zn, respectively, established by JECFA (2008). The results for the THQ and HI reported in Table 7 indicate that at both sites, As_{in}, assumed here to constitute 10% of total arsenic, was the primary contributor to the HI for all metals (Shengjin site: THQ = 18.110; 76.23% and Butrint site: THQ = 6.627; 64.17%). For all other metals analysed - except for Cd at both sites and Cr and Fe at the Shengjin site - the THQ values were found to be < 1, indicating that no adverse health effects are anticipated from exposure. Only Asin at the Shengjin site showed a THQ value greater than 10, suggesting a higher likelihood of adverse effects. However, since the Reference Doses values incorporate uncertainty factors, a THQ value cannot be directly equated with the probability of experiencing adverse health effects (Burger et al., 2015) It is also important to consider that chemical exposures typically involve mixtures of chemicals, while risk assessments are generally conducted for individual compounds (Burger et al., 2015; USEPA, 2001). This must be taken into account when

Table 6
Average metal concentrations (μg/g) in mussels collected at the Shengjin site (n = 44) and the Butrint site (n = 67).

Shengjin site					
Metal	Mean	SD	The amount of mussels *M. galloprovincialis* (g) that would need to be consumed per week by a 70-kg adult to exceed the JECFA limits.	Mean weekly metal exposure for average (125 g/week) and high (250 g/week) level mussel consumers (mg/week) and the percentage of prescribed PTWI values	
				125 g/week	250 g/week
Al	724.72	536.48	96.6	129.4% c	258.8% c
As_{tot}	136.80	43.95	28.7	435.5% d	871.1% d
As_{in}	13.68	4.40	286.6	43.6%	87.2%
Cd	4.34	3.38	112.9	110.7% e	221.4% e
Cr	7.91	5.41	18584.1	0.7%f	1.4%f
Cu	19.77	13.04	12392.5	1.0%g	2.0%g
Fe	1827.16	1778.66	214.5	58.3%h	116.6% h
Mg	19956.53	6672.86			
Ni	22.67	16.40	281.0	44.5%i	89.0%i
Pb	2.86	2.27	108.0a	115.7% l	231.5% l
			257.0 b	48.6% m	97.3% m
Zn	360.05	239.10	1360.9	9.2%n	18.4%n
Butrint site					
Al	129.15	126.53	542.0	23.1%c	46.1%c
As_{tot}	50.06	19.94	78.3	159.6% d	319.3% d
As_{in}	5.01	1.99	782.4	16.0%d	32.0%d
Cd	4.01	2.38	122.2	102.3% e	204.6% e
Cr	4.38	2.65	33561.6	0.4%f	0.8%f
Cu	21.72	9.75	11279.9	1.1%g	2.2%g
Fe	697.94	1089.46	561.7	22.3%h	44.5%h
Mg	12617.17	5831.22			
Ni	18.60	16.22	342.5	36.5%i	73.0%i
Pb	1.62	2.83	190.7a	65.6%l	131.1% l
			453.7 b	27.6% m	55.1% m
Zn	234.28	140.99	2091.5	6.0%n	12.0%n

a Chronic renal disease: 0.63 μg/kg of body weight/day.
b Effects on the systolic blood pressure: 1.50 μg/kg of body weight/day.
c PTWI = 60 mg/person/week.
d PTWI = 3.36 mg/person/week.
e PTWI = 0.42 mg/person/week.
f PTWI = 126 mg/person/week.
g PTWI = 210 mg/person/week.
h PTWI = 336 mg/person/week.
i PTWI = 5.46 mg/person/week.
l PTWI = 0.309 mg/person/week (chronic kidney disease).
m PTWI = 0.735 mg/person/week (effects on systolic blood pressure).
n PTWI = 420 mg/person/week.

interpreting the results. The U.S. Environmental Protection Agency (Burger et al., 2015; USEPA, 2010) provides guidance for assessing the effects of chemical mixtures. In the absence of sufficient data, the USEPA also recommends "component-based approaches". When chemicals have similar mechanisms and modes of action, additivity can be assumed, and the resulting hazard index (HI) calculations may be added to achieve an estimate of the combined toxicity. In this study, the HI for both sites was >1, primarily due to the accumulated concentration of Asin at both locations, which significantly elevated the HI values,

Table 7
Target hazard quotient and hazard index for toxic metals in both sampling site.

Metals	Shengjin site	Butrint site
	THQa	THQa
Al	0.288	0.051
As_{in}	18.110	6.627
Cd	1.724	1.593
Cr	1.047	0.580
Cu	0.196	0.216
Fe	1.037	0.396
Mg		
Ni	0.450	0.369
Pb	0.284	0.184
Zn	0.477	0.310
HIb	23.612	10.326

a Target hazard quotient.
b Hazard index.

indicating the need for different forms of intervention.

Interestingly, the concentrations of Fe, Cu, and Zn also varied with sampling depth. In particular, in Shengjin, the concentrations of these metals were higher at depths greater than 2 m, while in Butrint, the highest concentrations were found at shallower depths (<2 m), (Table 3). Tavoloni et al. (2021) also detected variations in Pb and Cr concentrations related to bathymetry, with greater quantities at 6–9 m depth for Pb and at 9–12 m for Cr. In the study by Nielsen (1974), Pb, Cd, Fe, and Zn values in cultured mussels (*Perna canaliculus*) varied with sampling depth. However, at another site with greater water column movement, the same elements did not exhibit depth-related gradients (Azizi et al., 2018). Among the many natural factors that influence the amount of metals in mussels, the location of the mussels in the intertidal area can generate variations in the bioavailability of the elements (Widdows and Donkin, 1992), although few studies have considered this aspect.

Of the metals investigated in the current study, Mg was by far the element with the highest average concentrations for both sites (Table 3). This observation is in line with that reported by Meloni et al. (2022), who found that out of 24 metals detected in mussels, Mg had the highest value (mean of 1095 mg/kg wet weight). However, we cannot comment on the potential risks from these exposures, as scientific authorities have not defined a dietary intake value, Mg not being a harmful metal.

Although zinc is an essential element for human health, high levels of dietary zinc can cause serious health problems, such as stomach cramps, skin irritations, vomiting, nausea, and anaemia. Very high levels of zinc can also damage the pancreas, disturb protein metabolism, and cause arteriosclerosis (Oyaro et al., 2005). The results regarding the correlation between elements (Table 8) showed that in the areas under study, the presence of certain metals exhibited strong correlations with others, specifically, were found between Al, As, Cr, and Fe, between As, Cr, and Mg, between Cr, Cu, Mg, and Pb, between Cd and Zn, between Cr, Fe, Ni, and Pb, between Cu and Ni, and between Mg and Pb (for all: $p < 0.01$).

The waste near the industry contains heavy metals and arsenic, which have had a strong negative impact on the environment and rivers. Industrial activities in these areas have been ongoing for more than 15 years, but the leaching of arsenic and other heavy metals into the groundwater and rivers continues, causing toxic effects in the aquatic system. The high content of arsenic in soil samples located in northern Albania is mainly associated with Fe–S, Cu–S, or Ni–S mineralization. Albania is exposed to high levels of heavy metal pollution, particularly from elements linked with mining operations and mineral dumps. Generally, areas in the western part of Albania remain exposed to high levels of heavy metal pollution, mostly linked with the oil and gas industry and shipping traffic, whereas areas in the eastern part of Albania remain exposed to high levels of heavy metal pollution linked mostly with mineral operations, mineral dumps, and the mineral processing industry (Lazo et al., 2018).

Table 8
Pearson correlation coefficient (r) between metal (μg/g).

	As	Cd	Cr	Cu	Fe	Mg	Ni	Pb	Zn
Al	**0.642**[a]	0.157	**0.850**[a]	0.300[a]	**0.746**[a]	0.420[a]	0.491[a]	0.378[a]	0.260[a]
As		0.250[a]	**0.510**[a]	0.128	0.408[a]	**0.653**[a]	0.243[b]	0.419[a]	0.330[a]
Cd			0.349[a]	0.330[a]	0.242[b]	0.353[a]	0.359[a]	0.406[a]	**0.587**[a]
Cr				0.473[a]	**0.829**[a]	0.407[a]	**0.592**[a]	**0.507**[a]	0.366[a]
Cu					0.411[a]	0.200[b]	**0.504**[a]	0.323[a]	0.391[a]
Fe						0.261[a]	0.490[a]	0.281[a]	0.292[a]
Mg							0.180	**0.649**[a]	0.305[a]
Ni								0.164	0.182
Pb									0.331[a]

Values in bold indicate strong correlations ($r \geq |0.5|$).
[a] Correlation is significant at the 0.01 level (2-tailed).
[b] Correlation is significant at the 0.05 level (2-tailed).

4. Conclusions

The metal concentrations detected in this study reveal a rather alarming situation. In Shengjin site, out of the 10 elements investigated, 5 exceeded the dietary intake values set by scientific authorities for a weekly consumption of 250 g of mussels. Cadmium content exceeded MRLs defined by European Regulations in 100% of the batches, and an average meal of mussels is sufficient to expose an adult weighing 70 kg to weekly cadmium levels well above the PTWI. Lead levels are also of concern, particularly in Shengjin, where high mussel consumers always exceed dietary intake values for nephrotoxic effects. Shengjin was, in fact, the site most polluted by heavy metals, with 8 out of 10 elements investigated having higher concentrations than Butrint. In this area, the degree of exposure to total arsenic for high mussel consumers is extremely worrying, exceeding the PTWI by more than 8 times.

In conclusion, we assume that the high metal content detected represents a very high health risk for mussel consumers, and the comparison with other studies from the Adriatic Coast supports this statement. The enlargement of uncontrolled industrial and domestic discharge, which is directly discharged into rivers, plays a key role in the pollution of the aquatic environment. Systematic periodic monitoring programs are required to monitor aquatic environment pollution and seafood quality in Albania.

CRediT authorship contribution statement

Enkeleda Ozuni: Writing – original draft, Investigation, Conceptualization. **Egon Andoni:** Writing – original draft, Investigation, Conceptualization. **Marta Castrica:** Writing – review & editing, Writing – original draft, Supervision, Data curation, Conceptualization. **Claudia M. Balzaretti:** Writing – review & editing, Visualization. **Gabriele Brecchia:** Writing – review & editing, Visualization. **Stella Agradi:** Writing – review & editing, Visualization. **Giulio Curone:** Writing – review & editing, Visualization. **Federica Di Cesare:** Writing – review & editing, Visualization. **Nour Elhouda Fehri:** Writing – review & editing, Visualization. **Blerina Luke:** Writing – review & editing, Visualization. **Mehmet Erman Or:** Writing – review & editing, Investigation, Formal analysis. **Esra Akkaya:** Writing – review & editing, Investigation, Formal analysis. **Oğuzhan Yavuz:** Writing – review & editing, Investigation, Formal analysis. **Laura Menchetti:** Writing – review & editing, Formal analysis, Data curation. **Lek Prendi:** Writing – review & editing, Investigation, Formal analysis. **Nural Pastacı Özsonacı:** Writing – review & editing, Investigation, Formal analysis. **Alev Meltem Ercan:** Writing – review & editing, Investigation, Formal analysis. **Fatma Ateş:** Writing – review & editing, Investigation, Formal analysis. **Dino Miraglia:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143689.

Data availability

Data will be made available on request.

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