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INTER-SPECIES VARIABILITY OF HEAVY METALS IN MARINE MOLLUSKS FROM THE ROMANIAN BLACK SEA: BIOACCUMULATION AND ECOTOXICOLOGICAL IMPLICATIONS

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This study investigates the inter-species variability in heavy metal bioaccumulation among three marine mollusk species (Mytilus galloprovincialis, Rapana venosa, and Anadara inaequivalvis) collected from the Romanian Black Sea sector in 2021. The concentrations of Cu, Cd, Pb, Ni, Cr, and Co were determined using graphite furnace atomic absorption spectrometry and expressed as total ionic content. The results indicate that most heavy metal levels were within expected natural variability ranges; however, a few cases of exceedances were observed for Cd. Pb retention was notably higher in Rapana venosa from Eforie Sud, while Cu concentrations were consistently elevated in Rapana venosa samples compared to the other two species. In contrast, Anadara inaequivalvis exhibited higher Ni and Co content, suggesting species-specific assimilation patterns. To better understand exposure pathways and uptake mechanisms, two standard metrics were applied: the bioconcentration factor to assess uptake from water, and the biota–sediment bioaccumulation factor to evaluate metal uptake from sediments. These metrics provided additional insights into the environmental availability and assimilation of toxic metals in the studied mollusks. These findings contribute to the ongoing evaluation of heavy metal contamination in the Romanian Black Sea and its ecological and human health implications.

Keywords: Romanian Black Sea, heavy metals, bioaccumulation, bioconcentration, marine mollusks, *Mytilus galloprovincialis*, *Rapana venosa*, *Anadara inaequivalvis*

1. Introduction

Heavy metals (HMs) contamination in marine environments remains a critical concern due to its potential ecological and human health risks. Marine

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organisms, particularly mollusks, are widely recognized as bioindicators of environmental pollution due to their ability to build
up contaminants from water and sediments through filter-feeding and bioaccumulation processes [1]. Among the HMs of concern, cadmium (Cd), lead (Pb), nickel (Ni), chromium (Cr), cobalt (Co), and copper (Cu) are particularly relevant due to their toxic effects, persistence in marine ecosystems, and potential for trophic transfer [2]

The Black Sea, a semi-enclosed basin with limited water exchange, is highly susceptible to anthropogenic pollution from riverine inputs, industrial discharges, and maritime activities [3]. Previous studies have shown that the Danube Delta has been severely affected by pollution, particularly with HMs and organic pollutants [4]. This is largely due to the transport of significant amounts of contaminants by the Danube River, which discharges into the Romanian Black Sea Coast (RBSC) [4]. However, the pressure of HMs pollution decreases significantly from the lower Danube towards the RBSC, supporting the conclusion that European industrial activity does not have a major impact on HMs levels in this coastal area [4]. This trend reflects the effectiveness of recent environmental regulations and the efforts made by upstream industries to reduce pollutant discharges into aquatic systems. Nevertheless, despite this overall improvement, key pollutants, such as HMs and organic compounds, still exceed regulatory thresholds near known pollution hotspots. These findings underscore the constant need for stricter regulations, enhanced regional cooperation, and more efficient waste management strategies to mitigate ongoing pollution challenges [3].

The Black Sea area that receives water and sediments from the Danube recorded low levels of HMs, suggesting that the Danube Delta system plays an important role in filtering out possible pollutants, which should be investigated in future studies [4]. The distribution of HMs in the constituents of the RBSC ecosystem highlights the differences between distinctive areas of the coastline, with slightly increased amount in the northern marine area under the influence of the Danube, while in the southern sector, the increased values, in certain areas, could be the result of various anthropogenic pressures (ports, wastewater discharges, industrial activities, seasonal tourism) [5]. Previous studies have highlighted the presence of HMs in water, sediments, and marine biota, raising concerns regarding their accumulation in commercially and ecologically important species [6–10].

Bivalve and gastropod mollusks, such as *Mytilus galloprovincialis*, *Rapana venosa*, and *Anadara inaequivalvis*, are commonly used in environmental monitoring programs due to their capacity to reflect local contamination levels [11]. These species exhibit different feeding behaviors, which influence their HMs uptake patterns, and special ecological roles. Mussels (*Mytilus galloprovincialis*) are filter feeders, accumulating contaminants from suspended particulate matter, while veined rapa whelk (*Rapana venosa)*, a predatory gastropod, accumulates metals through trophic transfer. *Anadara inaequivalvis*, a deposit feeder, can reflect sediment-associated contamination [12]. Understanding the species-specific variability in HMs accumulation in tissues is essential for assessing pollution sources, ecological risks, and potential impacts on seafood safety.

This study aims to evaluate the content of Cu, Cd, Pb, Ni, Cr, and Co in three mollusk species (*Mytilus galloprovincialis, Rapana venosa, and Anadara inaequivalvis*) collected from the RBSC in 2021, providing insights into their bioaccumulation patterns and potential health risks, emphasizing the inter-species variability in HMs content. To better understand contamination pathways and uptake mechanisms, two standard metrics were applied: the bioconcentration factor (BCF) to assess uptake from water, and the biota–sediment bioaccumulation factor (BAF) to evaluate contribution from sediments. These metrics provided additional insights into the environmental availability and assimilation of HMs in the studied mollusks, helping in emphasizing the key factors influencing HMs behavior, such as species-specific uptake, habitat conditions, and contamination sources. This research contributes to ongoing environmental monitoring efforts and supports risk assessment initiatives related to marine food safety and ecosystem health.

2. Materials and methods

2.1. Sampling and Preservation

Biota samples (*Mytilus galloprovincialis*, *Rapana venosa*, and *Anadara inaequivalvis*) were collected from the sampling transects Sf. Gheorghe (SG), Cazino Mamaia (CM), and Eforie Sud (ES) (bathymetric strip 20-30 m) during May-June 2021 in the framework of monitoring expeditions with R/V Steaua de Mare 1, using biological dredge equipment (Fig. 1). From the total catch, obtained by dredging along each transect at depths ranging from 20 to 30 meters, 5 to 10 individuals of the same species and of similar size (4-5 cm) were selected. All selected specimens were rinsed thoroughly with seawater, followed by a final wash with double distilled water to remove any external contaminants. The samples were then stored at - 20 °C until transported to the laboratory for further analyses. Water (surface horizon) and sediment samples (surface layer of 0–5 cm) were collected from the same locations and at the same time as biota. Water was sampled using Niskin bottles, while sediments were retrieved with a Van Veen grab. Both water and sediment samples were stored in acid-washed polyethylene containers and kept refrigerated on board until transport to the laboratory. Proper labeling was ensured using water-resistant markers and tracing paper to prevent loss of information [13].

2.2. Sample Processing and Digestion

Before analyses, the whole soft tissue of mollusks (composite samples of 5-10 individuals) was retained, while the shells were discarded. Tissues were then lyophilized and homogenized. The lyophilized tissues (dry mass) were weighed to ensure reproducibility of measurements. When expression of results on a wet weight (ww) basis was required (e.g., for BCF calculations), concentrations obtained on a dry weight (dw) basis were converted using a correction factor, considering the average dry substance content in mollusks of 20%. Digestion was carried out using 65 % Suprapure nitric acid (HNO_3) at 120 °C on an electric hot plate within sealed Teflon vessels to ensure complete mineralization. After digestion, the resulting solution was transferred into 100 mL volumetric flasks, ensuring complete recovery of the sample by rinsing the digestion vessel walls with deionized water. Seawater samples were acidified with HNO_3 to pH<2 and analyzed. Sediment samples were lyophilized, homogenized, and sieved to remove the coarse fraction (>2 mm), then digested with HNO_3 prior to analysis, similar with biota [14–16]. These prepared solutions were then used for subsequent chemical analyses to determine HMs concentrations.

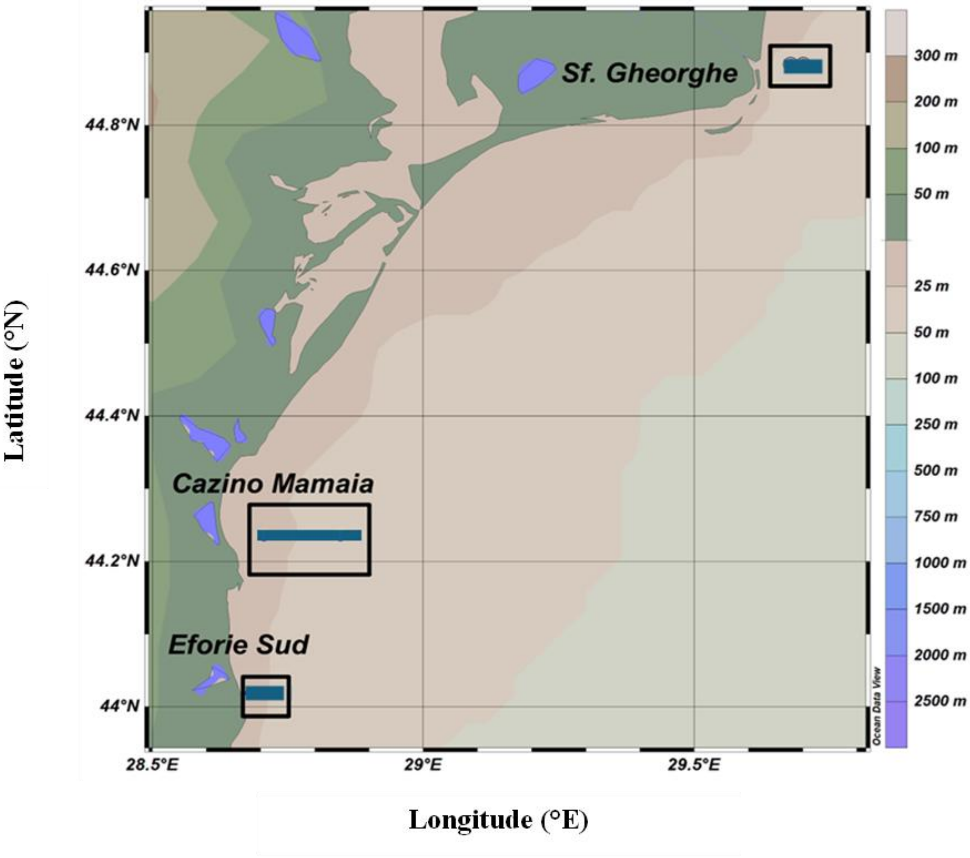

Fig. 1. Biota sampling transects along the Romanian Black Sea coast.

2.3. Analytical Determination of HMs Using Atomic Absorption Spectrometry

The determination of HMs in the prepared samples was performed using graphite furnace atomic absorption spectrometry (GF-AAS) [17]. The analysis was conducted with HR-CS ContrAA 800 G, Analytik Jena spectrometer (Jena, Germany), ensuring high sensitivity and precision in detecting metal concentrations. These instruments allow for accurate quantification of HMs in environmental and biological samples, the graphite furnace technology enhancing detection limits and minimizing matrix interferences. Following acid digestion, metals were present in solution in ionic form (as nitrates). Concentrations determined by GF-AAS therefore represent the total ionic content of Cu, Cd, Pb, Ni, Cr, and Co.

2.4. Bioaccumulation Assessment: BCF and BAF Calculations

To evaluate the accumulation potential of HMs in bivalves and gastropods, two standard metrics were applied: the Bioconcentration Factor (BCF) and the Biota–Sediment Bioaccumulation Factor (BAF). These factors offer insight into contaminant uptake from water and sediment, respectively, and are widely used in environmental risk assessment.

The BCF quantifies the extent to which an organism accumulates a contaminant directly from the surrounding water, excluding dietary intake. It was calculated using the formula:

$$BCF = \frac{Cbiota}{Cwater} \qquad (1)$$

where:

Cbiota is the amount of HM in biota (μg/kg ww);

Cwater is the amount of the same HM in seawater (μg/L).

This approach follows REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) Regulation (EC No 1907/2006) [18] and guidance provided by the European Chemicals Agency (ECHA) (ECHA, 2023) [19], which categorizes substances based on their bioaccumulative potential as :

- Non-bioaccumulative: BCF < 1000
- Bioaccumulative (B): 1000 ≤ BCF ≤ 5000
- Very Bioaccumulative (vB): BCF > 5000

The BAF evaluates the capacity of an organism to accumulate contaminants from sediments, including both direct and dietary exposure. It was calculated as:

$$BAF = \frac{Cbiota}{Csediment} \quad (2)$$

where:
Cbiota is the amount of the HM in biota (µg/g dw),
Csediment is the amount of the same HM in sediments (µg/g dw).

Unlike for BCF, REACH does not provide formal thresholds for BAF. However, the U.S. Environmental Protection Agency [20] considers that a BAF > 1 indicates, generally, significant contribution from sediments, though the interpretation is dependent on species traits and local sediment characteristics.

These factors were applied to all sampled species in this study to highlight interspecies differences and identify metals with elevated bioaccumulation potential from distinct environmental compartments.

3. Results and discussion

3.1 HMs spatial distribution

The mean, minimum and maximum concentrations of HMs in the 3 invertebrate species collected from the sampling transects Sf. Gheorghe, Cazino Mamaia, and Eforie Sud in 2021 generally fell within expected natural variation ranges (Table 1).

Table 1

Average HMs levels* in mollusk samples on each transect and their overall average, minimum and maximum values

Biota species	Transect	Cu (µg/g ww)	Cd (µg/g ww)	Pb (µg/g ww)	Ni (µg/g ww)	Cr (µg/g ww)	Co (µg/g ww)
Mytilus galloprovincialis	**SG**	0.77	0.32	0.77	1.02	1.21	0.01
Anadara inaequivalvis	**SG**	1.60	0.07	0.21	12.53	1.10	0.43
Anadara inaequivalvis	**CM**	1.80	0.17	0.09	6.80	0.72	0.18
Anadara inaequivalvis	**CM**	1.41	2.19	0.01	0.01	1.03	0.02
Mytilus galloprovincialis	**CM**	1.01	0.27	0.87	0.97	2.80	0.01
Rapana venosa	**CM**	6.02	0.11	0.07	0.10	1.36	0.01
Rapana venosa	**ES**	4.87	0.17	0.02	0.04	0.70	0.01
Rapana venosa	**ES**	6.43	0.21	1.31	0.01	1.42	0.01
Minimum		0.77	0.07	0.01	0.01	0.70	0.01
Maximum		6.43	2.19	1.31	12.53	2.80	0.43

Biota species	Transect	Cu (μg/g ww)	Cd (μg/g ww)	Pb (μg/g ww)	Ni (μg/g ww)	Cr (μg/g ww)	Co (μg/g ww)
Average		2.99	0.44	0.42	2.68	1.29	0.09

* Concentrations are expressed as total ionic forms of Cu, Cd, Pb, Ni, Cr, and Co, measured after mineralization of samples.

The measured Cd and Pb content in bivalves and gastropods harvested in 2021 were well below the maximum allowable concentrations (*MACs*) established by European regulations for human consumption (Commission Regulation (EU) 2023/915) [21]. The only exception was a single sample of *Anadara inaequivalvis* from Cazino Mamaia, which exceeded the Cd regulatory limit of 1 µg/g ww (Fig. 2).

Lead content was notably higher in a *Rapana venosa* sample from ES, followed by elevated measured values in mussels from SG and CM. In terms of copper content, all *Rapana venosa* samples exhibited higher levels compared to the other mollusk species. Conversely, *Anadara inaequivalvis* samples showed the highest levels of nickel and cobalt, indicating species-specific variations in HMs uptake (Fig. 2).

These findings highlight spatial and interspecies differences in HMs accumulation, suggesting the influence of environmental factors, habitat conditions, and different physiological pathways affecting this complex bioprocess among mollusk species (Fig. 3).

Proof of the influence of the water and sediments transported by the Danube River into the Black Sea is represented by the content of the HMs (Cu, Ni and Cr) found in mussels harvested in two locations, SG and CM (Fig. 3). Due to the local currents, induced by the surplus of water brought in the Black Sea which flows out through Bosphorus strait, the suspended solids, constituting the main feed for this bivalve, travel from SG to CM, thus contributing to the comparable levels of contaminants in the organisms living in these sites.

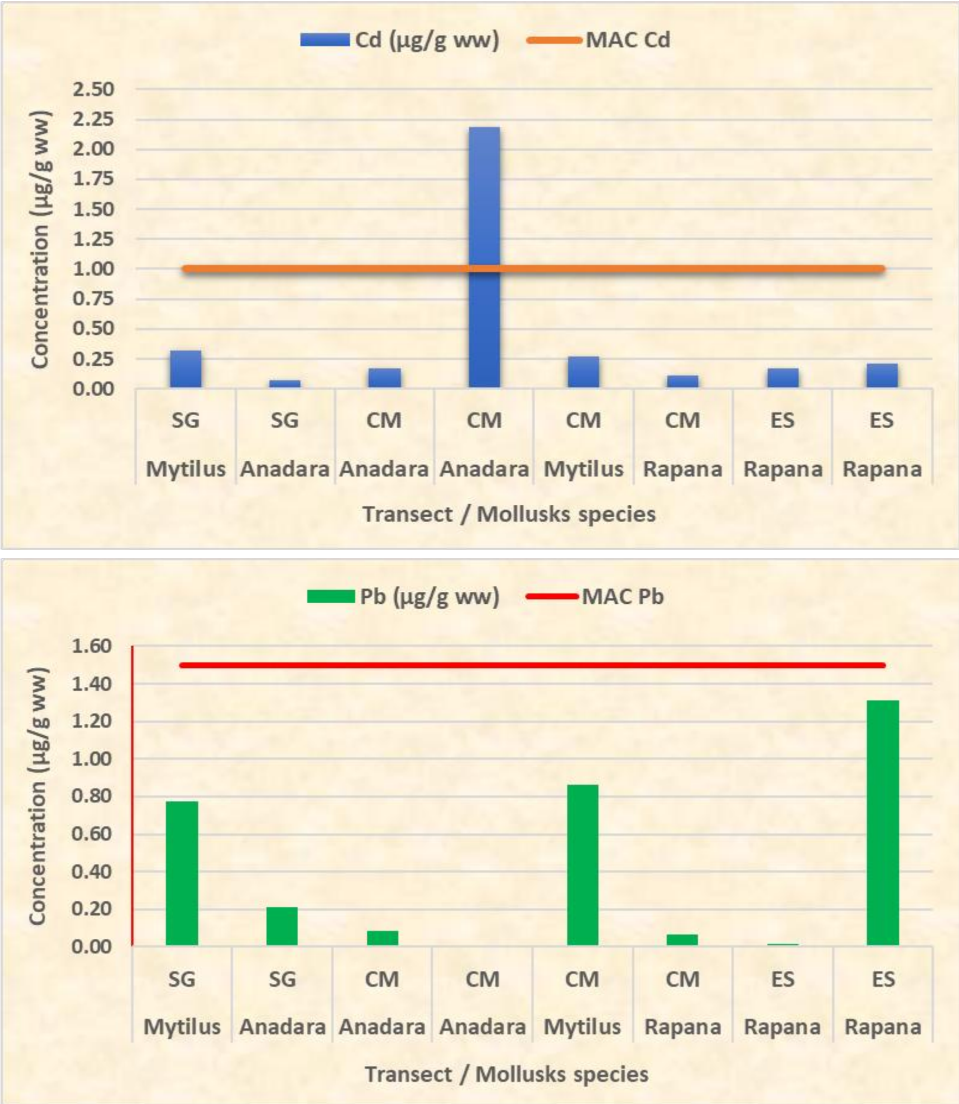

Fig. 2. Concentration levels of Cd and Pb (total ionic forms) in marine mollusks in 2021

Anadara, a species indicative of sediment-associated contamination, shows similar levels of contamination for three out of the four analyzed elements—Cu, Cr, and Co (total ionic forms)—at both SG and CM (Fig. 3), further supporting the link between water and sediment pollution. The notably high amount of Ni observed at SG may be linked to specific anthropogenic sources in the area, which could be contributing to higher Ni accumulation in the sediments. The lower levels of Ni and

Co bioaccumulated in the third *Anadara* sample from CM may be related to local sediment characteristics, such as a higher proportion of sandy material, which typically retains fewer contaminants compared to finer sediments.

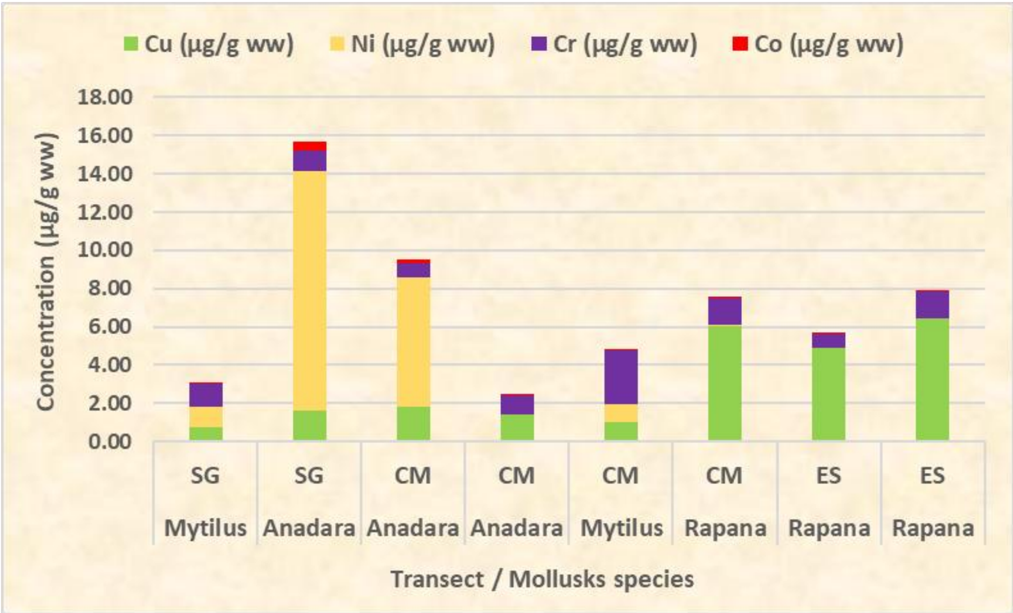

Fig. 3. Concentration levels of Cu, Ni, Cr, and Co (total ionic forms) in marine mollusks in 2021

The similar concentrations of Cu and Cr detected in *Rapana,* a species that accumulates HMs through trophic transfer, may suggest that the biota in both CM and ES is exposed to comparable levels of contamination.

Similar research confirms that the spatial heterogeneity of metal accumulation is also shaped by regional hydrodynamics and terrestrial inputs. Riverine systems such as the Danube and Dnieper deliver contaminants from industrial, agricultural, and urban sources, contributing to metal enrichment in coastal sediments and suspended matter [22,23]. This lateral transfer of pollutants into the Black Sea can be traced through mollusk tissue analyses, as demonstrated by comparable accumulation profiles in bivalves sampled along the RBSC [22,24,25].

A similar study conducted in 2019 [26,27] provides a comprehensive overview of HMs contamination in marine invertebrates across the Black Sea, revealing pronounced spatial variability and pollutant-specific patterns. Cadmium emerged as a consistent contaminant of concern, particularly in the northwestern sector. In Ukrainian waters, *Rapana venosa*, collected near Chornomorsk wastewater discharge area, exhibited Cd as high as 3.380 µg/g ww, exceeding the MAC_{Cd} (1.0 µg/g ww), while other mussels from the same region presented Cd ranging from 0.026 µg/g ww to 0.092 µg/g ww. In the Romanian sector, *Mytilus galloprovincialis* showed localized Cd exceedances as well, especially in the Danube-influenced areas. Turkish samples revealed regional hotspots, notably *Rapana venosa* from the Sakarya River area with an average Cd value of 2.728 µg/g ww. Pb concentrations, although generally below food safety thresholds (1.5 µg/g ww), were rather high in some areas: in Romanian deep-water (78 m) mussels showed higher Pb levels in comparison with other RO stations, and, also, Turkish mussels from Sakarya area, that recorded Pb average values of 0.28 µg/g ww [26,27].

Co was the most abundant HM in mollusks across all regions. In Ukrainian *Rapana*, Cu levels ranged between 9.52 and 19.1 µg/g ww, while mussels showed lower but still significant values (0.61–1.21 µg/g ww). Turkish veined rapa whelk from Sakarya had the highest Cu levels in the study, averaging 16.73 µg/g ww, with bivalves from the same site showing 1.214 µg/g ww. Romanian mussels under Danube influence also displayed slightly elevated Cu levels. Ni in Ukrainian mollusks ranged between 0.22–0.36 µg/g ww for *Rapana* and around 0.29–0.32 µg/g ww in mussels. Turkish bivalves from Sakarya reached 1.714 µg/g ww, among the highest Ni value reported. Chromium followed a similar trend, with Ukrainian veined rapa whelk showing values of 0.14–0.28 µg/g ww and mussels up to 0.25 µg/g ww; Turkish mussels showed 1.182 µg/g ww, while *gastropods* had moderate levels (0.324 µg/g ww). Cobalt, although the least abundant of the metals assessed, was detected in all regions. Romanian mussels from the southern coast (e.g., Constanța to Mangalia) had moderate Co levels, while Turkish ones reached 0.436 µg/g ww and *Rapana* recorded lower but measurable levels (0.081 µg/g ww) [26,27].

These previous regional findings demonstrate that while Cd and Pb pose the most immediate toxicological concern due to regulatory exceedances, in some areas outside RBSC, other metals such as Cu, Ni, and Cr are also present at appreciable levels that may contribute to chronic exposure risks. The elevated values observed in 2019 in the northwestern Black Sea, especially in the Ukrainian area, underscore the persistent impact of riverine discharges and urban sources, but, also, the dilution effect of the fresh water coming from Danube River, whereas Turkish waters showed localized but significant contamination linked to specific river mouths [23]. These results highlight the need for expanded regional monitoring and harmonized thresholds that include a broader suite of metals to better assess ecological and human health risks in mollusk-based food chains.

3.2 Bioaccumulation potential: BCF and BAF assessment

The BCF, calculated according to equation (1), provides critical insights into the assimilation behavior of HMs across different species and sampling transects (Tables 2;3;4).

The BCF threshold of 1000, as defined by REACH, signifies the onset of bioaccumulative behavior. *Anadara* consistently exhibits the highest number of BCF values exceeding this threshold, particularly for Ni and Cd, indicating it may be particularly sensitive to metal intake, possibly due to its filter-feeding strategy and physiology. *Rapana* shows notably high BCF values at the ES station, especially for Cd, which surpasses 6000, indicating very strong bioaccumulation, suggesting potential trophic transfer or species-specific retention mechanisms. *Mytilus*, also a filter-feeding organism, presented elevated BCFs for Cd and Pb, particularly at the SG and CM stations, suggesting a moderate but consistent tendency to accumulate these metals. This supports its role as a common bioindicator species in marine contamination monitoring.

Cd was consistently bioaccumulated by all three species, according to their BCFs, particularly by *Anadara*, which reached 12282.2 in CM station (classified as vB), and *Rapana* in ES (6328.3 – also vB). Mussels showed substantial bioaccumulation as well, with BCF values of 2043.6 (SG) and 2772.9 (CM), falling within the bioaccumulative/very bioaccumulative range. Veined rapa whelk from CM displayed a BCF of 1153.9, also exceeding the threshold for bioaccumulation.

Table 2

HMs concentrations* and BCF values across different species (*Mytilus*, *Anadara*,) at SG sampling transect

Non-bioaccumulative	B	vB

Element	Biota (µg/kg ww)			Water (µg/L)	BCF	
	Station	*Mytilus*	*Anadara*	Mean	*Mytilus*	*Anadara*
Cu	SG	772.60	1595.20	4.04	191.48	395.34
Cd	SG	318.80	68.92	0.16	2043.59	441.80
Pb	SG	773.80	213.00	0.59	1313.75	361.63
Ni	SG	1016.80	12534.00	1.11	916.86	11302.07
Cr	SG	1210.00	1096.80	7.34	164.96	149.53

* Concentrations are expressed as total ionic forms of Cu, Cd, Pb, Ni, and Cr, measured after mineralization of samples.

Table 3

HMs concentrations* and BCF values across different species (*Mytilus*, *Anadara*, *Rapana*) at CM sampling transect

Non-bioaccumulative	B	vB

Element	Biota (µg/kg ww)				Water (µg/L)	BCF		
	Station	*Mytilus*	*Anadara*	*Rapana*	Mean	*Mytilus*	*Anadara*	*Rapana*
Cu	CM	1008.60	1603.60	6024.00	6.30	160.17	254.66	956.65

Cd	CM	266.20	1179.09	110.78	0.10	2772.92	12282.19	1153.96
Pb	CM	864.80	49.20	64.94	0.80	1083.71	61.66	81.38
Ni	CM	968.80	3403.76	95.38	3.01	321.54	1129.69	31.66
Cr	CM	2804.00	872.60	1360.00	4.59	611.56	190.32	296.62

* Concentrations are expressed as total ionic forms of Cu, Cd, Pb, Ni, and Cr, measured after mineralization of samples.

Table 4

HMs concentrations* and BCF values for *Rapana* species at ES sampling transect

Non-bioaccumulative	B	vB

Element	**Biota (µg/kg ww)**		**Water (µg/L)**	**BCF**
	Station	***Rapana***	**Mean**	***Rapana***
Cu	ES	5649.00	7.11	794.74
Cd	ES	189.85	0.03	6328.33
Pb	ES	663.46	1.71	387.99
Ni	ES	26.18	3.44	7.62
Cr	ES	1061.80	4.16	255.24

* Concentrations are expressed as total ionic forms of Cu, Cd, Pb, Ni, and Cr, measured after mineralization of samples.

Ni showed a species-specific retention pattern, being highly bioaccumulative in *Anadara*, while much lower in *Mytilus* and minimal in *Rapana*. BCF values for Ni were extremely high in *Anadara*, especially in SG (11302.1, vB) and to a lesser extent in CM (1129.7, B). Mussels exhibited a value close to the bioaccumulation threshold in SG (916.9), which dropped significantly in CM (321.5). In contrast, veined rapa whelk accumulated very little Ni, with a BCF of only 31.7 in CM and 7.618 in ES.

Cu exhibited moderate accumulation, with *Rapana* showing slightly higher retention, possibly due to its trophic behavior, as a predatory gastropod. BCF values for Cu remained below the accumulation threshold for *Mytilus* (SG: 191.5, CM: 160.2) and *Anadara* (SG: 395.3, CM: 254.7). *Rapana* in CM, however, reached a value of 956.6, approaching the assimilation threshold.

Pb appears to be selectively accumulated, with *Mytilus* being the only organism indicating significant bioaccumulation potential, with a BCF of 1313.8 (SG) and 1083.709 (CM), exceeding the 1000 threshold. All other values for Pb remained below 1000 across species and stations.

Cr exhibited low accumulation in all species, although mussels showed relatively higher uptake. All Cr BCF values were below the threshold for bioaccumulation. The highest was observed in *Mytilus* from CM (611.6), followed by *Rapana* (296.6).

Overall, Cd and Ni emerged as the most concerning elements due to their high bioaccumulative potential, often exceeding the vB threshold (>5000), particularly in *Anadara* and *Rapana*. *Anadara* demonstrated exceptional sensitivity, supporting its inclusion in future biomonitoring programs for HMs pollution. *Mytilus*, with its moderate yet consistent BCFs, also remains a reliable bioindicator.

The BAF, calculated according to equation (2), reflects the extent to which organisms accumulate contaminants from surrounding sediments. Although no universal regulatory thresholds exist for BAF, like BCF under REACH, values exceeding 1.0 are generally interpreted as indicative of effective uptake from sediments.

Anadara showed the highest BAF values overall, especially for Cd and Ni, indicating high sediment uptake efficiency (Tables 5 and 6). *Rapana* exhibited elevated BAFs for Cd and Cu, consistent with its predatory behavior and potential for dietary exposure (Table 6 and 7). *Mytilus* had moderate BAFs for Cd and Cr, reinforcing its reliability as a bioindicator of metal pollution (Table 5 and 6).

Cd demonstrates high bioavailability and strong uptake from sediments across all species, with *Anadara* and *Rapana* showing very strong bioaccumulation at CM and ES respectively. These results align with the BCF patterns, reinforcing Cd as a key bioaccumulative metal. Thus, Cd showed high BAF values, particularly for: *Anadara* in CM (28.07) and SG (0.98), mussels in all stations: SG (4.55), CM (6.34) and veined rapa whelk in CM (2.64) and ES (5.58).

Ni uptake appears species-specific, with *Anadara* being better at bioaccumulating it, again, while *Rapana* showed minimal Ni bioaccumulation from sediments at CM (0.02) and ES (0.005). This matches the BCF trend and may reflect feeding mechanisms or metal binding specificity. *Anadara* in SG (1.57) and CM (0.75) exceeds or approaches the threshold for significant accumulation, whereas *Mytilus* remains low at all stations (SG: 0.13, CM: 0.21).

Cu shows moderate sediment-associated bioaccumulation, particularly in *Rapana*, likely due to dietary intake or metal-binding affinities, with BAF of 2.18 in CM, indicating elevated sedimentary Cu uptake. Both bivalve species exhibit lower but detectable values: SG (0.13–0.27), CM (0.36–0.58).

Table 5

HMs concentrations* and BAF values across different species (*Mytilus*, *Anadara*,) at SG sampling transect

					Non-bioaccumulative	Bioaccumulative
Element	**Biota (µg/g dw)**			**Sediments (µg/g dw)**	**BAF**	
	Station	***Mytilus***	***Anadara***	**Mean**	***Mytilus***	***Anadara***
Cu	SG	3.86	7.98	29.80	0.13	0.27
Cd	SG	1.59	0.35	0.35	4.55	0.98

Element	Biota (µg/g dw)			Sediments (µg/g dw)	BAF	
Pb	SG	3.87	1.07	33.41	0.12	0.03
Ni	SG	5.08	62.67	39.87	0.13	1.57
Cr	SG	6.05	5.48	38.55	0.16	0.14
Co	SG	0.07	2.17	10.03	0.01	0.22

* Concentrations are expressed as total ionic forms of Cu, Cd, Pb, Ni, Cr, and Co, measured after mineralization of samples.

Table 6

HMs concentrations* and BAF values across different species (*Mytilus*, *Anadara*, *Rapana*) at CM sampling transect

Non-bioaccumulative	Bioaccumulative

Element	Biota (µg/g dw)				Sediments (µg/g dw)	BAF		
	Station	*Mytilus*	*Anadara*	*Rapana*	Mean	*Mytilus*	*Anadara*	*Rapana*
Cu	CM	5.04	8.02	30.12	13.82	0.36	0.58	2.18
Cd	CM	1.33	5.90	0.55	0.21	6.34	28.07	2.64
Pb	CM	4.32	0.25	0.33	15.62	0.28	0.02	0.02
Ni	CM	4.84	17.02	0.48	22.82	0.21	0.75	0.02
Cr	CM	14.02	4.36	6.80	26.03	0.54	0.17	0.26
Co	CM	0.07	0.50	0.07	6.84	0.01	0.07	0.01

* Concentrations are expressed as total ionic forms of Cu, Cd, Pb, Ni, Cr, and Co, measured after mineralization of samples.

Cr is moderately bioaccumulated, with *Mytilus* showing a slightly higher capacity for uptake it from sediments, though BAF values stay below one. Thus, *Mytilus* in CM shows the highest BAF (0.54), followed by *Rapana* (0.26), whereas all other values remain <0.2.

Pb appears to have low sediment bioavailability, or is less efficiently accumulated, supporting findings from the BCF analysis. BAF values are consistently low (<0.3) for all species and stations. Only mussels show slightly elevated values (SG: 0.12, CM: 0.28).

Table 7

HMs concentrations* and BAF values for *Rapana* species at ES sampling transect

Non-bioaccumulative	Bioaccumulative

Element	Biota (µg/g dw)		Sediments (µg/g dw)	BAF
	Station	*Rapana*	Mean	*Rapana*
Cu	ES	28.25	15.73	1.80
Cd	ES	0.95	0.17	5.58
Pb	ES	3.317	18.38	0.18

Element	Biota (µg/g dw)		Sediments (µg/g dw)	BAF
Ni	ES	0.131	27.27	0.05
Cr	ES	5.309	33.51	0.16
Co	ES	0.062	7.9	0.01

* Concentrations are expressed as total ionic forms of Cu, Cd, Pb, Ni, Cr, and Co, measured after mineralization of samples.

Co had minimal uptake across all species and stations. These low BAF values suggest that Co remains largely unavailable from sediments or is biologically regulated. The only BAF value above 0.2 is for *Anadara* in SG (0.22). All other values are negligible (<0.1).

Overall, Cd stands out as the most bioaccumulative metal from sediments, across all species and stations. *Anadara* continues to be the most responsive species, followed by *Rapana*, for sediment-associated metal uptake. Metals like Pb and Co exhibit low sediment bioavailability in this study. These patterns underscore the importance of species-specific traits and sediment chemistry in controlling assimilation in tissues.

The observed inter-species differences in bioaccumulation reflect not only environmental exposure but also distinct trophic strategies. *Mytilus galloprovincialis*, as a filter feeder, primarily accumulates dissolved and particulate-bound metals from the water column, whereas *Rapana venosa*, a predatory gastropod, may be exposed to higher trophic transfer of metals such as Cd and Cu through dietary intake. The elevated BAF and BCF values for these metals in *Rapana* suggest the potential for trophic magnification, especially in areas influenced by sediment contamination. *Anadara inaequivalvis*, a deposit feeder, showed particularly high Ni and Cd retention, underscoring its vulnerability to sediment-bound contaminants. These trophic-level patterns highlight the importance of integrating ecological traits into biomonitoring frameworks and risk assessments for benthic and pelagic food webs.

Similar studies confirm that BCF and BAF are widely applied to quantify the uptake of HMs in aquatic organisms and offer insight into the environmental mobility and bioavailability of contaminants in marine ecosystems [28]. Mollusks, particularly bivalves and gastropods such as *Mytilus galloprovincialis*, *Rapana venosa*, and *Anadara spp.*, are recognized as effective bioindicators due to their ability to accumulate metals from both water and sediment compartments [22,28,29].

Variability in BCF and BAF values among mollusk species has been consistently observed, indicating species-specific accumulation patterns shaped by feeding strategy and physiology. Filter-feeding species such as *Mytilus galloprovincialis* are known to accumulate metals primarily from suspended particulates in the water column. In contrast, *Rapana venosa*, a carnivorous gastropod, and sediment-ingesting species like *Anadara tuberculosa* and *Anadara inaequivalvis* may acquire metals through trophic or benthic intake routes [30,31].

Several studies from the Black Sea basin have reported elevated content of Cd, Cu, and Pb in mollusks, with BCF and BAF values often exceeding standard thresholds for bioaccumulative behavior [28]. For example, mussels collected from the Turkish coast showed elevated levels of Cu and Cd [32], while *Rapana venosa* exhibited enhanced metal retention near riverine and wastewater discharge zones [23]. These results confirm the bioindicator role of mollusks and highlight the need to account for ecological traits when interpreting bioaccumulation data.

Notably, correlations between sediment and tissue metal concentrations support the use of BAF as an effective metric for evaluating benthic exposure, particularly in deposit-feeding biota [33]. The combined use of BCF and BAF enhances the understanding of contamination pathways and informs the selection of appropriate sentinel species. Moreover, the capacity of species like *Mytilus galloprovincialis* and *Rapana venosa* to reflect environmental gradients makes them suitable for long-term ecological assessments [34,35].

While this study provides useful information into HMs bioaccumulation in marine mollusks from the RBSC, some limitations should be acknowledged. First, sampling was restricted to three stations during a single campaign in 2021, which may not capture temporal variability or seasonal fluctuations in HMs concentrations. Second, the study used composite samples of individuals of similar sizes, which reduces variability but may overlook size- or age-related differences in bioaccumulation. Third, only six HMs were analyzed; other contaminants of emerging concern (e.g., mercury, arsenic, organic pollutants, microplastics) were not included, limiting the broader assessment of pollutant interactions. Finally, BCF and BAF values were calculated from measured concentrations in biota, water, and sediments at the same time, but without accounting for hydrodynamic changes or dietary preferences that may influence uptake pathways. Future research should therefore expand the spatial and temporal coverage, including additional pollutants, and integrating more detailed ecological and trophic analyses to strengthen environmental risk assessment.

4. Conclusions

The concentrations of HMs in the three marine species of invertebrates collected from the RBSC in 2021 were mostly within expected natural variability ranges. Cd and Pb levels were generally below their *MACs* for human consumption set by European legislation, with only a single exceedance observed in *Anadara* from CM.

The application of BCF and BAF factors provided critical insights into species-specific bioaccumulation behavior and contaminant bioavailability from water and sediment compartments. The analysis revealed that:

- Cd and Ni displayed the highest assimilation potential, often exceeding the thresholds for bioaccumulative (BCF > 1000) and very bioaccumulative substances (BCF > 5000) as defined by REACH/ECHA guidance. These findings were particularly evident for *Anadara*, which showed extremely elevated BCF values, and for *Rapana*, which recorded very high BCF for cadmium in ES.
- BAF factor confirmed the high sediment-associated uptake of Cd and Ni especially in *Anadara* and *Rapana*. Cd presented strong BAF values (>1.0) in nearly all species and stations, highlighting its environmental mobility and bioavailability. *Rapana* showed notable sediment uptake for Cu, likely linked to its feeding strategy.
- *Mytilus galloprovincialis*, traditionally used as a bioindicator species, displayed moderate but consistent bioaccumulation across most HMs, especially Cd and Pb, confirming its relevance for environmental monitoring in coastal ecosystems.

Overall, this integrated approach, combining direct HMs content measurements with BCF and BAF factors, enhances the understanding of metal bioaccumulation pathways in mollusks and supports species-specific risk assessment. *Anadara inaequivalvis*, due to its pronounced retention capacity, emerges as a designated candidate for future biomonitoring efforts in sediment-influenced areas.

The present results emphasize the importance of species-specific accumulation dynamics and environmental context in interpreting BCF and BAF data. Ongoing monitoring using mollusks from various ecological niches provides critical information for evaluating the bioavailability of HMs in the Black Sea and assessing the broader implications for ecosystem health. The findings reinforce the need for sustained regional monitoring and support the use of BCF/BAF as tools for evaluating metal bioavailability and ecological risks in marine environments.

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