Journal of
Marine Science
and Engineering

MDPI

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Integrated Sediment and Mussel Chemical Analysis for Environmental Quality Assessment in Rovinj's Coastal Waters (Northern Adriatic, Croatia)

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Abstract

Marine sediments are a key component of aquatic ecosystems, linking diverse water uses, functions, and services. Chemical contamination of sediments is a global concern, with many jurisdictions striving to prevent future pollution and manage existing contamination. This study evaluates the contamination status of Rovinj's coastal waters using an integrated approach that combines sediment and biota chemical analyses. Sediments were analyzed to assess long-term contaminant accumulation (D8.C1), while the Mediterranean mussel (*Mytilus galloprovincialis*) served as a bioindicator of bioavailable contaminants and their cumulative effects on marine habitats (D8.C2). Sediment samples were collected from five sites (S1–S5), and mussels were caged using Mussel Watch installations for approximately 120 days at a control site (Lim Bay) and within Rovinj harbor. Both matrices were analyzed for heavy metals (As, Cd, Cu, Cr, Hg, Ni, Pb, and Zn), polycyclic aromatic hydrocarbons (16 PAHs), and polychlorinated biphenyls (PCBs), following the EU Water Framework Directive. All sampled locations showed a reduction in sediment contamination relative to 2011 data, with most concentrations below ecotoxicological thresholds. Exceptions included elevated ΣPAH and PCB concentrations in the harbor (S1 = 3.18 mg/kg DW; 0.33 mg/kg DW) and marina (S2 = 3.64 mg/kg DW; 0.89 mg/kg DW), as well as Ni levels (S3 = 30 mg/kg DW; S4 = 34 mg/kg DW). Despite higher contaminant loads at some locations, mussel contaminant bioaccumulation remained limited, and their vitality and survival were only moderately affected in the harbor. Although localized increases in some contaminants were detected, all calculated Q_{PECm} values remained below 1.0, indicating no significant ecological risk. However, a moderate-to-high probability of toxic effects (P) may occur with long-term exposure for biota inhabiting harbor and marina areas. The results of this study demonstrate continued improvement in the environmental quality of Rovinj's coastal waters compared to the previous decade.

Keywords: sediment chemistry; mussels; bioavailability; heavy metals; organic contaminants; environmental monitoring; coastal contamination

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Academic Editor: Ryan J.K. Dunn

Received: 29 October 2025
Revised: 15 November 2025
Accepted: 18 November 2025
Published: 20 November 2025

Citation: Pelikan, J.; Grozić, K.; Privileggio, L.; Pavičić-Hamer, D.; Smodlaka Tanković, M.; Pikelj, K.; Glad, M.; Hamer, B. Integrated Sediment and Mussel Chemical Analysis for Environmental Quality Assessment in Rovinj's Coastal Waters (Northern Adriatic, Croatia). *J. Mar. Sci. Eng.* **2025**, *13*, 2212. https://doi.org/10.3390/jmse13112212

1. Introduction

Contaminants introduced into the marine environment can be found in the water column and tend to accumulate in sediments and marine organisms [1–3]. While sediments act as long-term reservoirs of contaminants, mussels reflect the bioavailability and bioaccumulation of contaminants within the water column. This combined approach enables the assessment of both immediate exposure risks and potential long-term environmental and human health impacts [4]. Marine sediments accumulate and retain contaminants over long periods [5]. Consequently, surface sediment deposits may represent a long-term environmental risk, as they serve as the final recipients and reservoirs of contaminants [5–7]. Understanding how contaminants interact and persist in the marine environment requires examining their distribution across different environmental compartments, such as sediments and biota. The Mediterranean blue mussel (*Mytilus galloprovincialis* Lamarck, 1819) is an important mariculture species with diverse functions in marine ecosystems. As a filter-feeder, it is a powerful bioindicator, frequently used for biomonitoring spatial distribution and temporal trends of contaminants in coastal waters. Additionally, it can be adopted in Mussel watch environmental quality assessment models to determine the pressure of distributed contaminants on the local marine biota [8,9].

Common harmful substances in the marine environment include oils and hydrocarbons, persistent organic pollutants (POPs), and heavy metal(loid)s, among others [10–12]. Regardless of their sources and type, contaminants pose significant threats to marine ecosystems and human health, requiring strict regulations and sustainable practices to mitigate their effects [2,13]. Given the critical role of sediments in marine environments, the European Union has implemented measures to prevent and manage marine pollution. The European Commission's Marine Strategy Framework Directive (MSFD) [14–17] aims to achieve and maintain Good Environmental Status (GES) across Europe's marine environments. The MSFD recommends that, considering the specific characteristics of each marine ecosystem, EU member states develop and adopt policies establishing critical contaminant limits. As a result, the method described by Bakke et al. [18], which includes threshold values for contaminants and their anticipated effects on biota, has been adopted for monitoring the eastern coast of the Adriatic Sea. This empirical approach classifies marine sediments into five categories based on the measured concentrations of present contaminants [19]. These regulatory frameworks provide a baseline for assessing regional marine systems such as the Adriatic Sea, where complex hydrodynamic and geological features influence contaminant behavior and accumulation. The Adriatic Sea is a semi-enclosed basin connected to the Mediterranean Sea. The northern part of the Adriatic Sea is the shallowest and is consequently heavily influenced by discharges from the Po River, while the central and southern Adriatic are more affected by Mediterranean waters [20]. The eastern Adriatic coast is primarily shaped of carbonate rocks without significant terrigenous sediment input [21]. The eastern Adriatic shelf is classified as a non-tropical carbonate-siliciclastic system, significantly influenced by in situ biogenic carbonate production predominantly found in the coarse-grained fraction [21–23]. Therefore, common minerals along the coast are calcite and aragonite, with terrigenous minerals (e.g., quartz, feldspar, detrital dolomite, and clay minerals) found in smaller quantities [21–23]. Despite these well-defined physical and chemical characteristics, comprehensive assessments of contamination levels and trends in the Adriatic Sea remained limited.

Recent analyses of national and regional metadata on marine sediment monitoring in the Adriatic Sea [24] have revealed differences in the reporting of contamination data. Moreover, research on the status of marine sediments and biota along the Adriatic coasts remains underrepresented at both local and broader scales [25–27]. To address these gaps, systematic and spatially integrated studies are needed to evaluate the concentrations and distributions of contaminants. This highlights the priority of monitoring hazardous substances, including metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and other indicators of marine pollution across marine environments. Equally important are GES assessments based on harmonized Sediment Quality Guidelines (SQGs), which enable the evaluation of ecological risks and compliance with regional and international marine protection frameworks. In this context, the present study contributes to this broader effort by investigating temporal trends and spatial variations in sediment contamination in the NE Adriatic Sea, focusing on coastal areas exposed to several presumed sources of contamination and the bioavailability of contaminants to marine biota in key areas.

2. Materials and Methods

2.1. Research Area, Sediment Collection, and Mussel Watch

Five sampling sites (Figure 1; Supplementary Information S1. Study area; Supplementary Figures S1–S3) were selected for investigation in the NE Adriatic Sea in the Rovinj coastal area (Istria, Croatia), each characterized by varying degrees of anthropogenic impact (Supplementary Information S1. Study area). These impacts primarily include maritime traffic, industrial activity, and urban runoff from the city of Rovinj. The specific sites were as follows: harbor (S1: 45°04′46.4″ N, 13°38′03.5″ E; 17 m depth) and marina/shipyard (S2: 45°04′37.3″ N, 13°37′59.6″ E; 6 m), both influenced by industrial, maritime and urban activities; a natural reserve and mariculture area Lim Bay Outer (S3: 45°08′02.0″ N, 13°37′31.4″ E; 31 m) and Lim Bay Middle (S4: 45°07′49.4″ N, 13°40′15.4″ E; 27 m), seasonally affected by maritime traffic; and a site located approximately 3 nautical miles west of Rovinj in the open sea (S5: 45°05′16.6″ N, 13°32′51.4″ E; 32 m), also seasonally affected by maritime traffic [10,26].

Figure 1. Sampling locations in the NE Adriatic Sea: S1—harbor, S2—marina/shipyard, S3—Lim Bay Out, S4—Lim Bay Middle, and S5—Open Sea location.

Surface sediment samples (0–3 cm) were collected from all investigated sites (Figure 1; Supplementary Information S1. Study area; Supplementary Figures S1–S3) in May 2022 using a Van Veen grab (KC Denmark, Silkeborg, Denmark) (mouth 0.25 m^2). As indicated, approximately 3 cm of surface sediment was taken through the window on the upper side of the grab. This approach would ensure to sample the contamination load related to recent events. Samples were collected as replicates (3 × 1 kg) and homogenized in one composite sample for further analysis. Samples were transferred in cooling boxes to the laboratory and stored at −80 °C until analysis.

Mussels (*Mytilus* sp.) are frequently adopted in contamination monitoring due to their sedentary lifestyle, high-filtration rates, and high tolerance to variable environmental conditions [8,9]. In this study, specimens of *Mytilus galloprovincialis* Lamarck, 1819 (Mollusca, Bivalvia), were obtained from a single mussel population cultivated in the mariculture area. Mussels of similar size (6–7 cm) were used in the Mussel watch experiment to obtain similar accumulation patterns of metal(loid)s related to age, size and variation in the filtration rates. At all the sites investigated, mussels were placed at depths between 3 and 5 m using cages. Cages were suspended in a limited geographical area from buoys (3 cages/site) and designed to allow free water flow while protecting mussels from predation [28,29]. Mussels were caged using the Mussel Watch methodology for approximately 120 days (April–July 2022) at the reference location (Lim Bay, S4) and closer vicinity to the Rovinj harbor (S1) where higher traffic-related activities are present [30–32]. At the end of the experiment, samples of soft mussel tissue were collected as pools of 40 mussel specimens (adult stage, average shell length 7 cm) per location. Collected mussel samples were lyophilized and stored at −80 °C until analysis.

2.2. Sediment and Mussel Characterization

Sediment samples were fractionated by wet sieving using a Retsch sieve set (Retsch GmbH, Haan, Germany) to obtain seven grain size fractions with mesh sizes of 4 mm, 2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.125 mm, and 0.063 mm. The separated mud fraction (<0.063 mm) was not further subdivided into silt and clay. All fractions were subsequently oven-dried and weighed [26]. Sediment grain size descriptive statistics and classifications were obtained using GRADISTAT software (v.8.0), following the procedures of Blott and Pye [33], and sediment was classified according to the Folk scheme [34].

Water content was determined by drying 50 g of homogenized sediment at 105 °C for 24 h in a circulating oven. The percentage of water content was calculated from the difference between wet and dry sample weights. Total organic matter (TOM) was determined by combustion of 10 g of dried sediment in a muffle furnace at 450 °C for 5 h. TOM content was calculated from the percentage weight loss of the sample.

Mussel mortality was assessed by counting the number of live specimens and empty shells or identified dead specimens. Average mussel mortality was expressed as a percentage of the total number of mussels present in cages. Mussel meat yield was determined at the end of the experimental period as percentage of fresh soft tissue weight in whole mussel weight [35]. Lipid content in mussel tissue was quantified using a two-step extraction with cyclohexane and propan-2-ol, as described by Smedes [36]. Briefly, samples were extracted by sequential solvent extraction and homogenization. The resulting phases were separated by centrifugation, and the upper layer was weighed.

2.3. Contaminant Analyses

Samples for the determination of metals (Cd, Cu, Cr, Ni, Pb, and Zn) and the metalloid (As) concentrations were prepared following modified standard methods EN 14084:2003 and EN 13804:2013 (for mussel tissue) and modified standard methods ISO 8288:1986 and

ISO 15586:2003 (for sediment) [37–40]. Briefly, approximately 1 g of dried (105 °C) samples (soft mussel tissue) or 0.5 g of dried (105 °C) samples (grain sized sediment fractions) were digested with 5 mL of concentrated HNO_3 (65%, Suprapur, Merck, Darmstadt, Germany) in a microwave digestion system, Anton Paar Multivawe 3000 (Anton Paar GmbH, Graz, Austria). After cooling, digested samples were transferred to volumetric flasks and ultrapure water (Siemens Water Technologies Corp, Warrendale, PA, USA) was added to the mark (25 mL for mussel tissue and 50 mL for sediment). Concentrations of selected metals were determined using an atomic absorption spectrometer, AAnalyst 800 (PerkinElmer, Waltham, MA, USA). For analysis of Hg, an advanced mercury analyzer was used (AMA 254, Leco, St Joseph, MI, USA). Metals were quantified using the external standards method, with calibration standards and blanks prepared in the same acid matrix. Analytical quality assurance was carried out using appropriate blanks and certified reference materials, and the analyses were performed in triplicate. The IAEA-407 reference material (fish tissue; International Atomic Energy Agency, Vienna, Austria) was used for validation of Cd, Cu, Cr, Ni, Pb, Zn, and As measurements, with mean recoveries between 88% and 104%. For validation of Hg measurements, reference material NIST 2976 (mussel tissue, National Institute of Standards and Technology, Gaithersburg, MD, USA) was used, with a mean recovery of 91%. IAEA 458 certified reference material (marine sediment sample, International Atomic Energy Agency, Vienna, Austria) was used for validation of the method for sediment analysis, with mean recoveries for Cd, Cu, Cr, Ni, Pb, Zn, As and Hg between 84% and 110%. Repeatability and reproducibility were under 15% (RSD). The quantitation limits were as follows: Cd 0.008 mg/kg; Cu 3.0 mg/kg; Cr 0.11 mg/kg; Ni 0.22 mg/kg; Pb 0.17 mg/kg; Zn 3.8 mg/kg; As 0.15 mg/kg; Hg 0.01 mg/kg for sediment. For mussel, quantitation limits were Pb 0.3 mg/kg; Cd 0.008 mg/kg; Hg 0.01 mg/kg; Ni 0.5 mg/kg; Cr 0.4 mg/kg; Zn 1 mg/kg; Cu 0.5 mg/kg; As 0.2 mg/kg. Expanded measurement uncertainty was under 15% for all measured metals. The concentrations of metals were expressed as mg/kg dry weight (DW).

PAHs were extracted from the samples using a Soxhlet extraction unit (Universal Extractor E800, BUCHI, Flawil, Switzerland) with approximately 250 mL of an organic solvent mixture (acetone/hexane, 1:1). About 5 g of dry sample (<0.5 mm sediment fraction) was weighed into the automatic extraction system, with each extraction cycle lasting 3 h. The extracts were purified by passing them through a column filled with silica gel and alumina, while traces of water were removed using anhydrous Na_2SO_4. The sorbents were then washed with a solvent mixture (dichloromethane/hexane, 8:2). The fraction containing PAHs was collected, evaporated to dryness, and the dry residue was dissolved in methanol. Determination of PAHs (naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, and indeno(1,2,3-cd)pyrene) was performed using a liquid chromatograph with UV and fluorescence detectors (HPLC, Agilent 1260/1290 Infinity, Santa Clara, CA, USA). PAHs were quantified using the external standards method. Analytical quality assurance was ensured using appropriate blanks and certified reference materials, and analyses were performed in triplicate. The IAEA 159A reference material (tuna fish flesh homogenate; International Atomic Energy Agency, Vienna, Austria) was used to validate PAH measurements, with mean recoveries between 73.8% and 125.4%. Analyses of PAHs were conducted in accordance with modified EPA methods: EPA 3541 for extraction, EPA 3603C for clean-up [41,42], in-house method M 160–200 Issue 1 (13 November 2019), and LC Varian Application Note Number 7 [43] for determination. Repeatability and reproducibility were below 20% (RSD). The quantitation limits were 1 μg/kg for all PAHs except 2 μg/kg for fluoranthene and 5 μg/kg for acenaphthylene, for both sediments and mussels. Expanded measurement uncertainty ranged from 15% to 40% for all measured PAHs. PAH concentrations were expressed as µg/kg DW.

The preparation of samples for the determination of PCBs was also performed in a Soxhlet extraction unit. These extracts were purified by passing them through a column containing different combinations of sorbents (acidic/alkaline), and traces of water were removed with anhydrous Na_2SO_4. The sorbents were washed with a mixture of solvents (dichloromethane/hexane, 8:2), and the fractions containing PCBs were collected and evaporated to dryness. The dry residue was dissolved in n-hexane. The PCBs (congeners: 28, 33, 52, 53, 77, 81, 101, 105, 110, 114, 118, 123, 126, 138, 149, 153, 156, 157, 167, 169, 170, 180, 189, and 194) in the samples were determined by gas chromatography with an ECD detector (SCION 436-GC, Scion Instruments, Goes, The Netherlands) or, for peak identity confirmation, by gas chromatography with an MS/MS detector (Agilent 7890B GC, Agilent 7000 GC/MS Triple Quad, Agilent Technologies, Santa Clara, CA, USA). PCBs were quantified using the external standards method. Analytical quality assurance was carried out using appropriate blanks and certified reference materials, and the analyses were performed in triplicate. The IAEA 435 reference material (tuna fish flesh homogenate; International Atomic Energy Agency, Vienna, Austria) was used for validation of PCB measurements, with mean recovery between 81.2% and 110.5%. The analyses of PCBs were carried out in accordance with modified EPA methods: EPA 3541 for extraction, EPA 3603C for clean-up, and EPA 1668C, as well as European standard method EN 6468:2002 for determination [41,42,44]. Repeatability and reproducibility were below 20% (RSD). The quantitation limits were 0.001 mg/kg for PCBs 53, 77, 105, 114, 118, 123, 126, 156, 157, 170, and 189; and 0.002 mg/kg for PCBs 28, 52, 101, 153, 138, 167, 169, 180, and 194. Expanded measurement uncertainty ranged from 30% to 52% for all measured PCBs. The concentrations of PCBs were expressed as mg/kg DW.

2.4. Good Environmental Status Assessment

The assessment of the GES of marine sediments was carried out by comparing measured contaminant concentrations with reference and threshold values established in various Sediment Quality Guidelines (SQGs) and toxicological studies. These comparisons provide a framework for evaluating the ecological risks associated with contamination. The effect-range approach (ERL/ERM) categorizes contaminant levels according to their potential biological effects on marine organisms. Threshold values derived from this approach are used to assess both the ecological risk of contaminants and their potential to cause adverse effects on benthic communities [45,46].

Globally, various SQGs have been developed to support sediment management and environmental protection. The French sediment quality guidelines (JORF n°0083 of 9 April 2024) are primarily focused on the reuse of dredged sediments. In this system, threshold values N1 and N2 define contamination levels relevant for sediment reuse or disposal. An equivalent to N3 was recently introduced in the regulation to improve decision-making regarding sediment disposal in marine environments [47].

The Norwegian SQGs [18] provide another widely recognized framework for assessing ecological risk in marine sediments, which is also adopted by Croatian national legislation for routine monitoring of marine sediments in the Adriatic Sea [48,49].

The United States Environmental Protection Agency (EPA) guidelines also employ the Effect Range Low (ERL) and Effect Range Median (ERM) concepts, developed from empirical observations of biological responses to several compounds across different sedimentary environments [46,50]. ERL represents the lower threshold, below which adverse biological effects are rarely observed, while ERM represents the upper threshold, above which adverse effects are frequently observed. Concentrations between ERL and ERM define an intermediate range of potential effects, where adverse outcomes may occur depending on site-specific factors such as contaminant type, bioavailability, and sediment characteristics. This approach facilitates the classification of sediments into distinct risk levels, supporting evidence-based decisions on dredging, disposal, or remediation.

Additionally, Long et al. [51] and MacDonald et al. [52,53] introduced a model for predicting sediment toxicity and establishing sediment quality benchmarks. This model defines Threshold Effect Levels (TELs), concentrations below which adverse effects on benthic organisms are unlikely, and Probable Effect Levels (PELs), concentrations above which adverse effects are likely to occur. Unlike the ERL/ERM system, this approach accounts for the combined toxicity of chemical mixtures rather than evaluating contaminants individually. Based on these SQG systems, sediments are classified as toxic when contaminant concentrations exceed ERM or PEL thresholds, and as non-toxic when concentrations fall below ERL or TEL values.

The sediment risk quotient $\mathrm{Q_{PECm}}$ is the sum of individual sediment contamination quotients, normalized by the PEC values and the number of contaminants analyzed, and is used to predict the risks of overall sediment matrix contamination to the environment. $\mathrm{Q_{PECm}}$ values greater than 1.0 indicate potentially high toxic effects [53]. Three equations were used for the ecological risk assessment for sediment data [26,53,54]:

$$QPECm = (\Sigma ni = 1\ (Ci/PECi))/n \tag{1}$$

where Ci is the measured concentrations for contaminant i (As, Cd, Cr, Cu, Ni, Pb, Hg, Zn, $\sum$PAHs, and $\sum$PCBs), $PECi$ is the predicted effect concentration of specific/sum contaminants and treated as Ci values, and n is the number of measured contaminants.

$$QPECm_{TOM} = \frac{1}{n}\sum\nolimits_{i=1}^{n} \frac{C_{i\times\frac{1\%}{TOM}}}{PEC_i} \tag{2}$$

where Ci is the measured concentrations for contaminant i (As, Cd, Cr, Cu, Ni, Pb, Hg, Zn, $\sum$PAHs, and $\sum$PCBs), $PECi$ is the predicted effect concentration of specific/sum contaminants, and n is the number of measured contaminants, TOM is the total organic content and normalization is expressed as 1% for all sediments.

$$P = \frac{1}{1 + e^{-(B0+B1\times\sum QPECm)}} \tag{3}$$

where P is the probability of observing a toxic effect, $B0$ is the intercept parameter, Bi is the slope parameter [44]. All values included in Equations (1)–(3) were scaled to equal units and same parameters were used for all sites to avoid site-different data.

2.5. Statistical Analysis

Prior to principal component analysis (PCA), numerical variables were transformed using the natural logarithm (scaled to unit variance). Data for PCA was further standardized using the standard z-score scaling. PCA was performed on contaminant concentration data comprising 26 indicators, including all analyzed metal(loid)s, individual PAHs, total PAHs, and total PCBs, representing contaminant concentrations in sediments and mussels.

Hierarchical clustering was applied to PCA scores using Ward's minimum variance method (ward.D2) and Euclidean distance. This method enables identifying groups of samples with similar contaminant profiles. Cluster assignments were visualized using dendrograms and projected onto the first two PCA components (PC1 and PC2). These components were selected as they captured the dominant variation in the dataset. Samples were enclosed in convex ellipses to highlight groupings. The hierarchical clustering was built on sediment data from this study, along with threshold values defined for each contamination category (Category 1–Category 4) as reported according to national legislation and as reported by Bakke [18].

Statistical analysis was conducted in the R environment (version 4.5.1; R Studio). All analyses were performed using base R functions, as well as the openxlsx, stats, FactoMineR, and FactoExtra packages [55,56].

3. Results and Discussion

3.1. Physico-Chemical Sediment Properties

Based on the grain size analysis (Figure 2, Table 1), the sediment samples were classified as gravelly muddy sand at site S5 (Open Sea), slightly gravelly muddy sand at sites S1 (Harbor) and S2 (Marina), and slightly sandy mud at sites S3 (Lim Bay Out) and S4 (Lim Bay Middle). These results (Table 1) are consistent with previous studies of marine sediments along the eastern Adriatic coast, which have reported a predominance of sandy sediments [21–23,57]. Sediments from Lim Bay contained the highest proportion of the finest fraction (Figure 2, Table 1), as previously described for this area [57,58]. According to Pikelj et al. [23] and the references therein, muddy sediments often contain higher amounts of organic matter. The results of this study are consistent with this observation, showing higher TOM content at sites dominated by the mud fraction. Owing to their small particle size, mud fractions can be easily transported over long distances and possess a large specific surface area, enabling the absorption of substantial amounts of both organic and inorganic matter, including nutrients and contaminants [59].

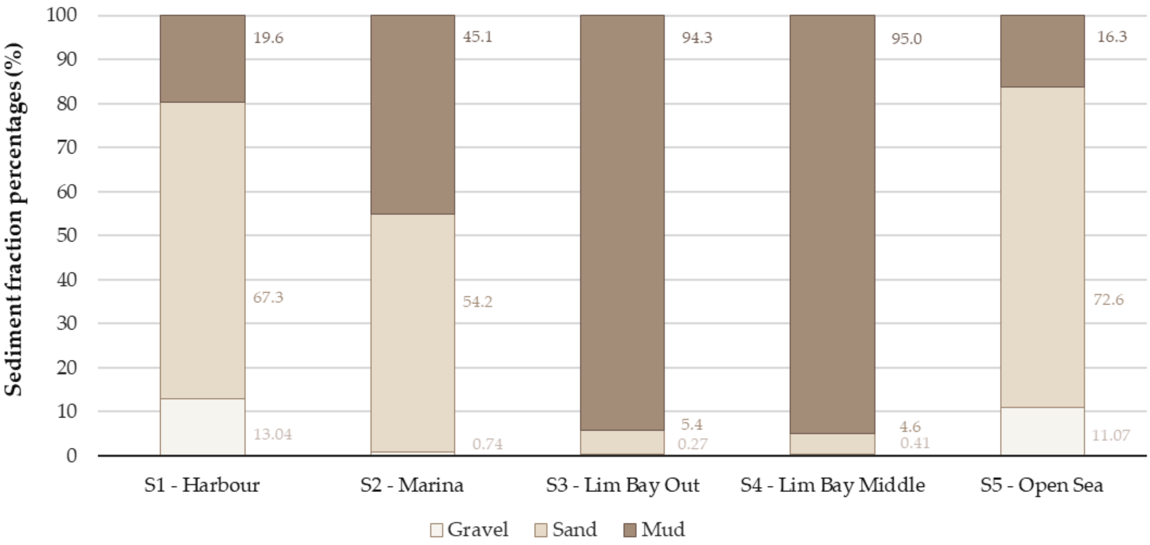

Figure 2. Sediment fraction percentages (%) of samples collected at investigated locations.

Table 1. Physico-chemical characteristics of sediments collected at investigated locations.

	Harbor (S1)	**Marina (S2)**	**Lim Bay Out (S3)**	**Lim Bay Middle (S4)**	**Open Sea (S5)**
Sediment class [1]	Slightly gravelly muddy sand	Slightly gravelly muddy sand	Slightly sandy mud	Slightly sandy mud	Gravelly muddy sand
Water content (%)	28.03	22.38	44.24	47.90	16.91
TOM (%)	1.88	1.20	2.77	3.11	0.98

[1] Sediment classification according to Folk [34].

The mussel *M. galloprovincialis* is a widely distributed sessile filter feeder throughout the Mediterranean region and holds substantial commercial importance. Due to its ecological and physiological characteristics, *M. galloprovincialis* has been extensively used as a bioindicator species in environmental monitoring programs such as Mussel Watch [60]. In the present study, the use of mussels as biomonitors revealed differences in mortality, meat yield and lipid content indices, with higher values observed in specimens from the mariculture–natural reserve area (Table 2). Such variations likely reflect differences in environmental quality, as contaminant uptake can adversely affect mussel vitality. These findings are consistent with previous studies reporting that increased contamination pressure induces a physiological and biochemical stress response in these highly tolerant pollution indicators [8,61].

Table 2. Mortality, meat yield, and lipid content of mussels (*Mytilus galloprovincialis*) caged at chosen pristine (Lim Bay) and traffic-/industrially affected location (Harbor).

Location	Mortality (%)	Meat Yield (%)	Lipid Content (%)
Lim Bay	<2.00	18.94 ± 2.60	3.69
Harbor	26.67	13.08 ± 1.74	5.50

3.2. Heavy Metals and Metalloids

Continuous monitoring of metal concentrations in marine sediments of the Adriatic Sea has predominantly been conducted near major harbors and regions under increased urbanization pressure [62–64]. In the present study, most analyzed elements (Pb, Zn, Cd, and Cr) were below contamination thresholds defined by all considered SQGs (Table 3). However, several elements exceeded guideline limits: As, Ni, and Hg were above the ERL; Cu, Ni, and Hg surpassed the TEL; and Ni and Hg exceeded the Category I threshold. Similar trends have been reported in other Adriatic harbors, where sediment metal concentrations frequently exceed background (Category I) levels [28]. Over the past decade, concentrations of metals exceeding SQG thresholds have markedly declined in the monitored areas [26], except for Ni, which showed a small relative increase (Table 3). The observed steadiness or slight increase in some metal concentrations across anthropogenically affected areas, and their differential accumulation in sediments compared to previous findings in the same study area [26] may be related to ongoing transport activities and urban waste inputs, which slow remediation processes in marine environments [65]. Nevertheless, the local establishment of waste-outfall collectors in recent years, along with the adoption of legal frameworks, substantially improved the overall environmental status.

Some metal and metalloid concentrations were relatively higher in slightly sandy mud sediments collected from the Lim Bay area (Table 3). Despite lower contaminant exposure, the preferential accumulation of potentially toxic elements in fine-grained sediments is well documented. Sojka et al. [66] reported strong correlations between metal concentrations, silt content, and organic matter, emphasizing the key role of fine particles and organic components in trace element binding. It should also be noted that, regardless of the relatively higher and variable concentrations observed in sediment matrices, the bioavailability of these metals to mussels (Table 3) may be limited due to their association with organic matter (Table 1). Ultimately, metal and metalloid bioavailability depends not only on sediment composition but also on the specific chemical properties of each element, prevailing environmental conditions, and metal-specific accumulation patterns [67,68]. These factors may explain the observed elemental partitioning between sediment and mussel matrices in this study (Table 3). The findings of this study (Table 3, Supplementary Figure S4) are consistent with previous research suggesting that elements exhibit distinct toxicokinetic and toxicodynamic behaviors, influencing their uptake mechanisms in ways that are not necessarily concentration dependent or directly correlated with their environmental levels [69]. Regarding overall trends in metal and metalloid concentrations in mussels from both industrial and pristine areas, a general decrease in their levels was observed compared with monitoring data collected a decade ago [8]. Nevertheless, data on contaminant levels in mussel tissues are available in food safety regulations (EC 2023/915) for Pb (1.5 mg/kg FW) and Cd (1.0 mg/kg FW). Despite being reported on a dry weight basis (FW:DW ratio 1:5), these metals had similar or lower concentrations in mussel tissues at both locations (Table 3).

Table 3. Heavy metals and metalloid concentration (mg/kg DW) in sediments (S) and mussels *Mytilus galloprovincialis* (M) in the present study sampled across the NE Adriatic Sea.

	Pb		**Zn**		**As**		**Cd**		**Cu**		**Ni**		**Cr**		**Hg**	
	S	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**
S1	26	0.4	47	134	7.9	16	0.1	1.0	25	3.3	16	1.2	26	1.0	0.3	0.1
S2	15	-	37	-	6.5	-	0.1	-	17	-	18	-	16	-	0.2	-
S3	21	-	62	-	5.9	-	0.1	-	15	-	30	-	39	-	0.1	-
S4	24	0.4	68	259	8.2	14	0.1	1.1	16	3.5	35	1.2	47	1.7	0.1	0.2
S5	11	-	43	-	4.4	-	0.1	-	13	-	12	-	19	-	0.1	-
Contaminant presence in sediments across the Adriatic Sea [1]																
R1	21–721		68–1050		7.7–34		0.1–3.4		25–372		71–154		63–124		0.05–6.9	
AS	10–164		23–353		-		0.08–2.36		8–85		-		-		0.1–6.2	
MS	119		387		-		0.07		79		25		32		-	
Sediment quality guidelines [2–5]																
N1	100	-	276	-	25	-	1.2	-	45	-	37	-	90	-	0.4	-
C (I)	30	-	150	-	20	-	0.25	-	35	-	30	-	70	-	0.15	-
C (II)	83	-	360	-	52	-	2.6	-	51	-	46	-	560	-	0.63	-
ERL	46.7	-	150	-	8.2	-	1.2	-	34	-	20.9	-	81	-	0.15	-
ERM	218	-	410	-	70	-	9.6	-	270	-	51.6	-	370	-	0.71	-
TEL	30.24	-	124	-	7.24	-	0.68	-	18.7	-	15.9	-	52.3	-	0.13	-
PEL	112.18	-	271	-	41.6	-	4.21	-	108.2	-	42.8	-	160.4	-	0.7	-

Abbreviations: S1—Harbor, S2—Marina, S3—Lim Bay Out, S4—Lim Bay Inside, S5—Open Sea, R1—Rijeka Harbor, AS—Adriatic Sea, MS—Mediterranean Sea; C—classification categories according to the national legislation. References: [1] Frančišković-Bilinski and Cukrov [70]; [2] Dorleon et al. [47]; [3] Bakke et al. [18]; [4] Long et al. [51]; and [5] Apitz et al. [71].

The decrease in the concentration of specific metals, such as Pb, across marine environments and biota has been linked to reductions in the use of leaded petrol and, consequently, changes in anthropogenic activities [72]. In contrast, the concentrations of other metals in marine biota may reflect their essential metabolic roles or natural biogeochemical cycling in seawater, rather than local pollution inputs [73,74]. For example, Zn is an essential metal required for enzymatic and physiological functions in mussels, whereas Cd is a non-essential xenobiotic metal that exhibits a high tendency to bioaccumulate despite its low concentrations in the marine environment [72,73]. In this study, Cd concentrations in sediments were low and relatively uniform (approximately 0.1 mg/kg DW) across all sites, regardless of sediment properties (Figure 1) or anthropogenic pressure (S1 and S2 vs. S3–S5). The Cd levels in mussel tissues were relatively higher (up to 1.1 mg/kg DW) compared to the sediment matrix, indicating bioaccumulation despite the low sedimentary background (Table 3; Supplementary Figure S4). Similar patterns have been reported elsewhere, where Cd distribution is governed primarily by natural oceanographic processes rather than anthropogenic inputs. The biogeochemical behavior of Cd is closely related to organic matter regeneration and nutrient-like cycling in seawater, with upwelling events transporting Cd-enriched waters and increasing its bioavailability [74]. Therefore, the elevated Cd concentrations observed in mussels relative to sediments in this study likely reflect natural oceanographic processes rather than direct anthropogenic contamination.

Arsenic showed a modest tendency to accumulate in mussel tissues (Table 3, Supplementary Figures S4 and S5). Sediment As concentrations were low (Category I—background levels; Table 3), while mussels had relatively higher levels (14–16 mg/kg DW), although these values appear to show a decreasing trend over time (30 mg/kg DW) [28]. Regional speciation studies indicate that arsenobetaine, a relatively non-toxic organic form involved in osmolyte regulation, predominates in mussel tissues [8,75]. Previous reports have reported no significant differences in total As between mussels from mariculture and port sites [8], suggesting that accumulation reflects potential biosynthesis pathways or natural biogeochemical cycling rather than anthropogenic inputs. Despite the positive trend in the decrease in this element, in both sediments and marine biota, limited knowledge on As highlights the need for further studies on its speciation and potential sources in the surrounding environment.

3.3. Polycyclic Aromatic Hydrocarbons and Polychlorinated Biphenyls

The Adriatic Sea, as a shallow and semi-enclosed basin, is particularly susceptible to anthropogenic pollution. Among various contaminants, PAHs constitute a significant portion of the total organic contamination load. Consequently, most monitoring and research activities have focused on high-risk areas such as harbors, marinas, wastewater outfalls, and coastal industrial sites. Bihari et al. [11] demonstrated that the toxicity of seawater and sediment organic extracts correlates strongly with PAH concentrations, suggesting that PAHs are the predominant toxic compounds in these environments. Similar results were observed in this study, where the analysis of heavy metals and metalloids, along with PAHs and PCBs indicated a dominance of PAHs in the contamination load (Tables 3–5, Figure 2). The contamination was primarily concentrated in harbor and marina sediments, whereas concentrations in the mariculture/natural reserve area and open sea locations predominantly reflected a good environmental status (Table 4). PAH levels in the marina and harbor sediments frequently exceeded the ERM and PEL thresholds, indicating a high potential for adverse biological effects. Bad sediment quality (category IV) in the marina and harbor was denoted by higher concentrations of benzo(a)anthracene, benzo(g,h,i)perylene, and indeno(1,2,3-c,d)pyrene. Moderate contamination levels (Category III) were observed for total PAHs as well as for anthracene, fluoranthene, pyrene, and benzo(b)fluoranthene. The lowest contribution to overall PAH contamination was associated with naphthalene, acenaphthene, fluorene, chrysene, benzo(k)fluoranthene, benzo(a)pyrene, and dibenzo(a,h)anthracene, which were generally below threshold values (Table 4). Elevated PAH levels in sediments exposed to intensified anthropogenic activity have also been reported in other harbors along the Adriatic coast. In comparison to the observed contamination load, larger and more industrialized harbors such as the nearby Rijeka exhibited higher contamination levels, with four out of five sampling sites classified as highly contaminated and ΣPAH concentrations exceeding 500 µg/kg DW [7,11,29,76,77]. Although PAH concentrations in the local marina and harbor remain relatively high and environmentally unfavorable (Table 4), their decline has been observed over the past decade (Supplementary Table S1), indicating gradual improvement in environmental quality and sediment safety [26]. Namely, past surveys of marine macroflora and sediment macrofauna have identified a historical increase in species diversity in the areas surrounding the local harbor and marina (indicated in this study as S1 and S2). However, past reports showed a higher dominance of nitrophilous species typically found in polluted waters and so-called harbor ecosystems with a dominance of species of wider ecological distribution [78]. In addition to these observations, the lower mussel vitality recorded at such sites (Table 2) suggests that additional mitigation efforts are still required, as elevated multi-xenobiotic contaminant loads in harbor and marina areas may continue to impact local marine biota of higher pollution sensitivity.

Table 4. Polycyclic aromatic hydrocarbons concentration (µg/kg DW) in sediments (S) and mussels *Mytilus galloprovincialis* (M) in the present study sampled across several locations in the NE Adriatic Sea.

PAHs (µg/kg DW)		**Sampling Sites**						
		S1		**S2**	**S3**	**S4**		**S5**
	N° Rings	**Harbor** **S**	**M**	**Marina**	**Lim Bay Out**	**Lim Bay Middle** **S**	**M**	**Open Sea**
Naphtalene	2	2.19 ^{b,e,g,i}	<1	1.36 ^{a,e,g,i}	<1 ^{a,e,g,i}	<1 ^{a,e,g,i}	<1	<1 ^{a,e,g,i}
Acenaphtylene	3	<5 ^{b,e,g,i}	<1	<5 ^{b,e,g,i}	<5 ^{b,e,g,i}	<5 ^{b,e,g,i}	<1	<5 ^{b,e,g,i}
Acenaphtene	3	22.30 ^{b,f,h,j}	0.78	24.40 ^{b,f,h,j}	<1 ^{a,e,g,i}	<1 ^{a,e,g,i}	0.76	<1 ^{a,e,g,i}
Fluorene	3	26.20 ^{b,f,h,j}	3.78	28.30 ^{b,f,h,j}	1.20 ^{a,e,g,i}	<1 ^{a,e,g,i}	2.97	<1 ^{a,e,g,i}
Phenanthrene (Phe)	3	346.00 $_{b,f,h,j}$	31.00	424.00 ^{b,f,h,j}	12.90 ^{b,e,g,i}	4.64 ^{a,e,g,i}	28.30	3.55 ^{a,e,g,i}
Anthracene (Ant)	3	71.90 ^{c,e,g,j}	0.57	87.80 ^{c,f,h,j}	1.99 ^{b,e,gi}	<1 ^{a,e,gi}	0.41	<1 ^{a,e,gi}
Fluoranthene (Flt)	4	609.00 $_{c,f,h,j}$	8.83	759.00 ^{c,f,h,j}	18.60 ^{b,e,g,i}	7.77 ^{a,e,g,i}	5.36	3.79 ^{a,e,g,i}
Pyrene (Pyr)	4	570.00 ^{c,f,g,j}	18.40	731.00 ^{c,f,h,j}	16.80 ^{b,e,g,i}	5.15 ^{a,e,g,i}	8.93	3.73 ^{a,e,g,i}
Chrysene	4	201.00 $_{b,e,g,j}$	<1	259.00 ^{b,e,g,j}	6.52 ^{b,e,g,i}	2.37 ^{a,e,g,i}	<1	1.24 ^{a,e,g,i}
Benzo(a)anthracene	4	275.00 $_{d,f,h,j}$	<1	304.00 ^{d,f,h,j}	7.00 ^{b,e,g,i}	1.98 ^{a,e,g,i}	<1	1.59 ^{a,e,g,i}
Benzo(b)fluoranthene	5	156.00 ^{c,e}	<1	278.00 ^{c,e}	18.30 ^{a,e}	6.03 ^{a,e}	<1	5.22 ^{a,e}
Benzo(k)fluoranthene	5	167.00 ^{b,e}	<1	167.00 ^{b,e}	5.63 ^{b,e}	2.40 ^{b,e}	<1	1.19 ^{b,e}
Benzo(a)pyrene	5	343.00 $_{b,e,g,j}$	<1	324.00 ^{b,e,g,j}	7.55 ^{b,e,g,i}	2.72 ^{a,e,g,i}	<1	2.17 ^{a,e,g,i}
Dibenzo(a,h)anthracene	5	16.50 ^{b,e,g,j}	<1	3.24 ^{a,e,g,i}	<1 ^{a,e,g,i}	<1 ^{a,e,g,i}	<1	<1 ^{a,e,g,i}
Benzo(g,h,i)perylene	6	240.00 ^{d,e}	<1	169.00 ^{d,e}	6.60 ^{a,e}	2.66 ^{a,e}	<1	2.03 ^{a,e}
Indeno(1,2,3-c,d)pyrene	6	133.00 ^{d,e}	<1	89.90 ^{d,e}	3.93 ^{a,e}	1.80 ^{a,e}	<1	<1 ^{a,e}
∑PAHs (µg/kg DW)		3179.00 $_{c,g,j}$	63.40	3641.00 ^{c,g,j}	107.00 ^{a,g,i}	37.50 ^{a,g,i}	46.70	24.50 ^{a,g,i}
Ant/(Ant + Phe)	3	0.17	-	0.17	0.13	0.10	-	0.12
Phe/Ant	3	4.81	-	4.83	6.48	9.28	-	7.10
Flt/(Flt + Pyr)	4	0.52	-	0.51	0.53	0.60	-	0.50
Flt/Pyr	4	1.07	-	1.04	1.11	1.51	-	1.02

Sediment Quality Guidelines	**National SQG**				**French SQG**		**NOAA/EPA**		**Consensus SQG**	
	C I ^{a}	**C II** ^{b}	**C III** ^{c}	**C IV** ^{d}	**N1** ^{e}	**N2** ^{f}	**ERL** ^{g}	**ERM** ^{h}	**TEL** ^{i}	**PEL** ^{j}
Naphtalene	<2	2–290	290–1000	1000–2000	160	1130	160	2100	34.57	390.64
Acenaphtylene	<1.6	1.6–33	33–85	85–850	40	340	44	640	5.87	127.87
Acenaphtene	<4.8	2.4–160	160–360	360–3600	15	260	16	500	6.71	88.9
Fluorene	<6.8	6.8–260	260–510	510–5100	20	280	19	540	21.17	144.35
Phenanthrene (Phe)	<6.8	6.8–500	500–1200	1200–2300	240	870	240	1500	86.68	543.53
Anthracene (Ant)	<1.2	1.2–31	31–100	100–1000	85	590	85.3	1100	46.85	245
Fluoranthene (Flt)	<8	8–170	170–1300	1300–2600	600	2850	600	5100	112.82	1493.54
Pyrene (Pyr)	<5.2	5.2–280	280–2800	2800–5600	500	1500	665	2600	152.66	1397.6
Chrysene	<4.4	4.4–280	280–280	280–560	380	1590	384	2800	107.77	845.98
Benzo(a)anthracene	<3.6	3.6–60	60–90	90–900	260	930	251	1600	74.83	692.53
Benzo(b)fluoranthene	46	46–240	240–490	490–4900	400	900	-	-	-	-
Benzo(k)fluoranthene	-	<210	210–480	480–4800	200	400	-	-	-	-
Benzo(a)pyrene	<6	6–420	420–830	830–4200	430	1015	430	1600	88.81	763.22
Dibenzo(a,h)anthracene	<12	12–590	590–1200	1200–12,000	60	160	63.4	260	6.22	134.61
Benzo(g,h,i)perylene	<18	18–21	21–31	31–310	1700	5650	-	-	-	-
Indeno(1,2,3-c,d)pyrene	<20	20–47	47–70	70–700	1700	5650	-	-	-	-
∑PAHs (µg/kg DW)	<300	300–2000	2000–6000	6000–20,000	300,000	2,000,000	4022	44,792	1684.1	16,770.4

References: Bakke et al. [18]; Dorleon et al. [47]; Long et al. [51]; and Apitz et al. [71]. Abbreviation: classification categories according to the national legislation. Letters a–j indicate the classification of specific contaminants according to provided sediment quality guidelines: a–d National SQG, e–f French SQG, g–h NOAA/EPA SQG, and i–j Consesus SQG with thresholds indicated in the table.

Table 5. Polychlorinated biphenyls concentration in sediments and mussels (*Mytilus galloprovincialis*) sampled across several locations in the NE Adriatic Sea.

Total PCBs (mg/kg DW) in Sediment Samples [1]		
Harbor (S1)	0.3283	
Marina (S2)	0.8903	
Lim Bay Out (S3)	0.0257	
Lim Bay Middle (S4)	<0.01	
Open Sea (S5)	<0.01	
SQGs (mg/kg DW) [2]		
Category (I)	0.005	
Category (II)	1.0	
ERM	0.400	
PEC	0.676	
ERL	0.050	
Individual congeners (mg/kg DW) in mussel samples [3]		
	Harbor (S1)	Lim Bay (S4)
PCB 53	<0.001	<0.001
PCB 52	0.059	0.038
PCB 101	0.007	0.003
PCB 81	0.013	0.007
PCB 77	0.004	<0.001
PCB 123	<0.001	<0.001
PCB 118	0.001	<0.001
PCB 114	<0.001	<0.001
PCB 153	0.007	0.004
PCB 105	<0.001	<0.001
PCB 138	0.004	0.002
PCB 126	<0.001	<0.001
PCB 167	<0.002	<0.002
PCB 156	<0.001	<0.001
PCB 157	<0.001	<0.001
PCB 180	<0.002	<0.002
PCB 169	<0.002	<0.002
PCB 170	<0.001	<0.001
PCB 189	<0.001	<0.001
PCB 194	<0.002	<0.002
Total PCBs	0.099	0.054

[1] Total PCBs calculated as Aroclor 1260, [2] Sediment quality guidelines for PCBs as reported by Bakke et al. [18] and MacDonald et al. [53], [3] Total PCBs calculated as individual congeners.

Clear guidelines on contaminant levels in marine biota are lacking in both the MSFD and GES frameworks. Concentration limits are instead established under food safety regulations (EC 2023/915), which specify a maximum level of 30 µg/kg for total PAHs on a wet weight (FW) basis. When compared with data from earlier monitoring activities conducted in the Lim Bay area [29], a decrease in the total concentration of PAHs in mussel tissues was detected. This reduction in contamination levels is consistent and probably related to the currently observed improvement in the environmental quality of the sediment matrices. In addition, the analysis of PAH concentrations in both matrices indicated that there is a generally higher accumulation of PAHs in sediment samples compared to mussel tissues (Supplementary Figure S5). An exception was observed for acenaphthene, which had higher tendency for accumulation in mussel tissues than in the sediment matrix (Supplementary Figure S5). The distribution of individual PAH compounds across marine sediments and biota is a significant factor in developing environmental quality guidelines. Increased bioaccumulation of specific contaminants may indicate either increased exposure during periods of elevated contamination pressure (such as tourism or marine industry activity) or an inherent capacity for bioaccumulation in marine organisms [29]. The relatively higher concentration of acenaphthene in mussel tissue vs. sediment, despite its lower log Kow and limited lipid affinity compared with higher-molecular-weight PAH [79], suggests its more recent introduction into the marine environment. This trend may result from its uptake via gills or ingestion, along with limited enzymatic degradation by cytochrome P450 or other metabolizing enzymes [80]. Events such as incomplete combustion or urban runoff may have contributed to these inputs. However, the relationships between contaminant occurrence, distribution among environmental compartments, and potential biological effects are complex. Comprehensive assessment therefore requires integrated, multi-matrix monitoring approaches capable of identifying both persistent and episodic contamination events [81,82]. Consequently, the contaminant levels measured in mussel tissues complement sediment and water analyses, offering an integrated assessment of short-term and cumulative contamination events.

PAH source diagnostics may contribute to understanding contamination pathways and facilitate their environmental interpretation. The observed ratios of specific PAH isomers Phe/Ant < 15, Flt/Pyr > 1, and Ant/(Ant + Phe) > 0.1 were aligned with pyrogenic origin of hydrocarbons (Table 4) [77]. The compositional profiles of PAHs were dominated by high-molecular-weight, combustion-derived compounds, consistent with previous research in the study area, indicating that a similar chemical profile has persisted over time [24]. As some sampling sites were located along a coastal area influenced by harbor and marina operations, boat traffic, urban runoff, and atmospheric deposition, the principal sources of PAHs are likely related to exhaust emissions, road traffic, and fuel combustion associated with both marine and terrestrial activities. Similar pyrogenic signatures have been reported in sediments near ports and urbanized coasts elsewhere [83]. Although such diagnostic ratios are widely recognized as effective tools for contamination fingerprinting [83], they are not mandatory indicators. Within the European environmental monitoring framework, they complement the assessment of absolute PAH concentrations, which are used to determine compliance with environmental quality standards under the WFD [84]. When combined, ratio-based source interpretation and concentration-based evaluation provide an integrated understanding of PAH behavior in marine environments. This integrated approach could improve the effectiveness of regional monitoring and management strategies, particularly in coastal areas under strong anthropogenic pressure, where tailored mitigation measures are essential to reduce impacts and preserve valuable marine ecosystems and biodiversity.

Polychlorinated biphenyls are persistent organic pollutants that pose significant environmental risks due to their toxicity, chemical stability, and strong bioaccumulation potential in marine ecosystems, particularly within Adriatic Sea sediments [64,85]. PCBs have historically been detected along the Adriatic coast, especially near industrial zones and urbanized areas [86]. Their concentrations commonly vary according to sediment grain size and organic matter content, with higher values typically associated with fine-grained, organic-rich sediments. This trend was not observed in the areas investigated in this work (Table 5) and may be related to their lower presence in zones such as protected areas and the open sea. When compared with SQG thresholds, measured PCB concentrations were considered of good quality according to national guidelines [18] but exceeded some ecological thresholds [53], indicating potential contamination levels across the study area (Table 5). These findings indicate an increase in PCB concentrations in sediments of the local marina and harbor compared to previous research conducted in the Rovinj coastal region (Supplementary Table S1) [26,77]. However, PCB concentrations remained generally low in mussel tissues (Table 5). The concentration of PCBs in the marine sediment samples from this study were similar or slightly increased when compared to previous measurements [26] highlighting the persistence of these compounds in the surrounding environments [87].

3.4. Contaminant Profiles Across Marine Environments

Multivariate analysis (PCA) (Figure 3; Supplementary Tables S1 and S2, Supplementary Figure S6) revealed distinct patterns of contamination across marine sediment and mussel samples. The first principal component (PC1), explaining 70% of the variance, was primarily associated with PAHs, including BaP, BghiP, BaA, BkF, CHR, and IcdP, all exhibiting high positive loadings (>0.8) and contributions (>5%). The second component (PC2), accounting for 25.3% of the variance, was dominated by heavy metal(loid)s such as Cr, Ni, As, Cd, and Pb, with strong loadings (greater than −0.8 or 0.8) and the highest contributions (>8%).

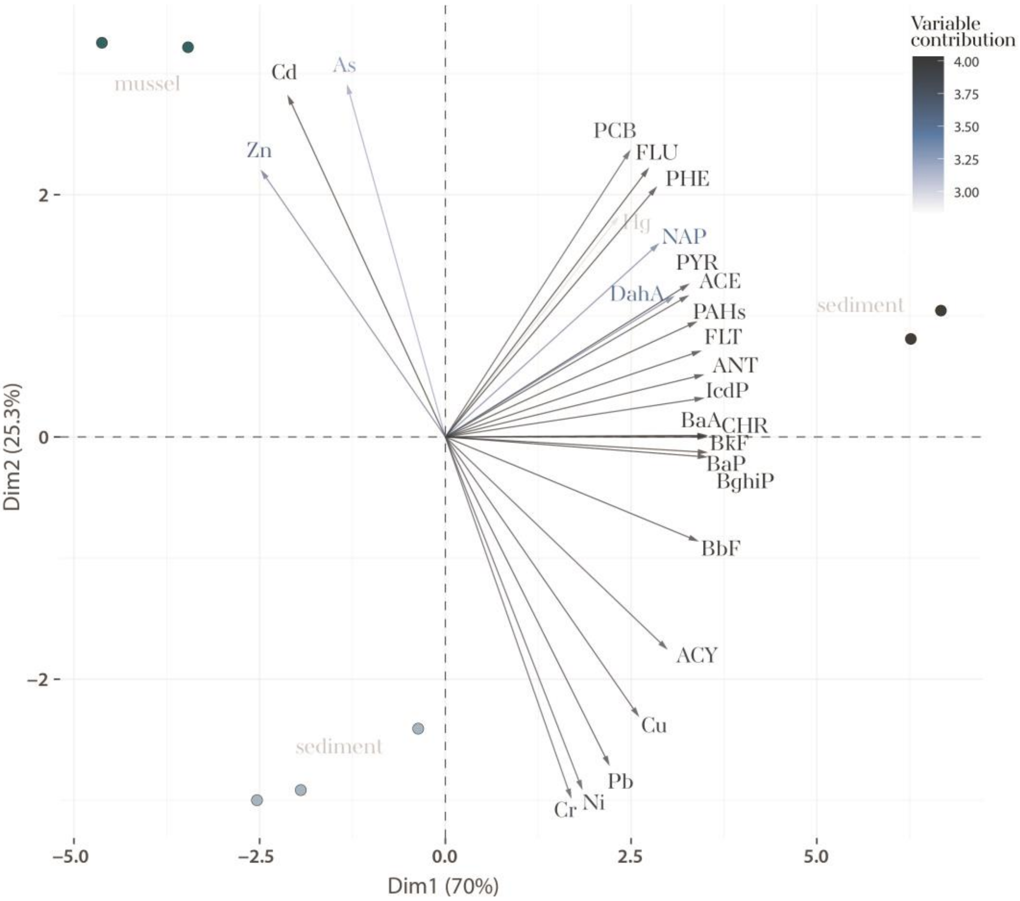

Figure 3. Principal Component Analysis (PCA) of contaminant profiles in marine sediments and mussels.

Spatial clustering of samples indicated clear environmental gradients. Mussel samples were associated with elevated PC2 scores suggesting bioaccumulation of metals. Sediments from harbor and marina sites have grouped with high molecular weight PAHs, PCBs, and metals (Cu, Pb, Cr, and Ni), reflecting combustion and antifouling-related inputs, whereas mussel samples correlated with Cd, Zn, and As, likely originating from natural sources. These patterns are consistent with previous studies [83], which highlighted pyrogenic and port-related contamination gradients along the Adriatic coast. Sediments sampled in other coastal areas (S3–S5) clustered with low scores on both components, confirming their pristine status.

The results of this study further confirm that contamination profiles of different matrices may provide a robust framework for revealing subtle differences in contamination patterns even when overall inputs are relatively low or derive from diverse, multi-xenobiotic sources. Multivariate analyses (Figure 3) may be adopted to differentiate pristine from anthropogenically influenced areas, offering an improved interpretation of contaminant distribution that reaches beyond threshold-oriented interpretation of concentration measurements. Although diagnostic ratios and multivariate approaches are not formally required for regulatory compliance, they complement assessments under the WFD and MSFD, providing critical source-oriented context for evaluating GES and informing site-specific management measures.

3.5. Toxic Effect Quotients

Sediments are typically composed of complex mixtures of contaminants, and the reliability of their quality assessment is improved when multiple SQGs are applied simultaneously. Therefore, conducting an additional evaluation of the predictive performance of SQGs can improve the interpretation of biological effects in relation to varying ranges of quotient values (Supplementary Tables S1–S3, Table 6, Figure 4) [53]. Despite generally low contamination and a sediment risk quotient (Q_{PECm}) value below the threshold of 1.0, the probability of toxic effects (P) remained high for the local harbor and marina (Table 6) [53,88]. The increased probability of toxic effects corresponds to elevated PAH and PCB concentrations in marina sediments, suggesting potential toxicity of the sediment matrix to the local environment (Table 6).

Table 6. Ecological risk indices of sediments sampled across several locations in the NE Adriatic Sea.

	Q_{PECm} [1]	normQ_{PECm} [2]	P [1]	P [2]	Interpretation of Both Q_{PECm}
Harbor (S1)	0.26	0.14	0.34	0.25	Moderate probability of toxic effects
Marina (S2)	0.56	0.44	0.84	0.82	High probability of toxic effects
Lim Bay Out (S3)	0.08	0.03	0.12	0.11	Low probability
Lim Bay Middle (S4)	0.04	0.02	0.09	0.10	Low probability
Open Sea (S5)	0.04	0.04	0.09	0.12	Low probability

[1] Q_{PECm} and probability of toxic effects (P) were calculated as the sum of all analyzed contaminants and corresponding PEC values as suggested by MacDonald et al. [53]. [2] normQ_{PECm} represents the calculation of sediment risk quotients using normalized contaminants' values (Supplementary Table S3).

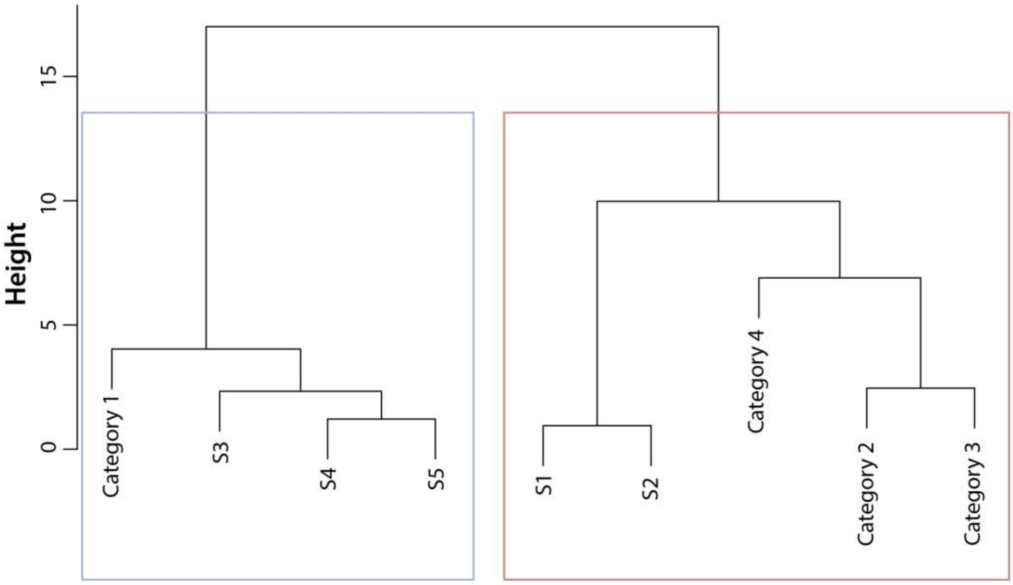

Figure 4. Hierarchical clustering dendogram of sediment contaminant profiles and classification according to national policy framework.

In addition to the sediment categorization proposed by policy frameworks (Supplementary Table S2), hierarchical clustering revealed two distinct groups of sediment contamination groups and contaminant categories (Figure 4). The analysis clearly separated pristine sites (S3–S5; Category I under national legislation) from more contaminated sites (S1 and S2; Categories II–IV), consistent with regulatory thresholds and calculated probability of toxic effects (Table 6). These findings may indicate that using multiple SQGs with probability-based assessments provides a more accurate evaluation of potential ecological impacts, supporting more informed environmental management and policy decisions for coastal and harbor ecosystems.

4. Conclusions

This study assesses sediment quality and contaminant distribution across distinct but closely located coastal environments in the northeastern Adriatic Sea. The results indicate spatial differences in sediment composition and contaminant loads, reflecting varying intensities of anthropogenic influence among the sites investigated.

While most metal concentrations were below guideline thresholds, elevated levels of As, Ni, Cu, and Hg in harbor and marina sediments suggest localized anthropogenic inputs. PAH contamination was highest in urbanized areas, with several compounds exceeding ecotoxicological benchmarks, indicating potential biological risks. PCB levels, though generally low, exceeded thresholds in some sites, reflecting their persistence.

Toxic effect quotient (Q_{PECm}) values were highest in harbor (0.26) and marina (0.56) sediments, primarily driven by PAH and PCB contamination, suggesting their potential toxicity. However, conducted TOM-normalization indicated lower contaminant values potentially indicating their lower bioavailability. Mussel biomonitoring complemented sediment analyses by indicating the bioavailable fraction of contaminants. Mussels accumulated metals and organic pollutants in patterns that were different from sediment profiles, revealing contaminant-specific bioaccumulation trends. This study demonstrates the value of combining chemical and biological monitoring, which can be replicated in other EU regions to strengthen MSFD implementation. Although environmental quality in the NE Adriatic Sea has improved in recent years, as reflected in contaminant categories based on SQGs and in general compliance with Croatian environmental legislation, several potential contamination hotspots persist near urban and harbor areas. According to national Croatian criteria, a total of 13 contaminants in the harbor area, 12 in the marina, and 12 in Lim Bay have fallen into more favorable ecological status categories over the past decade. Despite the continued presence of contaminants, the adoption of environmentally conscious measures, along with improvements in wastewater collection and treatment infrastructure, may have contributed to the observed progress in this coastal area.

The results of this study further indicate that determining the source and fate of contaminants is rather complex but represents a critical step in risk assessment for marine environments. Nevertheless, the overall distribution and retention of contaminants can be better understood through more frequent sediment-biota monitoring strategies that capture both chronically and acutely impacted areas. Limiting monitoring efforts or focusing exclusively on highly contaminated hotspots risks overlooking broader environmental dynamics, potentially allowing subtle degradation processes to proceed undetected. The frequency of environmental status-related monitoring activities can be rather low on national levels and can be underrepresented in some regions, while more frequent in others, requiring further improvements on a wider scale while considering the conservation and biodiversity status of an area. Despite being conducted on a limited scale, this study contributes to the overall evaluation of the environmental status in the Rovinj coastal area and contributes to the assessment of its status following the adoption of MSFD guidelines. The introduction and gradual improvement of wastewater collection systems have further contributed to the positive trends observed. The improvement of monitoring efforts, along with improvements in port-related and wastewater infrastructure, will be essential for maintaining and further promoting the environmental quality in this region.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse13112212/s1, Supplementary Information S1: Study area; Supplementary Figure S1: Wastewater treatment plant UPOV Cuvi with collection network scheme of Rovinj coastal area dating 2022.; Supplementary Figure S2: Natura 2000 Network—protected sites and habitats including the Aquatorium of Western Istria and Lim Bay areas; Supplementary Figure S3: Map of research sites with indication of historically significant sources of contamination; Supplementary Figure S4: Metal and metalloid concentrations (mg/kg DW) in sediments and mussels; Supplementary Figure S5: Polycyclic aromatic hydrocarbon concentrations (µg/kg DW) in sediments and mussels; Supplementary Table S1: Principal component loadings of variables and variable contributions for components included in the principal component analysis (PCA); Supplementary Table S2: Time comparison of contamination status in the investigated sites with national policy framework for marine sediments; Supplementary Figure S6: Variance (%) for all dimensions (PC) in principal component analysis (PCA); Supplementary Table S3: Contaminant data submitted to normalization with total organic content, expressed as TOM (%), for sediment samples collected across all research sites.

Author Contributions: Conceptualization, B.H. and M.G.; methodology, B.H., J.P., K.G., K.P., M.G. and M.S.T.; software, J.P., L.P., K.G., K.P., M.G. and B.H.; validation, B.H. and M.G.; formal analysis, J.P., D.P.-H., K.P., M.G. and B.H.; investigation, J.P., D.P.-H. and B.H.; resources, B.H., M.S.T., K.P. and M.G.; data curation, B.H., J.P. and K.G.; writing—original draft preparation, J.P., K.G., L.P., D.P.-H., K.P., M.S.T., M.G. and B.H.; writing—review and editing, B.H., K.G., D.P.-H., K.P., M.S.T., M.G. and J.P.; visualization, J.P., K.G., K.P. and B.H.; supervision of J.P., B.H. and M.G.; project administration, B.H., M.S.T., D.P.-H. and K.G.; funding acquisition, B.H. and M.S.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Croatian Science Foundation (HRZZ), ERA-NET BlueBio 2020 project MuMiFaST "Mussel Mitigation Feeds and Supply System Technological Development" (2021–2024), INTERREG Croatia-Italy project MARLESS "MARine Litter cross-border awarenESS and innovation actions" (2020–2023), and Region of Istria.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Acknowledgments: We gratefully acknowledge the technical support and help during the field and laboratory work of Andrej Jaklin, Ivan Radetić, Dario Devescovi, and other project Assistants.

Conflicts of Interest: The authors declare no conflicts of interest.

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