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From Waters to Fish: A Multi-Faceted Analysis of Contaminants Pollution Sources, Distribution Patterns, Ecological and Human Health Consequences

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Abstract: This study presents an extensive evaluation of the contamination levels in fish and mollusks, water, and sediments in the Black Sea over eight years, from 2016 to 2023. The primary aim was to find out what the concentrations and distribution patterns of heavy metals - HMs, polychlorinated biphenyls - PCBs, polycyclic aromatic hydrocarbons - PAHs, and other persistent organic pollutants - POPs in fish, water, and sediments of the Black Sea are, and the implications for marine ecosystem health and human safety. Data were collected through scientific cruises and the methodology involved systematic sampling across different regions of the Romanian Black Sea, followed by rigorous laboratory analyses to identify and quantify the presence of contaminants. The study also examined the temporal trends of these pollutants, providing insights into their sources, pathways, and persistence in the marine environment. Additionally, the research assessed the bioaccumulation of contaminants in various biota, offering a critical perspective on food safety and potential risks to human consumers. The findings reveal significant spatial highlighting areas of concern that require immediate attention and action. Notably, industrial discharge, agricultural runoff, and historical pollution hotspots were identified as major sources of contamination. The research underscores the need for enhanced monitoring and regulatory frameworks to mitigate pollution sources and safeguard the Black Sea ecosystem advocating for sustainable practices and effective management strategies to preserve marine resources in the Black Sea.

Keywords: contamination; bioaccumulation; fish and mollusks tissue; ecosystem components; heavy metals; organochlorine pesticides; polychlorinated biphenyls; polycyclic aromatic hydrocarbons.

Key Contribution: The key contribution of this study is its holistic approach to understanding the contamination dynamics in the Black Sea; offering insights into pollutant distribution patterns; sources; and impacts on marine and human health. This comprehensive analysis serves for informed decision-making and the development of targeted environmental management and pollution mitigation strategies.

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1. Introduction

The monitoring of the marine environment has particular importance in ensuring the security and safety of food [1]. This aspect is valid especially when it comes to fish and shellfish because they are, without a doubt, an integral part of any healthy and balanced diet organisms, like fish and shellfish, accumulate varying amounts of heavy metals and organic pollutants in their tissues depending on the species [3]. These pollutants can then be transferred up the food chain, sometimes exceeding safe limits for human consumption [3,4].

Hazardous substances are widespread in the marine environment. Many can be found in the Earth's crust and occur naturally in the seawater and sediments (HMs, PAHs). Synthetic hazardous substances such as OCPs and PCBs, are not found naturally in the environment. The main sources are generally waste/disposal, the burning of fossil fuels and industrial activities, including mining and production. Human activities have caused a general mobilization of these hazardous substances in the marine environment. The pathway of contamination is not always obvious, but it is primarily through riverine discharge and atmospheric deposition. Hence, although hot spots tend to be directly linked to human activities, the substances are also found in organisms that are collected far from point sources. The effects that some hazardous substances have on the environment and their potential risk to human health because of their toxic, bioaccumulative and persistent characteristics have led to considerable efforts (at the political, management and scientific levels) to address them. Specific policies and conventions aim to minimize the direct and indirect effects of these contaminants, generally by reducing emissions and discharges to the marine environment [5]

Heavy metals (HMs) pollution in marine environments is a significant global concern due to its adverse effects on marine ecosystems and human health. Understanding the sources, accumulation, and effects of heavy metals in marine ecosystems is essential for developing effective strategies to mitigate pollution and protect marine biodiversity. Studies have shown that heavy metals accumulate in coastal sediments [6], marine organisms [7], seawater, and living organisms [8]. The contamination of coastal waters by heavy metals poses risks to marine ecology and human health. Various sources contribute to heavy metals pollution, including industrial activities, urban and industrial waste discharge, agricultural runoff, and accidental spills of toxic chemicals [9]. The accumulation of heavy metals in marine organisms varies depending on pollution sources, elements, and species [10]. Research has highlighted the bioaccumulation of heavy metals in marine organisms, such as edible fish, emphasizing the dangerous threat these pollutants pose to human health [9]. Monitoring the concentration levels of heavy metals in marine environments is crucial to control pollution and protect water quality [11]. Furthermore, studies have investigated the impact of heavy metal accumulation on marine mollusks, emphasizing the negative effects on public health due to rising concentrations of heavy metals in aquatic environments [11]. Aquatic environments are unfortunately susceptible to contamination by various sources (natural and anthropogenic sources). Polycyclic aromatic hydrocarbons (PAHs) are natural fires, and natural gas eruptions. In addition to their natural formation is known, and it is known that perylene can be generated by diagenesis under anaerobic conditions and that naphthalene, phenanthrene and perylene can be produced naturally because of intense biological activity [13]. Anthropogenic sources of PAHs and POPs include industrial activities, agricultural chemicals (agrochemicals), water disposal, offshore activities, and maritime traffic [14,15]. Persistent organic pollutants (POPs) include two major types of chemicals: organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) [16]. Organochlorine pesticides (OCPs) are a category of chemicals phased out in the early 1980s due to their harmful properties [17] Examples include HCB (Hexachlorobenzene), Lindane, Heptachlor, and DDT (Dichlorodiphenyltrichloroethane) along with several others. These pesticides are not only highly toxic but also remarkably persistent in the environment. Their resistance to breakdown means they can linger for years or even decades in soil and sediments, and within living organisms (biota)[13,18].

PAHs are classes of pollutants that are particularly concerning because of their potential to harm marine life, with some PAHs even posing a carcinogenic threat. Due to the worldwide presence of PAHs in aquatic environments and their potential harm, monitoring for their presence is a global effort. Out of over the 100 polycyclic aromatic hydrocarbons (PAH) existing in the environment, 16 have been studied the most because they accumulate in living organisms and have harmful effects. These 16 PAHs include naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo(g,h,i)perylene, dibenzo(a,h)anthracene, and indeno(1,2,3-c, d) pyrene [19,20].

The European Union recognized this concern and included these pollutants on the list of priority hazardous substances within the Water Framework Directive 2000/60/EC [21].

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By analyzing the concentration of pollutants in different ecosystem components, such as water, sediment, and fish we can gain insights into the sources of their emissions [22]. They are concerning because they can harm both human health and the environment. Fish tend to store PAHs and POPs in their fatty tissue because they are lipophilic and very stable chemically. Therefore, fish are good indicators of pollution in coastal waters and they have been used for environmental monitoring [23]. Studies have shown that these pollutants can negatively impact marine life, hindering their growth and development, causing them to grow slower, and even leading to deformities in their embryos and larvae [24]. Chronic exposure to low levels of organic contaminants can disrupt the hormonal system in both animals and humans [4], polycyclic aromatic hydrocarbons (PAHs) found in the environment may not directly cause cancer, they can become carcinogenic after entering an organism [18]. Among PAHs, benzo[a]pyrene is the one with the strongest link to cancer in humans [25] Also, among sixteen priority PAHs analyzed, benzo[a]pyrene stands out as the only one with a maximum permissible limit set by European Regulation 915/2023/EC [26]. Studying HMs, POPs and PAHs in fish and mollusks tissues from the Black Sea is important for human health concerns. Thus, despite the well-established health benefits of fish consumption, concerns arise regarding the potential risks associated with frequent polluted fish intake. These risks stem from exposure to chemical pollutants found in fish and shellfish. By analyzing contaminants in fish and mollusks from the Black Sea, potential health risks associated with eating seafood can be assessed. Therefore, it is crucial to strike a balance in fish and mussels' consumption, maximizing the health benefits while minimizing the associated risks [4]. In the present study, risk evaluations were conducted for the first time to determine the potential hazards that may arise as a result of consuming mussels from the Romanian coast the Black Sea, similar with the approaches used in other Black Sea regions, by calculating the health risk indices including the estimated daily intake (EDI), target hazard quotient (THQ), total hazard quotient (TTHQ), and carcinogenic risk index (CRI) for heavy metals [27]. In the present study, risk evaluations were conducted for the first time to determine the potential hazards that may arise as a result of consuming mussels from the Romanian coast the Black Sea, similar with the approaches used in other Black Sea regions, by calculating the health risk indices including the estimated daily intake (EDI), target hazard quotient (THQ), total hazard quotient (TTHQ), and carcinogenic risk index (CRI) for heavy metals [27]

Another concern regards environmental safety. Due to its specific characteristics, morphological climatic, and hydrological properties, the Black Sea is highly susceptible to environmental damage caused by human activities which disrupts the balance of the marine ecosystem, putting the health of fish and shellfish at risk [18].

The contaminant types and concentrations within fish and mollusk tissues serve as a powerful knowledge for assessing the Black Sea's pollution state. It is essential for creating effective strategies to mitigate pollution and ensure a healthier marine environment. As the present paper aims to assess the concentrations and distribution patterns of heavy metals, persistent organic pollutants - POPs (polychlorinated biphenyls – PCBs and organochlorine pesticides - OCPs), and polycyclic aromatic hydrocarbons - PAHs, in mollusks, and fish of the Black Sea, and the implications for marine human safety and ecosystem health. Moreover, concentrations in water and sediments are assessed to identify potential sources of pollution. The results further contribute to understanding the pollution pathways and subsequently impact human health through seafood consumption.

2. Materials and Methods

A total number of 82 samples of mollusks (2 species) and fish (14 species), seawater, and sediments, were sampled from Romanian Black Sea sector (25 stations), during expeditions conducted by the National Institute for Marine Research and Development "Grigore Antipa" with the research vessel "Steaua de mare", during 2016-2023. The stations are located in the Romanian Exclusive Economic Zone (EEZ). The stations fall within the marine reporting regions within Marine Strategy Framework Directive:

Marine Reporting Unit - BLK_RO_RG_TT03: Northern stations, under the Danube's direct influence, up to 30 m depth isobath.

Marine Reporting Units - BLK_RO_RG_CT: Stations the neighborhood of harbor activities, shipping, tourism, up to 20 m depth isobath.

Marine Reporting Units - BLK_RO_RG_MT01: Stations in shelf waters, including maritime activities, vessel traffic, and industrial activities, from 30 m depth to 200 m (Figure 1).

The marine organisms sampled for this study were: mollusks - bivalve mollusks (*Mytilus galloprovincialis*), gastropods (*Rapana venosa*), and fish - pelagic species: *Sprattus sprattus* (sprat), *Engraulis encrasicolus* (anchovy), *Trachurus mediterraneus ponticus* (horse mackerel), *Chelon auratus* (golden gray mullet), Alosa immaculata (pontic shad), Belone belone (garfish), Sarda sarda (*atlantic bonito*), *Mullus barbatus ponticus* (blunt-snouted mullet), *Pomatomus saltatrix* (blufish), *Alosa tanaica* (Black Sea shad), and three benthic species, *Neogobius melanostomus* (round goby), *Psetta maeotica* (turbot) and *Squalus acanthias* (picked dogfish). The sampling locations were selected based on variations in ecological conditions and potential human activity impacts.

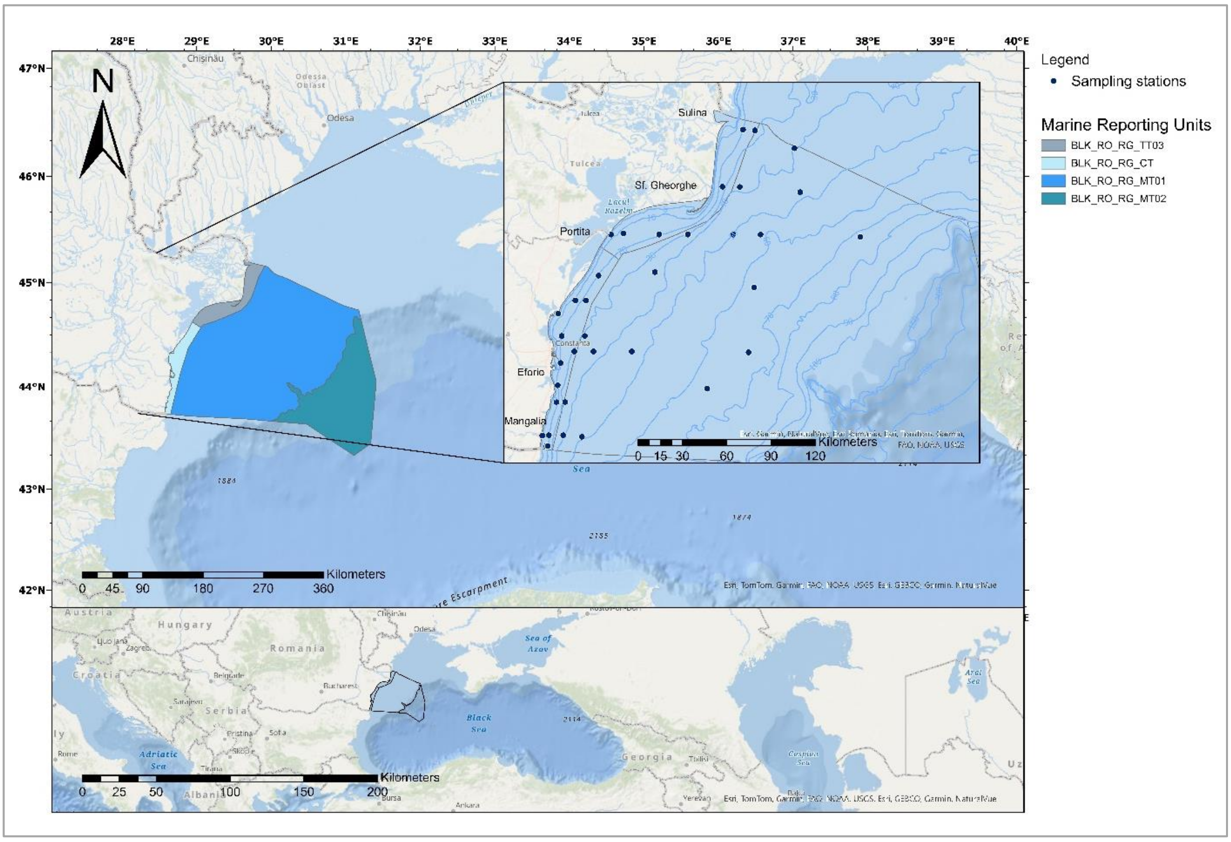

Figure 1. Map of sampling stations at the Romanian Black Sea Sector, 2016-2023.

2.1. Sampling and Preliminary Preparation Methods

Seawater samples were collected from the surface layer (1 meter below the surface) using Niskin bottles. The seawater samples were stored in a refrigerator (4-8 °C) for further analysis in the laboratory. Sediments were collected with a Van Veen bodengreifer; thickness of sampled sediment – 2 cm and stored at -20 ^{o}C. Mollusks were collected using a biological dredge and pelagic fish by pelagic trawl and benthic fish by bottom trawl. Following sampling, were stored in freezer boxes, and transported to the laboratory.

The mollusks and fish samples were washed on the research vessel, and these were measured and weighed in the laboratory. Whole mollusk bodies and fish muscle tissue were freeze-dried using a Labconco Freeze Dry System. Finally, the dried tissues were homogenized with an electric grinder.

2.2. Analytical Methods

Heavy metals in seawater, sediments, and biota

Seawater concentrations of copper (Cu), cadmium (Cd), lead (Pb), nickel (Ni), and chromium (Cr) were determined from unfiltered and acidified water samples (acidified up to pH = 2 with HNO_3 Ultrapure). For heavy metal (Cu, Cd, Pb, Ni, Cr) analyses in sediments and tissues, about 0.05 - 0.5 g of dry material was digested with 10 mL concentrated HNO_3 in sealed Teflon vessels on an electric hot plate at 120 °C. The solution was made up to 100 mL with deionized water (18.2 MΩ.cm, Millipore, Burlington, MA, USA). Heavy metal (HM) determinations were performed on a High-Resolution Continuum Source Atomic absorption spectrometer (HR-CS ContrAA 800 G equipment, Analytik Jena, Jena, Germany). Calibration was performed with working standards prepared from Merck stock solutions for each element in the following ranges: 0–50 µg/L (Cu), 0–10 µg/L (Cd), 0–25 µg/L (Pb), 0–50 µg/L (Ni), 0–50 µg/L (Cr). Each sample was measured in three parallel sub-samples, and the average value was reported. The method detection limits for HMs were, depending on element, between 0.001 and 0.01 µg/L. To ensure the accuracy of the analytical procedures, standard protocols were used [28,29]. Seawater concentrations of heavy metals are expressed as µg/L, sediments as µg/g dry weight (dws) and the tissue concentrations as µg/g tissue wet weight (wwt).

POPs (OCPs and PCBs) extraction in biota

Freeze-dried and crushed samples (approx. 2g) were microwave extracted with acetone/hexane (1/1) mixture. Internal standard 2,4,5-Trichlorobiphenyl was added to the samples for quantifying the overall recovery of the analytical procedures. Lipid removal was done by adding 10 ml of concentrated H_2SO_4 followed by cleanup with copper and fractionated on a florisil column. Finally, the samples were concentrated using nitrogen flow on a water bath and analyzed by GC-ECD.

PAHs extraction in biota

Unlike OCPs and PCBs, a separate method was used to prepare samples for analyzing 16 priority polycyclic aromatic hydrocarbons (naphthalene (Na), acenaphthylene (Ac), acenaphthene (Ace), fluorene (Flu), anthracene (An), phenanthrene (Ph), fluoranthene (Fln), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[g,h,i]perylene (BghiP), indeno[1,2,3-cd]pyrene (IP), and dibenzo[a,h]anthracene (DaA). These compounds are extracted from approx. 2g of freeze-dried biota samples by the Soxhlet method. Internal standard 9,10-dihydroanthracene was added to the samples for quantifying the overall recovery of the analytical procedures. Samples were Soxhlet extracted for 8 h with 250 ml methanol. The extracts were then saponified by adding 20 ml of 0.7 M KOH and 30 ml of water and refluxing for 2 h. The fraction containing PAH fraction was concentrated using a gentle stream of nitrogen to a final volume of 1 mL and analyzed by GC-MS.

POPs and PAHs extraction in seawater and sediments

Seawater samples are funnel extracted with a hexane/dichloromethane (3/1) mixture. Sediments samples were processing by freeze-drying, homogenization, and sieving for removing coarse fragments (> 0.5 mm), before extraction of POPs (OCPs and PCBs) and PAHs by microwave method. The extraction was followed by cleanup with copper and fractionated on a florisil column for OCPs and alumina/silica column for PAHs. Finally, the samples were concentrated using nitrogen flow on a water bath and analyzed by GC-ECD and GC-MS, respectively. Internal standard 2,4,5-Trichlorobiphenyl for POPs and 9,10-dihydroanthracene for PAHs were added to the samples for quantifying the overall recovery of the analytical procedures [25].

Gas-chromatographic conditions for organic pollutants

For OCPs and PCBs, the concentrated extracts were then analyzed using a gas chromatograph Perkin Elmer Clarus 500 equipped with electron capture detector (ECD). Working conditions for gas-chromatographic analysis were: Column: 30 m x 0.25 mm x 0.50 µm Elite-5MS; Carrier gas: helium, 1 ml/minute; Split/Spitless Injector (S/S), injection made in split mode; split flow 25 ml/minute; injector temperature: 330°C; detector gas: nitrogen; detector temperature, 330°C. Oven temperature program: 180° C (0 minute); 7°C/minute up to 230° C (10 minutes); 15°C C/minute up to 250°C (2 minutes).

Quantification of the specific OCPs and PCBs was achieved by comparing the sample peaks areas to those of known standards. Individual standard for 9 OCPs (Hexachlorobenzene, Lindane, Heptachlor, Aldrin, Dieldrin, Endrin, p,p'DDE, p,p'DDD, p,p'DDT) and 7 PCBs (PCB 28, PCB 52, PCB

101, PCB 118, PCB 138, PCB 152, PCB 180), dissolved in methanol with concentrations between 1000 - 5000 µg/mL, were used for the calibration curves for GC-ECD. The values of the retention times, the recovery coefficients, and the maximum admissible limits for OCPs and PCBs are represented in Tables 1s and 2s, respectively.

For PAHs the concentrated extract was then analyzed by GC/MS using a Perkin Elmer Clarus 690 system. The mass spectrometer operated at 70 eV scanning from m/z 47–400; the interface temperature was set at 330 C, the source temperature at 270 C. The extracts were injected in spitless mode and separated on Elite 35MS capillary column (5% diphenyl dimethyl polysiloxane; 30 m length x 0.32 mm i.d. x 0.25 lm film thickness). Helium served as the carrier gas, flowing at a constant rate of 1 ml/min, during the sample analysis. Initially injected at 100°C, the samples were heated in an oven following a two-stage program. In the first stage, the temperature ranges from 100°C to 250°C at a rate of 6°C per minute. The second stage increased the temperature further to 330°C at a faster rate of 10°C per minute. Once at 330°C, the oven temperature was held constant (isothermally) for 10 minutes. Data were gathered using the SIR technique, as described in Table 3s. Quantification of the specific polycyclic aromatic hydrocarbons (PAHs) was achieved by comparing the retention times of the sample peaks to those of known standards (Table 3s). A standard mixture, containing the 16 priority PAHs, dissolved in toluene in individual concentrations of 100 µg/mL, was used to calibrate the GC-MS.

2.3. Human Health Risk Assessment

Health risk indices including the Estimated Daily Intake (EDI) and the Estimated Weekly Intake (EWI), Target Hazard Quotient (THQ), Total Hazard Quotient (TTHQ), and Carcinogenic Risk Index (CRI) were assessed for heavy metals (Cu, Cd, Pb, Ni, Cr). Risk evaluations were conducted to determine the potential hazards that may arise because of consuming mussels (*M. galloprovincialis*) from the Romanian Black Sea coast during 2016 - 2023. This was determined by calculating the probability of a health hazard using likely exposure.

Estimated Daily Intake (EDI) and Estimated Weekly Intake (EWI)

The average daily intake of heavy metals (mg/kg/day) must be considered when calculating risk exposure. The estimated daily intake (EDI) is calculated based on element levels and the amounts of mussels consumed. The following equation was used to calculate the EDI of heavy metals [31,32]:

$$EDI = \frac{C_{metal} \times FIR}{BW}, mg/kg/day$$

where EDI is the estimated daily intake of heavy metal C_{metal} is the concentration of heavy metal in mussels' samples (whole tissues) (mg/kg, wet wt.), FIR (food ingestion rate) (kg/day) is the daily mean consumption of food item and BW is the average body weight (30 kg and 70 kg for children, and adults, respectively).

Information on the daily mean consumption of food item (FIR) was obtained from FAOSTAT [33]. Food supply quantity (kg/capita/year) for category "Mollusks, Other" for Romania, period 2010 – 2021, varied between 0.08 – 0.50 kg/capita/year, respectively 0.00022 – 0.00137 kg/day.

Estimated weekly intake (EWI) were found by multiplying EDI values by 7 [31,32].

Non-Carcinogenic Hazard (the Target Hazard Quotient (THQ)) and Hazardous Risk (Total Hazard Quotient (TTHQ))

The noncarcinogenic risk related to the consumption of mollusks and their associated heavy metals was evaluated using the target hazard quotient (THQ) or hazard index (HI), determined as the ratio of the calculated metal dosage (EDI mg/kg of body weight per day) to the reference dose (Rf. D. mg/kg/day) [34]:

$$THQ = \frac{EDI}{Rf.D.}$$

where Rf. D. is the Chronic Oral Reference Dose (mg/kg/day) which refers to the estimated maximum permissible health risk associated with daily human consumption of metals in food items (mussels). The Rf. D. values for Cd, Cu, Ni and Cr are 0.0001, 0.04, 0.02 and 0.003 mg/kg/day, respectively [35]. However, the Rf. D. value for Pb is not given.

If THQ (HI) > 1.0, the EDI of a particular metal exceeds the Rf. D., indicating that the metal is potentially hazardous. It is dependent on both metal levels and the amounts of mussel consumed.

The TTHQ estimates the cumulative risk associated with exposure to multiple heavy metals.

It is accepted that the likelihood of a health risk is inversely correlated with the combined impacts of metals on the same target organ. TTHQ >1 discloses potential chronic danger, while TTHQ <1 implies no potential health risk.

Carcinogenic Risk Index (CRI)

The CRI is one metric to measure the carcinogenic risk. The equation below represents CRI in terms of:

$$CRI = EDI \times CSF$$

The CSF (cancer slope factor) $(mg/kg/day)^{-1}$ establishes the risk associated with a lifetime average contaminant dose. CSF value is given for Pb, and this value is 0.0085 $(mg/kg/day)^{-1}$[36];[31].

If the CRI is less than 10^{-6}, it is deemed inconsequential; if the CRI is between 10^{-6} and 10^{-4}, it is acceptable or bearable; and if the CRI is greater than 10^{-4}, it is deemed significant.

4. Results

Heavy Metals

The statistical parameters provide insights into the distribution and variability of heavy metal concentrations in mussels (*Mytilus galloprovincialis*) (Table 1), gastropods (*Rapana venosa*) (Table 2) and fish (Table 3) from the Romanian Black Sea investigated during 2016 – 2023.

Based on Coefficient of Variation (CV) values greater than 100%, suggesting a significant dispersion in the data, Pb and Cr concentrations in mussels exhibited a high variability, with wide fluctuations around the average value (CV_{Pb} = 196.191%; CV_{Cr} = 119.599%), also indicating outliers or extreme values that contributed to these values. CV for Ni of 98.097% indicates a moderate variability, while the relative variability of Cu and Cd concentrations in mussels, 56.536% and 71.461%, respectively, is a moderate one. Positive skewness values suggests that the data for all metals tends to cluster toward the lower end, with a few extreme values pulling the mean to the right. Cu and Cr concentrations were characterized by a slightly right-skewed distribution, while Cd, Pb and Ni skewness values suggested a highly skewed distribution to the right. High positive kurtosis values were observed for Cd, Pb and Ni, that indicate heavy tails distribution (more extreme values), while low kurtosis value of Cr suggests a relatively normal distribution. Negative kurtosis value of Cu indicates a relatively flat distribution (Table 1). We noticed that cadmium extreme values (> 1 µg/g wwt) were measured in specimens from the Northern sector of the Romanian littoral, under the influence of river discharges.

Table 1. Variability of heavy metal concentrations in mussels (*Mytilus galloprovincialis*) - Romanian Black Sea, 2016 – 2023.

Descriptive Statistics (*Mytilus galloprovincialis*, 2016-2023)										
	N	Mean	Median	Min	Max	25th percentile	75th percentile	Coef. Var.	Skewness	Kurtosis
Cu (µg/	45	2.112	2.154	0.460	5.110	1.038	2.756	56.536	0.540	-0.311

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g ww)									
Cd (µg/g ww)	45	0.520	0.377	0.143	2.018	0.276	0.630	71.461	2.169	5.969
Pb (µg/g ww)	45	0.149	0.034	0.002	1.587	0.0192	0.102	196.19	3.445	13.486
Ni (µg/g ww)	45	1.005	0.758	0.143	5.744	0.450	1.140	98.097	3.090	12.160
Cr (µg/g ww)	45	0.957	0.390	0.080	4.384	0.233	1.169	119.59	1.748	2.091

Table 2 provides insights into the distribution and variability of heavy metal concentrations in *Rapana venosa* from the Romanian Black Sea during 2016 – 2021: copper and chromium concentrations exhibit moderate variability (55.47% CV_{Cu}, 81.921% CV_{Cr}), with a distribution shape resembling a normal curve; cadmium concentrations show high variability (100.65% CV), positively skewed (longer tail on the right side) and a distribution with pronounced peaks and heavier tails; lead concentrations exhibit extreme variability (245.91% CV), highly positive skewness (very long tail on the right side), with a distribution shape emphasizing both peak and tail behavior; nickel concentrations display substantial variability (85.49% CV), slight positive skewness, with a flatter distribution compared to the others (negative Kurtosis value) (Table 2).

Table 2. Variability of heavy metal concentrations in gastropod (*Rapana venosa)* - Romanian Black Sea, 2016 – 2021.

Descriptive Statistics (*Rapana venosa*, 2016 - 2021)										
	N	Mean	Median	Min	Max	25th percentile	75th percentile	Coef. Var.	Skewness	Kurtosis
Cu (µg/g ww)	15	5.994	5.2180	0.93	12.867	4.640	7.672	55.47	0.643	0.450
Cd (µg/g ww)	15	0.814	0.578	0.111	2.915	0.192	1.199	100.657	1.480	1.827
Pb (µg/g ww)	15	0.137	0.023	0.001	1.310	0.010	0.065	245.915	3.443	12.292
Ni	15	0.432	0.386	0.010	1.208	0.040	0.744	85.49	0.493	-0.612

(µg/g ww)										
Cr (µg/g ww)	15	0.843	0.5765	0.116	2.376	0.324	1.390	81.921	1.052	0.495

Overall, the heavy metal concentrations in fish from the Romanian Black Sea vary considerably. Lead (Pb) showed the highest variability, followed by nickel (Ni), cadmium (Cd), copper (Cu), and chromium (Cr). The positive skewness values for most metals indicate that the distribution of concentrations is skewed towards higher values. This suggests that a small number of fish may have high concentrations of heavy metals. Copper (Cu) and cadmium (Cd) still have a kurtosis value close to 0, indicating a mesokurtic distribution with normal tails. Lead (Pb), nickel (Ni), and chromium (Cr) all have positive kurtosis values, ranging from 2.736 to 10.949. This indicates a leptokurtic distribution with heavier tails compared to a normal distribution. Heavier tails suggest a higher prevalence of extreme values for these metals in the fish samples compared to a normal distribution (Table 3). For instance, Pb higher values (> 0.30 µg/g ww) were measured in some species of pelagic fish (E*ngraulis encrasicolus, Alosa caspia, Trachurus mediterraneus ponticus,* and *Belone belone*).

Table 3. Variability of heavy metal concentrations in pelagic and demersal fish - Romanian Black Sea, 2016 – 2019.

Descriptive Statistics (Fish, 2016 - 2019)										
	N	Mean	Median	Min	Max	25th percentile	75th percentile	Coef. Var.	Skewness	Kurtosis
Cu (µg/g ww)	21	2.990	2.592	0.565	7.907	1.275	4.725	68.518	0.770	-0.263
Cd (µg/g ww)	21	0.065	0.030	0.007	0.230	0.016	0.075	109.52	1.295	0.323
Pb (µg/g ww)	21	0.601	0.260	0.002	4.625	0.003	0.520	175.92	3.134	10.949
Ni (µg/g ww)	21	4.245	3.172	0.092	18.46	0.332	5.808	127.52	1.758	2.736
Cr (µg/g ww)	21	0.235	0.215	0.028	0.880	0.135	0.277	74.113	2.614	9.363

In seawater, most concentrations of heavy metals determined during 2016 – 2023 were within normal variability intervals, with the following values of percentile 75th: 10.263 µg/L Cu, 0.890 µg/L Cd, 9.370 µg/L Pb, 7.270 µg/L Ni and 4.160 µg/L Cr. Although, depending of sampling area and

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season, higher values were occasionally measured, so overall heavy metals levels varied within wide ranges: Cu 0.790-33.480 µg/L; Cd 0.001-2.070 µg/L; Pb 0.001-25.970 µg/L; Ni 0.010-75.380 µg/L; Cr 0.219-47.730 µg/L. High Coefficient of Variations values, such as those observed for nickel (CV 180.30%), and chromium (CV 152.68%) indicate that the metal concentrations vary significantly, with wide fluctuations around the average values. Positively skewed distributions were observed for all metals in seawater, indicating occurrence (sporadic) of extreme high values. For copper (Cu), the kurtosis value of 3.495 indicates leptokurtic behavior (heavier tails). This means that extreme copper concentrations occur more frequently than in a normal distribution. With a kurtosis of 0.637, cadmium's distribution is platykurtic (lighter tails), suggesting fewer extreme values, while lead's kurtosis of 1.028 is close to normal (mesokurtic), indicating a balanced distribution. For nickel (Ni) and chromium (Cr), the extremely high kurtosis (10.676 and 25.878 respectively) implies very heavy tails, with rare but extreme concentrations (Table 4S).

In sediments, most concentrations of heavy metals determined during 2016 – 2023 were within normal variability intervals, with the following values of percentile 75th: 43.750 µg/g d.w. Cu, 0.518 µg/g d.w. Cd, 20.830 µg/g d.w. Pb, 60.960 µg/g d.w. Ni and 47.841µg/g d.w Cr. Although, depending on sampling area, proximity of pollution sources and sediments granulometry, higher values were occasionally measured, so overall heavy metals levels varied within wide ranges: Cu 3.660-123.900 µg/g d.w; Cd 0.030-4.345 µg/g d.w.; Pb 1.350-65.362 µg/g d.w.; Ni 5.630-160.200 µg/g d.w.; Cr 6.290-98.730 µg/g d.w. Cadmium has the highest variability in sediments (CV 143.98%). All metals exhibit positively skewed distribution (longer tail on the right), but chromium has the least pronounced skew. Cadmium distribution stands out with extremely heavy tails (kurtosis 13.883), that means more extreme values than a normal distribution, followed by copper, while lead and nickel have moderately heavy tails. A kurtosis value of 0.355 indicates that the chromium distribution is closer to a normal distribution (mesokurtic), compared to the other metals (Table 5S).

Distribution of heavy metals in seawater and sediments during 2016-2023 highlighted the influence of localized sources that play a crucial role in shaping heavy metal levels in marine environment. First, the discharge zone of the Danube River significantly impacts heavy metal concentrations. As the Danube flows into the Black Sea, it carries dissolved and particulate matter, including heavy metals, thus sediments from the Northern sector of the Romanian littoral tend to accumulate higher metal levels. In the Southern sector, the Constanta and Mangalia port areas experience intense anthropogenic pressure. Discharges of wastewater, industrial runoff, and shipping activities contribute to metal pollution. Seawater and sediments in and around the port presented elevated heavy metal concentrations. Also, beside land-based sources, offshore activities (oil and gas platforms) could contribute with additional pressures. Increased naval traffic, especially in the recent period, could affect metal distribution patterns. Ships release ballast water, which can carry metals from one region to another, and thus water and sediments in heavily trafficked areas may also reflect this impact. (Figure 2, Figure 3).

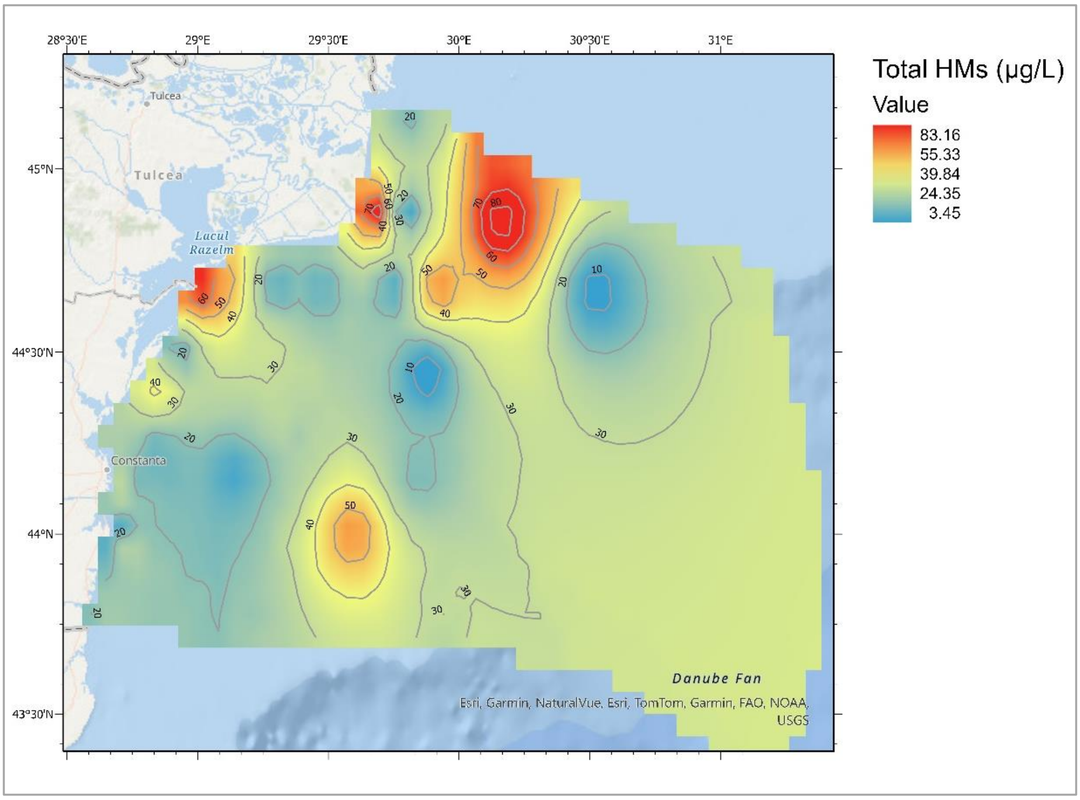

Figure 2. Distribution of total content HMs in seawater – Romanian Black Sea, 2016-2023.

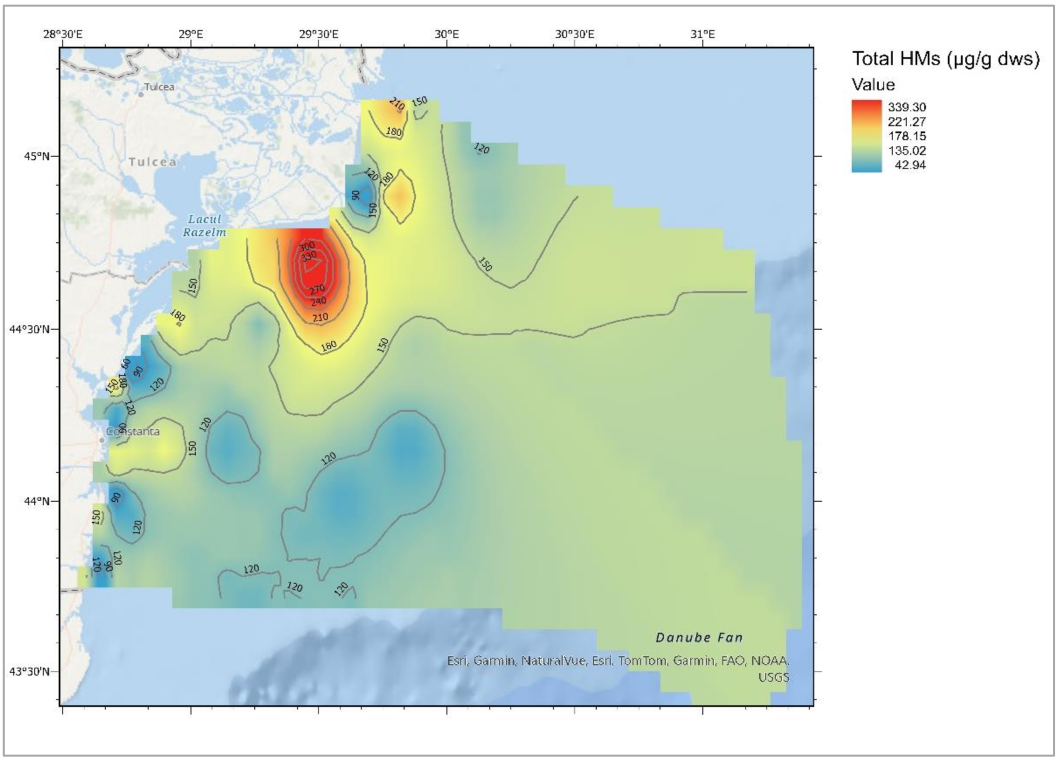

Figure 3. Distribution of total content HMs in sediments – Romanian Black Sea, 2016-2023.

The levels of heavy metals in both seawater and sediments significantly impact the bioaccumulation of these elements in mollusks and fish. Heavy metals exist in seawater in dissolved and particulate forms, and the bioavailability of these metals depends on their concentration and chemical speciation. Over time, metals could accumulate in the tissues of marine organisms. Sediments act as sinks for heavy metals associated with particles and benthic organisms (mollusks and fish) which accumulate metals from sediments through their diet and direct contact. Under certain conditions (e.g., low oxygen), sediments release previously sorbed metals back into the water column, making them available in the pelagic habitat. Mollusks and fish are part of food webs, and they transfer accumulated metals to higher trophic levels (e.g., predators). Humans consume seafood, including mollusks and fish, as part of their diet. Elevated metal concentrations in contaminated seafood pose health risks to humans, especially when consumed over extended periods.

Human Health Risk Assessment

The heavy metal (Cd, Pb) values measured in mussels and fish were compared to the concentrations permitted by European Commission Regulation (EU) 2023/915 [26] for consumed seafood. In mollusks, maximum admissible concentrations (MACs) of 1 µg/g ww Cd were surpassed in 6% of mussels, respectively in 30% of gastropods, most of the contaminated samples being found in the area under the influence of Danube discharge (Figure 1S). The number of Pb values surpassing MACs of 1.50 µg/g ww Pb was insignificant in mollusks (below 0.3% of samples). In summary, while some Cd contamination was observed in both mussels and gastropods, the Pb levels remained well below the established limits. Monitoring and managing contamination sources, especially near the Danube discharge area or various hot spots, are essential to ensure the safety of consumed seafood.

In pelagic and demersal fish, MAC for Cd of 0.05 µg/g ww was surpassed in 35% of samples, whereas MAC for Pb of 0.30 µg/g ww was surpassed in 48 % of samples. These findings highlight the importance of monitoring heavy metal levels in fish to ensure the safety of seafood consumption. (Figure 4).

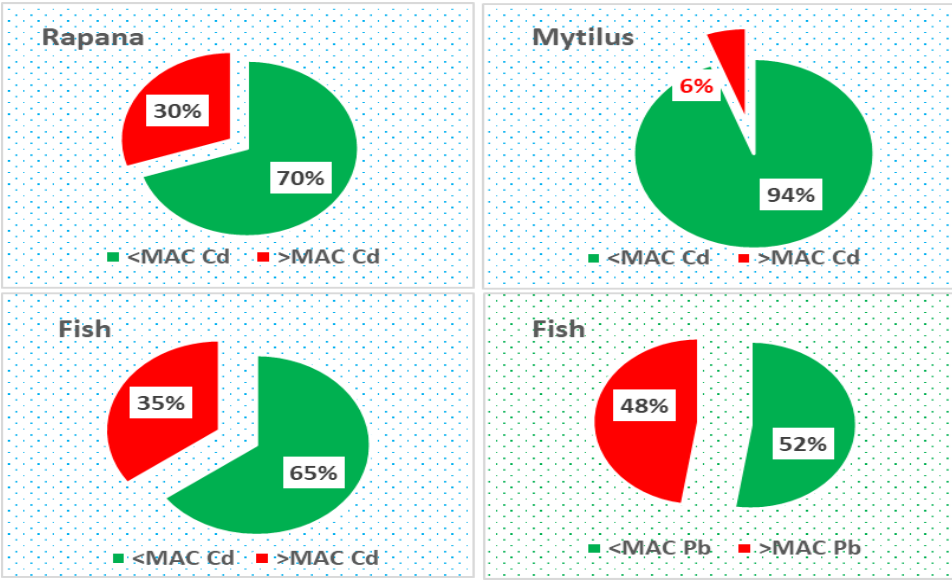

Figure 4. Heavy metals (Cd, Pb) values measured in mollusks (*Rapana venosa* and *Mytilus galloprovincialis*) and pelagic and demersal fish, compared to the values permitted by European Commission Regulation (EU) 2023/915 for consumed seafood, 2016-2023.

The present study investigated the estimated daily intake (EDI), Target Hazard Quotients (THQs), Total Hazard Quotient (TTHQ) and Carcinogenic Risk Index (CRI) of heavy metals in two distinct groups (children and adults) consuming mussels (*M. galloprovincialis*), harvested from the Romanian coast of the Black Sea during the study period spanning from 2016 to 2023.

Our findings are summarized in Table 4. Notably, the EDI rates for copper (Cu), cadmium (Cd), chromium (Cr), nickel (Ni), and lead (Pb) in mussels were consistently below the Chronic Oral Reference Dose (Rf. D.) for both children and adults, suggesting safe consumption levels. The calculated exposure values by food item (mussels) (EDIs) were compared and found below the

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health-based guidance values provided by European Food Safety Authority (EFSA). For Pb, the benchmark dose level (BMDL10) of 0.63 µg/ kg b.w. per day is considered as health-based guidance value (HBGV) by EFSA Panel on Contaminants in the Food Chain (CONTAM Panel), value confirmed by Joint FAO/WHO Expert Committee on Food Additives (JECFA) as well [37]. For Ni, tolerable daily intake (TDI) of 13 µg/kg b.w. per day was established by recent decision by EFSA CONTAM Panel after the European Commission asked EFSA to update its previous Opinion on nickel in food and drinking water, considering new occurrence data [38]. For Cd, tolerable weekly intake (TWI) of 2.5 µg/kg b.w. per week is recommended by CONTAM Panel, which is equivalent to 0.36 µg/kg b.w. per day [39].

Table 4 also provides the Target Hazard Quotients (THQs) and Total Hazard Quotient (TTHQ) values for copper (Cu), cadmium (Cd), chromium (Cr), and nickel (Ni) in mussels consumed by both children and adults. If the THQ exceeds 1.0, it indicates that the estimated daily intake (EDI) of a specific metal surpasses the Chronic Oral Reference Dose (Rf. D.), suggesting potential health hazards.

The THQ is influenced by both metal concentrations and the quantities of mussel consumption. Metals found in mussels along the Romanian Black Sea coast result from a combination of anthropogenic activities and natural processes. These factors include industrial and urban wastewater discharge, riverine input, and atmospheric deposition. These sources introduce a variety of metals into the marine environment, leading to elevated concentrations in specific areas. Based on the findings from our current study, mussel consumption poses no risks to consumers. The calculated Target Hazard Quotients (THQs) for children and adults indicate that the estimated values remain below concern.

The Total Hazard Quotient (TTHQ) assesses the cumulative risk associated with exposure to multiple heavy metals. It is widely accepted that the likelihood of health risks is inversely correlated with the combined impacts of these metals on the same target organ. Specifically:

- TTHQ > 1: Indicates potential chronic danger, suggesting that the combined effects of metals exceed safe levels.
- TTHQ < 1: Implies no potential health risk, as the cumulative impact remains below critical thresholds.

In our study, the TTHQ values for the metals found in mussels along the Romanian coast of the Black Sea were consistently lower than 1. This result indicates that there are no adverse effects for consumers associated with heavy metal exposure from consuming mussels.

Furthermore, our findings reveal that the calculated Carcinogenic Risk Index (CRI) associated with lead (Pb) exposure is negligible. This information is presented in Table 4. If the CRI is less than 10^{-6}, it is deemed inconsequential; if the CRI is between 10^{-6} and 10^{-4}, it is acceptable or bearable; and if the CRI is greater than 10^{-4}, it is deemed significant.

Table 4. The EDIs and EWIs for all heavy metals, THQs and TTHQ for Cu, Cd, Cr and Ni, and carcinogenic risk index (CRIs) for Pb in two groups consuming mussels (M. galloprovincialis) from Romanian coasts of the Black Sea (2016-2023).

Metal	**EDIs**		**EWIs**		**THQs**		**CRIs**	
	Children	Adults	Children	Adults	Children	Adults	Children	Adults
Copper (Cu)	8.27E-05	3.54E-05	5.79E-04	2.48E-04	2.07E-03	8.86E-04		
Cadmium (Cd)	1.89E-05	8.09E-06	1.32E-04	5.66E-05	1.89E-01	8.09E-02		
Chromium (Cr)	3.88E-05	1.66E-05	2.72E-04	1.17E-04	1.29E-02	5.55E-03		
Nickel (Ni)	4.12E-05	1.77E-05	2.88E-04	1.24E-04	2.06E-03	8.83E-04		

Lead (Pb)	1.05E-05	4.52E-06	7.38E-05	3.16E-05			8.96E-08	3.84E-08
TTHQ					2.06E-01	8.81E-02		

EDI: Estimated daily intake (mg/kg/day); EWI: Estimated weekly intake; Target hazard quotient: THQ; Total hazard quotient: TTHQ; Carcinogenic Risk Index: CRI.

Persistent organic pollutants – POPs (PCBs and OCPs) in biota, seawater, and sediments

The statistical parameters provide insights into the variability of POPs concentrations in mussels *(M. galloprovincialis)* (Table 5, Table 6), gastropods *(Rapana venosa*) (Table 7, Table 8) and fish (Table 9, Table 10) from the Romanian Black Sea investigated during 2016 – 2023.

Table 5. Variability of OCPs concentrations in mussels (*M. galloprovincialis*) - Romanian Black Sea, 2016 – 2023.

Descriptive Statistics (*M. galloprovincialis*, 2016 - 2023)								
	N	Mean	Median	Min	Max	25th percentile	75th percentile	Coef. Var.
HCB (µg/g ww)	46	0.0335	0.00021	0.00008	0.4505	0.00008	0.0176	0.0858
Lindane (µg/g ww)	46	0.3312	0.00324	0.00006	7.3458	0.00006	0.1041	1.2112
Heptachlor (µg/g ww)	46	0.4533	0.00005	0.00005	9.0988	0.00005	0.0415	1.5225
Aldrin (µg/g ww)	46	0.0050	0.00005	0.00005	0.0577	0.00005	0.0019	0.0121
Dieldrin (µg/g ww)	46	0.0827	0.00032	0.00005	1.4328	0.00003	0.0137	0.2893
Endrin (µg/g ww)	46	0.0857	0.00212	0.00006	0.7117	0.00006	0.0893	0.1721
p,p' DDE (µg/g ww)	46	0.0589	0.00041	0.00003	1.1550	0.00003	0.0103	0.2073
p,p' DDD (µg/g ww)	46	0.5189	0.00089	0.00003	15.1661	0.00003	0.0553	2.2678
p,p' DDT (µg/g wwt	46	0.1560	0.00003	0.00003	5.3864	0.00003	0.0025	0.8001

The OCPs mean concentrations in mussels ranged from 0.005 to 0.5189 µg/g ww. Most of compounds have levels below 1 µg/g ww. The dominant compounds were, p,p' DDD, Heptachlor and Lindane, having the highest overall concentrations among the studied pollutants (p,p' DDD - 15.1661 µg/g ww, Heptachlor - 9.0988 µg/g ww and Lindane - 7.3458 µg/g ww). HCB has the lowest coefficient of variation, indicating more consistent concentrations across samples, whereas p, p' DDD shows high variability (Table 5).

Although the PCBs mean concentrations in mussels (*M. galloprovincialis)* are lower than OCPs, ranging from 0.0045 to 0.0856 µg/g ww, they also represent a possible threat for marine life. PCB 28, PCB 52 and PCB 138 have the highest overall mean concentration (0.0497, 0.0555 and 0.0856 µg/g ww) and the greatest coefficient of variation. The least encountered was PCB 180 for which were recorded the lowest values (mean concentration - 0.0045 µg/g ww, maximum concentration - 0.0547 µg/g ww)(Table 6). The detection of PCBs in mussels warrants further investigation due to their potential for bioaccumulation within the food chain.

Table 6. Variability of PCBs concentrations in mussels (*M. galloprovincialis*) - Romanian Black Sea, 2016 – 2023.

Descriptive Statistics (*M. galloprovincialis*, 2016 - 2023)								
	N	Mean	Median	Min	Max	25th percentile	75th percentile	Coef. Var.
PCB28 (µg/g ww)	46	0.0497	0.0001	0.00006	1.3773	0.00006	0.0011	0.2139
PCB52 (µg/g ww)	46	0.0555	0.0003	0.00005	0.9289	0.00005	0.0540	0.1515
PCB101 (µg/g ww)	46	0.0195	0.0004	0.00009	0.1863	0.00009	0.0091	0.0458
PCB118 (µg/g ww)	46	0.0188	0.0005	0.00006	0.1260	0.00006	0.0296	0.0310
PCB153 (µg/g ww)	46	0.0068	0.0001	0.00009	0.0688	0.00009	0.0010	0.0182
PCB138 (µg/g ww)	46	0.0856	0.0001	0.00011	2.1570	0.00011	0.0047	0.3439
PCB180 (µg/g ww)	46	0.0045	0.0002	0.00005	0.0547	0.00005	0.0048	0.0102

The highest mean values and coefficients of variation in the *Rapana venosa* were recorded for p,p' DDD (1.2885 µg/g ww), p,p' DDT (0.4965 µg/g ww) and Dieldrin (0.3487 µg/g ww). On the opposite, p,p' DDE has the lowest values (mean concentration - 0.0029 µg/g ww, maximum concentration - 0.0185 µg/g ww)(Table 7).

PCBs concentrations in gastropods ranged from 0.0001 to 0.1169 µg/g ww with the highest average concentration (0.0204 µg/g ww) and the most extensive range (from 0.0001 µg/g ww to 0.1169 µg/g ww) recorded for PCB 52. PCB 118 and PCB 138 have the lowest average concentrations (around 0.004 µg/g ww) and very low maximum concentrations (around 0.003 µg/g ww)(Table 8).

Table 7. Variability of OCPs concentrations in gastopods (*Rapana venosa* - Romanian Black Sea, 2016 – 2021).

Descriptive Statistics (*Rapana venosa*, 2016 - 2021)								
	N	Mean	Median	Min	Max	25th percentile	75th percentile	Coef. Var.
HCB (µg/g ww)	15	0.0084	0.0037	0.0001	0.0323	0.0003	0.0161	0.0105
Lindane (µg/g ww)	15	0.0596	0.0044	0.0001	0.3320	0.0003	0.1252	0.1009
Heptachlor (µg/g ww)	15	0.2029	0.0062	0.0001	1.2954	0.0009	0.0977	0.4271
Aldrin (µg/g ww)	15	0.1592	0.0020	0.0001	1.2146	0.0001	0.0925	0.3744
Dieldrin (µg/g ww)	15	0.3487	0.0238	0.0001	1.9202	0.0001	0.3265	0.6307
Endrin (µg/g ww)	15	0.2421	0.0168	0.0001	1.3636	0.0077	0.2814	0.4306
p,p' DDE (µg/g ww)	15	0.0029	0.0001	0.0001	0.0185	0.0001	0.0026	0.0057
p,p' DDD (µg/g ww)	15	1.2885	0.0020	0.0001	10.6067	0.0001	0.6253	2.9083

p,p' DDT (µg/g ww)	15	0.4965	0.0289	0.0001	3.7823	0.0001	0.0983	1.1062

Table 8. Variability of PCBs concentrations in gastopods (Rapana venosa - Romanian Black Sea, 2016 – 2021).

Descriptive Statistics (*Rapana venosa*, 2016 - 2021)								
	N	Mean	Median	Min	Max	25th percentile	75th percentile	Coef. Var.
PCB28 (µg/g ww)	15	0.0043	0.0005	0.0001	0.0290	0.0001	0.0016	0.0100
PCB52 (µg/g ww)	15	0.0204	0.0063	0.0001	0.1169	0.0012	0.0417	0.0330
PCB101 (µg/g ww)	15	0.0055	0.0005	0.0002	0.0383	0.0002	0.0061	0.0111
PCB118 (µg/g ww)	15	0.0044	0.0001	0.0001	0.0300	0.0001	0.0023	0.0099
PCB153 (µg/g ww)	15	0.0074	0.0002	0.0002	0.0549	0.0002	0.0017	0.0164
PCB138 (µg/g ww)	15	0.0084	0.0002	0.0002	0.0545	0.0002	0.0044	0.0177
PCB180 (µg/g ww)	15	0.0090	0.0002	0.0001	0.0692	0.0001	0.0069	0.0189

Overall, the POPs concentrations in fish from the Romanian Black Sea vary considerably. Most pesticides show very high coefficients of variation, indicating significant variability in pesticide concentrations across the fish samples (Table 9).

Table 10 summarizes the levels of seven polychlorinated biphenyls (PCBs) found in fish samples collected between 2016 and 2019. Most PCBs show very high coefficients of variation, indicating significant variability in PCB concentrations across the fish samples.

Table 9. Variability of OCPs concentrations in pelagic and demersal fish - Romanian Black Sea, 2016 – 2019.

Descriptive Statistics (Fish, 2016 - 2019)								
	N	Mean	Median	Min	Max	25th percentile	75th percentile	Coef. Var.
HCB (µg/g ww)	21	0.0315	0.0197	0.0001	0.2223	0.0001	0.0285	0.0537
Lindane (µg/g ww)	21	0.0100	0.0001	0.0001	0.0875	0.0001	0.0049	0.0210
Heptachlor (µg/g ww)	21	0.0251	0.0020	0.0001	0.1974	0.0001	0.0271	0.0483
Aldrin (µg/g ww)	21	0.0089	0.0001	0.0001	0.0844	0.0001	0.0020	0.0205
Dieldrin (µg/g ww)	21	0.0536	0.0101	0.0001	0.5735	0.0001	0.0283	0.1333
Endrin (µg/g ww)	21	0.0326	0.0040	0.0001	0.2291	0.0001	0.0107	0.0663
p,p' DDE (µg/g ww)	21	0.0155	0.0033	0.0001	0.1288	0.0019	0.0096	0.0312
p,p' DDD (µg/g ww)	21	0.0688	0.0053	0.0001	0.4481	0.0031	0.0406	0.1272

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p,p' DDT (µg/g wwt	21	0.0309	0.0028	0.0001	0.2195	0.0008	0.0081	0.0659

Table 10. Variability of PCBs concentrations in pelagic and demersal fish - Romanian Black Sea investigated during 2016 – 2019.

Descriptive Statistics (Fish, 2016 - 2019)								
	N	Mean	Median	Min	Max	25th percentile	75th percentile	Coef. Var.
PCB28 (µg/g ww)	21	0.0108	0.0008	0.0001	0.0516	0.0001	0.0164	0.0163
PCB52 (µg/g ww)	21	0.0147	0.0014	0.0001	0.1636	0.0001	0.0074	0.0370
PCB101 (µg/g ww)	21	0.0260	0.0006	0.0002	0.2390	0.0004	0.0138	0.0649
PCB118 (µg/g ww)	21	0.0342	0.0008	0.0001	0.1939	0.0001	0.0822	0.0579
PCB153 (µg/g ww)	21	0.0265	0.0003	0.0002	0.2220	0.0002	0.0052	0.0558
PCB138 (µg/g ww)	21	0.0205	0.0002	0.0002	0.1126	0.0002	0.0485	0.0335
PCB180 (µg/g ww)	21	0.0082	0.0005	0.0001	0.1262	0.0001	0.0025	0.0274

OCPs values exceeding the maximum admissible level for human consumption stipulated by national legislation (Order 147/2004) [32], were recorded mostly in mollusks (for HCB, Lindane, Dieldrin, Endrin, Heptachlor and Total DDT), but also in fish (for HCB, Dieldrin).

The study found that the maximum admissible concentrations (MACs) for various contaminants were exceeded in mussels as follows: HCB (5%), Lindane (19%), Dieldrin (11%), Endrin (22%), Heptachlor (27%), and Total DDT (30%) (Figure 5). In gastropods, the maximum admissible concentrations (MACs) were exceeded as follows: Heptachlor (21%), Aldrin (14%), Dieldrin (29%), Endrin (21%), and Total DDT (29%) (Figure 6).

The PCBs concentrations were measured in mussels, gastropods, and fish and were compared to the values (Sum of 6 PCBs) stipulated by European Commission Regulation (EU) 2023/915 for consumed seafood[26]. MAC of 0.075 µg/g ww Sum of 6 PCBs was surpassed in 27% of mussels, 29 % in gastropods and mostly in fish (43%) (Figure 7).

These findings highlight the importance of monitoring POPs (OCP and PCBs) levels in seafood to ensure the safety of consumption.

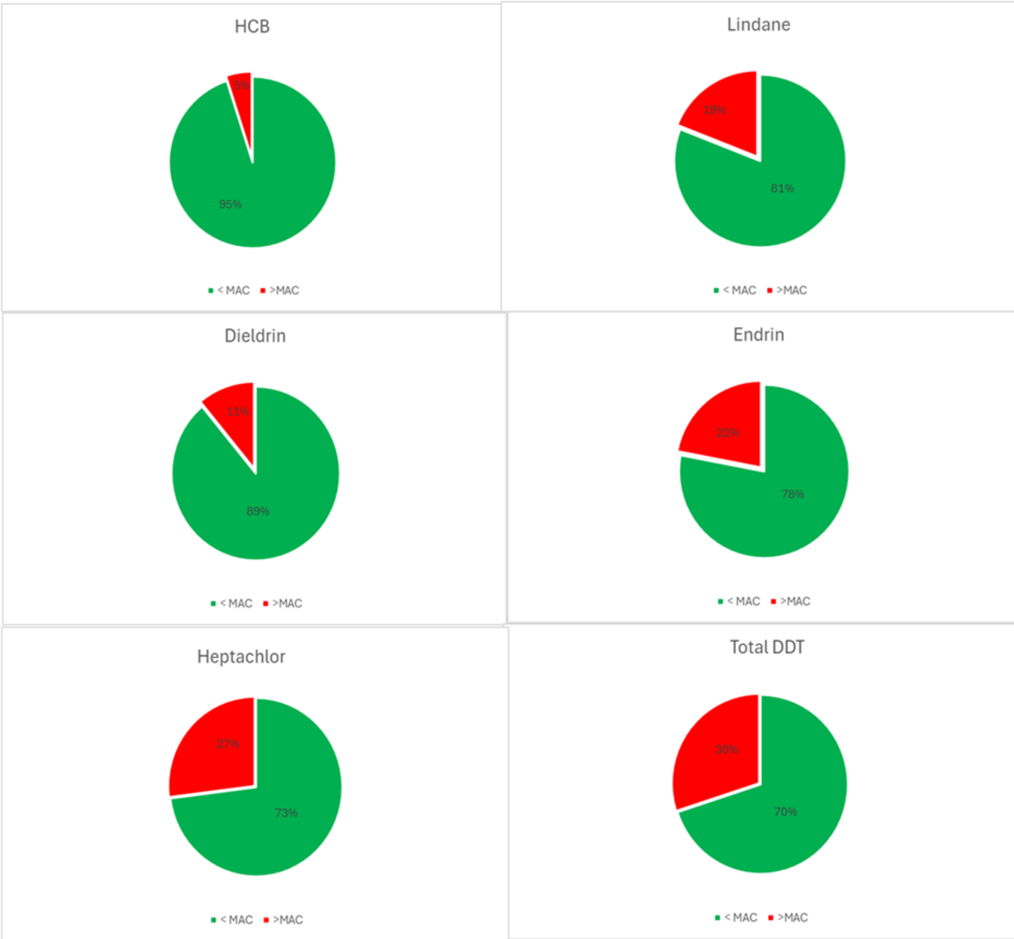

Figure 5. OCPs (HCB, Lindane, Dieldrin, Endrin, Heptachlor and Total DDT) values measured in mussels compared to the values permitted Order 147/2004, 2016-2023.

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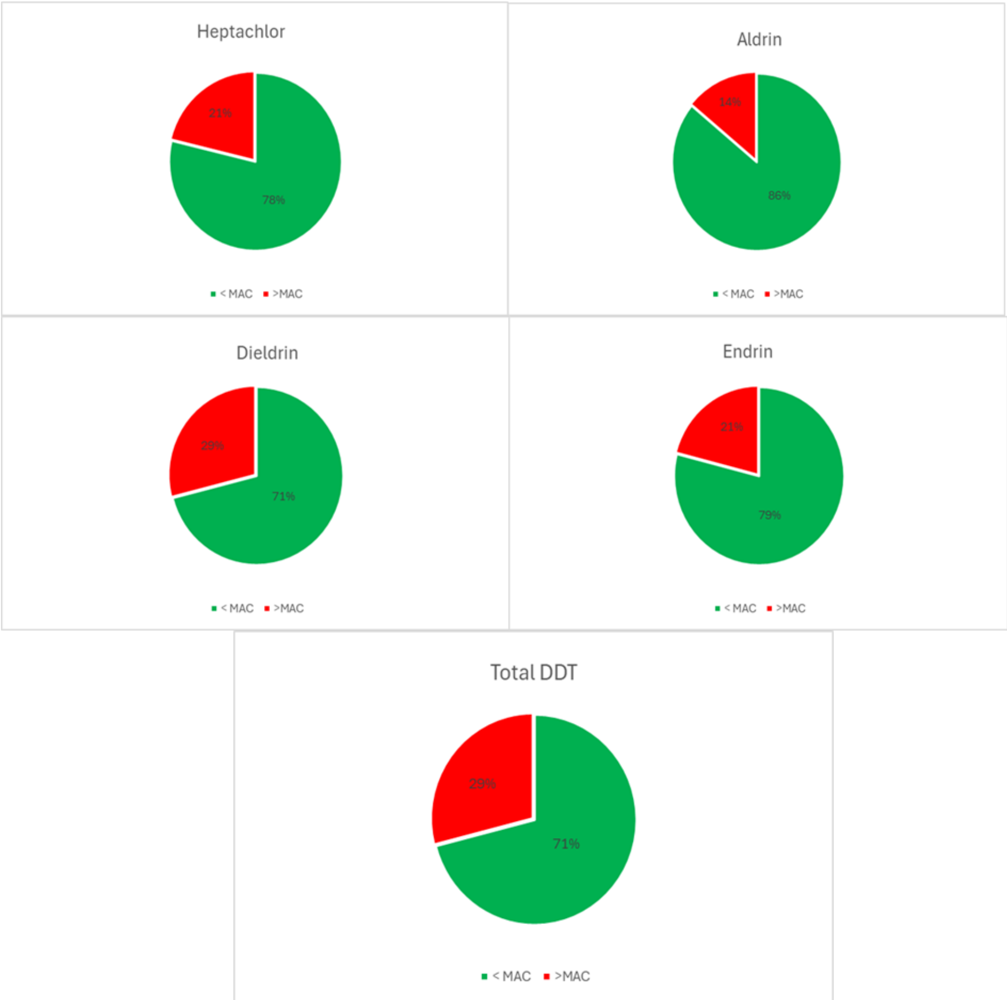

Figure 6. OCPs (Heptachlor, Aldrin, Dieldrin, Endrin and Total DDT) values measured in gastropods compared to the values permitted Order 147/2004, 2016-2023.

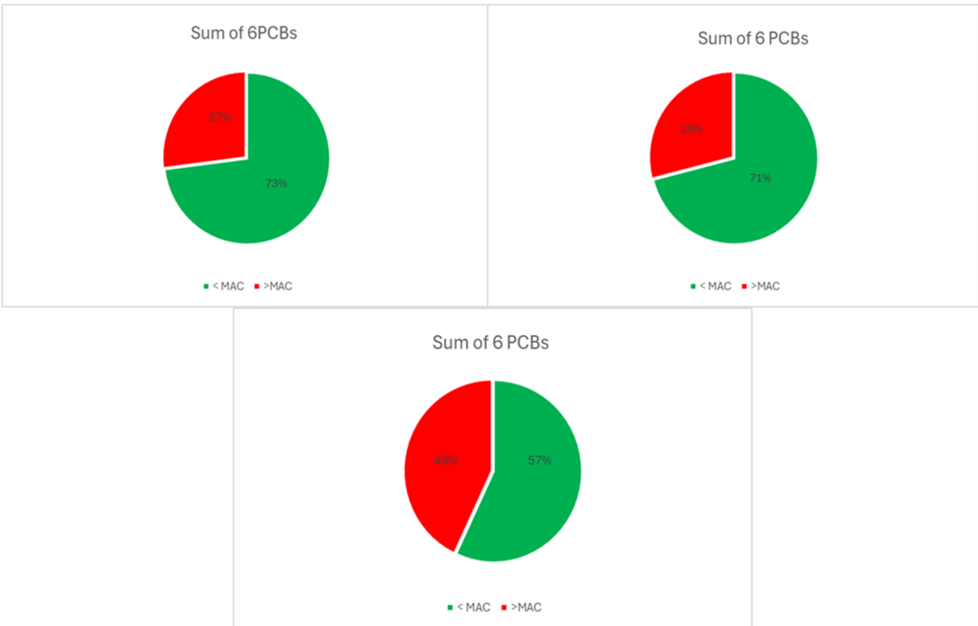

Figure 7. PCBs (Sum of 6 PCBs) values measured in mussels, gastropods, and fish, compared to the values permitted by European Commission Regulation (EU) 2023/915 for consumed seafood, 2016-2023.

High concentrations of DDT and metabolites were detected in biota from the Constanta and Mangalia areas, indicating significant local contamination (Figure 8). These elevated levels in aquatic organisms suggest a persistent presence of DDT in these port regions, likely due to historical usage and ongoing inputs from maritime activities. The port of Constanta is a cereal hub in the Black Sea while both Constanta and Mangalia are major hubs for maritime transport and industrial activities, which can contribute to the introduction and persistence of such contaminants in the local marine environment.

In contrast, biota from the broader shelf area exhibited moderate DDT concentrations. This indicates a more diffuse but widespread contamination across the shelf. The moderate levels suggest that DDT is present throughout the shelf region. The entire shelf area is influenced by various activities, including maritime transport, and possibly atmospheric deposition, all contributing to the observed DDT levels in marine organisms. The presence of DDT in biota across these regions is concerning due to its persistence, bioaccumulative nature, and potential for causing adverse effects on wildlife and human health. DDT, despite being banned or restricted in many countries, continues to persist in the environment and bioaccumulate in the food web, leading to higher concentrations in higher trophic levels.

High concentrations of PCBs were detected in biota from shelf waters under the influence of rivers from the northwestern part of the Black Sea (Danube, Dnieper, Dniester) and Mangalia areas indicating local contamination (Figure 9), probably due to industrial activities related to Mangalia harbor.

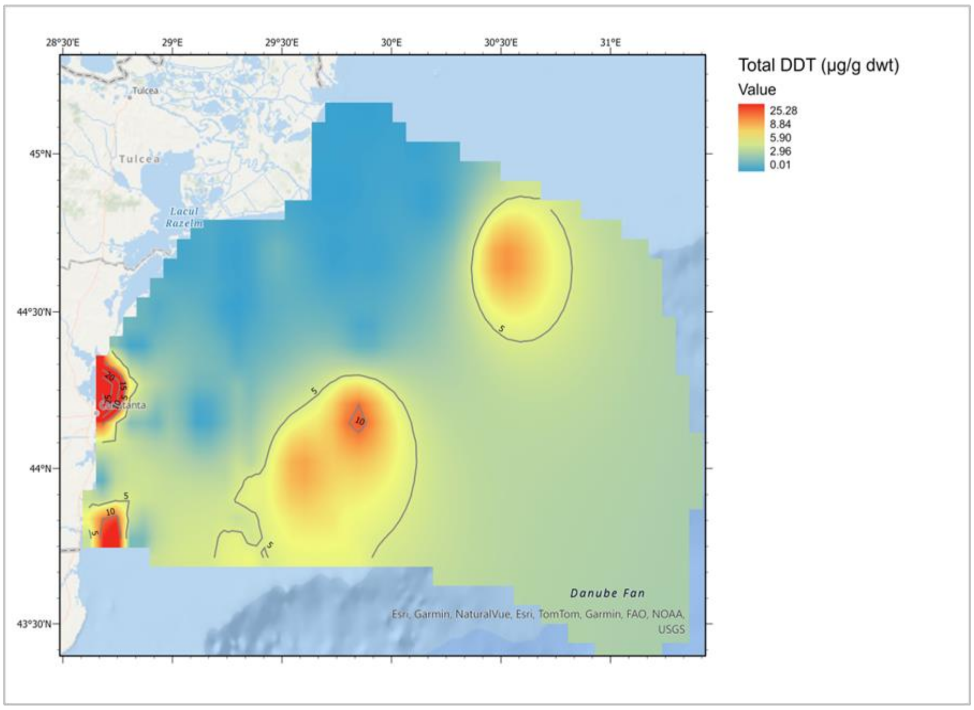

Figure 8. Total DDT content in biota – Romanian Black Sea, 2016-2023.

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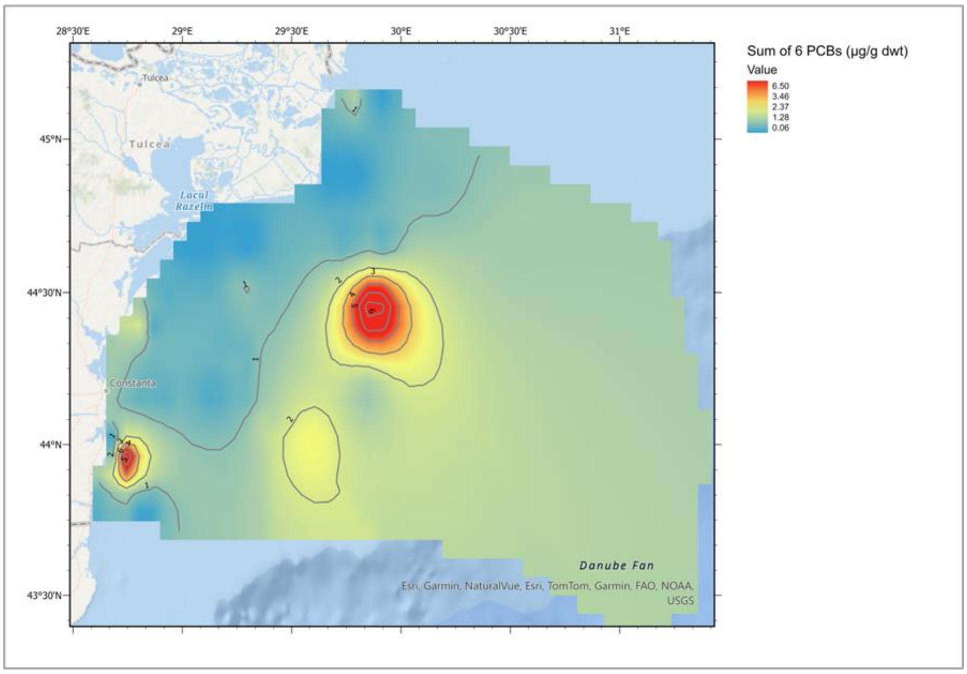

Figure 9. Sum of 6 PCB in biota, Romanian Black Sea, 2016-2023.

The analysis of organochlorine pesticides (OCPs) in seawater reveals two primary sources of contamination. The first source is identified in the Sfântu Gheorghe arm (Figure 10) of the Danube, located near an important agricultural area, Dunavat-Murighiol (2.538 ha) [41]. This proximity to intensive farming activities suggests that agricultural runoff is a significant contributor to the presence of OCPs in this part of the water system even though their use is forbidden in the Danube Delta. The use of pesticides in crop cultivation likely leads to their leaching and washing into the river, especially during rainfall or irrigation events, resulting in elevated concentrations in the water (Table 6S).

The analysis of organochlorine pesticides (OCPs) in sediments suggests notable patterns of accumulation in specific areas. The highest levels of OCP accumulation are also observed in the northern shelf region (Figure 11). This area, influenced by various hydrodynamic and anthropogenic factors, appears to be a significant sink for these persistent contaminants. The sediment here likely captures and retains OCPs transported by water currents, leading to high concentrations over time (Table 8S). In addition to the northern shelf, other spots with elevated OCP levels are identified near the Sfântu Gheorghe arm and the southern shelf. Near the Sfântu Gheorghe arm, the accumulation of OCPs in sediments is consistent with the observed sources of contamination in the water column, primarily due to agricultural runoff. The sediments act as a repository for these pesticides, which settle out of the water and become part of the benthic environment. Overall, the sediment data indicate that the northern shelf is the primary area of OCP accumulation, with additional significant spots near Sfântu Gheorghe and the southern shelf.

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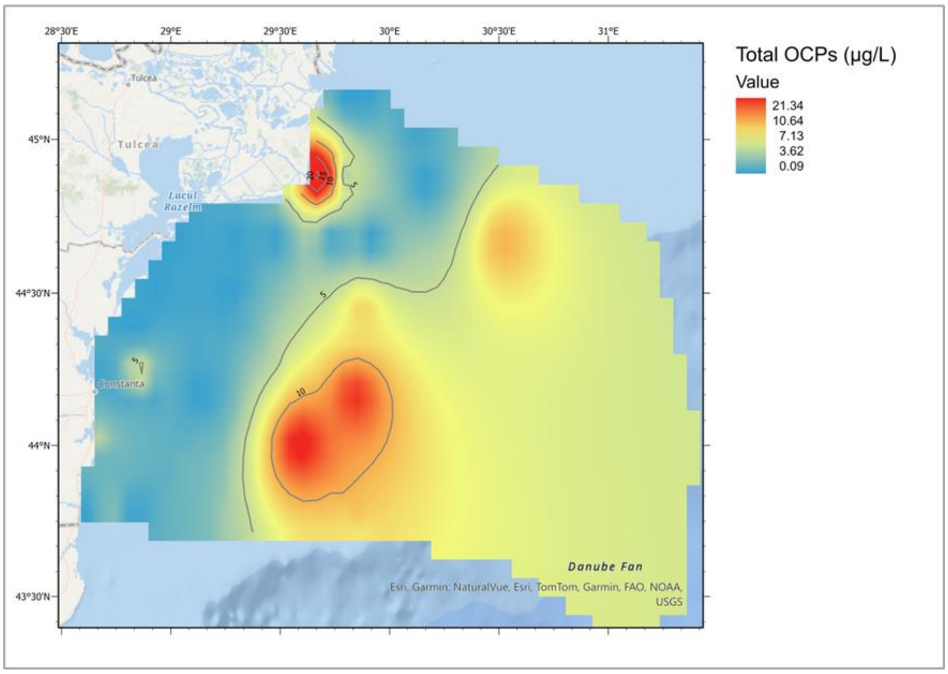

Figure 10. Total OCPs content in seawater – Romanian Black Sea, 2016-2023.

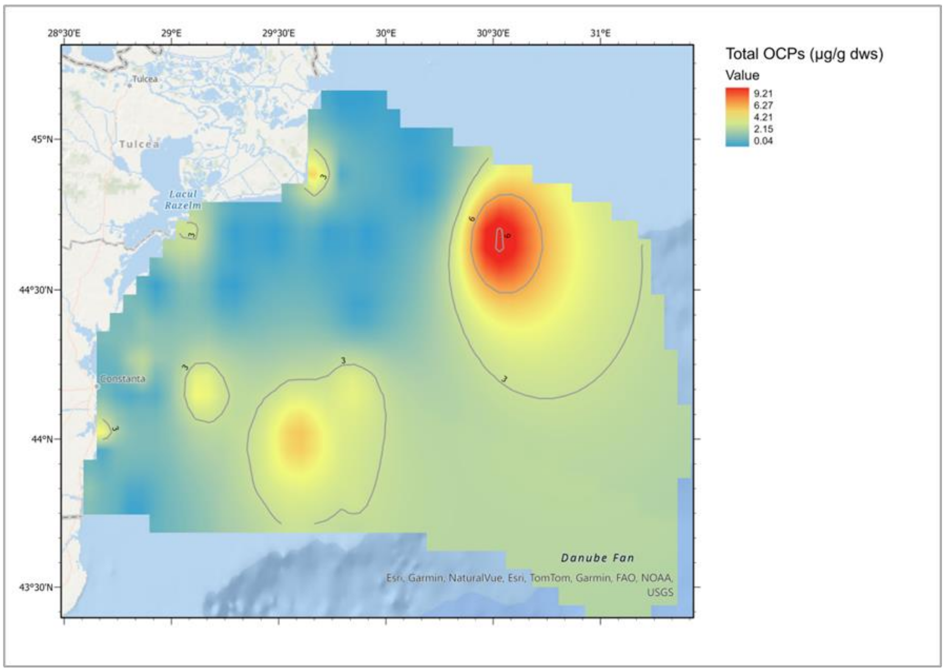

Figure 11. Total OCPs content in sediments – Romanain Black Sea, 2016-2023.

The analysis of PCBs indicates that the highest concentrations (Table 7S) were found in the seawater from the Mangalia area. This suggests that Mangalia is a significant hotspot for PCB contamination. The presence of these high concentrations can be attributed to several factors, including historical industrial activities, ongoing maritime operations, and potential local sources of PCB discharge. Mangalia, being a key port and industrial zone, has a history of activities that could have introduced PCBs into the marine environment. These include shipbuilding, repairs, and various manufacturing processes that historically used PCBs for their chemical stability and insulating properties. Despite the ban on PCB production and use in many countries, these contaminants persist in the environment due to their resistance to degradation.

Although the sediments in Mangalia did not show high values of contaminants (Figure 12), Mangalia is a notable spot for high levels of contaminants in biota as well. This discrepancy suggests that PCBs and possibly other pollutants, are more bioavailable and are being readily taken up by marine organisms, even if they are not as concentrated in the sediments (Table 9S).

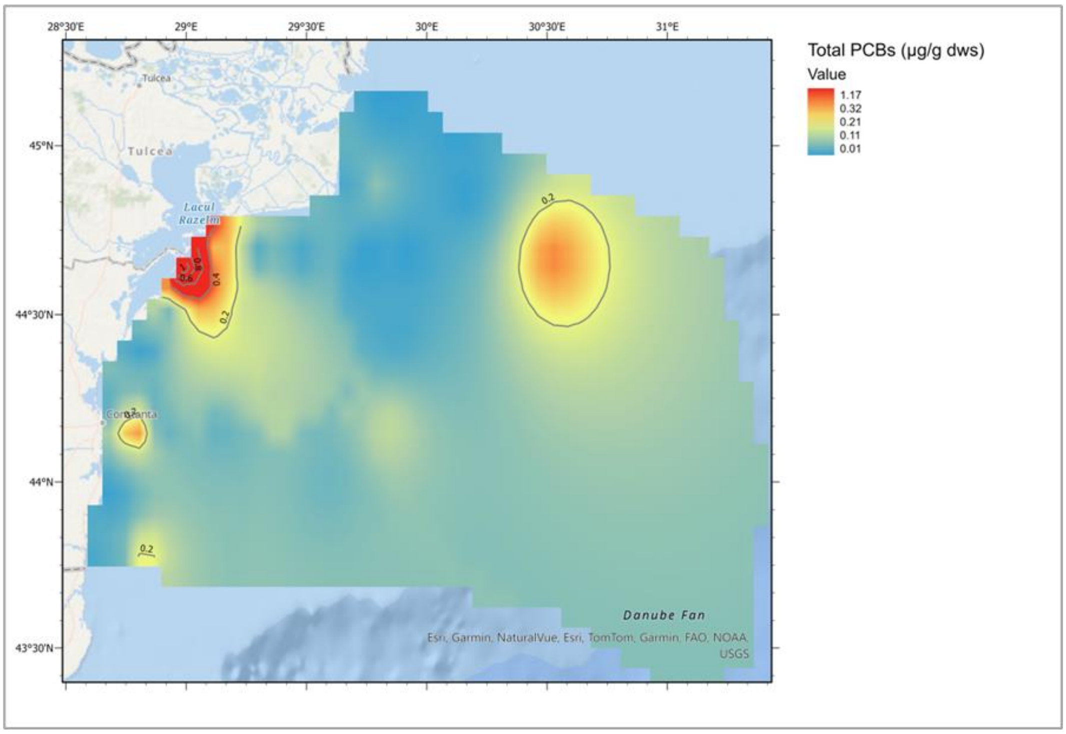

Figure 12. Total PCBs in sediments – Romanian Black Sea, 2016-2023.

Organic pollutants – PAHs in biota, seawater, and sediments

The statistical parameters provide insights into the variability of PAHs concentrations in mussels (*M. galloprovincialis*) (Table 11), gastropods (*Rapana venosa*) (Table 12) and fish (Table 13) from the Romanian Black Sea investigated during 2016 – 2023.

Table 11. Variability of PAHs concentrations in mussels (*M. galloprovincialis*) - Romanian Black Sea, 2016 – 2023.

Variable	Descriptive Statistics (*M. galloprovincialis*, 2016-2023)					
	Mean	Median	Min.	Max.	75th percentile	Coef. Var.
Naphthalene (µg/g ww)	3.23E-4	1.3E-5	nd	3.088E-3	2.23E-3	246.2413
Acenaphtylene (µg/g ww)	1.00E-5	0.000	nd	2.65E-4	0.000	501.5951
Acenaphtene (µg/g ww)	1.00E-5	0.000	nd	2.82E-4	0.000	495.4992
Fluorene (µg/g ww)	9.10E-4	0.000	nd	9.169E-3	2.5E-5	297.9722
Phenanthrene (µg/g ww)	1.385E-3	2.0E-6	nd	1.1100E-2	1.332E-3	214.7091

Anthracene (µg/g ww)	4.80E-4	0.000	nd	1.0778E-2	1.61E-4	408.3381
Fluoranthene (µg/g ww)	1.59E-4	0.000	nd	1.214E-3	1.0E-5	235.3721
Pyrene (µg/g ww)	4.57E-4	0.000	nd	7.539E-3	1.6E-5	321.3973
Benzo[a]anthracene (µg/g ww)	2.32E-4	0.000	nd	6.865E-3	0.000	540.3274
Crysene (µg/g ww)	1.4E-5	0.000	nd	3.05E-4	0.000	393.7274
Benzo[b]fluoranthene (µg/g ww)	1.12E-4	0.000	nd	1.463E-3	0.000	267.7644
Benzo[k]fluoranthene (µg/g ww)	3.3E-5	0.000	nd	5.89E-4	0.000	384.8164
Benzo[a]pyrene (µg/g ww)	7.7E-5	0.000	nd	8.87E-4	4.0E-6	292.5008
Benzo(g,h,i) perylene (µg/g ww)	1.8E-5	0.000	nd	2.31E-4	0.000	326.2723
Dibenzo(a,h) anthracene (µg/g ww)	1.5E-5	0.000	nd	2.28E-4	0.000	372.3739
Indeno (1,2,3-c, d) pyrene (µg/g ww)	1.8E-5	0.000	nd	2.58E-4	0.000	369.8438

nd – not detected.

For all PAHs, the minimum concentration indicated that the PAH was not detected in mussels. The median concentration is generally lower than the mean for most PAHs, suggesting a right-skewed distribution where there are more frequent lower values than higher values. Fluorene is the dominant compound with the highest overall mean concentration (9.10E-4 µg/g ww). Pyrene, benzo[a]anthracene, and chrysene follow with relatively high mean concentrations compared to other PAHs. Acenaphthylene, acenaphthene, and benzo(g,h,i) perylene have the lowest mean concentrations (all around 1.0E-5).

All PAHs in mussels show very high coefficients of variation, ranging from 246.24 for naphthalene to 501.59 for acenaphthylene showing significant variations of PAHs within the mussel samples.

Table 12. Variability of PAHs concentrations in gastropods (*Rapana venosa*) - Romanian Black Sea, 2016 – 2021.

Variable	Descriptive Statistics (*Rapana venosa*, 2016-2021)						
	Mean	Median	Min.	Max.	25th percentile	75th percentile	Coef. Var.
Naphthalene (µg/g ww)	2.772E-3	1.5E-6	nd	12.087E-2	1.5E-6	2.928E-3	152.4665
Acenaphtylene (µg/g ww)	1.5E-6	1.5E-6	nd	1.5E-6	1.5E-6	1.5E-6	0.000

Acenaphtene (µg/g ww)	1.94E-4	1.5E-6	nd	1.622E-3	1.5E-6	1.5E-6	276.7241
Fluorene (µg/g ww)	1.602E-3	1.5E-6	nd	9.835E-3	1.5E-6	1.5E-6	213.6379
Phenanthrene (µg/g ww)	3.4381E-2	1.800E-3	nd	0.135450	1.5E-6	0.049054	143.8718
Anthracene (µg/g ww)	0.001062	1.5E-6	nd	8.889E-3	1.5E-6	1.5E-6	276.8563
Fluoranthene (µg/g ww)	1.909E-3	1.5E-6	nd	0.011486	1.5E-6	2.597E-3	198.5881
Pyrene (µg/g ww)	2.751E-3	1.5E-6	nd	0.017988	1.5E-6	0.002387	214.8892
Benzo[a]anthracene (µg/g ww)	2.29E-4	1.5E-6	nd	1.937E-3	1.5E-6	1.5E-6	280.2920
Crysene (µg/g ww)	5.3E-5	1.5E-6	nd	3.54E-4	1.5E-6	1.5E-6	214.5570
Benzo[b]fluoranthene (µg/g ww)	1.499E-3	1.5E-6	nd	0.011766	1.5E-6	1.5E-6	259.3468
Benzo[k]fluoranthene (µg/g ww)	2.00E-4	1.5E-6	nd	1.681E-3	1.5E-6	1.5E-6	277.4948
Benzo[a]pyrene (µg/g ww)	4.659E-3	8.3E-5	nd	3.7211E-2	1.5E-6	2.173E-3	262.7476
Benzo (g,h, i) perylene (µg/g ww)	2.821E-3	1.5E-6	nd	0.023204	1.5E-6	2.98E-4	271.7314
Dibenzo(a,h) anthracene (µg/g ww)	1.609E-3	1.5E-6	nd	0.014360	1.5E-6	1.5E-6	297.2002
Indeno (1,2,3-c, d) pyrene (µg/g ww)	2.246E-3	1.5E-6	nd	0.020095	1.5E-6	1.5E-6	297.9945

nd – not detected.

PAH analysis in gastropods revealed that mostly PAHs are not detected. This suggests they are present in very small concentration. For most PAHs, the median concentration is like the minimum value, indicating that a large portion of the samples have very low levels. Phenanthrene is the dominant compound with a mean concentration (3.4381E-2 µg/g ww) almost an order of magnitude higher than other compounds.

The coefficient of variation is very high for most PAHs. This implies a large variation in concentration across the samples, with some having much higher levels than others.

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Table 13. Variability of PAHs concentrations in pelagic and demersal fish - Romanian Black Sea, 2016 – 2019.

Variable	Descriptive Statistics (Fish, 2016-2019)						
	Mean	Median	Min.	Max.	25th percentile	75th percentile	Coef. Var.
Naphthalene (µg/g ww)	1.6683E-2	3.501E-3	2.5E-5	7.7349E-2	1.25E-5	0.015594	181.4329
Acenaphtylene (µg/g ww)	3.065E-3	5.26E-4	2.5E-5	0.011294	9.4E-5	5.924E-3	151.0249
Acenaphtene (µg/g ww)	3.523E-3	6.10E-4	2.5E-5	0.012942	1.44E-4	6.804E-3	150.4601
Fluorene (µg/g ww)	3.670E-3	6.63E-4	2.5E-5	0.013670	2.00E-4	6.799E-3	150.9471
Phenanthrene (µg/g ww)	0.019458	0,008764	2.38E-3	0.071160	3.188E-3	0.024633	138.1410
Anthracene (µg/g ww)	0.015274	2.609E-3	1.0E-5	0.078800	1.00E-4	7.528E-3	204.7746
Fluoranthene (µg/g ww)	3.425E-3	1.89E-4	2.5E-5	0.013209	6.3E-5	6.879E-3	160.6858
Pyrene (µg/g ww)	3.558E-3	6.55E-4	2.5E-5	0.013076	1.00E-4	6.837E-3	150.2645
Benzo[a]anthracene (µg/g ww)	3.614E-3	6.60E-4	2.5E-5	0.013280	1.68E-4	6.891E-3	149.9080
Crysene (µg/g ww)	3.585E-3	6.53E-4	2.5E-5	0.013139	1.54E-4	6.887E-3	149.7954
Benzo[b]fluoranthene (µg/g ww)	2.369E-3	4.27E-4	2.5E-5	8.647E-3	8.1E-5	4.605E-3	149.6779
Benzo[k]fluoranthene (µg/g ww)	2.488E-3	4.58E-4	2.5E-5	9.068E-3	1.46E-4	4.773E-3	148.7542
Benzo[a]pyrene (µg/g ww)	2.585E-3	4.58E-4	2.5E-5	9.466E-3	1.25E-4	4.976E-3	149.7488
Benzo (g,h, i) perylene (µg/g ww)	1.059E-4	1.60E-4	1.0E-5	5.192E-4	2.5E-5	8.19E-4	193.1788
Dibenzo(a,h) anthracene (µg/g ww)	1.127E-3	1.76E-4	1.0E-5	5.523E-3	2.5E-5	864E-4	193.0662
Indeno (1,2,3-c,d)pyrene (µg/g ww)	2.740E-3	4.98E-4	2.5E-5	1.0115E-2	2.5E-5	5.282E-3	151.2229

Overall, the PAHs concentrations in fish from the Romanian Black Sea vary considerably. PAHs analysis in fish, reveald that all have very low minimum concentrations and higher maximum concentrations, suggesting a wide range of values across the samples. The coefficient of variation is very high for most PAHs, indicating significant variability in concentrations. Phenanthrene is the dominant compound PAH based on the mean concentration (0.01945 µg/g ww). Benzo(g,h,i) perylene and Dibenzo(a,h) anthracene have the highest coefficient of variation, meaning their concentrations fluctuate the most compared to their average.

PAHs values (Benzo[a]pyrene, Sum of PAHs: benzo(a) pyrene, benzo(a) anthracene, benzo(b) fluoranthene and chrysene) were compared to the values permitted by European Commission

Regulation (EU) 2023/915 [26] for consumed seafood. In mollusks, maximum admissible concentrations (MACs) of 0.05 µg/g ww benzo(a)pyrene were surpassed in 19 % of mussels, respectively in 1 % of gastropods. MAC for sum of PAHs of 0.030 µg/g ww was surpassed in 0.09 % of mussels. Considering that the legislation in force does not provide maximum permissible limits in relation to human consumption for fresh fish, the limits provided for fresh mollusks were used. In pelagic and demersal fish, MAC for benzo(a)pyrene of 0.05 µg/g ww was surpassed in 33 % of samples, and MAC for sum of PAHs: benzo(a) pyrene, benzo(a) anthracene, benzo(b) fluoranthene and chrysene) of 0.030 µg/g ww was surpassed in 33 % of samples. These findings highlight the importance of monitoring organic pollutants levels in fish to ensure the safety of seafood consumption (Figure 13).

Figure 13. PAHs (Benzo(a)pyrene, Sum of PAH: benzo(a)pyrene, benzo(a) anthracene, benzo(b) fluoranthene and chrysene) values measured in mussels and fish, compared to the values permitted by European Commission Regulation (EU) 2023/915 for consumed seafood, 2016-2023.

PAHs analysis in biota revealed high concentrations of PAHs in the northern shelf area (Figure 14) indicating a significant bioaccumulation risk, potentially impacting the food web and ecosystem health. The source of these high PAH concentrations in biota appears to be other rivers in the region that contribute to the PAH load, carrying contaminants from upstream industrial or urban areas. Additionally, maritime transport activities in the area could be a significant source, as ships often release various pollutants, including PAHs, through their exhaust, bilge water, and operational discharges.

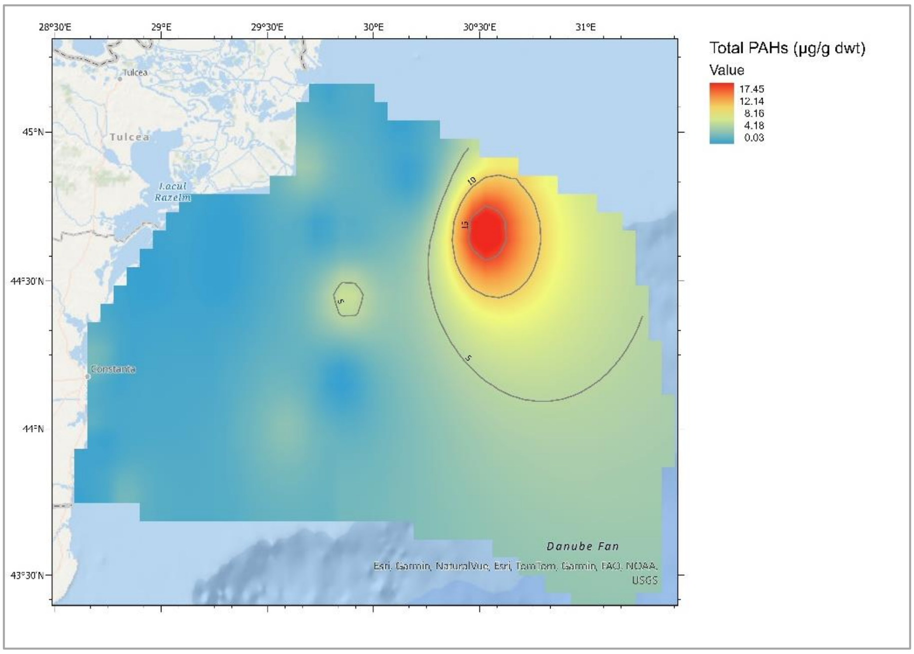

Figure 14. Distribution of total content of PAHs in biota – Romanian Black Sea, 2016-2023.

The analysis revealed that the highest concentrations of PAHs in seawater are predominantly sourced from the Danube River (Figure 15). This suggests that the Danube is the primary pathway introducing these contaminants into the aquatic environment. In contrast, the PAHs found in sediments serve as markers for accumulation, indicating areas where these compounds settle and persist over time. Notably, the sediments from the Constanta and Mangalia ports vicinity also show significant concentrations of PAHs, pointing to these locations as notable areas of PAH accumulation (Figure 16).

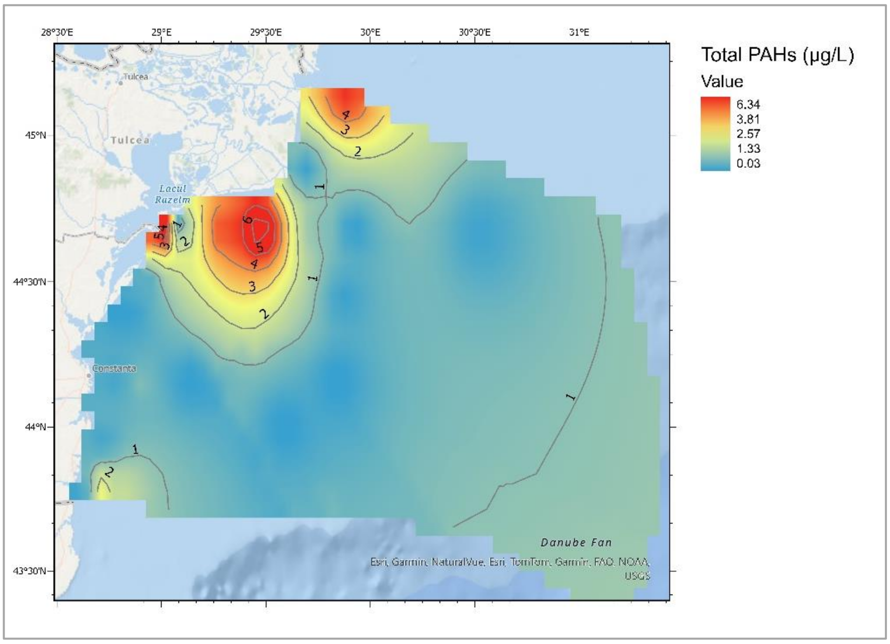

Figure 15. Distribution of total content PAHs in seawater – Romanian Black Sea, 2016-2023.

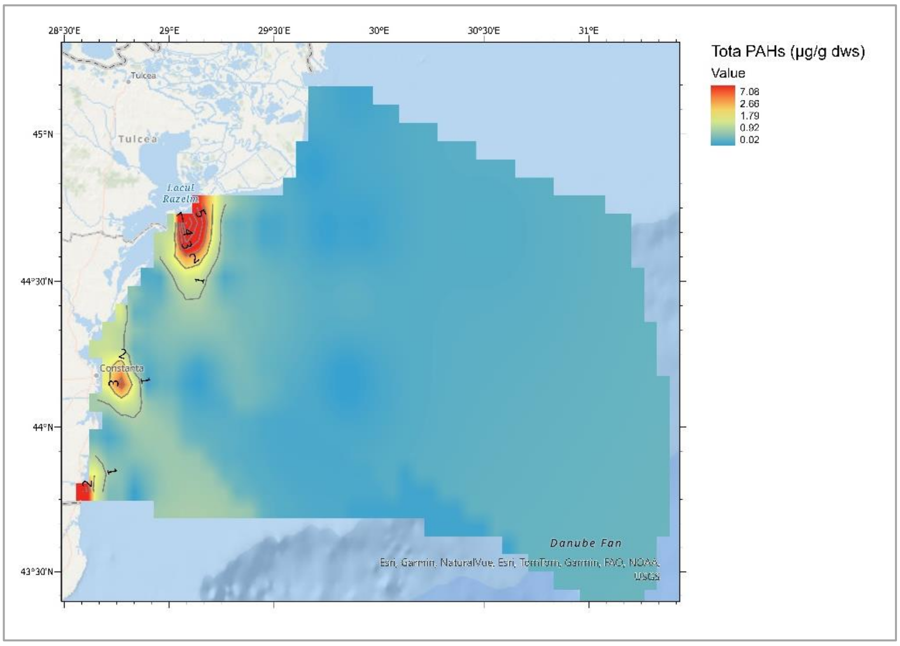

Figure 16. Distribution of total content of PAHs in sediments – Romanian Black Sea, 2016-2023.

5. Discussion

Our findings indicate that the levels of copper (Cu), cadmium (Cd), chromium (Cr), nickel (Ni), and lead (Pb) in mussels (*M. galloprovincialis*) investigated during 2016 – 2023 remain within safe limits, as evidenced by the calculated Target Hazard Quotients (THQs) and the Total Hazard Quotient (TTHQ). Therefore, based on our preliminary results, heavy metal exposure through consumption of mussels does not pose any significant health risks to people. Our findings align with previous studies involving mussels from the Turkish coast of the Black Sea that highlighted that the concentration of heavy metals in mussels is safe for people's intake in terms of their toxicity, according to the estimated daily intake (EDI). The target hazard quotients (THQs) in metals were also found <1, which implies no threat to consumers [27,42,43].

In comparison to the values (MAC) permitted by European Commission Regulation (EU) 2023/915 for consumed seafood, we noticed that while Cd contamination was observed in both mussels (6% of samples) and gastropods (30% of samples) investigated, the Pb levels remained well below the established limits. In pelagic and demersal fish investigated during 2016 - 2023, MAC for Cd of 0.05 µg/g ww Cd was surpassed in 35% of samples, whereas MAC for Pb of 0.30 µg/g ww Pb was surpassed in 48 % of samples. These findings constitute additional arguments for continuous monitoring of marine environment abiotic and biotic components, especially near the Danube discharge area or various hot spots where elevated heavy metals levels were measured, activity that is essential to ensure the safety of consumed seafood. Areas near river discharge, industrial zones, wastewater discharge points, and shipping routes are more likely to have elevated contaminants levels. Coastal regions with historical pollution or heavy human activity should also receive special attention.

Similar studies have focused on the bioaccumulation of heavy metals in mollusks and other marine organisms in the Black Sea region. An ecological risk assessment of heavy metals in surface sediments and mussel samples along the mid-Black Sea coast of Turkey was conducted, highlighting the effects of anthropogenic activities on metal accumulation. Higher levels of heavy metals were found in sediments and mussels compared to water, indicating accumulation. Mussel samples from Samsun city harbor showed the highest metal concentrations, suggesting urban influence, and the presence of cadmium and lead suggests ongoing pollution from domestic and industrial sources [44]

Heavy metal accumulation in various marine organisms along the Romanian Black Sea coast, focusing on areas with human influence was investigated. Higher levels of lead, copper, and cadmium were found in sediments near harbors and wastewater treatment plants. Based on calculated bioconcentration factors, algae accumulated copper most effectively, while mollusks (mussels) and demersal fish (red mullet) concentrated copper, cadmium, lead, and nickel to varying degrees. While the study didn't find widespread exceeding of safety limits for cadmium and lead in organisms, the bioaccumulation observed indicates potential concerns, especially for bottom-feeders [45].

A study examined the levels of various heavy metals in three seafood benthic species (mussels, whelks, and crabs) from the Black Sea coast of Turkey [46]. Measurable levels of aluminum, arsenic, copper, zinc, iron, and cadmium were found in all three organisms. Mercury and lead were rarely detected. Overall, the levels of most metals detected were below the safety limits set by international organizations for human consumption. Only the cadmium level measured in the veined Rapa whelk (*Rapana venosa*) approached the limit.

Another study investigated the risk of consuming mussels and whelks from the Black Sea's Varna Bay due to potential heavy metal contamination (lead, cadmium, and mercury) [47] Cadmium was the highest metal found in both mussels and whelks, followed by lead and then mercury. Despite the presence of these metals, the estimated daily intake (EDI) and hazard quotients (THQ & HI) for adults consuming these seafood items were all below established safety limits.

Several studies have been conducted to assess the bioaccumulation of heavy metals in fish species from various regions, including the Black Sea. Heavy metal concentrations in fish from the Mediterranean Sea and the potential health risks to consumers were assessed. Fish caught near a petrochemical area in Siracusa had the highest levels of cadmium, lead, and chromium. While some metal concentrations exceeded European regulations, the estimated human intake was below safe limits set by European authorities. There was also no indication of carcinogenic risk. Overall, the study suggests some areas of concern for heavy metal pollution, particularly near industrial sites. However, based on the metals measured and estimated intake, the risk to human health from consuming these fish appears to be low [48]. Investigations on the nutritional value and bioaccumulation of heavy metals in muscle tissues of commercially important marine fish species from the Red Sea highlighted significant variations in heavy metal concentrations within and between fish species. The levels of some heavy metals (chromium, iron, nickel, and cadmium) in the muscle tissue exceeded recommended safety standards, suggesting contamination in the Red Sea environment. While these fish are a good source of protein, the presence of heavy metals above safe levels makes them unsafe for consumption [49].

Investigations on tissue bioaccumulation of heavy metals in grey mullet from the Black Sea and the Ionian Sea found that the levels of various elements in the fish tissues from the two regions differed significantly, suggesting that the two sea environments have varying degrees of pollution [50]. A comprehensive study in the Black Sea region of Turkey was conducted to determine radioactivity levels and heavy metal concentrations in common fish species, concluding that fish consumption poses no threat to human health [51]. Measured levels of arsenic, manganese, iron, chromium, nickel, zinc, copper, and lead in fish Species: anchovy, trout, bluefin, and whiting were all below the recommended daily intake limits set by international organizations.

A study focused on cadmium (Cd), lead (Pb), organochlorine pesticides (OCPs), and PCBs in anchovy muscle tissue from the Romanian Black Sea coast found some cases of exceedances of European Union's safety limits for human consumption for cadmium and lead, while OCPs and PCBs levels were not considered a threat to consumers [52]. Another study on selected fish species (sprat, horse mackerel, gobies, shad, bonito, bluefish, and grey mullet) from the Bulgarian Black Sea found that shad and sprat had the highest levels of copper, zinc, and lead, horse mackerel had the highest mercury, and bonito accumulated the most arsenic, but the metal concentrations in the fish muscle were within acceptable limits for human consumption [53].

The bioconcentration of essential and nonessential elements in Black Sea turbot in relation to fish gender was evaluated, contributing to the characterization of heavy metals pollution in the Romanian Black Sea coast. The levels of toxic metals in the muscle tissue were lower than expected, considering that the study area (Constanta) is potentially impacted by human activity. The study also suggests that the specific environment and diet of the fish influence how much of an element they take up [54].

Our findings evinced that concentrations of organochlorine pesticides were low in fish compared with values recorded for mollusks species where these compounds varied widely. Gastropods showed particularly high levels of Dieldrin and Endrin but also high levels of Lindane and total DDT. Similar concentrations of Heptachlor, total DDT, were also found in mussels (*M. galloprovincialis*). This is probably because their feeding behavior as mussels filter large quantities of water, and gastropods represent the next trophic level of the food chain feeding with mussels. Mussel species are widely regarded as some of the most valuable sentinels and biological indicators of pollution. This is due to their ideal combination of characteristics: they are sessile filter feeders that accumulate contaminants in their tissues and maintain stable populations in many locations [55].

Except DDT, Heptachlor and Dieldrin the levels of most OCPs remain within the safe limits, as the surpassing of the values permitted by national legislation (Order 147/2004) is lower than 25% in all studied species. PCBs are accumulated mainly in fish where the maximum admissible levels stipulated by European Commission Regulation (EU) 2023/915 for consumed seafood, were exceeded in 43% of samples.

PAHs analysis in biota highlighted average concentrations ranged from 1.00E-5 to to 1.39E-03 µg/g ww in *M. galloprovincialis,* from 1.5E-6 to 4.659E-3 µg/g ww in Rapana venosa and from 1.059E-4 to 0.01945 µg/g ww fish. Total mean PAHs concentrations in *M. galloprovincialis* were 4.25E-03µg/g ww, the dominant compounds being Fluorene with the highest overall mean concentration (9.10E-4 µg/g ww). According to studies on mussels *(M.galloprovincialis)* in the Mediterranean Sea, the amount of individual polyaromatic compounds (PAHs) ranged between 0.025 and 0.068 µg/g ww. Naphthalene is the dominant compund, with an average concentration of 0.04093 µg/kg ww [56]. The values are higher compared to those of individual compounds measured in mollusks from the Romanian Black Sea coast. *M. galloprovincialis* could be used as indicators of pollution, thus a comparison of PAH levels in mussels from different seas was conducted to provide a clearer picture of the PAH contamination in the study area (Table 14).

In comparison to the values (MAC) permitted by European Commission Regulation (EU) 2023/915 for consumed seafood, we noticed that while Benzo(a)pyrene contamination was observed in both mussels (19 % of samples) and gastropods (1% of samples) investigated, the Sum of PAHs (benzo(a)pyrene, benzo(a) anthracene, benzo(b) fluoranthene and chrysene) levels remained well below the established limits. In pelagic and demersal fish investigated during 2016 - 2023, MAC for benzo(a)pyrene of 0.005 µg/g ww was surpassed in 33% of samples, whereas MAC for Sum of PAHs (benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene) of 0.030 µg/g ww Pb was surpassed in 33 % of samples.

Table 14. PAHs levels measured in mussels (M. galloprovincialis) from different seas (ng/g dw).

Location	Concentration range	References
Prince Islands (Marmara,Turkey)	664-9083	Balcıoğlu[57]
Eastern Aegean Coast (Turkey)	29.4-64.2	Küçüksezgin et al.[58]
Iberian Mediterranean Coastal areas (Spain)	75-390	Leon et al.[59]
Bizerte lagoon (north Tunisia)	107.4-430.7	
Ionian Sea (Italy)	14.8-645.3	Barhoumi et al.[56]
Saronikos Gulf (Greece)	1480-2400	Storelli et al.[60]
Black Sea coast (Romania)	1.0E-5-0.0073926	Valavanidis et al.[61] Present study

Recent data on chemical contamination (HMs, PAHs, OCPs, PCBs) of marine organisms (mussels, veined rapa whelk, pelagic and demersal fish) from various Black Sea regions (Ukraine, Romania, Bulgaria and Turkey) were collected [62]. The HELCOM integrated

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hazardous substances assessment tool (CHASE)[63] was tested on this data set and the overall scores evinced sub-regional differences in the status results, with worse status predominating in the north-western part of the Black Sea (rivers influenced coastal areas and hotspots) and better status in the open sea area and in the southern part of the Black Sea. Across the investigated biota samples, the test assessment showed a range of status results from "bad" to "high", almost half (46%) of biota samples being „unaffected by hazardous substances" state ("good" and "high" status), whereas the remaining 54% of biota samples are „affected by hazardous substances" state ("bad", "poor" and "moderate")[64].

The cumulative effect of heavy metals, POPs and PAHs found in mollusks and fish should be considered for further studies. Excessive consumption, especially over long periods, may lead to health risks for humans. Individual factors such as age, weight, and existing health conditions should be considered. Transparent communication between scientists, policymakers, and the public is vital, to provide clear guidelines on safe consumption levels based on scientific evidence and to emphasize that occasional consumption of seafood within recommended limits is safe.

Continuous monitoring of heavy metal, POPs and PAHs concentrations in mussels and fish is crucial. Regular sampling and analysis allow us to track any fluctuations or trends over time. Environmental agencies, research institutions, and seafood industry stakeholders should collaborate to establish monitoring programs that can help identify potential contamination sources and assess the overall health of marine ecosystems.

6. Conclusions

This eight-year study reveals significant spatial variations in the levels of hazardous substances (HMs, PAHs, OCPs and PCBs) in the seawater, sediments, and biota across the Romanian Black Sea coast. Areas with high levels of pollutants were identified, as Danube influenced area, harbors, or areas affected by wastewaters discharges, intensified maritime traffic, offshore oil and gas platform, s.a. Bioaccumulation of contaminants in various marine organisms (mollusks and fish) raises concerns about food safety and potential risks to human health. Specific pollutants exceeding safe limits were identified during our investigations (cadmium, lead, heptachlor, dieldrin, total DDT, sum of 6 PCBs, benzo (a) pyrene, sum of PAHs: benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene).

Although our results showed that heavy metal exposure through consumption of mussels does not pose any significant health risks to people, according to the estimated daily intake (EDI) and the target hazard quotients (THQs), the cumulative effect of HMs, POPs and PAHs found in mollusks and fish should be considered for further studies on risk assessment. Excessive consumption, especially over long periods, may lead to health risks for humans. Transparent communication between scientists, policymakers, and the public is vital, to provide clear guidelines on safe consumption levels based on scientific evidence and to emphasize that occasional consumption of seafood within recommended limits is safe.

Our findings constitute additional arguments for continuous monitoring of marine environment abiotic and biotic components, especially near the Danube discharge area or various hot spots where elevated hazardous substances levels were measured, to ensure the safety of consumed seafood. Areas near river discharge, industrial zones, wastewater discharge points, and shipping routes are more likely to have elevated contaminants levels. Coastal regions with historical pollution or heavy human activity should also receive special attention.

This study offers a comprehensive analysis that can be used for informed decision-making regarding environmental management and pollution mitigation in the Black Sea and it emphasizes the urgency for action to protect the marine environment and human health.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org., **Figure S1**: Distribution of cadmium in biota, during 2016-2023; **Table S1:** Retention time, monitored ion, linearity, and limits of detection (LOD) of OCPs for biota (mollusks and fish); **Table S2**: Retention time, monitored ion, linearity, and limits of detection (LOD) of PCBs for biota (mollusks and fish); **Table S3**: Retention time, monitored ion, linearity, and limits of detection

(LOD) of PAHs for biota (mollusks and fish); **Table S4**: Variability of heavy metal concentrations in seawater from the Romanian Black Sea investigated during 2016 – 2023; **Table S5**: Variability of heavy metal concentrations in sediments from the Romanian Black Sea investigated during 2016 – 2023; **Table S6**: Variability of OCPs concentrations in seawater from the Romanian Black Sea investigated during 2016 – 2023; **Table S7**: Variability of PCBs concentrations in seawater from the Romanian Black Sea investigated during 2016 – 2023; **Table S8**: Variability of OCPs concentrations in sediments from the Romanian Black Sea investigated during 2016 – 2023; **Table S9**: Variability of PCBs concentrations in sediments from the Romanian Black Sea investigated during 2016 – 2023.

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