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Screening for Organic Pollutants in the Black Sea Turbot (*Scophthalmus maeoticus*)

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Abstract: The health of aquatic organisms can be affected due to anthropogenic activities and limited actions to reduce the pollution of the Black Sea. The accumulation of organic pollutants (OPs) in the aquatic environment occurs in water, sediment, and then biota. The turbot (*Scophthalmus maeoticus*) is a benthic fish of commercial interest scarcely studied in the Black Sea region, and none of the studies researched OP concentrations in its main tissues. In this paper, polycyclic aromatic hydrocarbons (PAHs) and POPs, organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) were determined in water, sediment, and turbot muscles, gills, gonads, and livers, to research their accumulation level. The determinations were made with gas chromatography on turbots sampled in 2021 from the Romanian Black Sea waters. OCPs—p,p'DDT and its metabolites p,p'DDE, p,p'DDD—are dominant in the turbot tissues. From PAHs, benzo(g,h, i)perylene was the dominant compound, while for PCBs it was PCB 52. The OPs' presence in the wild turbot is due to river input, dredging and coastal rehabilitation works, industrial activities and contaminated food and poses a risk to human health due to the exceeding maximum allowable concentration for human consumption in Romania and the European Union.

Keywords: organic pollutants; turbot; Black Sea; fish tissue; organochlorine pesticides; polychlorinated biphenyls; polycyclic aromatic hydrocarbons

Key Contribution: The screening for Organic Pollutants in the Black Sea turbot (*Scophthalmus maeoticus*) showed the presence of PAHs and persistent organic pollutants (POPs) (OCPs and PCBs) in all tissues (muscle, liver, gills and gonads) at levels that emphasize bioaccumulation and risk to human health.

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1. Introduction

The pollution of the marine environment has represented a growing problem over the years [1–6]. One of the important causes of marine environment degradation is the use of POPs (Persistent Organic Pollutants) [7], a group of highly toxic and persistent chemicals that can cause a wide range of human health and environmental problems [8]. Although PAHs (polycyclic aromatic hydrocarbons) are not typically included in the category of POPs (persistent organic pollutants), they were included in this study because they share some similarities [9].

It has been demonstrated that the exposure of fish to pollutants can lead to endocrine disruption, which can have toxic effects through their interference with the normal functioning of the endocrine system, which is responsible for regulating various physiological processes in the body. These disruptors can mimic or block the actions of natural hormones, leading to a range of negative effects such as interferences in reproduction, changes in behavior, immune system suppression, developmental abnormalities and interference with metabolism [10–15].

Anthropogenic sources of PAHs and POPs include:

Industrial processes: the manufacture of chemicals, and oil and gas extraction [16–19].

Waste disposal: improper disposal of waste. This includes landfill sites, the open burning of waste, and the dumping of waste into water bodies [19].

Agricultural practices: Pesticides and herbicides used in agriculture can contain POPs. Runoffs from farms can also carry POPs into nearby water bodies [20].

Transportation: Vehicles and ships emit PAHs and POPs, particularly from their exhaust systems [21].

Consumer products: Some consumer products such as electronics, furniture, and clothing contain POPs. When these products are disposed of, the POPs can be released into the environment [22].

Incineration: The burning of waste, including medical waste and hazardous waste, can release PAHs and POPs into the air [23–25].

These anthropogenic sources of PAHs and POPs have contributed to their widespread distribution in the environment, including in remote regions far from their source through different pathways—aerial, terrestrial and aquatic. Thus, their presence and the severity of pollution with POPs have become evident with their detection in areas such as the Arctic Zone, where they have never been used or produced, at levels that pose risks to both wildlife [15] and human health [13].

Some of the most important and used chemicals belonging to the category of POPS are organochlorine pesticides (OCPs), and polychlorinated biphenyls (PCBs).

Organochlorine pesticides (OCPs) are a class of pesticides heavily used until the early 1980s. They include HCB (Hexachlorobenzene), Lindane, Heptachlor, Aldrin, p,p'DDE (Dichlorodiphenylethylene), Dieldrin, Endrin, p,p' DDD (Dichlorodiphenyldichloroethylene), and p,p' DDT (Dichlorodiphenyltrichloroethane). They are highly toxic, difficult to biodegrade and have a high persistence, having a long half-life in the environment and biota (years or decades in soil/sediments) [6]. OCPs accumulate in tissues rich in animal lipids and become soluble in adipose tissues, rather than entering the aqueous environment of cells. Because of this, they are persistent in biota and bioconcentrate in food chains [26–28]. They are responsible for a series of adverse effects recorded on human and animal health such as cancer, neurological disorders, and disorders of the reproductive and endocrine systems [29].

Polychlorinated biphenyls (PCBs) are environmental contaminants associated with the combustion processes of plastic materials containing chlorine. There are no known natural sources of polychlorinated biphenyls [30]. They were synthesized for the first time in 1929 and after the awareness of their negative effects, industrial production was stopped in the USA in 1970 [6,28,31]. Their use has been realized as coolants, lubricants, hydraulic fluids, plasticizers, and additives in dyes [32]. PCBs studied in this work are PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180.

Additionally, there are more than 100 polycyclic aromatic hydrocarbons (PAHs), but 16 are most-studied due to their bioaccumulation and adverse effects—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 [33,34]. PAHs in the marine environment can be released through industrial wastewater and sewage discharges, oil spills, and maritime traffic. Generally, the aquatic environment is stressed by tons of accidentally spilt oil, harming the aquatic populations [30,31,35,36].

PAHs from the environment are not active and are incapable of causing carcinogenesis; only after entering an organism, they are metabolically transformed into carcinogenic forms [37]. The PAH that is the most carcinogenic to humans is Benzo[a]pyrene [38]. Therefore, the importance of monitoring the marine environment, including the commercial species, has been demonstrated to show the connection between their contamination and their effects at various levels to biological organization [39,40] and human health [29].

The Black Sea has six riparian countries—Bulgaria, Georgia, Romania, Russia, Turkey, and Ukraine, thus uniting two continents. It is a semi-enclosed sea, communication with neighboring seas being achieved through straits. Thus, the Kerch Strait connects with the Sea of Azov through the Bosphorus Strait, the Black Sea communicates with the Marmara Sea, and further through the Dardanelles Strait, the connection is made with the Aegean Sea, followed by the Mediterranean Sea [39,40].

The Black Sea ecosystem is characterized by specific morphological, climatic, and hydrological properties thus making it vulnerable to the action of anthropogenic pressures. The results of the increase in populations in the coastal area for the benefit of its ecosystem services consist of the development of maritime transport, tourism, and industrialization [41]. Due to anthropogenic activities, many pressures are generated on the marine ecosystem such as the introduction of pollutants, loss of natural reserves, and introduction of nutrients [42,43].

The turbot (*Scophthalmus maeoticus*) is a benthic fish that lives on the bottom of the Black Sea. It is widespread in European waters, from the East Atlantic to Morocco, and from the North-East Atlantic to the Arctic Circle. It is also found in the Baltic Sea and the Mediterranean Sea [44]. The turbot lives at depths of about 100 m and comes close to the shore at a depth of 20–30 m to spawn. The Black Sea turbot's diet consists of mollusks, crustaceans, flounder, horse mackerel and gobies [45]. The turbot has a high commercial value, which is why in some countries it is also grown in aquaculture [46].

In the case of the Black Sea's Romanian waters, turbot catches recorded for the years 2021 and 2022 were approximately 75 tons/year. These quantities were exceeded only by rapana whelk (*Rapana venosa*) and mussels (*Mytilus galloprovincialis*) [47,48]. It is an attractive fish with white meat and a special taste after preparation that makes it a very popular fish for human consumption. However, once the pollutants reach the aquatic environment, they can accumulate into marine organisms, and over time can cause damage to human health following fish consumption [29].

With only two EU member states (Romania and Bulgaria), not all European legislation is recognized and applied by the riparian countries. Instead, they all are contracting parties of the Convention on the Protection of the Black Sea Against Pollution (Bucharest, 1992) and should implement its requirements [49]. However, the common monitoring program includes PCBs and PAHs.

On the other hand, the most important European legislation for the ecological assessment of marine ecosystems is the Marine Strategy Framework Directive (MSFD) which aims at maintaining and creating the state of the marine environment by preventing the long-term deterioration of marine ecosystems [50]. MSFD dedicated two descriptors—descriptor 8 (Concentrations of contaminants give no effects) and descriptor 9 (Contaminants in seafood are below safe levels)—to evaluate the presence and effects of pollutants in the marine environment [51–53]. The last assessment of the Romanian Black Sea highlighted the non-Good Environmental Status (GES) for the contaminants in water and sediments whilst for biota the status was "Good" [54].

There are few studies focused on how water or sediments contaminated with polycyclic aromatic hydrocarbons influence the development of turbot [55,56]. The available studies carried out so far in the Black Sea aimed to determine PAHs, OCPs and PCBs either in water, sediments [57–59], or biota, especially mollusks [60–63]. According to available information, only one study that included turbot as a species for the determination of one persistent organic pollutants group—PCBs quantified in different fish species [64]—was carried out in the Black Sea region.

Therefore, studying PAHs and POPs in turbot tissues from the Black Sea is important for several reasons:

Environmental concerns: the Black Sea is known for its high levels of pollution [65], which can have a detrimental effect on the marine ecosystem and the health of the fish living in it [66]. Thus, understanding the type and concentration of contaminants in turbot tissues can provide important information about the level of pollution in the Black Sea and help in developing strategies to mitigate the pollution.

Food safety: Turbot is a popular fish for human consumption, and its consumption can expose people to contaminants such as PAHs and POPs, which can be harmful to human health. Studying the contaminants in turbot tissues can help identify potential health risks associated with consuming this fish from the Black Sea.

Consequently, the present paper aims to quantify OP levels in turbot tissues from the Romanian waters of the Black Sea by analyzing PAHs and POPs (OCPs, and PCBs), evaluating them in relation to their content in the marine environment (water and sediment), and to assess them against national and European legislation regarding food standards for human consumption. The results are also important for future works on understanding the impact of pollution on the marine ecosystem and its effects on the physiology of aquatic organisms under the stress of a cocktail of pollutants, especially the turbot's (*Scophthalmus maeoticus*) reproductive capacity, and the presence of oxidative stress through plasma biomarker analysis from the same fish.

2. Materials and Methods

Seven turbot specimens were sampled during the expeditions carried out by the National Institute for Marine Research and Development "Grigore Antipa", Constanta with R/V "Steaua de mare" in April and May of 2021. The sampling area is the northwestern/western part of the Black Sea (Figure 1).

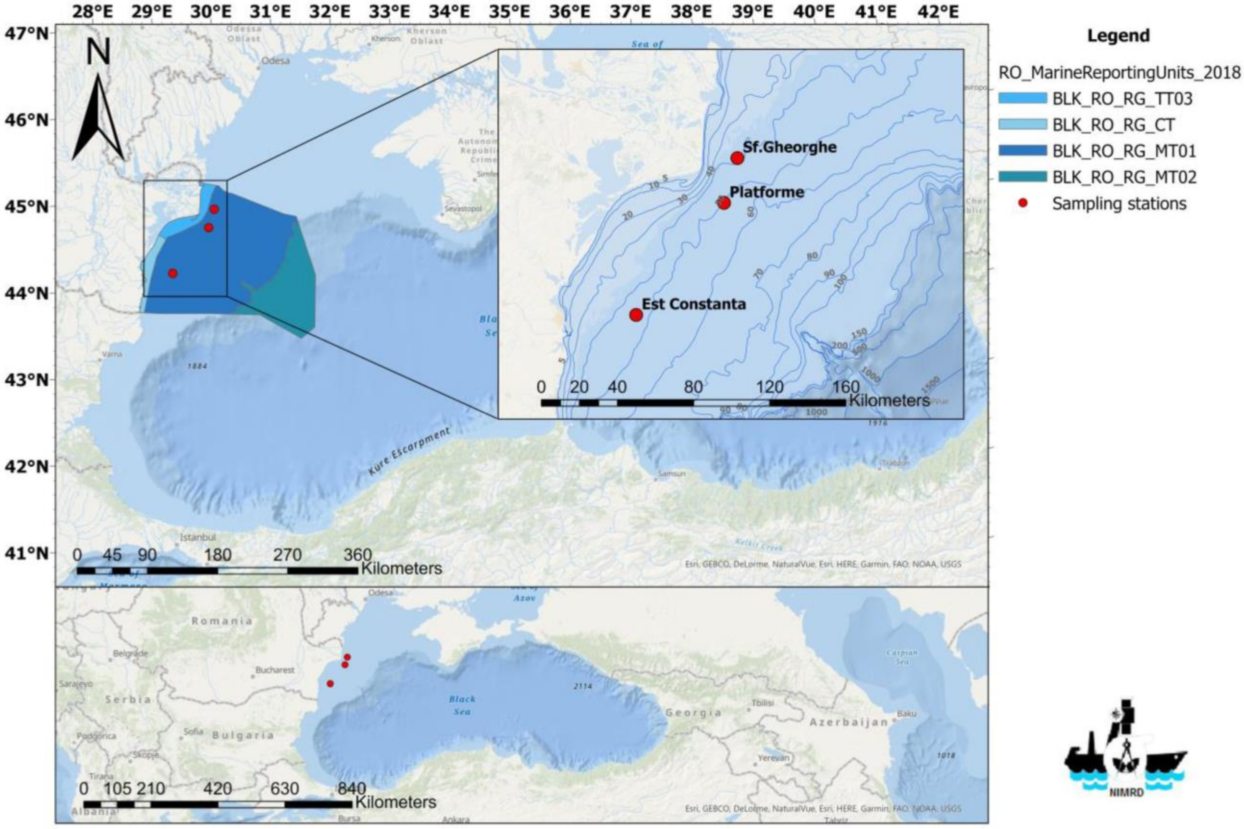

Figure 1. Network stations for turbot (*Scophthalmus maeoticus*) sampling at the Romanian Black Sea coast.

During April–May 2021, 7 fish were sampled and for each turbot specimen, 10 g of each of four types of tissue (muscle, liver, gills, gonads) were weighed resulting in N = 27 samples, with one gonad not being sampled. The stations, their bottom depths, fish sexes and weights are specified in Table 1, from north to south. In this study, the sampling of blood and brain tissue from the turbot specimens was not taken into account, because the determination method used requires a larger amount of sample than that existing in the specimen.

Table 1. Sampling stations and turbot (*Scophthalmus maeoticus*) characteristics.

Nr. Crt.	Sampling Station	Collection Date (dd/mm/yyyy)	Bottom Depth (m)	Turbot Sex	Weight (g)	Humidity (%)
1	Sf. Gheorghe	13 May 2021	50.5	F	1100	78.3
2	Sf. Gheorghe	13 May 2021	50.5	F	2400	75.6
3	Sf. Gheorghe	13 May 2021	43.3	F	6400	79.2
4	Sf. Gheorghe	13 May 2021	40.5	F	5350	77.4
5	Platforms	13 April 2021	51.1	F	4900	81.0
6	Est Constanta	13 May 2021	40.3	M	3650	77.2
7	Est Constanta	13 May 2021	40.3	F	2400	76.1

No approval from the National Institute for Marine Research and Development "Grigore Antipa" Ethics Committee was required, as no targeted fishing was performed to obtain the tissue samples and no animals were sacrificed for this purpose. All turbot (*Scophthalmus maeoticus*) specimens from which tissue was collected originated from the "Pilot Study Aiming at Obtaining Scientific Evidence for the Exemption of Turbot from the Landing Obligation, in Accordance with Commission Delegated Regulation C (2021) 2065/25 August 2021" (funded and approved by the Romanian National Agency for Fisheries and Aquaculture—NAFA). The study involved scientific fishing, in compliance with Romanian legislation (NAFA Scientific Fishing Regulation) and aimed at calculating the survivability of turbot caught with gillnets (turbot survival rate 81.67%). The remaining dead specimens (18.33%) were used for various laboratory analyses, among which was the current research.

On visual examination, all fish had intact skin and mucus and did not exhibit any indications of lesions, asymmetry, or deformities.

The samples were analyzed according to IAEA-MEL Marine Environmental Studies Laboratory [67]. The tissues were frozen after sampling. The preliminary stages of analysis for OPs consisted of lyophilization of frozen matrices and comminution (Figure S1 Supplementary Material).

2.1. PAHs Extraction

Freeze-dried and crushed samples were prepared for extraction. The extraction was a Soxhlet method. A mass of 2 g of tissue was weighed in an extraction cartridge. The Soxhlet installation was prepared with 200 mL of methanol each in the boiling flasks. The extractor with the extraction cartridge was attached and connected to the refrigerant. The extraction took place for 8 h, after which 20 mL of KOH 0.7 M and 30 mL of distilled water were added and boiled in the bath for another 2 h (Figure S2 Supplementary Materials). Then, liquid–liquid separation was performed, with a volume of hexane to separate the phase in which PAHs are concentrated. Next, the concentration stage at the rotary evaporator was conducted. The extract passed through the silica gel and alumina column for better filtration of the PAHs, followed by concentration in a water bath and under nitrogen flow [67].

After all these steps, the ampoules were ready to be injected into the gas chromatograph coupled with the mass spectrometer.

To identify and quantify PAHs a Perkin Elmer Clarus 690 GC/MS system was used. The analytical conditions used were the following: capillary column Elite 35 MS, stationary phase: Dimethylpolysiloxane (35% Diphenyl), length 30 m, internal diameter 0.32 mm, film thickness 0.25 mm; carrier gas—helium, rate—1 cm^3/min, injector split/splitless in split mode, split flow 15 cm^3/min, sample volume—2 µL, injector temperature—300 °C, temperature program—initial temperature 100 °C, 5 min, heating rate—6 °C/min, first isotherm—250 °C for 0 min, heating rate—10 °C/min, second isotherm—330 °C for 10 min, ionization—E +70 eV, interface temperature—330 °C, temperature of source—270 °C, data collection method—SIR (Table 2).

Table 2. Retention time, monitored ion, linearity, and limits of detection (LOD) of PAHs for biota.

PAHs	Retention Time	m/z	Linearity (R^2)	LOD µg/g
Naphthalene	7.25	128	0.9885	0.0001
Acenaphthylene	13.70	152	0.9937	0.0001
Acenaphthene	14.50	154	0.9933	0.0001
Fluorene	16.57	166	0.9945	0.0001
Phenanthrene	20.36	178	0.9922	0.0001
Anthracene	20.56	178	0.9968	0.0001
Fluoranthene	25.04	202	0.9923	0.0001
Pyrene	25.87	202	0.9980	0.0001
Benzo[a]anthracene	30.74	228	0.9972	0.0001
Chrysene	30.90	228	0.9946	0.0001
Benzo[b]fluoranthene	33.80	252	0.9840	0.0001
Benzo[k]fluoranthene	34.00	252	0.9734	0.0001
Benzo[a]pyrene	34.68	252	0.9858	0.0001
Benzo(g,h,i)perylene	37.20	276	0.9765	0.0001
Dibenzo(a,h)anthracene	37.40	278	0.9823	0.0001
Indeno(1,2,3-c,d)pyrene	37.74	276	0.9756	0.0001

Internal standard 9,10 dihydroanthracene was added to the samples for quantifying the overall recovery of the analytical procedures. The PAHs were identified by the characteristic ions and the retention times (Figure S3 Supplementary Material).

2.2. OCP and PCB Extraction

The processing of samples for the determination of OCPs and PCBs was carried out by a different method from the PAH determination. Thus, 2 g of the lyophilized sample was weighed and placed in containers for microwave extraction, over which 30 mL of solvent was added. The extracts were collected in the separatory funnel, and hexane and sulfuric acid are added to separate the lipids with the help of the separatory funnels. Next, concentration was performed in a Kuderna Denish water bath, with the extracts passing through the column with florisil and Na_2SO_4 followed by their concentration under nitrogen flow [67]. The detection of OCPs and PCBs was carried out with a Perkin Elmer Clarus 500 gas chromatograph coupled with an electron capture detector (ECD). The analytical conditions used were the following: capillary column Elite 1, stationary phase: Dimethylpolysiloxane, length 30 m, internal diameter 0.25 mm, film thickness 0.25 mm; carrier gas—nitrogen, rate—1 cm^3/min, sample volume—1 µL, injector temperature—300 °C, temperature program—initial temperature 180 °C, 10 min, heating rate—7 °C/min, first isotherm—230 °C for 10 min, heating rate—15 °C/min, second isotherm—250 °C for 2 min, ionization.

Internal standard 2,4,5 trichlorobiphenyl was added to the samples for quantifying the overall recovery of the analytical procedure [67]. The values of the retention times, the recovery coefficients, and the maximum admissible limits for OCPs and PCBs are represented in Tables 3 and 4, respectively.

Table 3. Retention time, linearity, and limits of detection (LOD) for OCPs for biota.

OCPs	Retention Time	Linearity (R^2)	LOD µg/g
Hexacholorbenzen	3.176	0.9925	0.0005
Lindan	5.118	0.9909	0.0004
Heptachlor	7.060	0.9909	0.0003
Aldrin	7.886	0.9587	0.0003
Dieldrin	11.220	0.9860	0.0003
Endrin	12.200	0.9945	0.0004
p,p′DDE	10.800	0.9751	0.0002
p,p′DDD	12.790	0.9917	0.0002
p,p′DDT	15.100	0.9909	0.0002

Table 4. Retention time, linearity, and limits of detection (LOD) for PCBs for biota.

PCBs	Retention Time	Linearity (R^2)	LOD µg/g
PCB28	18.827	0.9996	0.0004
PCB52	20.801	0.9979	0.0003
PCB101	25.880	0.9988	0.0006
PCB118	29.591	0.9992	0.0004
PCB153	30.819	0.9994	0.0006
PCB138	32.362	0.9952	0.0007
PCB180	36.257	0.9992	0.0003

Thus, the gas chromatographic analysis resulted in a set of N = 864 data corresponding to the 7 fish, 4 types of tissue for 6 of them and 3 types of tissue for one (no gonads), and 32 individual compounds (16 individual PAHs, 9 OCPs and 7 PCBs).

In addition, OP (PAHs, OCPs and PCBs) data were used regarding water and sediments analyzed in the laboratory, sampled from the same area, in the cruise carried out on 27–30 May 2021 (Table 5). The gas chromatographic analysis methods were the same as those described previously. Each area (N = 3) corresponds to a set of results (N = 32) representing N = 96 values for each matrix (water, sediments), N = 192 in total.

Table 5. Environmental data (surface water and sediments) sampling stations and associated pressures.

Nr. Crt.	Sampling Station	Collection Date	Bottom Depth (m)	Associated Pressures
1	Sf. Gheorghe	27 May 2021	40	Danube's input
2	Platforms	28 May 2021	50	Danube's input, offshore activities, shipping and port activities
3	Est Constanta	30 May 2021	44	Shipping and port activities Dredging and coastal rehabilitation works

For better visualization and analysis of the data, the following programs were used: ArcGIS Desktop Advanced 10.7, Microsoft Excel, and Statistica 14.0.1.25 (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results

3.1. POP Concentrations in the Marine Environment

3.1.1. Water and Sediments

Based on our data, OCPs were found to be the dominant POPs (µg/L) present in the Black Sea waters. Thus, the concentrations of individual PAHs in water were found to range from 0.0001–0.0746, the sum of the 16 analyzed compounds being between 0.0442 and 0.2297. The pesticides' (OCPs) individual levels oscillated between 0.002 and 12.4375 while their sum was in the range of 11.1621–31.1169. In the case of polychlorinated biphenyls, the concentrations in water fluctuated between 0.0023 and 0.0144, the sum of the nine compounds falling within the range 0.0367–0.0545. Thus, the maximum concentrations of OCPs in the Black Sea waters exceeded by 167 to 863 times the maximum concentrations of PAHs and PCBs, respectively, and prevailed in the Danube's influence area (Figure 2).

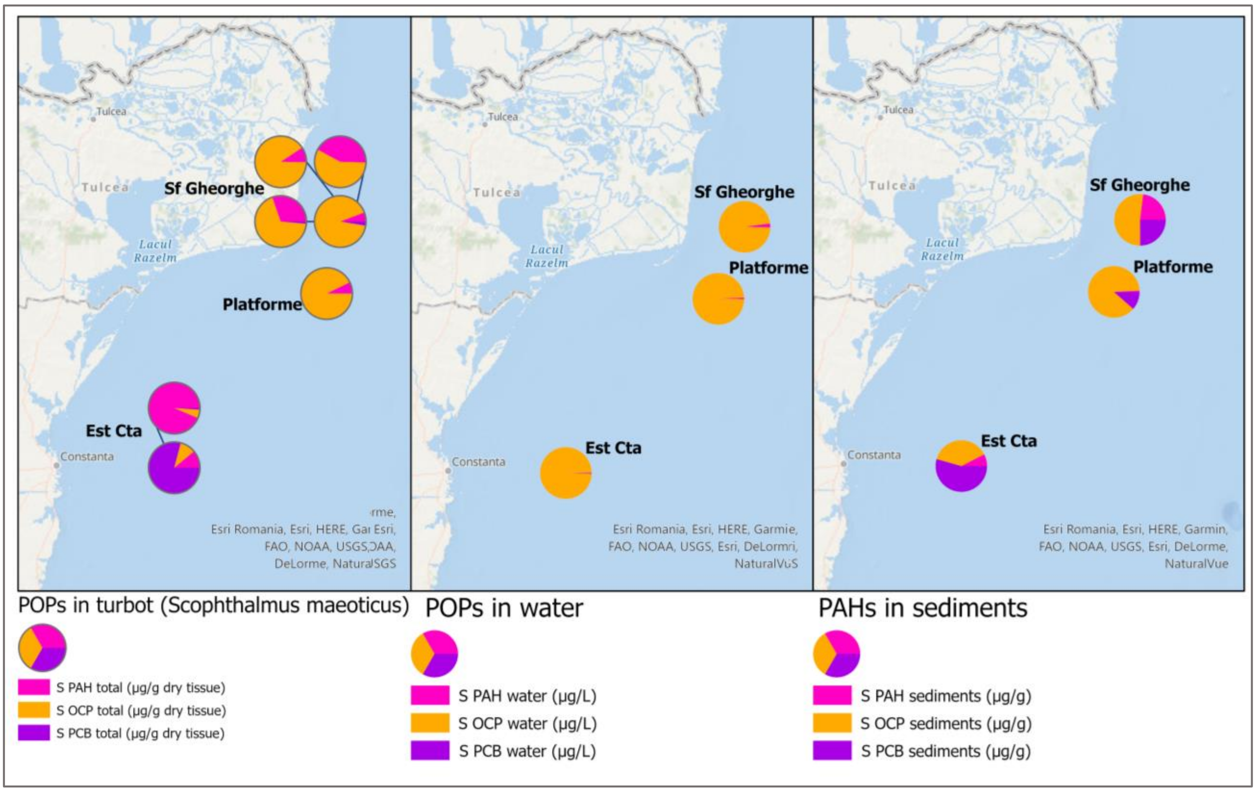

Figure 2. Spatial distribution of POPs' dominance in the Black Sea's turbot (N = 7) (*Scophthalmus maeoticus*) tissues (N = 864), water and sediments (N = 192).

POPs typically attach themselves to particle debris and eventually build up in the underlying sediments [68,69]. Because of this, sediments are frequently thought of as the last sink for anthropogenic pollutants in the marine environment and as a measure of contaminant loading. To learn more about the effects of human activity on the marine environment, it is therefore vital to investigate the presence of organic contaminants in sediments. Sediments may develop connections with a variety of anthropogenic pollutants and, through resuspending particulate matter, may serve not only as sinks for chemical contaminants but also as sources of such pollutants.

When it comes to sediments, there were higher concentrations of OCPs (µg/g), which predominated, too. The levels of quantified PAHs varied between 0.0001 and 0.0148 and the sum of the 16 compounds analyzed was from 0.0054 to 0.0731. The organochlorine pesticides varied in the range of 0.002–1.0462 and their sum oscillated in the range of 0.0273–2.3141. The variation of PCBs was in the range of 0.003–0.1531 and their total concentration oscillated between 0.039 and 0.3024 (Figure 2).

Compared to another study carried out on the Black Sea sediments, in the present study the maximum concentrations determined for PAHs were 100 times lower, while the concentrations of pesticides and biphenyls were 165 times, and 13 times higher, respectively [28].

3.1.2. Fish

Unfortunately, the presence of POPs was detected in all analyzed tissues in varying quantities (Figure 2).

Thus, the amount of PAH individual components (µg/g dry wt.) per specimen varied between 0.0003 and 401.4864 (mean 11.7098, std. dev. 43.0657). The total PAH tissue content (µg/g dry wt.) was relatively homogeneous, with the highest levels in the liver and gonads (liver 8.0355–47.5983; gonads 8.7514–314.9637; muscle 8.0355–47.5983; gills 6.5796–57.2838). The dominant compound, on average, was Benzo(g,h,i)perylene (109.1242) (Figure 3).

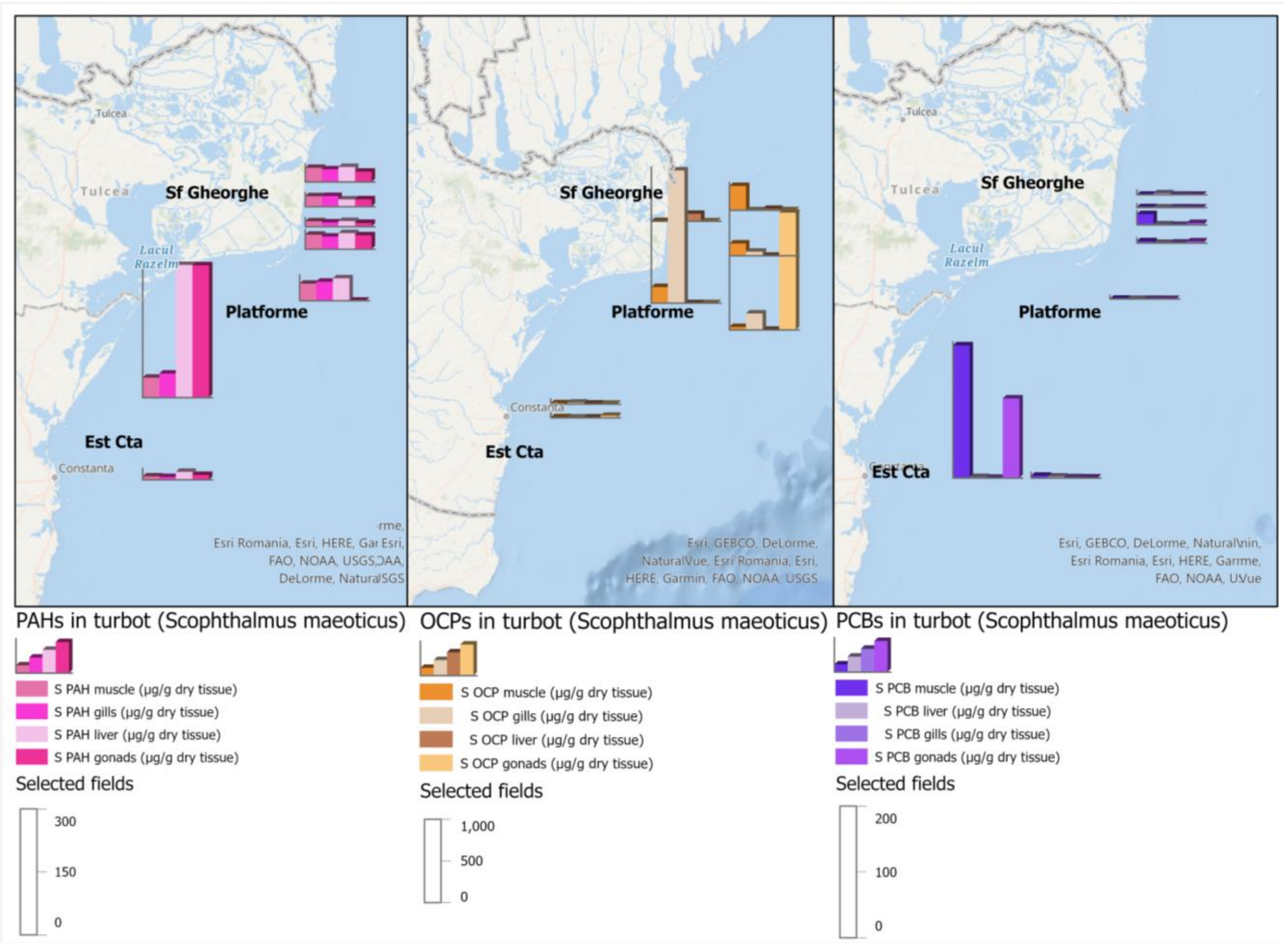

Figure 3. OPs concentrations in (N = 7) turbot (*Scophthalmus maeoticus*) tissues (N = 864).

OCPs (µg/g dry wt.) were present at maximum levels exceeding the other contaminants by two to five times varying by individual compounds, in the range 0.0008–1239.255, and were distributed differently in the tissues (liver 0.0023–89.2733; gonads 0.0028–979.1358; muscle 2.8509–291.2888 and the highest range was found in the gills 0.4454–1592.0597). They were dominated by p,p′DDD which reached an average concentration of 198.1173. High concentrations of the metabolites p,p′DDD and p,p′DDE demonstrate a long exposure time under the action of these pesticides [70,71] (Figure 3).

The levels of PCBs (µg/g dry wt.) on the specimen were in the range 0.0018–215.4668 and were distributed in tissues as follows: the lowest maximum concentration was determined in the gills 0.0612–1.3713, followed by the liver with 0.0775–2.3323, and gonads with 0.0192–120.9852. The highest concentration was determined in muscle 0.1074–201.6239. Overall, PCB52 predominated, being present at an average level of 32.5495 (Figure 3).

Studies carried out on the Black Sea coast in bream [72], shad and grey mullet [73], and in 2017 in species such as garfish, red mullet, turbot, and grey mullet [64], showed lower concentrations (in the order of nanograms) in the PCB and the DDT groups than those determined in this study.

In the case of PAHs, the predominant compound was Benzo(g,h,i)perylene and its sources can be represented by combustion processes (pyrolytic origin), and by emissions released as a result of sea, air and land transport [74]. Among the polychlorinated biphenyls, the dominant compound was PCB 52. From OCPs, the main compound was the metabolite p,p′DDD whose presence shows that its parent p,p′DDT had been present in the environment for a long time [75].

4. Discussion

Principal Component Analysis (PCA) for the presence of OPs in marine waters and sediments revealed that 100% of the sum of squares was explained by all the extracted components with the highest contribution from PAHs in sediments and OCPs in water. (Figure 4). Moreover, according to the analysis of variance (ANOVA) there are significant differences between the content of the PAHs, OCPs and PCBs in water ($p = 1.2067 \times 10^{-6}$) due to the raised levels of OCPs, and a comparable content in sediments ($p = 0.2935$) (Tables S1 and S2 Supplementary Material).

Analyzing the links between OP content in the environment and tissues, significant correlations were observed between different parameters (Tables S3–S8 Supplementary Material). For example, in the Danube's influence area (Sf. Gheorghe) negative significant correlations between fluoranthene and pyrene tissue content with naphthalene in sediments were found potentially as the result of naphthalene biotransformation [76,77].

In the case of OCPs, the best correlation between sediment content and tissues was observed for p,p′DD and p,p′DDT with HCB, Lindan, Aldrin, Dieldrin and Endrin, and it is most probably a link to the source as long as they are different chemical compounds that are not directly related to each other, it being unlikely for Dieldrin to be metabolized into DDT in fish.

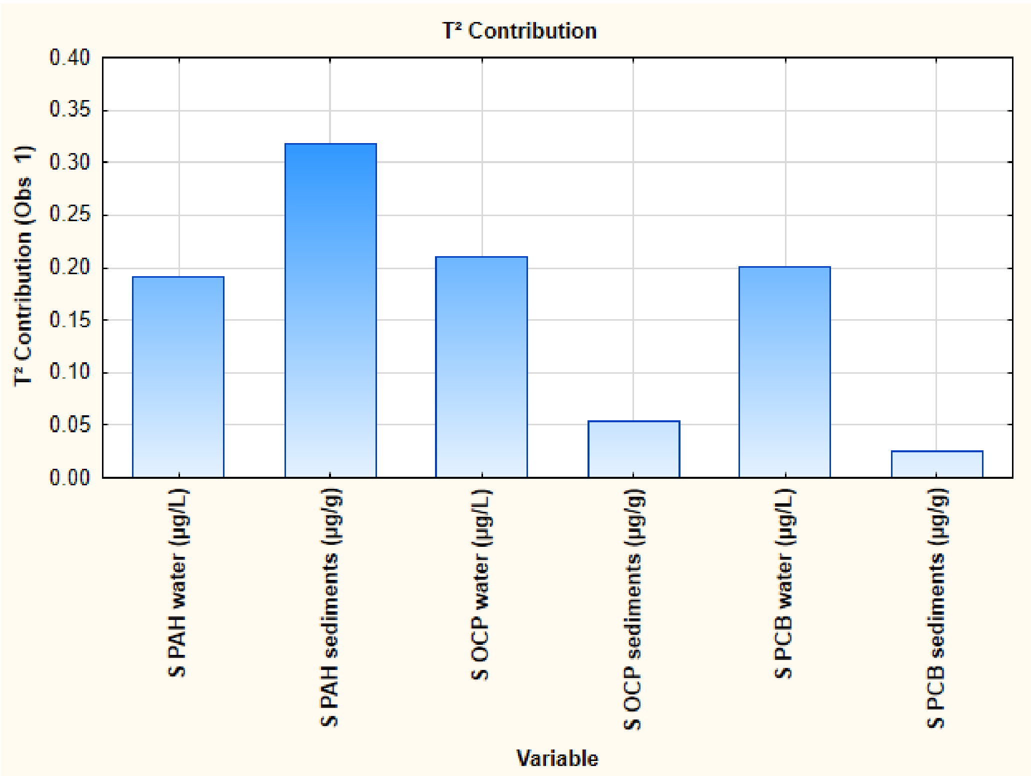

Figure 4. *Cont.*

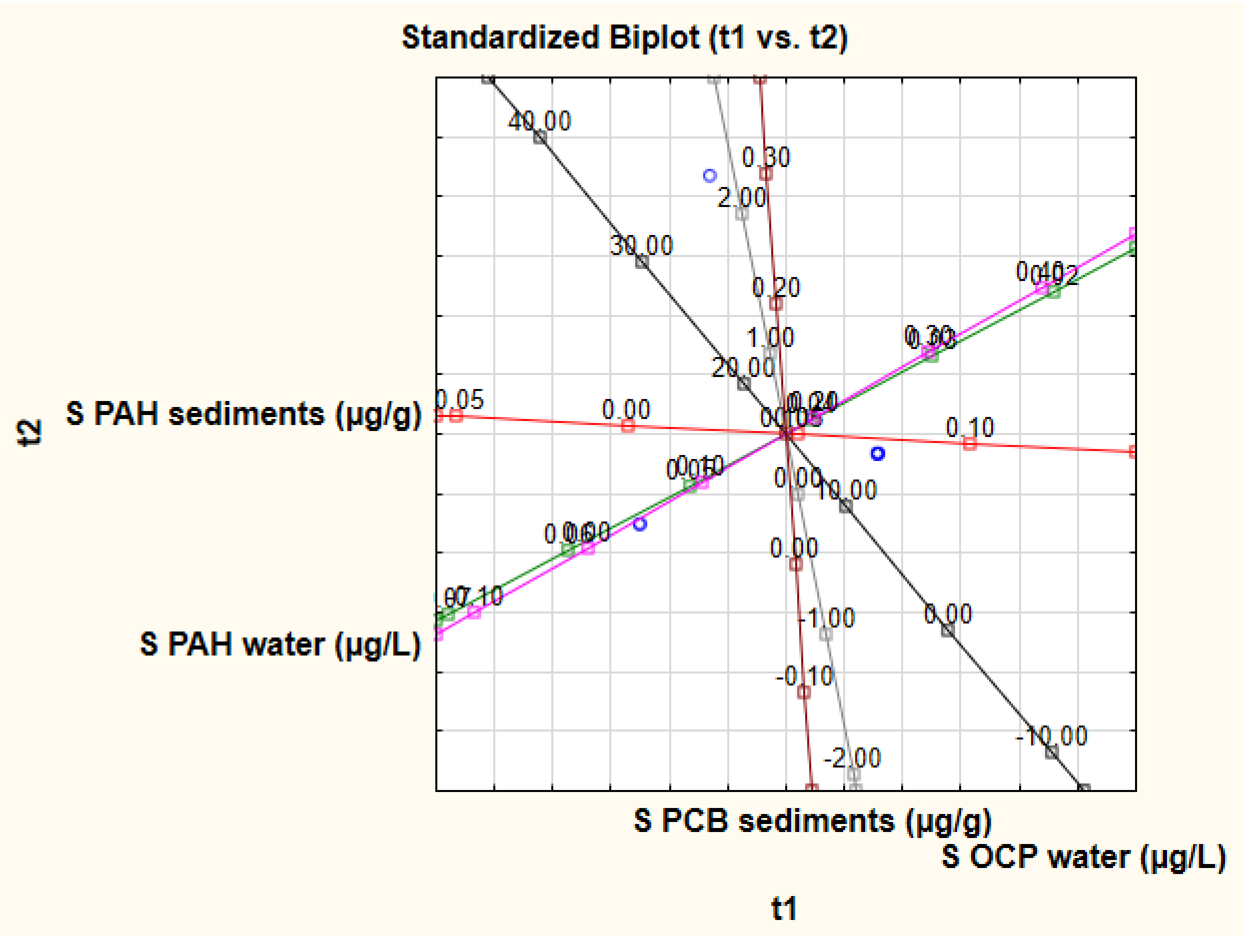

Figure 4. Variable contribution and PCA standardized biplot for POPs in water and marine sediments in the Black Sea.

No significant correlations between the tissue and environmental matrix were found in the PCB cases. One of the major ways that OPs may enter the Black Sea waters is by coastal drainage and estuarine runoff via the major rivers Danube, Dniester, and Dnieper [78,79]. This was observed in the case of PAHs that recorded the maximum values in the waters sampled from the stations located in front of the Danube Mouths.

Significant correlations were observed only between PAHs and OCPs concentrations in water and sediments from the same stations (Figure 5). On the other hand, the presence of some synthetic compounds, such as PCBs, in sediments could have only two sources—through different discharges or atmospheric deposition—which could represent an important pathway not studied in the present work.

Statistically (ANOVA, $p = 0.06$), the highest concentrations observed in turbot (Figure 3) were those of OCPs (Table S9 Supplementary Material). Among these, the maximum, an extreme value of p,p′DDT, was observed in the gills of the specimen from the station neighboring both the riverine input and offshore activities (station Platforme). Although it was also considered by us to be of an extremely high value, we chose to report it in the absence of other data from turbot gills, to be validated with subsequent data. As in other studies, the predominant compounds were the pattern p,p′DDT and its metabolites, p,p′DDD and p,p′DDE [62,80]. This indicates the fact that sources of pollution can be either the continental runoff by river inputs (from the drainage of agricultural lands), the sediment legacies or the already-contaminated food of the turbot specimens. Higher concentrations of the metabolites p,p′DDD and p,p′DDE indicate that the presence of these pesticides in the marine environment has been around for a long time, even if they are no longer used at present. It should be mentioned that DDT is known to have estrogenic effects on fish.

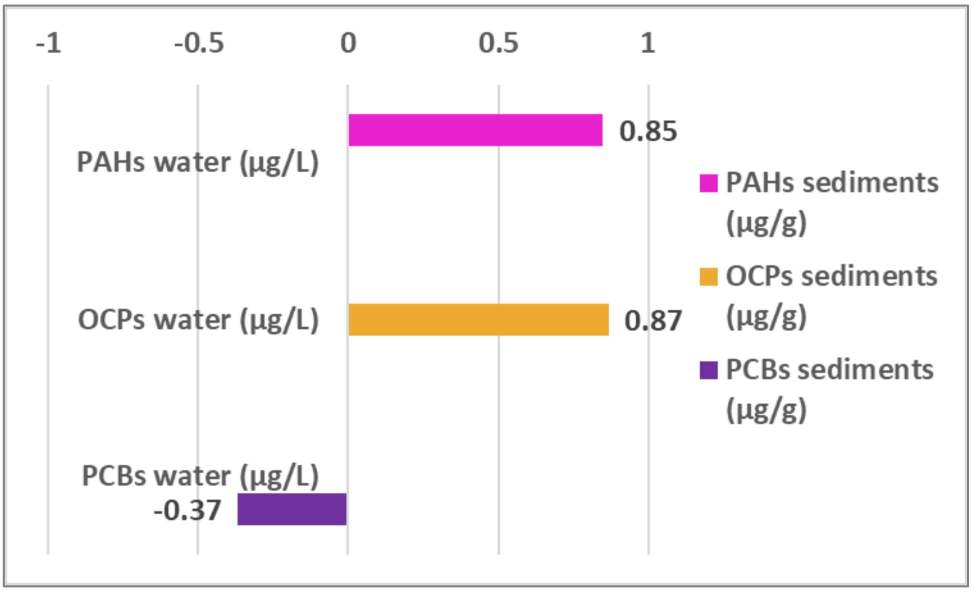

Figure 5. Correlation coefficients between water and sediment content of OPs.

There is a close connection between the pollutant values and the fish's food. This has also been highlighted in other studies through the bioaccumulation of pollutants in the mollusks and fish it feeds on [28,63,64]. In the present case, due to the large range of correlation significance between the environmental and fish data, we also turn our attention to the accumulation of pollutants in food.

Thus, the use of persistent pesticides may accumulate in fish or shellfish to varying extents depending on their physicochemical properties and their resistance to metabolism. As a result of such discharges and their accumulation at each trophic level, concentrations of certain chemicals in fish tissue may be up to 10 times greater than those found in the surrounding waters [81]. The PCB concentrations in turbot were similar to those in the other study carried out on the Black Sea coast [64]. PCB 52 was the dominant compound in the present study, and also in the study of POP determination in mollusks from the Black Sea coast [72]. Pollutant concentrations in pelagic fish from the Black Sea coast are 10 times lower than those determined by the present study in turbot [64]. Regarding PAH concentrations in mollusks, the previous works found them to be lower than those determined in turbot [61] suggesting their bioaccumulation in the food web.

The biota–sediment accumulation factor (BSAF) was also determined from field measurements and calculated using Equation (1) [82].

$$BSAF = \frac{c_{biota}}{c_{sediment}} \tag{1}$$

where c_{biota} is the chemical concentration in the turbot tissues and $c_{sediment}$ is the chemical concentration in sediment from the stations where fish were collected in the field. BSAF is the expression of all exposure routes (i.e., dietary, water, and contact with sediments via skin and ingestion), and they can be based on residues in the whole organism, muscle, liver, or any other tissue [82–84]. Excluding two *BSAF* extreme values (from PAHs and PCBs) the data showed exposure to PAHs > OCPs > PCBs in turbot (Figure 6). However, there is no significant difference between hazards and different groups of OPs (ANOVA, $p = 0.3367$).

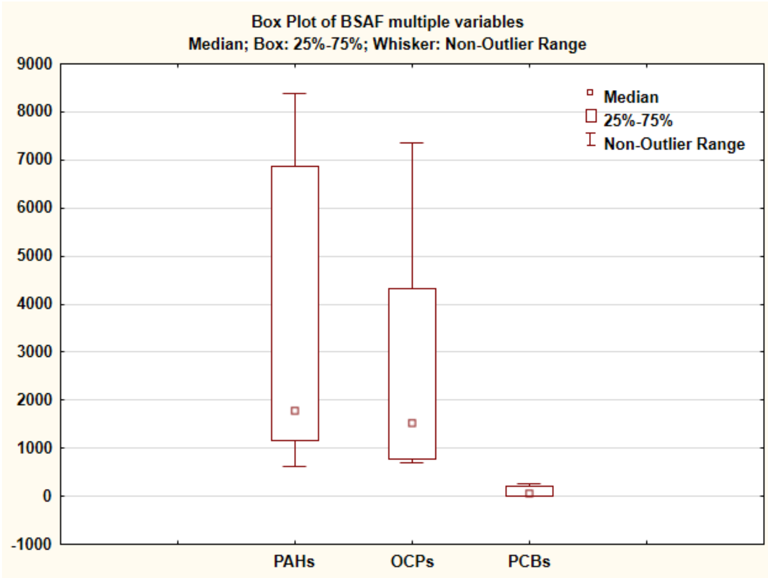

Figure 6. The biota–sediment accumulation factor (BSAF, N = 224) variability for OPs in the Black Sea.

Comparing the four types of tissues, muscle tissue, gills, gonads, and liver, the highest concentrations of PAHS were found in the liver and gonads, those of PCBs in the muscle followed by the gonads, and those of OCPs in the gills followed by the gonads. The results were statistically checked (ANOVA) and it was found that there were no significant differences between accumulation in biota for PAHs (p = 0.6395), OCPs (p = 0.5137) and PCBs (p = 0.4793). However, statistical differences appeared when comparing the levels of organic pollutants in different types of tissue. Thus, we found significant differences (ANOVA) only in the case of the liver (p = 0.0302), the levels in gills (p = 0.0617), gonads (p = 0.0720) and muscle (p = 0.0996) being comparable. The average concentration in the liver followed the same pattern as in the case of BSAF, respectively, PAHs > OCPs > PCBs.

Regarding the risk to human health, we evaluated the results obtained in the fish in relation to European and national quality standards (Table 6).

Table 6. Maximum Allowable Concentrations of OPs in fish according to Romanian and European legislation.

Legislation	Compound	OPs Group	Value (μg/g Wet Tissue)	
COMMISSION REGULATION (EC) 1881/2006 setting maximum levels for certain contaminants in foodstuffs	Benzo[a]pyrene	PAH	0.002	Fish fillets, other than smoked fish
Order 147/2004 for the approval of veterinary sanitary and food safety rules regarding pesticide residues in animal and non-animal products and veterinary drug residues in animal products.	Aldrin	OCP	0.02	Fish for human consumption (fillets)
	Dieldrin		0.2	
	Sum DDD, DDE, DDT		0.1	
	Endrin		0.05	
	HCB		0.02	
	Heptachlor		0.02	
	Lindan		0.1	

Table 6. *Cont.*

Legislation	Compound	OPs Group	Value (µg/g Wet Tissue)	
COMMISSION REGULATION (EC) 1881/2006 setting maximum levels for certain contaminants in foodstuffs, amended by Regulation 1259/2011 as regards maximum levels of dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs	Sum PCB28, 52, 101, 138, 153, 180	PCB	0.075	Fish products and fillets of fish

The results highlighted a potential risk for human consumption due to predominant exceedances, expressed as % of fish (N = 7, N = 78 analyses) (Figure 7). Overall, the maximum allowable concentrations were exceeded in all specimens for different compounds. Consuming fish contaminated with PAHs, OCPs, and PCBs can pose significant health risks to humans. These contaminants are known to be toxic and can accumulate in the body over time, potentially leading to various health problems [75,85]. It should be mentioned that DDT is known as a risk factor for breast cancer [75].

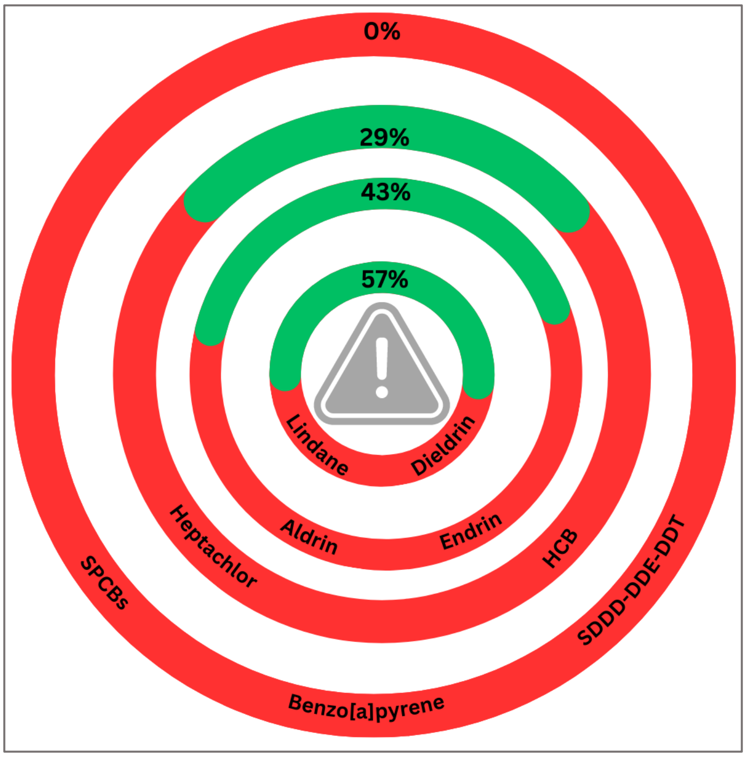

Figure 7. Turbot (*Scophthalmus maeoticus*) tissues OP contents and percentages of allowable concentrations in Romanian Black Sea.

5. Conclusions

The screening for organic pollutants in the Black Sea turbot (*Scophthalmus maeoticus*), a benthic species, showed the presence of PAHs and persistent organic pollutants (POPs)

(OCPs and PCBs) in all tissues (muscle, liver, gills and gonads) at levels that emphasize bioaccumulation and risk to human health.

Although the content in the sediments of each group is statistically comparable, it was observed that the highest level of biota-sediment accumulation factor is due to the presence of high-molecular-weight PAHs, substances with high potential for accumulation in sediments. On the other hand, although it is banned in many countries, including all EU Member States, pp'DDT reached an extreme value in turbots' gills sampled in an area in which the impact of agriculture cumulates via river inputs, as well as oil and gas activities. Its metabolites pp'DDD and pp'DDE were also present at high levels, their presence also indicating possible bioaccumulation in the food chain. Another potential source is maritime transport through the maintenance of ships with paints containing p,p'DDT [86]. However, it was decided to report the value despite its high level, as we lacked data from turbot gills and plan to confirm its accuracy with future data.

The bioaccumulation of biota-sediment factor showed the same pattern as its accumulation in the turbots' liver.

The assessment against national and European legislation concerning contaminants in food highlighted the presence in all specimens of benzo[a]pyrene, sum of PCB28, 52, 101, 138, 153 and 180, and sum of DDT, DDD and DDE at levels that exceed the maximum allowable concentrations. Thus, consuming fish contaminated with these contaminants can be harmful to human health and should be avoided or limited.

It is important to monitor OP levels in aquatic ecosystems and implement measures to reduce their use and runoff to protect the health of benthic fish, other aquatic organisms, and humans. Moreover, studies addressing the combined effects of numerous PAHs and POPs (the "cocktail effect") should be carried out due to serious concerns regarding fish population declines and the potential for chronic human exposure to contaminated seafood.

Future research should be directed towards effects on the physiology of aquatic organisms under the stress of a mix of pollutants, especially focusing on the turbot's (*Scophthalmus maeoticus*) reproductive capacity, and the presence of oxidative stress through plasma biomarker analysis from these fish.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes8050265/s1, Figure S1: The lyophilization stages of turbot tissues; Figure S2: PAHs extraction steps; Figure S3: Standard solutions chromatogram of PAHs; Figure S4: The stages of PCBs and OCPs extraction; Table S1: ANOVA results for Water samples; Table S2: ANOVA results for Sediment samples; Table S3: Correlations coefficients between PAHs individual components in dry tissues and their content in seawater; Table S4: Correlations coefficients between PAHs individual components in dry tissues and their content in sediments; Table S5: Correlations coefficients between OCPs individual components in dry tissues and their content in seawater; Table S6: Correlations coefficients between OCPs individual components in dry tissues and their content in sediments; Table S7: Correlations coefficients between PCBs individual components in dry tissues and their content in seawater; Table S8: Correlations coefficients between PCBs individual components in dry tissues and their content in sediments; Table S9: ANOVA results for the total content of OPs in turbot tissues samples.

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Institutional Review Board Statement: No approval from NIMRD's Ethics Committee was required, as no targeted fishing was performed in order to obtain the tissue samples and no animals were sacrificed for this purpose. All turbot (*Scophthalmus maeoticus*) specimens from which tissue was collected resulted from the "Pilot Study Aiming at Obtaining Scientific Evidence for the Exemption of Turbot from the Landing Obligation, in Accordance with Commission Delegated Regulation C (2021) 2065/25.08.2021" (funded and approved by the Romanian National Agency for Fisheries and

Aquaculture—NAFA). The study involved scientific fishing, in compliance with Romanian legislation (NAFA Scientific Fishing Regulation), and aimed at calculating the survivability of turbot caught with gillnets (turbot survival rate 81.67%). The remaining dead specimens (18.33%) were used for various laboratory analyses, among which included the current research.

Data Availability Statement: Data are belonging to National Institute for Marine Research and Development "Grigore Antipa"—NIMRD and can be accessed by requirement to http://www.nodc.ro/data_policy_nimrd.php.

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