Bulgarian Chemical Communications, Volume 56, Special Issue D2 (pp. 10-19) 2024 DOI: 10.34049/bcc.56.D.S2P22

Persistent pollutants in marine organisms: assessment of the state of the Black Sea environment

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Received: November 3, 2023; Revised: April 11, 2024

In the last decades, the residues of persistent pollutants such as organochlorine pesticides and polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in marine ecosystems and continue to bioaccumulate in animal tissues. The present study investigated the presence of pollutants in biota with the aim to assess the current environmental state of the Black Sea using benthic fish species as sentinel organisms. The biota samples: goby (*Neogobius melanostomus*), grey mullet (*Mugil cephalus*) and turbot (*Psetta maxima maeotica*) were collected from different sites along the Bulgarian Black Sea coast in the period 2021 – 2022. The concentrations of 13 PAHs and organochlorine pesticides such as DDTs and its metabolites, hexachlorocyclohexane and its isomers (HCHs), hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD) were determined in fish tissues by simultaneous extraction of persistent compounds in an accelerated solvent extractor (ASE) and were detected by gas chromatography with mass spectrometry (GC-MS).

The highest levels of PAHs in fish were found in goby samples: 14.2 ng/g ww (wet weight). The results showed that low-molecular weight (LMW) PAHs (3 and 4 aromatic rings) were predominant accounting 94% of total PAH levels, suggesting petrogenic origin of pollution. Benzo(a)pyrene was not detected in fish samples from the Bulgarian Black Sea coast. Lindane and other HCHs isomers have very low concentrations in all samples investigated. DDT is present mainly in the form of its metabolites p,p'- DDE and p,p'- DDD, suggesting contamination in the past. The HCB and HCBD levels in the fish species did not exceed the EQS of the Directive 2013/39/ EU. These results confirm that the persistent organic pollutants continue to be present in the Black Sea marine environment.

Keywords: polycyclic aromatic hydrocarbons, organochlorine pesticides, fish, the Black Sea

INTRODUCTION

Various studies have shown that persistent pollutants such as organochlorine pesticides and polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in different parts of marine ecosystems (water column, sediment and biota) and exhibit different types of toxicity to humans and marine organisms depending on their persistence, mobility and bioavailability [1-3].

The Marine Strategy Framework Directive (MSFD) aims at maintaining the state of the marine environment by preventing the long-term deterioration of marine ecosystems [4]. Assessments on the environmental status of marine waters and the anthropogenic pressures were based on the Good Environmental Status (GES) definitions. Determination of the GES which defines the environmental quality to be assessed for 11 descriptors: these are either descriptors for pressure (non-indigenous species, eutrophication, hydrographical changes, contaminants in the environment, contaminants in the seafood, marine litter and underwater noise) or 'State' descriptors (biodiversity, commercially exploited fish and shellfish, food webs and sea-floor integrity) [4]. Evaluation of the presence, control and effects of pollutants in the marine organisms according to MSFD was considered by descriptor 8 (Concentrations of contaminants give no effects) and descriptor 9 (Contaminants in seafood are below safe levels) [3, 4].

Plastic particles, pharmaceuticals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides and other endocrine disruptors, are only some examples of persistent pollutants that are permanently present in the coastal ecosystems [2, 5, 6]. The physicochemical properties of pollutants such as molecular size, high liposolubility and volatility, determine their availability, distribution and environmental persistence in the aquatic ecosystems and biota [1, 7].

The EU Directive 2008/105/EC established the Environmental Quality Standards (EQS) for 33 priority substances and other 8 pollutants with the aim to achieve a good chemical status of surface

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waters [8, 9]. The EQS were set for prey tissue (ww) and EU member states were being able to choose "the most appropriate indicator from among fish, molluscs, crustaceans and other biota" [8]. Fish concentrate pollutants in their tissues directly from water, but also through their diet, thus enabling the assessment of the transfer of pollutants through the trophic web [10].

The specific morphological, climatic and hydrological properties of the Black Sea as a semi-enclosed water basin make it very vulnerable to the impact of anthropogenic pollution. The Bulgarian Black Sea coast is subject to high levels of pollution from various sources – inflow of large rivers, agriculture, intensive shipping, tourism and recreation [11]. The war in Ukraine, which has been going on for almost two years, also poses an environmental risk and is likely to have long-term negative consequences for the Black Sea ecosystem.

PAHs are ubiquitous pollutants in shallower and coastal waters, especially in areas highly subjected to anthropogenic inputs: harbors and river mouths [12, 13]. Of the hundreds of known PAHs, 16 have been listed as priority pollutants by the United States Environmental Protection Agency (US EPA) [2, 14]. The International Agency for Research on Cancer (IARC) has classified sixteen PAHs as probable or possible human carcinogens which can cause mutagenic effects in humans and animal species [15, 16].

HCB and HCBD were pointed as priority substances under EC Directive 2013/39/EU [17]. The main source of this hydrophobic and highly persistent compound HCB today is the chemical industry from which this compound can be emitted as a product in high-temperature processes [18]. Hexachlorobutadiene (HCBD) is currently generated in large quantities as an unintentional by-product in the manufacture of other chlorinated hydrocarbons and polymers [19, 20]. DDT (1,1,1-trichloro - 2, 2 - bis (4-chlorophenyl) ethane) and its metabolites (DDTs) rapidly accumulated in living organisms due to their high lipophilicity [21]. DDT was among the initial persistent organic pollutants listed under the Stockholm Convention (2001) [22] and continues to be used for control of malaria in tropical and subtropical countries [23]. Hexachlorocyclohexane (HCH) isomers were added to the list of the Stockholm Convention in 2009 [22]. Lindan (γ-HCH) and the isomers α-HCH and β-HCH were indicated by the Integrated Risk Information System (IRIS) online database of the Environmental Protection Agency (EPA) as carcinogenetic for humans [24].

Data on the presence and distribution of persistent contaminants in fish and especially edible fish species are important not only from ecological, but also human health perspective [25]. To our knowledge, the data on levels of PAHs in fish from the Bulgarian Black Sea coast are very scarce in the literature. The study investigated the presence of persistent organic pollutants in biota with the aim to assess the current environmental state of the Black Sea using benthic fish species goby (*Neogobius melanostomus*), grey mullet (*Mugil cephalus*) and turbot (*Psetta maxima maeotica*) as sentinel organisms.

MATERIALS AND METHODS

Sampling

Benthic fish species were sampled from different stations of the Black Sea coast of Bulgaria: goby (*Neogobius melanostomus*), grey mullet (*Mugil cephalus*) and turbot (*Psetta maxima maeotica*). Samples were caught by local professional fishermen in the period Spring 2021 – Spring 2022. All fish samples were transported into the laboratory in foam boxes filled with ice. In the laboratory, for every fish species a pooled sample of muscle tissue from each individual was compiled by filleting and dissecting. The muscle fish tissue from 10-12 individuals (except turbot) was homogenized by a blender.

Analytical method

The concentrations of 13 PAHs and organochlorine pesticides such as DDTs and its metabolites, hexachlorocyclohexane and its isomers (HCHs), hexachlorobenzene and hexachloro-butadiene were determined in fish tissues with a mixture of hexane:acetone by simultaneous extraction of POPs in an accelerated solvent extractor. The accelerated solvent extraction (ASE) method requires a small amount of organic reagent, and the extraction can be performed rapidly.

A cellulose filter (Thermo Scientific) was placed at the bottom of a 66 mL extraction cell, followed by 10 g Al_2O_3 (acid, Brockmann Activity 1) and another cellulose filter. A 4 g sample was homogenized with an equal weight (10 g) of Thermo Scientific Dionex ASE Prep DE (Thermo Scientific) in a mortar and transferred into the extraction cell. Into this mixture, 25 µL hexane solution containing the two internal standards (PCB30, PCB204 and 9,10 dihydroanthracene, Dr. Ehrenstorfer Laboratory, Augsburg, Germany, 10 µg/mL) was added for quantifying the overall recovery of the analytical procedures.

Optimized ASE parameters (4:1, v/v n-hexane/acetone, 80 °C, 1500 psi, 10 min static time, two-cycle extraction, and 90 % rinse volume). Total extraction time and total solvent volume per sample: ~ 30 min and ~ 100 mL, respectively. The extracts were collected in 250 mL vials and were treated with sodium sulfate to remove any possible humidity. After filtration, the organic phase was concentrated to dryness on a rotary evaporator (Hei-Vap Precision Heidolph, Heidolph Instruments GmbH & CO. KG, Germany). The lipid content of each sample was measured gravimetrically.

The clean-up of the samples was conducted according to the previously described method [5]. The extract was cleaned-up on a self-packed multilayer glass column filled with neutral silica and acid silica. PAHs and OCPs were eluted with 10 mL of n-hexane followed by 20 mL of n-hexane/dichloromethane (9:1 v/v). The purified extracts were concentrated to near dryness, reconstituted in 0.5 mL of n-hexane and submitted to analysis by GC-MS. The GC conditions are summarized below.

The analytical determination of individual compounds was carried out on a gas chromatograph GC FOCUS with a POLARIS Q Ion Trap mass spectrometer (Thermo Electron Corporation, USA). A TG-5ms capillary column (Thermo Electron Corporation, USA) with a length of 30 m, 0.25 mm ID and a film thickness of 0.25 μm was used for GC separation of individual compounds. The temperature program for separation of PAHs was as follows: 40°C (1 min), 40°C/min to 130°C (3 min), 12°C/min to 180°C, 7°C/min to 280°C, 10°C/min to 310°C and a final hold for 5.0 min. For DDTs, HCHs, HCB and HCBD determination, the oven was programmed as follows: 60°C (1 min), 30°C/min to 180°C, 5°C/min to 260°C, 30°C/min to 290°C and final hold - 3.0 min. Helium at a flow rate of 1 mL/min was used as carrier gas.

For instrument calibration, recovery determination and quantification of compounds were used pure reference standard solutions: EPA 525 PAH Mix B, 500 μg/mL of each component in acetone (Sigma Aldrich, USA) and EPA 625/CLP Pesticides Mix 2000 μg/mL each component in hexane: toluene (1:1) (Supelco, USA). GC-MS was applied to the analysis of compounds: 13 PAHs: acenaphthylene (ACL), anthracene (AN), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbFA), benzo[k]fluoranthene (BkFA), benzo[ghi]perylene (BghiP), benzo[a]pyrene (BaP), chrysene (CHR), dibenzo[a,h]anthracene (DbahA), fluorene (FL), indeno[1,2,3-cd]pyrene (IP), phenanthrene (PHE) and pyrene (PY). Organochlorine compounds (OCs): p,p'-DDT, p,p'-DDD and p,p'-DDE, HCB, HCBD; isomers of hexachlorocyclohexane (HCH): γ-HCH (Lindane), α-HCH, β-HCH and δ-HCH. All measurements were performed in triplicate in order to ensure the accuracy of the analytical procedures. Multi-level calibration curves (range 5 – 100 ng/mL) were used for the quantification and good linearity ($R^2 > 0.996$) was achieved for the tested intervals that included the whole concentration range found in the samples (Table 1).

The identification of target analytes by GC with Ion Trap - MSn (IT-MSn) detection was based on a selected parent ion and the whole mass spectrum of its daughter ions. The IT-MSn detection was performed by isolation of the selected parent (precursor) ion for each compound inside the Ion trap MS analyzer followed by application of an adequate excitation voltage for its subsequent fragmentation to its daughter ions (Figs. 1 and 2). Parent ions were selected from the EI-MS spectra (Full scan) based on high m/z values and the peak abundance as well as the chromatographic signal obtained after its isolation in the Ion trap [7]. The PAHs and organochlorine compounds were identified by the relative retention time and the intensity ratios of the monitored extracted ions for GC-MS - Table 1.

Quality control

Quality control procedures included procedural blanks, analysis of replicate samples, use of recovery surrogates, and analysis of certified reference materials BCR - 598 (DDTs in Cod liver oil) - Institute for Reference Materials and Measurements, European Commission). Recovery of DDTs from certified reference material varied in the range 85 - 109% for individual congeners. The blanks did not contain traces of contaminants. The repeatability of the method (evaluated as the relative standard deviation, RSD) was <20%, calculated on 6 replicates of sample at the lowest spiked level.

The method limits of detection (LOD) were calculated as 3 times the standard deviation, based on the low concentrations of PAHs and OCs in fish tissue. The LOQ is the analyte concentration corresponding to ten times the standard deviation. The limit of detection (LOD) of the method was from 0.02 to 0.15 ng/g and the limit of quantification (LOQ) from 0.07 to 0.5 ng/g ww.

Statistical analysis

The statistical analysis of the data was based on the comparison of average values by a t-test. The p-value below 0.05 was considered statistically significant ($p<0.05$). Concentrations below LODs were considered as LOD/2 for all statistical analyses.

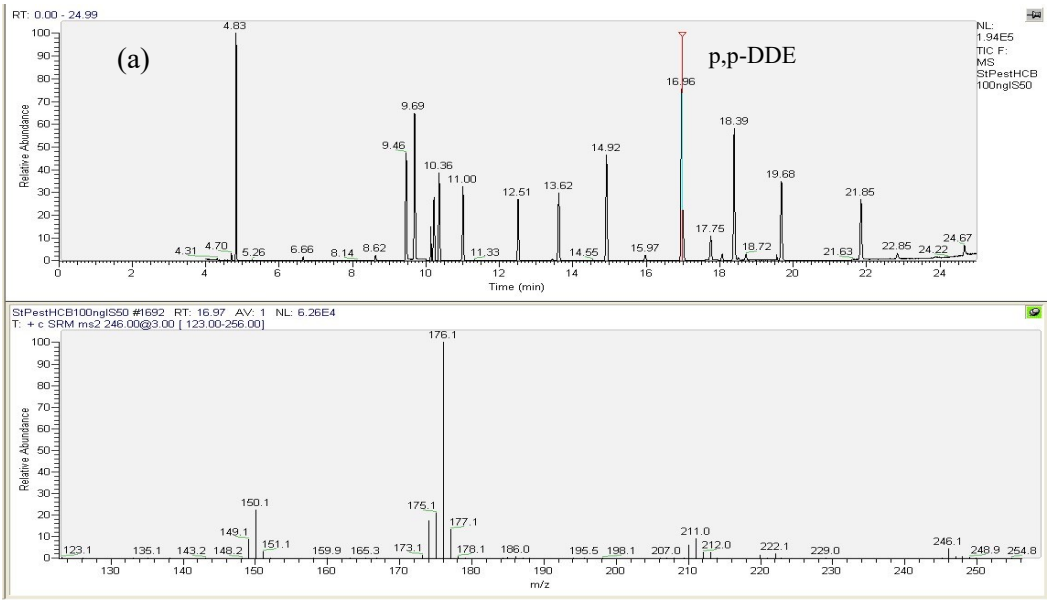
All statistical tests were performed using the SPSS V19.0 package for Windows (SPSS Inc., Chicago, IL, USA).

The chromatograms and mass spectra of reference standard solutions of OCPs and PAHs and fish extract samples are presented in Figs. 1(a, b) and 2(a, b), respectively.

Table 1. Retention time, linearity (correlation coefficient), precursor ions, extracted ions, recovery of individual organochlorine compounds and PAHs.

Compounds	Retention time (s)	Linearity	Recovery	Precursor ions	Extracted ions selected
	min	R^2	(%)	m/z	m/z
HCBD	4.79	0.9966	97.5	225	153.0, 190.0, 225.0
HCB	9.67	0.9979	99.1	284	214.0, 249.0, 284.0
α-HCH	9.44	0.9968	96.5	181	109; 145; 147
γ-HCH	10.34	0.9977	97.8	181	109; 145; 147
β-HCH	10.14	0.9976	86.9	181	109; 145; 147
δ-HCH	10.99	0.9991	89.3	181	109; 145; 147
p,p' - DDE	16.94	0.9956	98.1	246	176.1; 150.1
p,p' - DDD	18.37	0.9985	96.2	235	165.1; 199.1
p,p' - DDT	19.66	0.9999	95.9	235	165.1; 199.1
ACL	9.47	0.9984	97.5	152.1	98.1; 126.1; 152.1
FL	11.08	0.9991	96.1	165.1	139.1; 165.2
PHE	13.56	0.9992	96.3	178.1	98.1; 152.1; 176.1
AN	13.68	0.9997	91.4	178.1	98.1; 152.1; 176.1
PY	17.73	0.9998	93.9	202.2	122.1; 174.1; 200.1
BaA*	21.63	0.9986	89.3	228.2	146.0; 200.1; 226.1
CHR*	21.76	0.9978	88.1	228.2	170.1; 202.1; 224.1
BbFA*	24.99	0.9971	86.2	252.2	193.1; 179.1; 224.1
BkFA	25.06	0.9995	85.9	252.2	193.1; 179.1; 224.1
BaP*	25.85	0.9986	87.5	252.2	193.1; 179.1; 224.1
IP	28.52	0.9978	89.1	276.2	222.1; 248.2; 274.2
DBahA	28.60	0.9998	84.5	276.2	224.2; 248.2; 274.2
BghiP	29.13	0.9965	83.8	276.2	222.1; 248.2; 274.2

* - group of the priority 4PAHs; PAHs: polycyclic aromatic hydrocarbons



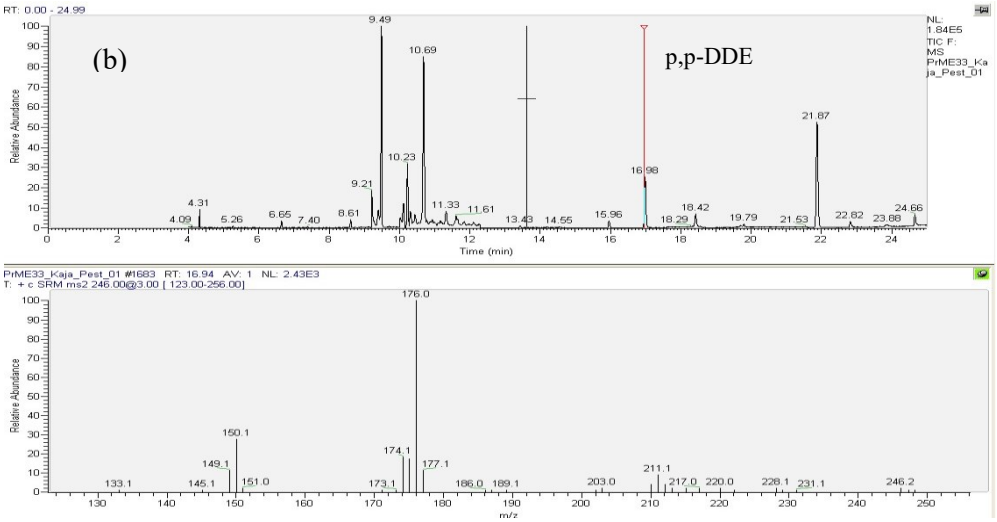


Figure 1. Chromatograms of reference standard solutions of OCPs 100 ng/mL (a) and fish extract sample (b); mass spectra of DDE in standard and sample.

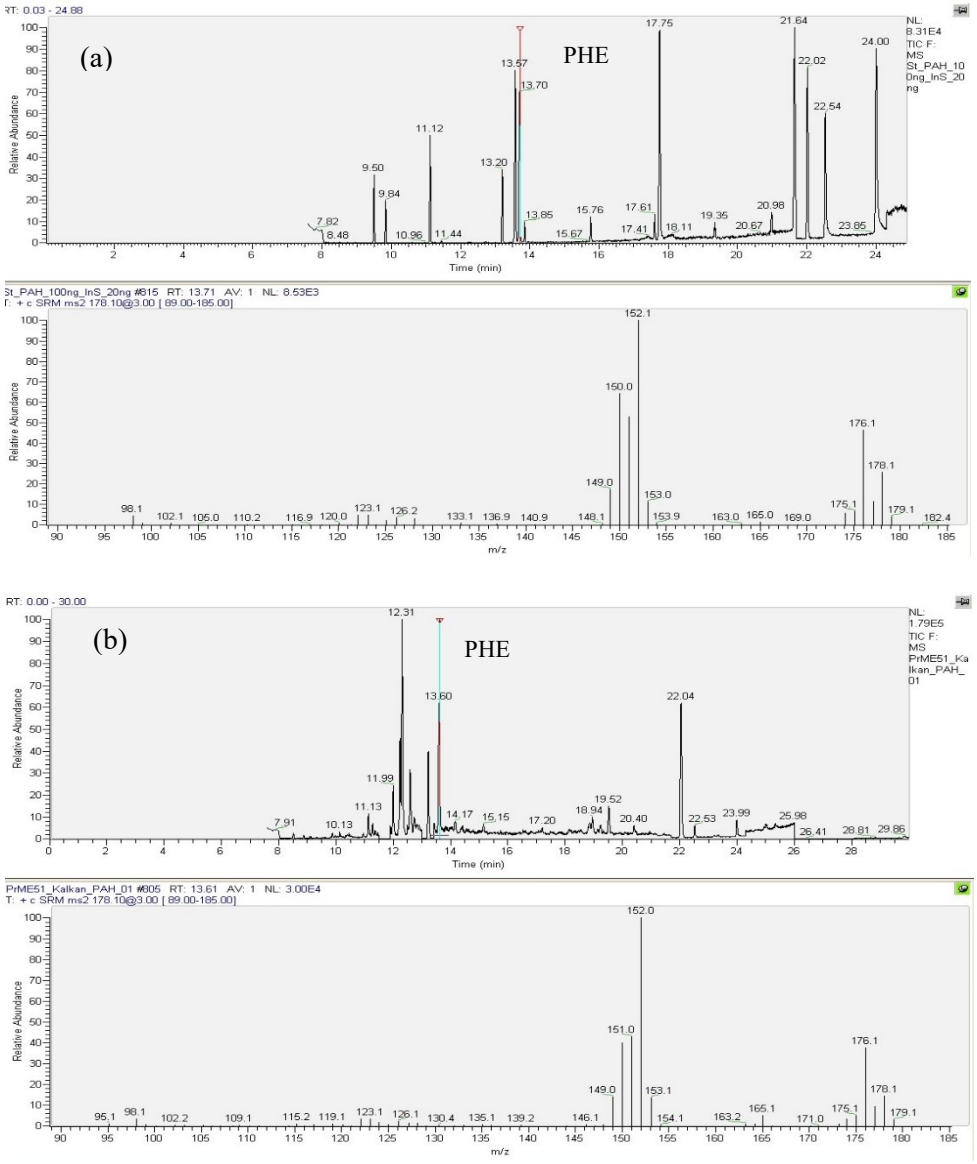


Figure 2. Chromatograms of reference standard solutions of PAHs, 100 ng/mL (a) and fish extract sample (b); mass spectra of PHE in standard and sample.

RESULTS AND DISCUSSION

The assessment of the state of the marine environment regarding the pressure of priority pollutants in the biota was made in accordance with the Marine Strategy under Descriptors 8, 9 and 10 and the Environmental Quality Standards (EQS) for priority substances [4, 8].

Organochlorine compounds (OCs)

The obtained results for the mean concentrations of organochlorine compounds in different fish species from the Black Sea are summarized in Table 2.

Hexachlorobenzene (HCB) is regulated as a hazardous priority pollutant by the Water Framework Directive (WFD) and is ubiquitously distributed in the environment and assumed to mildly biomagnify in aquatic food webs [26]. The highest HCB level was found in turbot, 0.18 ng/g of ww. HCB was detected in only 20% of the goby samples, while in the grey mullet samples, HCB was determined in 75% of the samples examined. HCBD was not found in the investigated fish species goby, grey mullet and turbot both from the North and the South sampling areas. To protect the most sensitive organisms from harmful effects of hazardous substances, Environmental Quality Standards (EQS) have been developed within the European Commission [8, 17]. The HCB and HCBD concentrations in the fish species did not exceed the EQS of the Directive 2013/39/ EU - 10 and 55 ng/g (μg/kg) ww for biota, respectively [17]. Based on the results obtained for HCH and HCBD in biota, we could conclude that a good chemical status of the marine environment has been achieved.

In general, our results were lower than the data reported in recent years by a number of authors: Spanish authors reported HCB in salmon and mackerel from Mediterranean Sea 1.68 and 0.80 ng/g ww, respectively [27].

Among compounds of the OCs class, the highest quantified value of the sum of DDT and metabolites was 7.73 ng/g ww and was found in turbot samples from the north part (Krapec, cape Kaliakra) of the Black Sea coast. The main metabolite p,p'-DDE was present in much higher concentrations than the other DDTs, while p,p'-DDT was detected in only 12% of the analyzed samples at levels close to the LOQ. This suggests that recently these pesticides have not been used in agriculture after their ban.

The γ isomer of HCHs (Lindane) is one of the most used insecticides in the past. It was considered as carcinogenic to humans (IARC group 1) for professional exposures [15, 28]. The highest value of Lindane was found in a sample of turbot at 1.22 ng/g ww and the sum of the three HCH isomers was quantified as 2.93 ng/g ww. The levels of HCHs in fish samples (mean value 2.5 ng/g ww) were found higher than results reported for sea bass from Lake Como, Italy [28].

The comparison of OCs levels by sampling area was made with aim of assessing the current state of organochlorine pollution along the Bulgarian part of the Black Sea coast (Fig. 3).

Table 2. Lipid content (%) and mean concentrations of HCB, HCBD, HCHs and DDTs, (ng/g ww) determined in fish species from the Black Sea coast

Fish species	Goby (N=5)	Grey mullet (N=5)	Turbot (N=3)
Lipids, %	0.40±0.01	2.3±0.21	1.2±0.10
HCB	0.08±0.01	0.11±0.01	0.18±0.02
HCBD	nd	nd	nd
p,p' - DDE	1.08±0.16	2.56±0.27	4.52±0.56
p,p' - DDD	0.66±0.08	1.42±0.16	3.01±0.36
p,p' - DDT	nd	0.56±0.08	0.20±0.02
Sum DDTs	1.74	4.54	7.73
α-HCH	1.06±0.12	0.48±0.06	1.05±0.09
β-HCH	nd	nd	nd
γ-HCH	0.77±0.09	0.46±0.06	1.22±0.14
δ-HCH	0.95±0.09	0.85±0.10	0.66±0.07
Sum HCHs	2.78	1.79	2.93

N – number of samples, nd – not detected

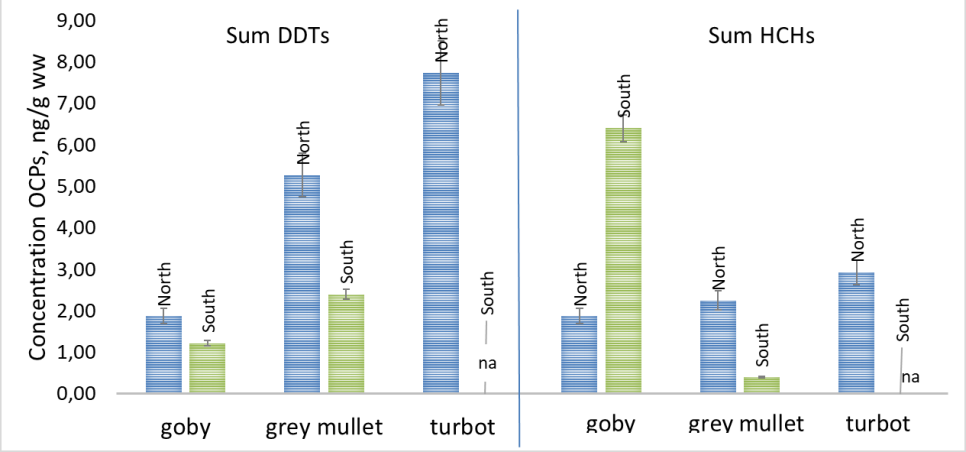


Figure 3. Comparison of DDTs and HCHs levels, ng/g ww in fish from North and South coast of the Black Sea.

Table 3. Individual PAHs concentrations (mean values, ng/g ww) in fish species from the Bulgarian Black Sea coast

PAH compounds	Aromatic rings	Goby (N=5)	Grey mullet (N=5)	Turbot (N=3)
ACL	3	0.40±0.03	0.18±0.02	0.49±0.03
FL	3	2.34±0.15	1.80±0.19	1.87±0.22
PHE	3	11.01±1.13	10.30±1.12	10.08±0.92
AN	3	nd	nd	nd
PY	4	0.42±0.05	nd	1.25 ± 0.14
BaA*	4	nd	nd	nd
CHR*	4	nd	nd	nd
BbFA*	5	nd	nd	nd
BkFA	5	nd	nd	nd
BaP*	5	nd	nd	nd
IP	6	nd	nd	nd
DBahA	5	nd	nd	nd
BghiP	6	nd	nd	nd

nd – not detected; * - group of the priority 4PAHs; PAHs: polycyclic aromatic hydrocarbons

The sum of DDT and its metabolites (Sum DDTs) was found higher in fish samples from the North coast of the Black Sea (mean 4.96 ng/g ww) than in samples from South sampling sites – 1.81 ng/g ww ($p < 0.002$). In contrast, the sum of the three HCH isomers in goby samples from the South sampling area (south of Cape Emine) was quantified higher than HCHs levels in goby from the North area (6.40 and 1.86 ng/g ww, respectively).

Levels of PAHs in fish

The distribution pattern of individual PAHs showed similar profiles in all fish species investigated and was as follows: phenanthrene > fluorene > pyrene > acenaphthylene (Table 3). In all samples anthracene, chrysene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, and dibenz[a,h]anthracene showed levels below the detection limit (LOD) of the method. Phenanthrene is one of the most widespread PAHs in the environment, due to its stable structure and persistence. Due to its high lipophilicity, phenanthrene was the most predominant PAH component in fish tissues (11.01 ng/g ww in goby). Four of the polycyclic hydrocarbons have been identified as indicators by EFSA (designated as 4 PAHs - benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene) [29]. Benzo[a]pyrene, known as the most toxic PAH, was not detected in fish samples from the Black Sea, Bulgaria.

The results showed that low-molecular weight (LMW) PAHs (3 and 4 rings) were predominant (accounting 94% of total PAH levels), while high-molecular weight (HMW) PAHs (5- and 6- rings) were below LOD and not detected in the fish samples investigated (Table 3). PAHs are produced by a variety of sources: LMW PAHs are defined as petrogenic compounds (resulting from spillage of

diesel and fuel oil), and HMW PAHs are products of the incomplete combustion of organic matter - they have pyrolytic origin [30].

The ratio of LMW and HMW PAHs indicates the sources of PAHs pollution in the environment [31]. Our results showed that the ratio LMW/HMW PAHs is higher than 1 (mean 19.4), suggesting that PAH pollution of the Bulgarian Black Sea coast was predominantly of petrogenic origin.

The low levels of HMW PAHs found in the present study are logical because it has been found that fish have the ability to metabolize PAHs, in contrast to clams that accumulate them in their tissues [32, 33]. However, they can be used to assess the current state of the marine environment.

The mean PAHs levels in fish species sampled from the northern (north of Cape Emine) and southern (south of Cape Emine) locations of the Bulgarian Black Sea coast are presented in Fig. 4. Comparison of the levels of PAHs in fish by sampling area was made and statistical analysis showed that there is no statistically significant difference in the levels of PAHs in grey mullet from the North and South regions.

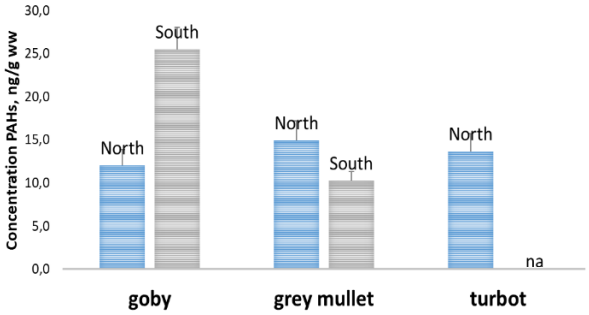


Figure 4. Total PAHs levels, ng/g ww in fish from different sampling regions.

The highest level of Sum PAHs in fish was found in goby samples from Chernomorets (southern sampling area). This result is consistent with a recent study on PAHs in white clam (*Donax trunculus*) [34] which revealed a higher concentration of these pollutants in samples from the Sozopol area. The authors suggest that these levels have been related to marine fuel spill incidents. This confirms that the PAH levels found in the Black Sea biota have a petrogenic origin.

Comparison with other studies on the Black Sea

Recent studies carried out on the Black Sea coast in species such as goby, turbot and red mullet [36] and in turbot in 2021 [3] showed higher concentrations in the DDT and the HCH groups than those determined in the present study. Romanian researchers [36] in 2019 determined concentrations of OCPs in fish (*Neogobius melanostomus, Psetta maeotica*, and *Mullus barbatus ponticus*) from the southern part of the Romanian Black Sea coast (Mangalia region): mean values for HCB - 66.06 ng/g ww, Lindane – 19.72 ng/g ww and sum DDT and its metabolites - 23.11 ng/g ww). The results of the present study were lower than the data from the Romanian project [36].

The findings in our study regarding PAHs were comparable with results obtained from a recent international project on pollution monitoring of the Black Sea. The most important contributors to PAH components in fish from Yeşilırmak and Sakarya Rivers (the Black Sea coast of Turkey) were phenanthrene (43%) and naphthalene (20%) and their distribution profile corresponds to a petrogenic origin of contamination [36]. Total PAH in fish samples showed a distribution between 30.4-285.7 µg/kg ww in Sakarya River and 25.6-842.6 µg/kg ww in Yeşilırmak [36].

Recent study reported PAHs concentrations from 0.001 to 147.45 ± 9.28 µg/kg ww in pelagic fish species (*Sprattus sprattus* and *Trachurus mediterraneus ponticus*) and from 0.0001 to 45.73 ± 5.28 µg/kg in benthic fish species (*Neogobius cephalarges*) from the Romanian Black Sea coast [35]. The average sum of 13PAHs in fish species from the Bulgarian Black Sea coast was 13.38 ng/g ww and it is comparable to the results of a recent study by Romanian scientists within the ANEMONE project, which found an average value for the sum of 9 PAHs - 8.15 ng/g ww [36]. The results in our study were lower than the data from a Spanish investigation in the period 2011 – 2018: 16 PAHs were measured in fatty tissues from Mediterranean dolphins and marine turtles (100±59 and 136±47 ng/g ww, respectively) and the tissue pattern of PAHs eminently suggested a petrogenic origin [13].

CONCLUSION

The pollution assessment of the Bulgarian Black Sea showed a good chemical status with regard to HCB and HCBD in benthic fish species, which were found in low levels and did not exceed the European EQS. The organochlorine pesticide DDT was present mainly in the form of its metabolites p,p'-DDE and p,p'- DDD in all samples investigated, suggesting contamination in the past. Low-molecular weight (LMW) PAHs (3 and 4 aromatic rings) were predominant, suggesting petrogenic origin of pollution. The most toxic PAH compound benzo[a]pyrene was not detected in fish samples from the Bulgarian Black Sea coast. In general, concentrations of HCB, HCHs, DDTs, and PAHs in fish species goby, grey mullet and turbot from the

Black Sea were found lower than levels measured in the fish species by other studies.

The monitoring data of OCPs and PAHs levels in the Black Sea ecosystem is important with a view to implementing measures to reduce their widespread distribution and protecting the biodiversity of aquatic organisms and human health. Future research should be directed towards the combined effects of numerous POPs due to serious concerns regarding the potential chronic human exposure.

Acknowledgement: *This work was supported by the Maritime Affairs and Fisheries Programme 2014-2020 co-financed by the European Union through the European Maritime Affairs and Fisheries Fund. Project No BG14MFOP001-6.004-0006, contract No МДР-ИП-01-13/25.01.2021.*

REFERENCES

1. M. C. Vagi, A. S. Petsas, M. N. Kostopoulou, *Water.* **13** (18), 2488 (2021). https://doi.org/10.3390/w13182488
2. C. L. Chițescu, A. Ene, E.-I. Geana, A. M. Vasile, C.T. Ciucure, *Applied Sciences*, **11(20)**, 9721 (2021) https://doi.org/10.3390/app11209721
3. D. Danilov, L. Dediu, N. A. Damir, V. Coatu, L. Lazar, *Fishes*, **8 (5)**, 265 (2023). https://doi.org/10.3390/fishes8050265
4. European Commission, Directive 2008/56/EC, Establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive), *Off. J. Eur. Comm.*, **L 164/19**, 1 (2008).
5. S. K. Georgieva, Zl. V. Peteva, *Bulgarian Chemical Communications*, **49** (Special Issue G), 205 (2017)
6. S. K. Georgieva, Z. V. Peteva, M. D. Stancheva, *BioRisk*, **20**, 59 (2023). https://doi.org/10.3897/biorisk.20.97555
7. R. Serrano, M. Barreda, E. Pitarch, F. Hernández, *J. Sep. Sci.,* **26,** 75 (2003).
8. European Commission, Directive 2008 /105/ EC, Environmental Quality Standards in the Field of Water Policy, *Off. J. Eur. Union*, **L348**, 84 (2008)
9. L. I. Majoros, R. Lava, M. Ricci, B. Binici, F. Sandor, A. Held, H. Emons, *Talanta*, **116**, 251 (2013). https://doi.org/10.1016/j.talanta.2013.04.080
10. A. T. Fisk, K. A. Hobson, R.J . Norstrom, *Environ Sci Technol*, **35**, 732 (2001). https://doi.org/10.1021/es001459w
11. A. Simeonova, R. Chuturkova, V. Yaneva, *Marine Pollution Bulletin*, **119** (1), 110 (2017). https://doi.org/10.1016/j.marpolbul.2017.03.035
12. N. Berrojalbiz, J. Dachs, M. J. Ojeda, M. C. Valle, J. Castro-Jimenez, J. Wollgast, et al., *Global Biogeochem*. Cycles **25 (4)**, (2011). https://doi.org/10.1029/2010GB003775
13. G. López-Berenguer, A. Acosta-Dacal, O. P. Luzardo, J. Peñalver, E. Martínez-López, *Chemosphere*, **336**, 139306 (2023). https://doi.org/10.1016/j.chemosphere.2023.139306
14. U.S. EPA. Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons (PAH). Available online: https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=49732 (accessed on 17 July 2021).
15. IARC, Monographs on the evaluation of carcinogenic risks to humans. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. Lyon, (2010). http://monographs.iarc.fr/ENG/Monographs/vol92/mono92.pdf
16. M. Ferrante, G. Zanghì, A. Cristaldi, C. Copat, A. Grasso, M. Fiore, S.S. Signorelli, P. Zuccarello, G. Oliveri Conti, *Food and Chemical Toxicology*, **115**, 385 (2018). https://doi.org/10.1016/j.fct.2018.03.024
17. European Commission Directive 2013/39/EU as regards priority substances in the field of water policy *Off. J. Eur. Union*, **L 226**/1, (2013). https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:226:0001:0017:en:PDF, (accessed on 12/08/2013)
18. F. M. Jaward, N. J. Farrar, T. Harner, A. J. Sweetman, K. C. Jones, *Environ. Sci. and Technol.*, **38**, 34 (2004). https://doi.org/10.1021/es034705n
19. J. E. Balmer, H. Hung, K. Vorkamp, R. J. Letcher, D. C.G. Muir, *Emerging Contaminants*, **5**, 116 (2019). https://doi.org/10.1016/j.emcon.2019.03.002
20. M. D. Jürgens, A.C. Johnson, K. C. Jones, D. Hughes, A. J. Lawlor, *Sci. of the Total Environ.,* **461–462,** 441 (2013). https://doi.org/10.1016/j.scitotenv.2013.05.007
21. W. Masmoudi, M. S. Romdhane, S. Kherriji, M. E. Cafsi, *Food Chem.,* **105**, 72 (2007). https://doi.org/10.1016/j.foodchem.2007.03.037
22. Stockholm Convention on Persistent Organic Pollutants (POPS)-Texts and Annexes. United Nations Environ Program. Available online: http://chm.pops.int/TheConvention/ThePOPs/AllPOPs/tabid/2509/Default.aspx (accessed on 22 June 2022).
23. H. van den Berg, G. Manuweera, F. Konradsen, *Malar J.*, **16**, 401 (2017). https://doi.org/10.1186/s12936-017-2050-2
24. IRIS. Available online: https://cfpub.epa.gov/ncea/iris/search/index.cfm (accessed on 22 June 2022).
25. K. Macgregor, I. W. Oliver, L. Harris, I. M. Ridgway, *Environmental Pollution* **158**, 2402 (2010). https://doi.org/10.1016/j.envpol.2010.04.005
26. I. J. Allan, B. Vrana, A. Ruus, *Environmental Science & Technology* **56 (12)**, 7945 (2022). https://doi.org/10.1021/acs.est.2c00714
27. G. Falcó, J. M. Llobet, A. Bocio, J. L. Domingo, *The Science of the Total Environment*, **389**(2-3), 289 (2008). https://doi.org/10.1016/j.scitotenv.2007.09.025
28. G. Mosconi, F. Di Cesare, F. Arioli, M. Nobile, D. E. A. Tedesco, L .M. Chiesa, S. Panseri. *Foods*, **11(15)**, 2241 (2022). https://doi.org/10.3390/foods11152241
29. European Food Safety Authority (EFSA). *EFSA J.*, **6**, 724 (2008). https://doi.org/10.2903/j.efsa.2008.724

30. K. Srogi, *Environ. Chem. Lett*., **5**, 169 (2007). https://doi.org/10.1007/s10311-007-0095-0
31. R. Mercogliano, S. Santonicola, A. De Felice, A. Anastasio, N. Murru, M. C. Ferrante, M. L. Cortesi, *Mar. Pollut. Bull*., **104(1-2)**, 386 (2016) https://doi.org/10.1016/j.marpolbul.2016.01.015
32. T. Cappello, M. Maisano, A. D'Agata, A.Natalotto, A. Mauceri, S. Fasulo, *Mar. Environ. Res.*, **91,** 52 (2013). https://doi.org/10.1016/j.marenvres.2012.12.010
33. S. Fasulo, F. Iacono, T. Cappello, C. Corsaro, M. Maisano, A. D'Agata, et al., *Ecotoxicol Environ. Saf*., Oct; **84**, 139 (2012) https://doi.org/10.1016/j.ecoenv.2012.07.001
34. S. K. Georgieva, M. D. Stancheva, Z. V. Peteva, T. I. Ivanova, A. V. Alexandrova, *BioRisk,* **17**, 95 (2022) https://doi.org/10.3897/biorisk.17.77343
35. N.-A. Damir, V. Coatu, E. D. Pantea, M. Galațchi, E. Botez S. Birghilă, *Polycyclic Aromatic Compounds*, **42 (10)**, 7595 (2022) https://doi.org/10.1080/10406638.2021.2006243
36. Project ANEMONE Deliverable 3.1, "Hazardous substances assessment in Black Sea biota", A. Oros (ed.). CD PRESS, p. 96, 2021.ISBN 978-606-528-531-6