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Organochlorine pesticides and PCBs in marine fish

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Abstract Organochlorine pesticides (such as 1,1,1-trichloro - 2,2 - bis (4-chlorophenyl) ethane (DDT) and its metabolites) and polychlorinated biphenyls (PCBs) are classified as Persistent Organic Pollutants (POPs) and are present in the contamination pattern of marine environments world-wide. Concentrations of PCBs and DDTs were measured in two marine species: garfish (*Belone belone*) and red mullet (*Mullus barbatus*). Samples were collected from Black Sea, Bulgaria during 2007 – 2010. The DDTs and PCBs were determined by gas chromatography - mass spectrometry.
Concentrations in muscle tissue of garfish ranged from 80.89 to 118.04 ng/g wet weight for total DDTs. DDTs concentration in red mullet was found 104.59 ng/g ww. PCB concentrations in garfish varied in the range of 40.04 and 65.62 ng/g ww. In muscle tissue of red mullet PCB concentrations were found 34.12 ng/g ww. The levels of DDTs and PCBs in garfish and red mullet from the Black Sea were comparable with those found in other marine ecosystem.

Keywords: fish, DDT, PCB, Black Sea, Bulgaria

1. Introduction

PCBs and selected organochlorine pesticides are a group of chemicals that have attracted considerable attention due to their high toxicity, persistence in the environment, and ability to bioaccumulate. The combination of these properties means that organisms at the upper levels of the food chain can potentially be exposed to concentrations sufficient to cause adverse effects. [1,2] In biological systems, several of these chemicals are potentially carcinogenic and may cause alternations in endocrine, reproductive and nervous systems [3]. For these reasons, most countries have restricted or banned the use of PCBs and DDTs since 1970s. DDT (1,1,1-trichloro - 2,2 - bis (4-chlorophenyl) ethane) is a chlorinated pesticide widely used in the past to control the spread of insects and other agricultural peststicides. In the environment DDT metabolised slowly and the metabolite DDE is particularly persistent compound. Polychlorinated biphenyls have been widely used by a large variety of industries over the past 50 years. However, several studies have demonstrated that they are toxic to a variety of marine organisms [4].
Fish is a suitable indicator for the environmental pollution monitoring because they concentrate pollutants in their tissues directly from water, but also through their diet, thus enabling the assessment of transfer of pollutants through the trophic web [1,5]. Data on the presence and distribution of organohalogenated contaminants in fish and especially edible fish species are therefore important not only from ecological, but also human health perspective [6,7].

Red mullet are non-migratory species and feed mainly with benthic invertebrates (crustaceans, worms, and molluscs). The garfish *(Belone belone)* is a pelagic, oceanodromous needlefish found in marine waters of the Mediterranean Sea, the Baltic Sea, etc. The fish lives close to the surface, has a migratory pattern and feed mainly with small fish.

The purpose of this study was to determine the levels of persistent organochlorine contaminants in garfish and red mullet from the Bulgarian Black Sea coast and to monitor the accumulation of these pollutants during the period 2007 – 2010.

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2. Experimental

2.1. *Sampling and sample preparation*

Samples (garfish and red mullet) were collected from Varna to Burgas of Bulgarian Black Sea coast during 2007 – 2010 (**Fig. 1**). The samples were transferred immediately to the laboratory in foam boxes filled with ice and were stored in a freezer (-20°C) until analysis. The edible tissues of the each fish was homogenized using a blender; pools of about 300 g were made with fillets taken from several individual fish. The fish species was selected according to their characteristic feeding behavior.

2.2. DDTs and PCBs analysis

The analytical method for determination of residues of DDT and PCB was based on method BDS EN 1528:2001. Briefly, the edible tissues of fish were homogenized and 10 g were taken for extraction. Each sample was spiked with internal standards PCB 30 and PCB 204. The compounds were extracted with hexane / dichloromethane (3/1, v/v) in a Soxhlet apparatus. After lipid determination, the extract was cleaned-up on a glass column packed with neutral and acid silica. PCBs and DDTs were eluted with 80 ml n-hexane followed by 50 mL n-hexan/ dichloromethane (80:20 v/v). The eluates were concentrated to near dryness and reconstituted in 0.5 cm^3 in hexane.

Gas chromatographic analysis of the DDTs and PCBs were carried out by GC FOCUS (Thermo Electron Corporation, USA) using POLARIS Q Ion Trap mass spectrometer and equipped with an AI 3000 autosampler. Experimental MS parameters are the following: the Ion source and Transfer line temperatures were 220°C and 250°C, respectively. The splitless Injector temperature was 250°C. For DDTs 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 with a final hold for 3.0 min. The PCBs experimental temperature program - 90°C for 1 min, then programmed 30°C/min to 180°C, 2°C/min to 270°C, 30°C/min to 290°C with a final hold for 3.0 min. Splitless injections of 1 µl were performed using a TR-5ms capillary column coated with cross-linked 5% phenyl methyl siloxane with a length of 30 m, 0.25 mm ID and a film thickness of 0.25 µm. Helium was applied as carrier gas at a flow of 1 mL/min.

The selectivity of the IT–MS method was based on the appropriate selection of parent ions for the detection of each analyte by mass spectrometry extracted ion mode.

Fig. 1. Black Sea map

Pure reference standard solutions (EPA 625/CLP Pesticides Mix 2000 µg/mL - Supelco and PCB Mix 20 - Dr. Ehrenstorfer Laboratory) (see **Fig.2** and **3**), were used for instrument calibration, recovery determination and quantification of compounds. Measured compounds were p,p'-DDT, p,p'-DDD and p,p'-DDE, PCB congeners: IUPAC № 28, 31, 52, 77, 101, 105, 118, 126, 128, 138, 153, 156, 169, 170, 180). The quality control was performed by regular analyses of procedural blanks and certified reference materials: BCR - 598 (DDTs in Cod liver oil) and BB350 (PCBs in Fish oil) – Institute for Reference Materials and Measurements, European commission.

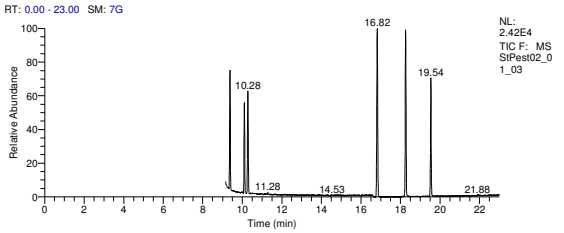

Fig. 2. GC-MS chromatogram of DDTs standard solution, 100 ng/mL

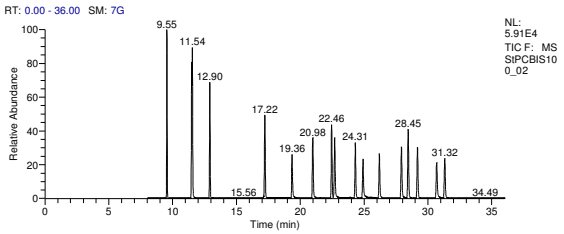

Fig. 3. GC-MS chromatogram of PCB standard solution, 100 ng/mL

3. Results and Discussions

3.1. DDT and its metabolites

The lipid content and average concentrations of DDT and its metabolites DDE and DDD in the edible parts of investigated species from the Black Sea coast of Bulgaria are shown in **Table 1.**

Lipid determination was performed on an aliquot of the extract (1/5th) before clean up. The average lipid percentage in garfish was 8.79 % and 15.9 % in red mullet. The lipid content of fish tissue is influenced by several factors, such as sex, age, species, nourishment and spawning status [8].

Figure 4 shows the GC-MS chromatogram of DDTs detected in garfish sample.

The main metabolite p,p'-DDE was the most frequently detected compound in the marine species investigated and was present in much higher concentrations than the other DDTs (ranging from 44.14 to 74.90 ng/g wet weight (ww)). p,p'-DDD concentration in garfish was found at levels ranging from 30.06 to 36.38 ng/g ww and in red mullet – from 22.20 to 47.50 ng/g ww. In garfish p,p'-DDT concentrations varied between 3.03 and 12.57 ng/g ww and in red mullet were found in the range 3.13 – 8.19 ng/g ww.

In all investigated samples, the residues were found in the order of DDE > DDD > DDT and this is in agreement with the results of other authors [9-11]. This suggests that recently these pesticides have not been used in agriculture after their ban. Thus, p,p'-DDE was the principal form of DDT in all the species studied, constituting from 54.5% to 67.8% of the total DDTs (Table 1). These findings are not surprising considering the high chemical stability and hydrophobicity of p,p'-DDE and its long half-life and persistence in both abiotic and biotic components of the aquatic ecosystems [12].

In the present study lipid based results are given for comparison with other studies (Table 1). Levels of ∑DDTs in garfish (average 1101.71 ng/g lipids) and red mullet (average 648.4 ng/g lipids) were found comparable than levels measured in the similar fish species from other seas - Marmara Sea, Adriatic Sea, Mediterranean Sea and Belgian North Sea [8, 10, 12, 13]. The benthic red mullet has been largely used as an indication of level of contamination [10]. A total burden of 1700 ng/g fat of DDTs was reported in striped red mullet from the Black Sea [14]. In the present study total DDT contamination was measured as 648.4 ng/g fat in red mullet from Bulgarian coast of the Black Sea. In red mullet from the Marmara Sea, the total DDT was previously reported as 1262 ng/g fat [13] and 731.42 ng/g fat [10]. ∑DDTs in garfish from Konya markets (Turkey) were found 0.0591 µg/g wet weight, but in this research DDD and DDT were the prevalent contaminants [15].

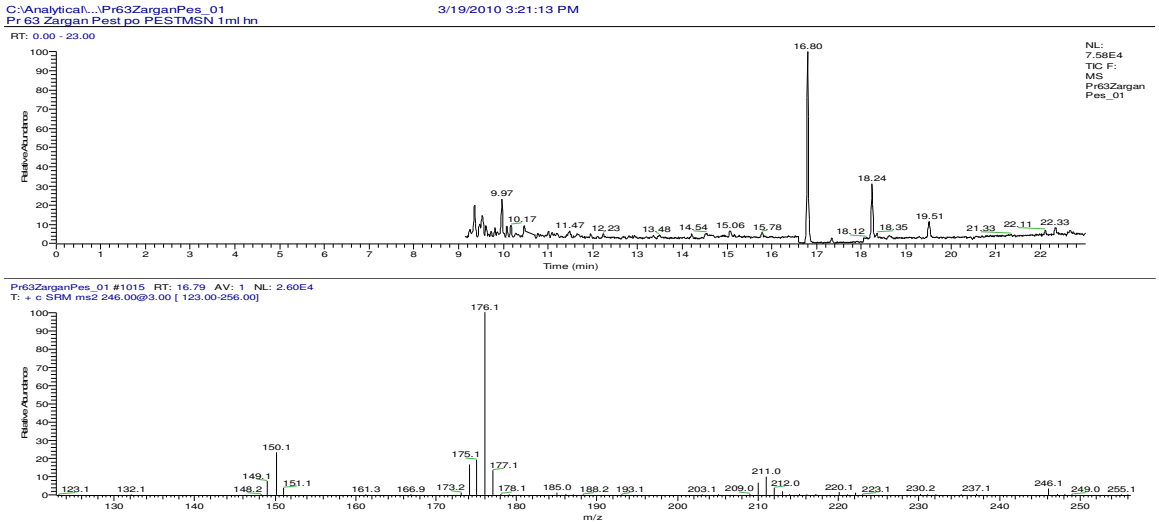

Fig. 4. GC-MS chromatogram and MS spectra of DDTs in fish tissues of garfish

Table 1. Lipid content (%) and concentrations of DDT and its metabolites (ng/g ww and ng/g lipids)

Fish species	Year	Lipids, %	p,p'-DDE, ng/g ww	p,p'-DDD, ng/g ww	p,p'-DDT, ng/g ww	∑DDT, ng/g ww	∑DDT, ng/g lipids
Garfish (*Belone belone*)	2007	10.47	69.09	36.38	12.57	118.04	1127.9
	2008	8.76	44.14	30.06	6.68	80.89	923.0
	2009	7.15	56.18	30.10	3.30	89.91	1254.1
Red mullet (*Mullus barbatus*)	2009	17.49	74.90	47.50	8.19	130.58	746.7
	2010	14.29	53.28	22.20	3.13	78.61	550.2

3.2. PCBs levels

The fifteen congeners of PCB were analyzed including the set of 7 indicators PCBs (IUPAC No 28, 52, 101, 118, 138, 153, 180). **Figure 5** shows the GC-MS chromatogram of PCBs detected in red mullet sample.

The concentration of individual PCB congeners measured in fish species are shown in Table 2. ∑PCBs were found at concentrations ranging between 22.82 ng/g ww in red mullet and 65.62 ng/g ww in garfish (calculated as the sum of all the investigated congeners). The sum of the seven indicator congeners constituted more than 82% of the ∑PCBs.

The PCB pattern found in fish showed a predominance of PCB 153 followed by PCB 138, 180 and 118 for indicator PCBs. The predominance of hexachlorinated PCBs in marine fish species, especially PCB 153 and PCB 138, has been reported by several authors for different coastal areas in the Mediterranean [16], in the Adriatic Sea [12, 17] and in Marmara Sea [10].

Table 2. PCBs (ng/g ww) concentration levels determined in fish species collected from the Black Sea

PCB congeners	Garfish (*Belone belone*)			Red mullet (*Mullus barbatus*)	
	2007	2008	2009	2009	2010
28*+31	4.58	3.14	3.82	3.47	3.20
52*	4.22	2.90	3.44	1.64	nd
101*	4.45	3.50	4.12	nd	nd
77	nd	nd	nd	nd	nd
118*	5.72	5.47	5.42	3.45	nd
153*	9.85	9.27	14.96	15.34	9.11
105	4.15	3.56	3.98	1.47	nd
138*	7.60	8.56	15.11	7.52	4.98
126	nd	nd	nd	4.98	nd
128	nd	nd	3.30	1.35	nd
156	nd	nd	nd	nd	nd
180*	3.99	3.64	7.35	6.46	5.53
169	nd	nd	nd	nd	nd
170	nd	nd	4.13	5.02	nd
∑PCBs, ng/g ww	44.56	40.04	65.62	45.72	22.82
∑Indicator *PCBs, ng/g ww	40.41	36.48	54.22	37.89	22.82

*Indicator PCB, nd – not detection

Although there were differences among the marine species, PCB patterns were always dominated by a large contribution from the hepta-, hexa-, and pentachlorinated PCBs 180, 153, 138, and 118. PCB 153 was the dominant congener in investigated species (**Table 2**).

The 153, 138, 180 and 118 congeners turned out to be the most abundant also due to their high lipophilicity, stability and persistence that facilitate the adsorption to sediments and the accumulation in the aquatic ecosystem [16].

The experimental results indicate that ∑PCB contamination of garfish (mean 600.4 ng/g lipids) and in red mullet (mean 210.6 ng/g lipids) from the Bulgarian Black Sea coast is comparable to the results from the Marmara Sea, where average concentrations (sum of seven PCB congeners) in fish were found in the range from 209.36 to 508.71 ng/g fat [10]. The levels of indicators PCBs found in present study are lower than the results of fish species from Gulf of Naples, the Mediterranean Sea (1005.3 - 17 259.0 ng/g lipid weight) reported by Naso, S. et al. [16]. The low levels of PCBs observed in fish tissues correspond with the fact that no industrial PCBs production in Bulgaria.

Actually in Bulgaria, a maximum residue limit (MRL) for PCBs in fish is not yet established. However, the European Union has recommended a tolerance limit of 200 ng/g fat weight, calculated as the sum of the seven target PCBs in terrestrial edible animals but not in fish [18]. Our values for indicators PCBs in garfish exceeded the MRL of 200 ng/g fat weight, but we cannot take into account this limit for the evaluation of the contamination in our fish samples.

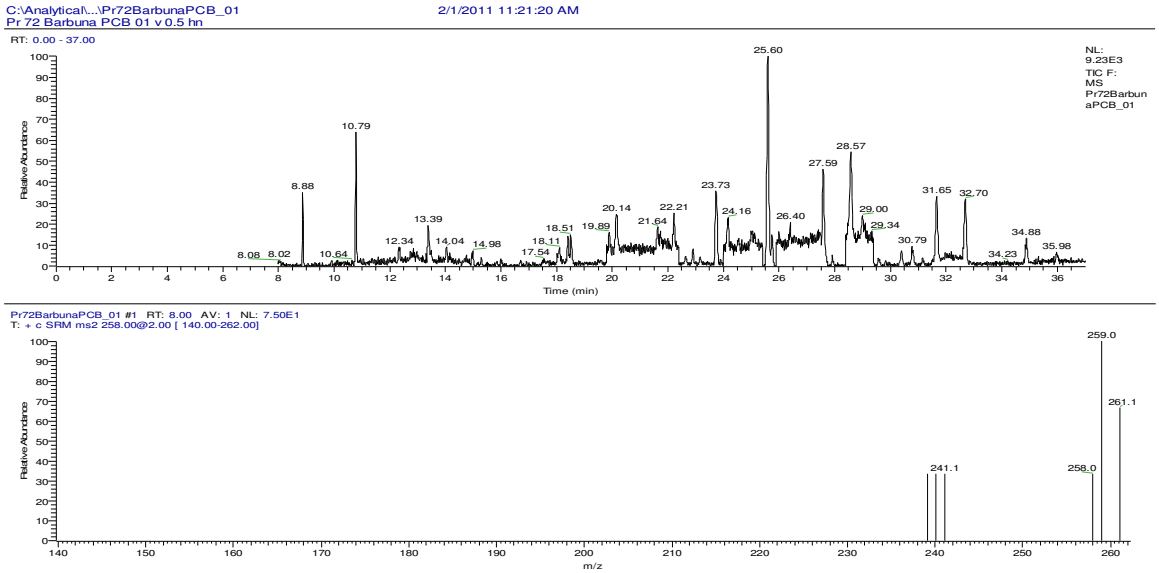

Fig. 5. GC-MS chromatogram and MS spectra of PCBs in fish tissues of red mullet

4. Conclusions

Concentrations of DDTs and PCBs were measured in two marine species: garfish (*Belone belone*) and red mullet (*Mullus barbatus*), collected from Black Sea, Bulgaria. DDTs are prevalent contaminants in investigated fish species - garfish and red mullet.

DDTs were found in detectable levels in muscle tissues of garfish (mean 96.28 ng/g ww) and of red mullet (mean 104.60 ng/g ww). The main metabolite p,p'-DDE was the most frequently detected compound and was present in higher concentrations than the other DDTs. In all investigated samples the residues of DDT were found in the following order DDE >DDD >DDT, showing the lack of significant fresh input of p,p'-DDT from Bulgarian coast during 2007-2010 and reflecting the fact that countries around the Black sea banned the use of DDT in the early 1970s.

PCBs were found at concentrations 50.07 ng/g ww in garfish and 34.27 ng/g ww in red mullet. The most abundant PCB congeners in all fish species were the indicator PCBs constituting more than 82% of the total amount of PCBs.

The levels of DDTs and PCBs in garfish and red mullet from Bulgarian Black Sea coast were comparable to those found in fish from the neighboring seas – the Marmara Sea, the Aegean Sea and the Mediterranean Sea.

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