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Organochlorine contaminants (PCBs, DDTs, HCB & HBDE) in fish from the Lake Varna and Lake Beloslav, Bulgaria

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Abstract. Concentrations of organochlorine compounds such as polychlorinated biphenyls (PCBs), DDT and its metabolites, hexachlorobenzene (HCB) and hexachlorobutadiene (HBDE) were determined in three fish species: goby *(Neogobius melanostomus),* golden grey mullet *(Mugil auratus)* and silverside *(Atherina boyeri).* Samples were collected from the Lake Varna and the Lake Beloslav in 2014. The edible fish tissues were analyzed in order to investigate the presence of pollutants in species from the lakes near Varna City, Bulgaria and compared the results to the levels in other aquatic ecosystems. The fifteen congeners of PCBs, HCB, HCBD, DDT and its two main metabolites DDE and DDD were determined by capillary gas chromatography system with mass spectrometry detection. The OCPs levels in the wild fish were found in the order DDTs > PCBs. The other contaminants HCB and HCBD were not detected or were below the analytical detection limit. Among the pesticides, essentially only the metabolites *p, p*'- DDE and *p, p*'- DDD were found.

The concentrations of DDTs were determined from 2.66 to 17.97 ng/g wet weight and PCBs concentrations were found from 0.43 to 8.05 ng/g ww (in goby and golden grey mullet, respectively). The sum of the six Indicator PCBs did not exceed the European maximum limit 75 ng/g wet weight. The concentrations of DDTs and PCBs were found lower compared to those in similar fish species from other aquatic ecosystems.

Keywords: PCBs, DDTs, HCB, HCBD, fish, Bulgaria.

1. Introduction

Organochlorine compounds (OC) such as polychlorinated biphenyls (PCBs), 1, 1, 1-trichloro - 2, 2 - bis(4-chlorophenyl)ethane (DDTs) and its metabolites, hexachlorobenzene (HCB) and hexachlorobutadien (HBDE) are widely distributed environmental pollutants. Due to their stable structure and lipophilic character, they tend to concentrate and magnify in the food chain particularly associated with fat [1].

Various studies have proved that organochlorine compounds exhibit several types of toxicity in humans and marine organisms, especially the neurotoxicity [2, 3]. For these reasons, most countries have restricted or banned the use of PCBs and organochlorine pesticides since 1970s. Polychlorinated biphenyls have been widely used by a large variety of industries over the past 50 years. DDT is a chlorinated pesticide widely used in the past to control the spread of insects and other agricultural pests. In the environment DDT metabolized slowly and the metabolite DDE is particularly persistent compound.

Hexachlorobenzene (HCB) is a hydrophobic and highly persistent chemical [4]. In 2002 it remained a consistent and widespread atmospheric contaminant across Europe [5]. HCBD was mainly used as a solvent in the production of rubber and other polymers. Other uses were in agriculture as a seed dressing, in hydraulic fluids and a number of other industrial processes [6]. HCB and HCBD are also named as priority substances under the EU Water Framework Directive [7].

Fish 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 [2].

Lake Varna is the largest by volume and deepest lake along the Bulgarian Black Sea Coast, divided from the sea by a 2 km-wide strip of sand and having an area of 17 km², maximal depth 19 m, and

a volume of 166 million m³. A number of rivers pour into the lake, including Devnya and Provadiyska that empty near the western shores of Lake Beloslav, which is connected to Lake Varna.

To our knowledge, no data on levels of organohalogenated pollutants in fish in this aquatic ecosystem are present in the literature.

The aim of the present study was to investigate the presence of organohalogenated contaminants PCBs, DDTs, HCB and HCBD in several fish species from Varna Lake and Beloslav Lake, lakes with great economical importance for the region.

2. Experimental

2.1. *Sampling and sample preparation*

Three wild fish species: goby *(Neogobius melanostomus)*, golden grey mullet *(Mugil auratus)* and silverside *(Atherina boyeri)* were caught by local professional fishermen by net along the Lake Varna and the Lake Beloslav between September and November 2014 (**Fig.** 1). Samples were immediately transferred to the laboratory in foam boxes filled with ice and were stored in a freezer (-18°C) until analysis. The edible tissue of 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.

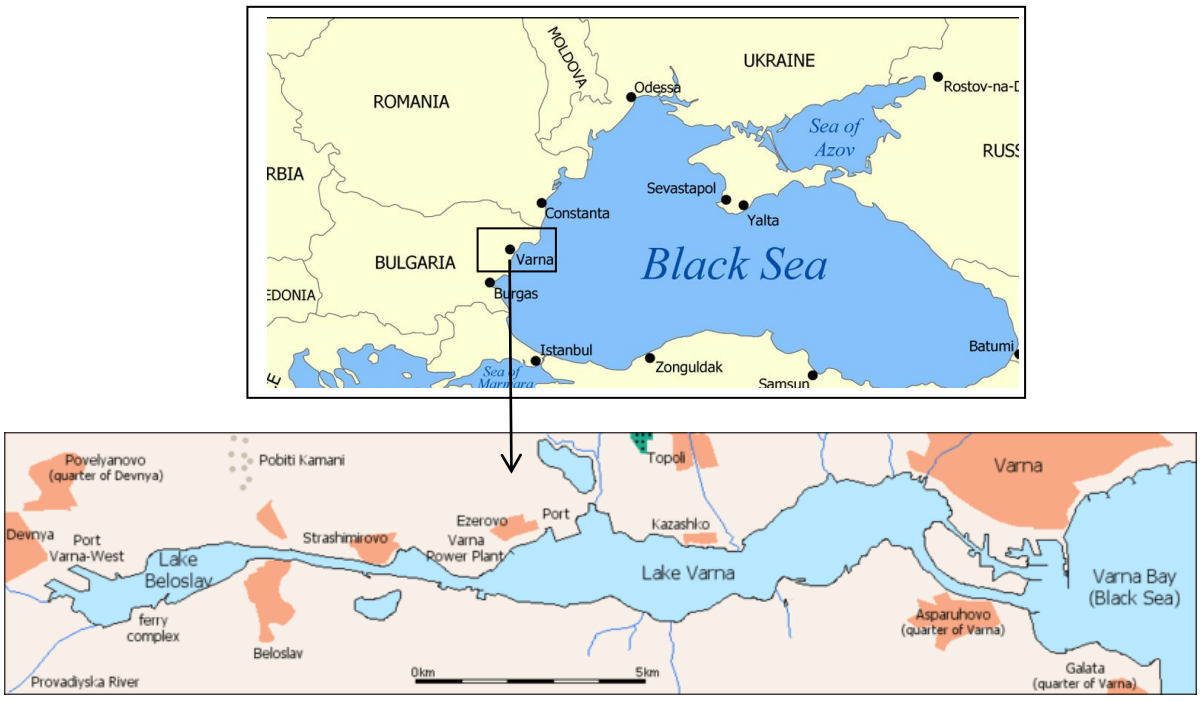

Fig. 1. The Lake Varna and Lake Beloslav map.

2.2. *Chemical analysis*

Samples were prepared according to a previously described method [8]. Briefly, the edible tissues of fish were homogenized and twenty grams 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 Soxhlet Extractor. 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, PCBs, HCB and HCBD 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 mass spectrometry 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, 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 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% phenylmethylsiloxane 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.

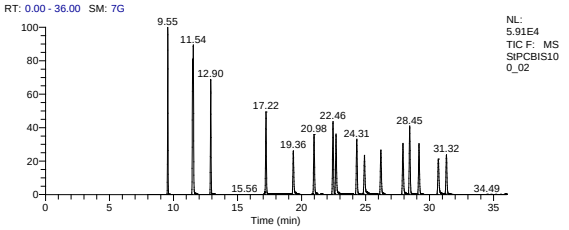

Fig.2. GC-MS chromatogram of PCB standard solution, 100 ng/ml.

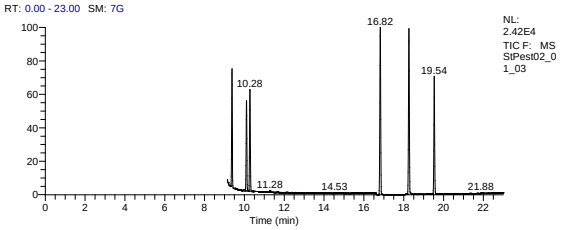

Fig. 3. GC-MS chromatogram of DDTs standard solution, 100 ng/ml.

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, HCB, HCBD and PCB congeners: IUPAC № 28, 31, 52, 77, 101, 105, 118, 126, 128, 138, 153, 156, 169, 170, 180).

Each sample was analyzed three times and was taken an average of the results obtained. The limits of quantification (LOQ) varied for individual OCs from 0.2 to 0.5 ng/g ww.

2.3. Quality control

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. Procedural blanks and a spiked sample with standards were analyzed between each 5 samples to monitor possible laboratory contamination. Blanks did not contain traces of contaminants.

3. Results and Discussions

3.1. PCBs levels

The concentration of individual PCB congeners measured in fish species are shown in **Table 1**. The set of 6PCBs (IUPAC No 28, 52, 101, 138, 153, 180) are defined by WHO as important for evaluating the risk to human health and are called indicator PCB (noted with * in **Table 1**).

The ΣPCBs (calculated as the sum of all the investigated congeners) varied in the range between 0.42 ng/g ww in goby (Lake Varna) and 8.05 ng/g ww in golden grey mullet.

The PCB pattern found in fish showed a predominance of PCB 153 followed by PCB 138 for indicator PCBs. The predominance of hexachlorinated PCBs in fish species, especially PCB 153 and PCB 138, has been reported by several authors for different coastal areas in the Mediterranean [9], in the Adriatic Sea [10, 11] and in Marmara Sea [12].

The higher levels of PCBs in golden grey mullet compared to other fish species may be due to its higher lipid content.

The contamination degree with PCBs of fish samples from the Lake Varna was found lower than PCB levels found in other countries. PCB concentrations found by Erdogrul *et al*., 2005 in carp and wels muscle from the Kahramanmaras, Turkey ranged between nd - 4.8 and 0.39 - 42.3 ng/g ww, respectively [13]. Barbel and chub from rivers of the North of Luxembourg contained Σ PCBs ranging from 29.6 to 158.2 ng/g ww and from 21.7 to 195.3

ng/g ww, respectively - reported by Boscher, A. *et al*., 2010 [14].

Table 1. PCBs (ng/g ww) concentration levels determined in fish species collected from the Black Sea.

	Lake Varna		**Lake Beloslav**		
PCB congeners	**Goby***(Neogobius melanostomus)*	**Golden grey mullet***(Mugil auratus)*	**Silverside** *(Atherina boyeri)*	**Golden grey mullet***(Mugil auratus)*	**Goby***(Neogobius melanostomus)*
28*+31	0.15	1.05	0.83	0.64	0.41
52*	0.10	1.31	0.94	0.50	0.26
101*	nd	1.46	0.74	0.54	0.21
77	nd	nd	nd	nd	nd
118	nd	nd	nd	nd	0.28
153*	0.17	2.63	1.48	0.87	0.41
105	nd	1.60	nd	nd	nd
138*	nd	nd	nd	nd	nd
126	nd	nd	nd	nd	nd
128	nd	nd	nd	nd	nd
156	nd	nd	nd	nd	nd
180*	nd	nd	nd	nd	nd
169	nd	nd	nd	nd	nd
170	nd	nd	nd	nd	nd
∑PCBs, ng/g ww	0.42	8.05	3.99	2.55	1.58

*Indicator PCB, nd – not detection

The European Union has recommended a maximum level of 75 ng/g wet weight, calculated as the sum of the six I-PCBs in muscle meat of fish [15]. Our results for I-PCBs in all fish species (max values 8.05 ng/g ww in golden grey mullet) did not exceed this limit. The toxic dl-PCBs in all samples showed concentrations below LOD.

3.2. DDTs, HCB, HCBD levels

DDTs were the prevalent organochlorine contaminants found in the investigated fish species from Lake Varna and Lake Beloslav. Lipid contents and concentrations of *p, p*'- DDE, *p, p*'- DDD, *p, p*'- DDT, HCB, HCBD found in the samples, are present in **Table 2**. The lipid percentage ranged from 0.4% in goby to 8.5% in golden grey mullet. The lipids in fish tissue are influenced by several factors, such as sex, age, species, nourishment and spawning status [16].

The main metabolite *p, p′* - DDE was the abundant organochlorine contaminant in all samples. The metabolite *p, p*'- DDE constituted more than 75% of the ΣDDTs for each species, followed by *p, p*'- DDD (20–25%). The concentrations of *p, p*'- DDE varied in the range from 1.57 to 13.45 ng/g wet weight (ww) in goby and golden grey mullet samples respectively. DDT did not found at detectable levels in investigate fish species.

Many authors assess the chronology of DDT inputs by using the DDE/ΣDDTs ratio [17]. In the present study, this value was calculated between 0.6 and 0.8 in goby and silverside, respectively. This suggests that these pesticides have not been recently used in agriculture after their ban. In all tested samples, the residues were found in the order of DDE > DDD > DDT and this is in agreement with the results of other studies [18, 19].

Table 2. Lipid content (%) and concentrations of DDT and its metabolites, HCB and HCBD (ng/g ww).

	Lake Varna		**Lake Beloslav**		
Fish species	**Goby***(Neogobius melanostomus)*	**Golden grey mullet***(Mugil auratus)*	**Silverside** *(Atherina boyeri)*	**Golden grey mullet***(Mugil auratus)*	**Goby***(Neogobius melanostomus)*
Lipids, %	0.4	8.5	6.1	4.2	0.5
HCB	< LOD	< LOD	< LOD	< LOD	< LOD
HCBD	< LOD	< LOD	< LOD	< LOD	< LOD
p,p' - DDE	2.04	13.45	5.44	3.52	1.57
p,p' - DDD	0.62	4.52	1.36	1.14	1.15
p,p' - DDT	< LOD	< LOD	< LOD	< LOD	< LOD
Σ DDTs	**2.66**	**17.97**	**6.79**	**4.65**	**2.72**

The concentrations of DDTs in investigated samples goby, golden grey mullet and silverside were found lower than those reported by Erdogrul, Ö., 2005 for carp from the Kahramanmaras, Turkey (median 77.4 ng/g ww) [13], and those reported by Covaci, A. *et al*., 2006 from Danube Delta (2 847 ng/g lipid weight) [18]. Perch sampled from coastal waters of Latvia [20] was found to contain 180-1100 ng/g lw DDTs and pikeperch from Danube Delta had concentration of DDTs 4 829 ng/g lw [18].

Regarding other organochlorine compounds determined concentrations of HCB and HCBD were all below detectable levels and did not exceed the European EQS of 10μg/kg and 55 μg/kg, respectively. In a recent study of wild fish from four English rivers HCB was a maximum of 6 μg/kg in some eels [6].

In a recent survey of eels in Scotland [21] HCBD was only detected in one of150 samples at detection limits of either 1 or 3 μg/kg and the authors of a French study also failed to detect any HCBD in fish [22].

4. Conclusions

The analysis of fish tissues of goby, golden grey mullet and silverside showed a mean total load of DDT pollutants 8.78 ng/g ww, respectively. DDTs were the predominant organohalogenated contaminants in all species, with the *p, p*'- DDE contributing to more than 75% to the total DDTs. In all samples DDT was present mainly in the form of its metabolites *p, p*'- DDE and *p, p*'- DDD, suggesting previous contamination.

The mean residue concentrations of PCBs in fish tissue quantified in our study were found 3.32 ng/g ww. The residues of PCBs varied in the range between 0.42 ng/g ww in goby (Lake Varna) and 8.05 ng/g ww in golden grey mullet. The very low levels of PCBs observed in fish tissues correspond with the fact that no industrial PCBs production in Bulgaria.

In general, concentrations of DDTs, HCB, HCBD and PCBs in fish species: goby, golden grey mullet and silverside from the Lake Varna and Lake Beloslav were found lower or similar to levels measured in the fish species from European rivers or other lakes.

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