PERSISTENT ORGANIC POLLUTANTS - PCBs AND DDTs IN FISH FROM DANUBE RIVER AND FROM BLACK SEA, BULGARIA

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Persistent organochlorine pollutants (POPs) like polychlorinated biphenyls (PCBs) and DDT residues (DDTs) can still be a problem for the aquatic environment and the human health. PCBs and DDTs were determined in three freshwater fish species: common carp *(Cyprinus carpio),* catfish *(Silurus glanis),* pike-perch *(Sander lucioperca)* and two marine fish: shad *(Alosa pontica pontica)* and grey mullet *(Mugil cephalus).* The freshwater fish samples were collected from the Danube River and from Black Sea, Bulgaria in 2010. The POPs were analyzed in order to investigate the presence of PCBs and DDTs in fish species from Danube River and compared the results to the levels in marine fish species from Black Sea. The fifteen congeners of PCBs, p,p'-DDT and its two main metabolites p,p'-DDE and p,p'-DDD were determined by capillary gas chromatography system with mass spectrometry detection. DDTs were the predominant contaminants in investigated species, with the p,p'- DDE contributing to more than 67% to the total DDTs. In freshwater fish concentrations of DDTs were found from 19.2 to 30.3 ng/g ww and PCBs concentrations - from 6.2 to 12.6 ng/g ww. The highest levels of PCBs and DDTs were determined in shad. The levels of DDTs and PCBs were determined lower than those found in similar fish species from other aquatic ecosystems.

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Introduction

Polychlorinated biphenyls (PCB) and organochlorine pesticides, such as dichlorodiphenyltrichloroethane (DDT) and its metabolites DDE and DDD, are compounds classified as persistent organic pollutants (POPs), capable of remaining in the environment and able to be transported, to accumulate in animal tissues over long periods of time (UNEP 2001). When released into the environment, POPs may be transported by air or water to areas that are often rather distant from the place of origin (Gouina et al., 2005). 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 (Fisk et al., 2001). Data on the presence and distribution of organohalogenated contaminants in fish species are therefore important not only from ecological, but also human health perspective (Binelli & Provini, 2004; Smith & Gangoli, 2002). In biological systems, several of these chemicals are potentially carcinogenic and may cause alternations in endocrine, reproductive and nervous systems (Langer et

al., 2003). The river Danube, the second longest river in Europe, flows through several countries from where it receives discharges of agricultural, industrial, and urban effluents (Woitke et al., 2003). The Black Sea receives freshwater inputs from some of the largest rivers in Europe: the Danube, Dniester and Dnieper. There is very little information available on the highly persistent PCBs and DDTs in fish from Danube River (Covaci et al., 2006) and from the Black Sea (Tanabe et al., 1997; Stoichev et al., 2007).

The aim of the study was to determinate the presence of PCBs and DDTs in fish from the Danube River and from the Black Sea and a comparison between freshwater and marine fish species.

Materials and methods

The fish species were selected according to their importance to human consumption in Bulgaria. Three freshwater fish species: common carp *(Cyprinus carpio),* catfish *(Silurus glanis)* and pike-perch *(Sander lucioperca)* were obtained from fishermen along the Danube River, Silistra, Bulgaria between September and November 2010. Marine fish - shad *(Alosa pontica pontica)* and grey mullet *(Mugil cephalus)* were collected from Bulgarian Black Sea coast (near cape Kaliakra) in 2010 (Figure 1).

Figure 1: Sampling areas



Source: Authors using Gagnon (2011)

Common carp is omnivorous - can eat water plants, but prefer to scavenge the bottom for insects, crustaceans, crayfish and benthic worms. Catfish prefer to feed on crayfish, clams, worms, seed and small fish. Pike-perch is an excellent predatory fish in the early years. Grey mullet is an omnivorous scavenger feeding mainly on organic matter as well as plankton, benthic organisms and algae and therefore may be a good tool for monitoring. Shad migrates to middle reaches of large rivers (like Danube River) for spawning. It feeds on a wide variety of zooplankton and small fish. The samples were transferred immediately to the laboratory in foam boxes filled with ice and were stored in a

freezer (-20ºC) until analysis. Each sample of selected fish species is composed of edible tissues of several individuals.

The analytical method for determination of PCB and DDTs was based on US EPA 1668a. Briefly, the homogenized tissues of fish (20 g) were taken for extraction. Each sample was spiked with internal standards PCB 30 and PCB 204. The PCB and DDTs were extracted with hexane / dichloromethane (3/1, v/v) in Soxhlet apparatus. The solvent was carefully evaporated and the lipid content was determined gravimetrically of an aliquot of the extract (1/5th). The extract was cleaned-up on a glass column packed with neutral and acid silica. After elution with 80 ml n-hexane followed by 50 ml n-hexan/dichloromethane (80:20) the cleaned extract was concentrated to approximately 0.5 ml. 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. Splitless injections of 1 µl were performed using a TR-5MS capillary column (30 m x 0.25 mm x 0.25 µm). Helium was applied as carrier gas at a flow of 1 ml/min. Pure reference standard solutions (PCB Mix 20 - Dr. Ehrenstorfer Laboratory and EPA 625/CLP Pesticides Mix 2000 µg/ml - Supelco), were used for instrument calibration, recovery determination and quantification of compounds. Measured compounds were: p,p'-DDT and its metabolites: 1,1,- dichloro- 2,2-bis (4-chlorophenyl) ethane (p,p'-DDD) and 1,1,- dichloro- 2,2-bis (4-chlorophenyl) ethylene (p,p'-DDE) and 15 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 PCBs and DDTs from 0.2 to 0.5 ng/g wet weight (ww).

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. Recovery of DDTs from certified reference material BCR – 598 were: DDE – 97.5%, DDD – 103.4% and DDT – 85.4%. PCBs recovery varied in the range 85 -109% for individual congeners. The statistical analysis of the data was based on the comparison of average values by a t-test and a significance level of $p<0.05$ was used. All statistical tests were performed using SPSS 16 software.

Results and discussion

The set of 6 indicator PCBs IUPAC No 28, 52, 101, 138, 153, 180 (Indicator PCBs) was recommended by the European Union for assessing the pollution by PCBs (Commission of the EC, 1999). The concentrations of individual PCBs congeners measured in fish species are shown in Table 1.

Sum PCBs were found in all the marine species at concentrations ranging between 5.91 ng/g ww and 47.81 ng/g ww. Although there were differences among the fish species, PCB pattern found in fish showed a predominance of the hepta-, hexa-, and pentachlorinated PCBs 180, 153, 138, and 118. PCB 153 was the dominant congener in all investigated species. The 153, 138, 180 and 118 congeners turned out to be the most abundant also due to their high lipophilicity, stability and persistence in the aquatic ecosystem. The predominance of PCB 153 and 138 in marine fish species has been reported by several authors for different coastal areas in the Mediterranean Sea (Naso et al., 2005), in the Adriatic Sea (Bayarri et al., 2001; Perugini et al., 2004) and in Marmara Sea (Coelhan et al., 2006).

The sum of the six indicator PCBs constituted from 72% to 87% of the total PCBs. The lowest concentration of the indicator PCBs was found in common carp and grey mullet samples and the

highest concentration - in shad (34.6 ng/g ww). Shad contained significantly higher PCBs concentrations than in other fish species ($p<0.05$). The European Union has recommended a maximum level of 75 ng/g wet weight, calculated as the sum of six indicator PCBs in muscle meat of fish (European Commission, 2011). Our results for PCBs in all fish species did not exceed this limit.

Table 1: PCBs and DDTs concentrations (ng/g ww) determined in fish species

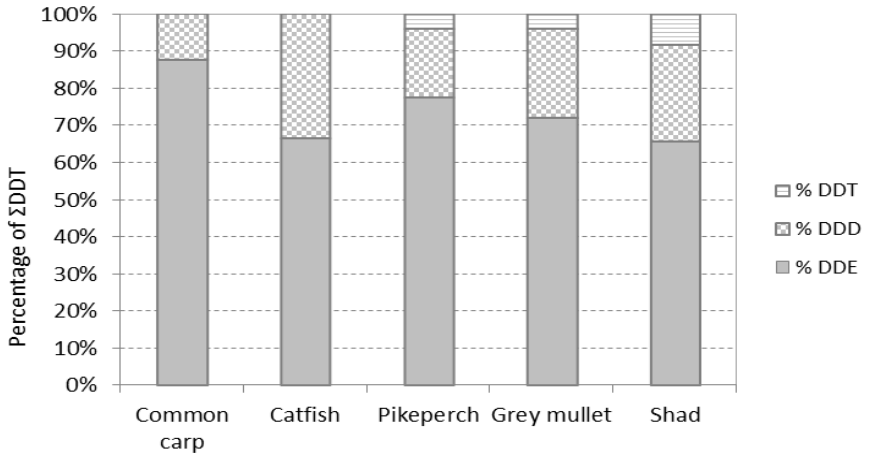
Fish species / Concentration, ng/g ww	Freshwater fish, Danube River			Marine fish, Black Sea	
	common carp	catfish	pikeperch	grey mullet	shad
Lipids, %	1.9	2.8	1.7	8.4	18.1
PCB28*+31	0.68	< 0.2	0.70	0.77	1.84
PCB52*	< 0.2	0.87	0.87	0.68	2.57
PCB101*	0.97	0.95	0.83	< 0.2	3.89
PCB77	nd	nd	nd	nd	nd
PCB118	< 0.2	1.06	1.12	< 0.2	7.05
PCB153*	1.51	4.00	2.70	1.34	12.63
PCB105	< 0.2	0.88	0.50	< 0.2	2.50
PCB138*	1.20	2.64	1.48	1.21	9.13
PCB126	nd	nd	nd	nd	nd
PCB128	< 0.2	< 0.2	< 0.2	nd	0.46
PCB156	< 0.2	< 0.2	< 0.2	nd	nd
PCB180*	0.89	1.30	1.07	1.16	4.48
PCB169	nd	nd	nd	nd	nd
PCB170	0.81	0.85	0.79	0.75	3.26
Sum PCBs	6.06	12.55	10.05	5.91	47.81
Sum Indicator PCBs	5.25	9.77	7.65	5.16	34.60
p,p' – DDE	16.80	20.13	16.07	41.75	141.19
p,p' – DDD	2.36	10.15	3.87	10.09	58.12
p,p' – DDT	nd	nd	0.85	2.34	17.69
Sum DDTs	19.16	30.28	20.78	54.18	217.00

Note: *Indicator PCB, nd – not detected
Source: Authors

The contamination degree with PCBs of the freshwater fish samples from the Danube River, Bulgaria was lower than PCB levels found in other regions. PCB concentrations found by Erdogrul et al. (2005) in carp and catfish muscle from the Kahramanmaras, Turkey ranged between 0.39 - 42.3 ng/g ww. Barbel and chub from rivers of the North of Luxembourg contained 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 et al (2010). PCB concentrations in European flounder from the Scheldt estuary (Ael et al., 2012) were higher (median 105 ng/g ww) than our results for freshwater fish. The levels of indicator PCBs found in grey mullet are lower than the results of fish species from Tunis Bay (Masmoudi et al., 2007).

The differences in feeding preferences and lipid content of common carp, catfish, pike-perch, grey mullet and shad justify the large range of observed DDTs levels (Table 1). Major DDTs metabolite p,p′-DDE was the abundant organochlorine contaminant in all samples (Table 1). The metabolite p,p'-DDE constituted more than 67% of the Sum DDTs for each species (Figure 2).

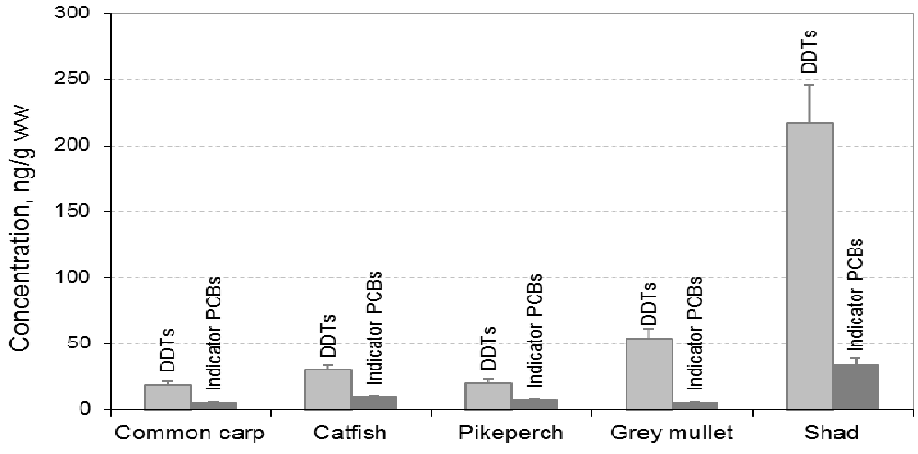
Figure 2: Distribution pattern of DDE, DDD and DDT in fish. Each compound refers to Sum DDTs.



Source: Authors

In our study, the p,p'-DDE / p,p'-DDT ratio in the investigated species correlated with exposure to DDT in the past (high p,p'-DDE concentrations and low p,p'-DDT content). p,p'-DDT were measured in all fish species in very low amounts (from nd to 8.3%). DDTs in common carp, catfish and pike-perch muscle samples were measured 19.2, 30.3 and 20.8 ng/g ww, respectively. DDTs concentrations in shad were found significantly higher than in other investigated fish species ($p<0.05$) – Figure 3.

Figure 3: Sum Indicator PCBs and Sum DDTs in fish species



Source: Authors

The concentrations of DDTs in carp samples from Danube River were found lower than those reported by Erdogrul et al (2005) for carp from the Kahramanmaras, Turkey (median 77.4 ng/g ww) and those reported by Covaci et al (2006) from Danube Delta (2847 ng/g lipid weight). Perch sampled from coastal waters of Latvia (Olsson et al., 2009) was found to contain 180 - 1100 ng/g lw DDTs and pikeperch from Danube Delta had concentration of DDTs 4829 ng/g lw (Covaci et al., 2006).

The levels of DDTs detected in fish species from the Black Sea, Bulgaria were comparable to those reported for fish from other aquatic ecosystems (Ferreira et al., 2004; Naso et al., 2005; Coelhan et al., 2006; Stoichev et al., 2007; Kalyoncu et al., 2009; Georgieva S., 2012).

Conclusion

PCBs and DDTs were determined in three freshwater fish species - common carp, catfish, pike-perch and two marine fish - shad and grey mullet. Our results showed the highest PCB concentrations in shad (47.8 ng/g ww). The concentrations of DDTs were found from 19.2 to 217.0 ng/g ww in common carp and shad, respectively. PCBs and DDTs residues in freshwater fish were lower than concentrations in marine fish (shad). Levels of PCBs and DDTs in common carp, catfish, pike-perch, grey mullet and shad were found lower or comparable than levels measured in the similar fish species from other aquatic ecosystems. Our results for sum of indicator PCBs in all fish species did not exceed the recommended maximum level of 75 ng/g ww.

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