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Assessment of Organic Pollutants Accumulation in Fish from Maritsa River Basin (Bulgaria)

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Abstract. Two selected fish species were sampled from 5 sites within the Maritsa River Basin in the period 2013-2014 with the aim to assess the effects of pesticide industry and intensive agriculture. Sampling sites were located in the middle of the Maritsa River Basin (near Plovdiv City, Bulgaria) and the watersheds of the Chepelarska and Stryama Rivers. Methods for analysis of certain priority substances and specific pollutants were applied in order to establish trends in the accumulation in fish, as required by the Directive 2008/60/EC and Directive 2008/105/EC. The reported data for organic pollutants are the first for the studied river basin. The selection of indicators (priority substances and specific pollutants) provides particular guidelines for planning future monitoring for assessment of river chemical and ecological status.

Key words: fish, Maritsa River, PCBs, OCPs, PAHs**.**

Introduction

Maritsa River is the largest on the Balkan Peninsula; it is the biggest river in terms of discharge volume and the third longest river in Bulgaria. It emerges from springs in the Rila mountain range and its basin is a cross-border watershed for Bulgaria, Greece and Turkey. The number of the tributaries of Maritsa River is about 100. The most significant among them are the rivers Chepinska, Topolnitsa, Luda Yana, Vacha, Chepelarska, Stryama, Sazliyka, Arda, Tundzha, Ergene.

Determining organic compounds in freshwater fish enables the identification of the contaminant origin in local regions. Polychlorinated biphenyls (PCBs) are toxic chemicals which precipitate in soil and water. They are mixtures of synthetic and organic chemical substances which share similar chemical structure. PCBs had been derived for their very high flash points and were widely deployed as fire-extinguishing agents, electrical insulators and plasticizers, mainly in electrical apparatuses. Most often they are introduced into the environment through defective equipment, illegal discharge, scavenge oil from electrical equipment, as well as hazardous waste. They are a family of 209 synthetic molecules composed of a biphenyl nucleus with chlorine at any, or all, of the 10 available sites (McFarland & Clarke, 1989). The ortho-, meta- and para positions are important in determining the chemical properties of PCB, such as high dielectric constants, non-flammability, hydrophobic quality and chemical stability. The usefulness of these properties led PCBs to be used in mixtures for a wide range of

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applications from the 1930s onwards (BREIVIK *et al*., 2002; BEYER & BIZIUK, 2009). However, after their toxicity became recognized, PCBs were progressively banned in most developed countries during the 1980s (LETZ, 1983). Due to their large-scale production, extensive use and environmental persistence, these compounds have accumulated in many ecosystems all over the world; in addition in aquatic environments they are trapped in sediments. PCB contamination continues to be a problem as these compounds can be transferred from the sediment to the lower trophic levels of an ecosystem through microbial and bottom-feeder uptake.

Organochlorine pesticides (OCPs) have been widely used in the past, but because of their high persistency in environment and accumulation in the food chain, they can still arouse topical concerns about human health

Polyaromatic hydrocarbons (PAHs) are a large group of organic substances with two or more benzene cores. They are characterized by low solubility in water, but high solubility in fats. The polycyclic aromatic hydrocarbons are produced mainly by incomplete combustion of coal and diesel fuel. There are several hundred PAHs. These compounds are absorbed by organisms mainly through the respiratory system, but may also be assimilated together with water and food. The most thorough study of the carcinogenic effect is that of inhaled benzo[a]pyrene (BaP), essential source of which is tobacco smoke.

The aim of the research was to identify the extent of accumulation of organic pollutants in "fish" matrix in selected river valleys and monitoring stations along the Maritsa River Basin.

Material and Methods

The monitoring of fish was accomplished in accordance with Guidance document No. 25 on chemical monitoring of sediment and biota to the common implementation strategy for the Directive 2008/105/EC (EC, 2010).

The research was conducted at 5 monitoring stations located in the Maritsa River Basin and two of its major tributaries – Chepelarska and Stryama River (Fig. 1).

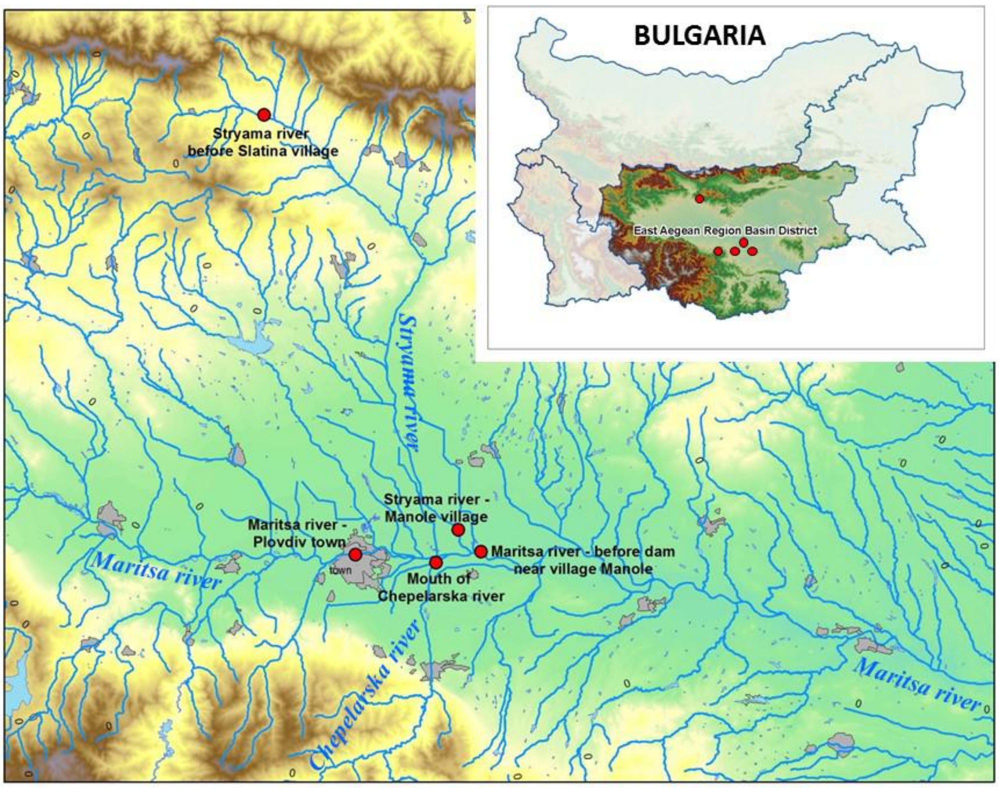

Fig 1. Map of the studied region.

The selection of the stations was made in compliance with the key criteria and the best practices of the strategy for sampling of fish specified in Guidance document No. 7 of the Water Frame Directive (EC, 2003). Thus, the stations were selected upon a preliminary analysis of the information about the anthropogenic pressure from point and diffuse sources of organic pollutants, the typological characteristics of the rivers, the composition of the bottom substrate (pebbles, gravel, sand, slime, clay), the hydrological characteristics (water runoff, water level) and accessibility for sampling. The geographic characteristics of the monitoring stations (watershed, river, location, geographic coordinates) were specified below.

Stryama river, the village of Slatina (N42.6902; E24.56917**)** was selected as a potential reference site for national type R5 Semi-mountain rivers in Ecoregion 7, slightly influenced by anthropogenic pressure and limited impact on the quality elements: extensive agriculture and forestry in the vicinity of the monitoring station; low pressure from domestic discharges in the catchment area of the site (the town of Klissura); limited automobile traffic; good chemical and good to high ecological status identified as a reference point for type R5 in the RBMP of East Aegean River Basin district. Substratum was consisted mainly by pebbles, gravel and sand, and organic slime where the current is sluggish. Moderate to significant water runoff, moderate to high current velocity, water level was 0.2-0.4 m.

Stryama river, the village of Manole (N42.1871; E24.91314) – type R13 Small and medium-sized lowland rivers in Ecoregion 7, located in a region with settlements in the catchment area of the of monitoring station; intensive agriculture; cumulative pressure from small settlements (<2000 p.e.); automobile traffic; good chemical and moderate ecological status; substrate – mostly sand and at places where the current is sluggish – organic slime. Moderate to significant water runoff, moderate to low current velocity, water level was 0.3-0.6 m.

Chepelarska River mouth, Kemera bridge area (N42.1457; E24.87722) – type R5 Semi-intensive rivers in Ecoregion 7, a site of significant anthropogenic pressure from industrial plants producing pesticides and metals ("Agria" Ltd., "KCM" Ltd.); discharge of untreated waste water from large settlements (Assenovgrad Town > 1000 p.e.); intensive agriculture; automobile traffic; poor chemical status (metals) and poor ecological status; substrate – mostly gravel and sand, and where the current is sluggish – organic slime. Moderate to significant water runoff, moderate to low current velocity, water level: 0.3-0.5 m.

Maritsa River, upstream of Plovdiv City (42.1608; 24.95124) – type R12 Large lowland rivers in Ecoregion 7, site of combined point and diffuse pressure from the upper and middle part of the catchment area of the Maritsa river, before discharge of waste water from Plovdiv City; discharge of treated waste water from large settlements in the catchment area on the monitoring station (Pazardzhik >1000 p.e., Stamboliyski town 2000-10000 p.e.); advanced industry (pulp and paper, food and flavor industry); intensive agriculture; intensive automobile traffic; good chemical and moderate to good ecological status; substrate – mostly sand and gravel with depositions of organic slime at places of sluggish current; significant water runoff, moderate to low current velocity, water level: 0.4-1.2 m.

Maritsa river, dam near the village of Manole (N42.1529; E24.74322) – type R12 Large lowland rivers in Ecoregion 7; a monitoring station of significant cumulative anthropogenic pressure from all the sources in the catchment area of the other monitoring stations (discharge of waste water from large settlements, industrial enterprises and intensive agriculture; discharge of treated waste water) from large settlements in the catchment area of the monitoring station (Plovdiv City – 300000 p.e., Assenovgrad Town >10000 p.e., a large number of settlements <2000 p.e.); combined pressure from developed industry (industrial zones of the city of Plovdiv, "Agria" Ltd., "KCM" Ltd.); intensive agriculture in the catchment areas of the surveyed rivers – the Maritsa, Stryama and Chepelarska rivers; limited automobile traffic; good chemical and moderate ecological status; substrate – massive deposition of organic slime in the area of the monitoring station before the dam near the village of Manole, sand; significant water runoff, low velocity of the current, water level: 1.0-3.0 m.

Selection of biota (fish) for analysis. Two species were selected for their importance to local human fish consumption: *Barbus cyclolepis* Heckel, 1837 - a freshwater fish in the family Cyprinidae. It is found in Bulgaria, Grece, and Turkey. Its natural habitats are river and intermittent rivers. It is not considered a threatened species by the IUCN (CRIVELLI, 2006). *Squalius orpheus* Kottelat & Economidis, 2006, which belongs to a genus of fish in the family Cyprinidae found in Europe and Asia, with "LC - least concern" status, same as the previous species, according to the IUCN Red List (FREYHOF & KOTTELAT, 2008).

Sampling. The sampling was accomplished three times in the period between June and October 2013-2014. The sampling was done in compliance with the EN 14011 European Standart "Water quality – Sampling of fish with electricity" (CEN, 2003).

Transportation and sieving. Samples (upon sieving) were transferred into preliminary cleaned brown glass containers for organic pollutants. They were transported in a cooled state to the laboratory (at 4°C) within a period not exceeding 6 hours. Cooling was accomplished by using cool boxes and freezer block inserts.

Storage. The sifted samples of fish were kept in containers frozen at -20°C.

Analytical methods. A 10 g sample was extracted with organic solvent hexane: acetone (1:1), by microwave decomposition under a programmed furnace temperature of 120°C detainment time 25. The extract obtained was concentrated, then subjected to a purification procedure with silica gel and again concentrated. The sample was analyzed using gas chromatography – mass spectrometry (GC-MS) equipment.

Gas chromatographic analysis of the OCP, DDTs and PCBs were carried out by GC (Agilent 7890B/5977A/MSD).

Quality control. The procedures for quality assurance include validation of the methods by routine in-house procedures and independent external procedures (participation in inter-laboratory tests). Use was made of certified reference materials: standard referent material 1947 Lake Michigan fish tissue - NIST, BCR 682-70G mussel tissue, standard referent material 2974a-Organics in Freeze-Dried Mussel Tissue - NIST, Pesticide mix 13 - Dr. Ehrenstorfer, PAH - Dr. Ehrenstorfer.

Selection of organic pollutants to be monitored. Analysis of synthetic compounds was made (pesticides, medicinal preparations, industrial pollutants), dissociated in the event of pollution from point and diffusive sources, atmospheric depositions, which are taken into account in the assessment of the chemical and ecological status of the water bodies (e.g. priority substances according to Directive 2008/105/EC (EC, 2008) and Directive 2013/39/EC (EC, 2013) and specific pollutants in compliance with the approved list and the EQS of the Bulgarian legislation.

Results and Discussion

In the fish *Barbus cyclolepis* the concentrations of all tree PCBs (PCB 52, PCB 153, PCB 180) were below the limit of quantification (<1 μg kg^{-1} w.w) at all monitoring stations. In addition, in the he fish *Squalius orpheus* the concentrations of all tree PCBs (PCB 138, PCB 153, PCB 180) were also below the limit of quantification (<1 μg kg^{-1} w.w) at all monitoring stations.

However, the results for 6 PCB_s in all fish showed concentrations exceeding the limit of quantification was - PCB 28, PCB 52, PCB 101, PCB 138 (Table 1) and (Fig. 2, 3).

Table 1. Minimum, average and maximum concentrations of 6 measured PCB in *Barbus cyclolepis* and *Squalius orpheus*, μg kg^{-1} w.w.

PCB	***Barbus cyclolepis*** (n=15)			***Squalius orpheus*** (n=15)		
	min	**max**	**average**	**min**	**max**	**average**
PCB 28	3,38	8,20	5,15	1,74	6,15	3,30
PCB 52		<1		5,82	6,00	5,93
PCB 101		2,37	2,08	1,49	1,94	1,71
PCB 138		2,82	2,58		<1	
PCB 153		<1			<1	
PCB 180		<1			<1	

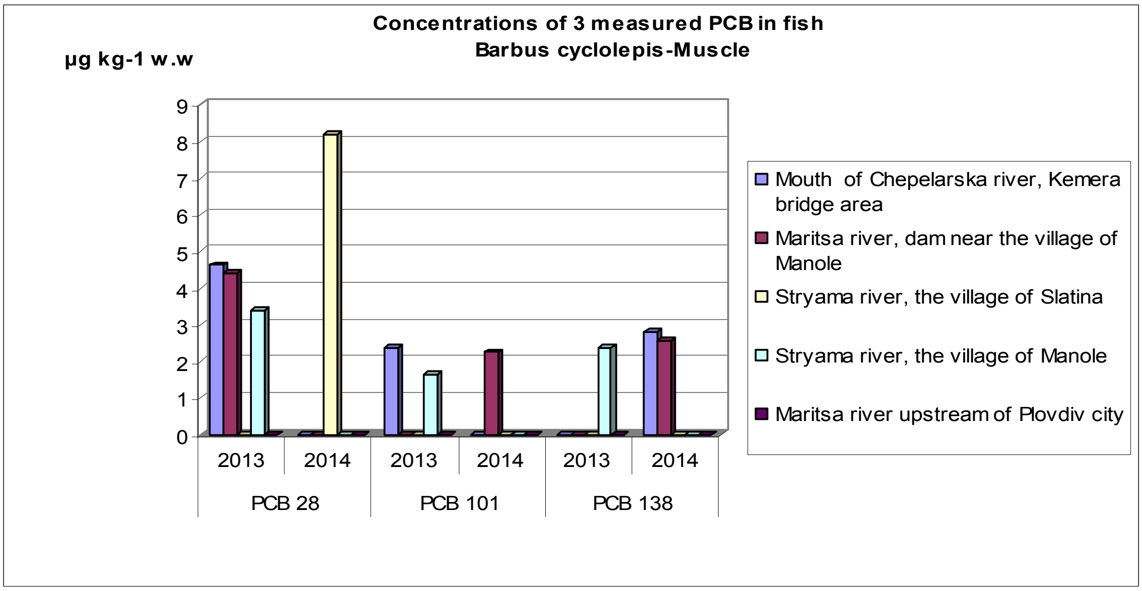

Fig. 2. Concentrations of measured PCB in fish *Barbus cyclolepis* - muscle.

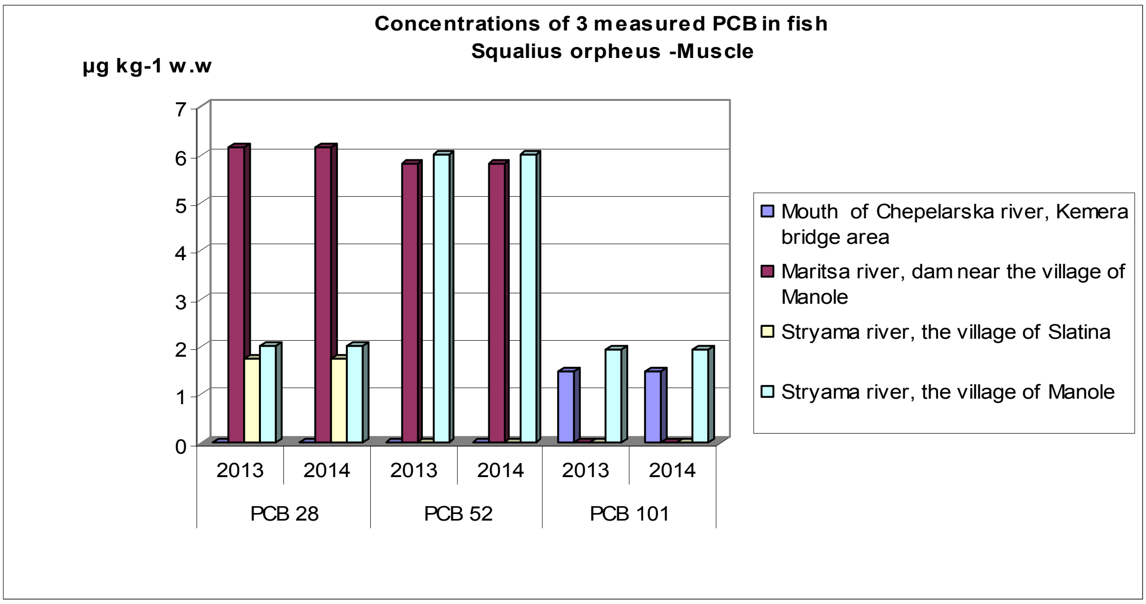

Fig. 3. Concentrations of measured PCB in fish *Squalius orpheus* - muscle.

Table 2. Detected minimum, maximum and average values of studied 24 OCPs in *Barbus cyclolepis* and *Squalius orpheus*, μg kg^{-1} w.w.

OCP	***Barbus cyclolepis*** (n=15)			***Squalius orpheus*** (n=15)		
	min	**max**	**average**	**min**	**max**	**average**
HCB		<1			<1	
α-HCH	2,41	2,63	6,86	2,63	6,86	3,95
β-HCH	9,96	3,32	41,31	3,32	41,31	19,99
γ-HCH	7,71	4,51	22,08	4,51	22,08	12,59
δ-HCH	3,40	3,48	5,97	3,48	5,97	3,05
ε-HCH		<1		3,00	3,39	3,00
Heptachlor		<2			<2	
Aldrin		<2			<2	
Isodrin	12,90	12,42	66,98	12,42	66,98	38,59
Dieldrin	4,42	4,36	73,00	4,36	73,00	24,14
Endrin		<2			<2	
Chlordan		<2			<2	
trans-chlordan		<2			<2	
Metoxychlor		<2			<2	
Mirex		<2			<2	
α-Endosulfane		<3			<3	
β-Endosulfane		<3			<3	
Heptachlor		<2			<2	
o,p-DDE		<1			<1	
p,p-DDE		<1			<1	
o,p-DDT		<1			<1	
p,p-DDT		<2			<2	
o,p-DDD	3,72	4,21	32,85	4,21	32,85	11,32
p,p-DDD		<3		5,71	18,45	5,71

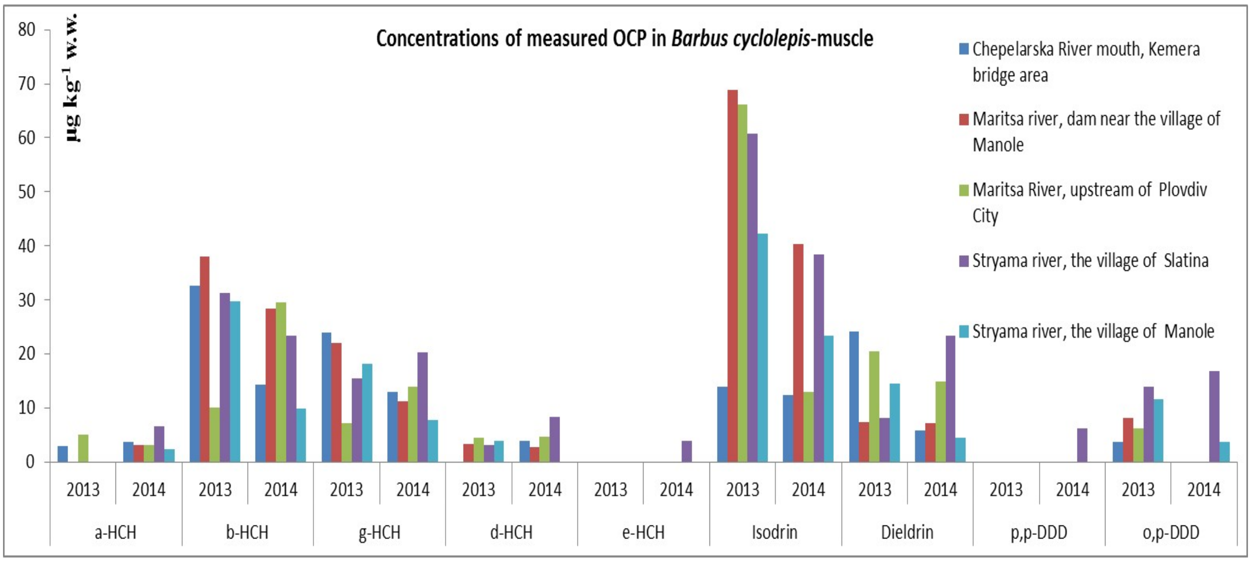

Fig. 4. Concentrations of measured OCP in *Barbus cyclolepis* - muscle.

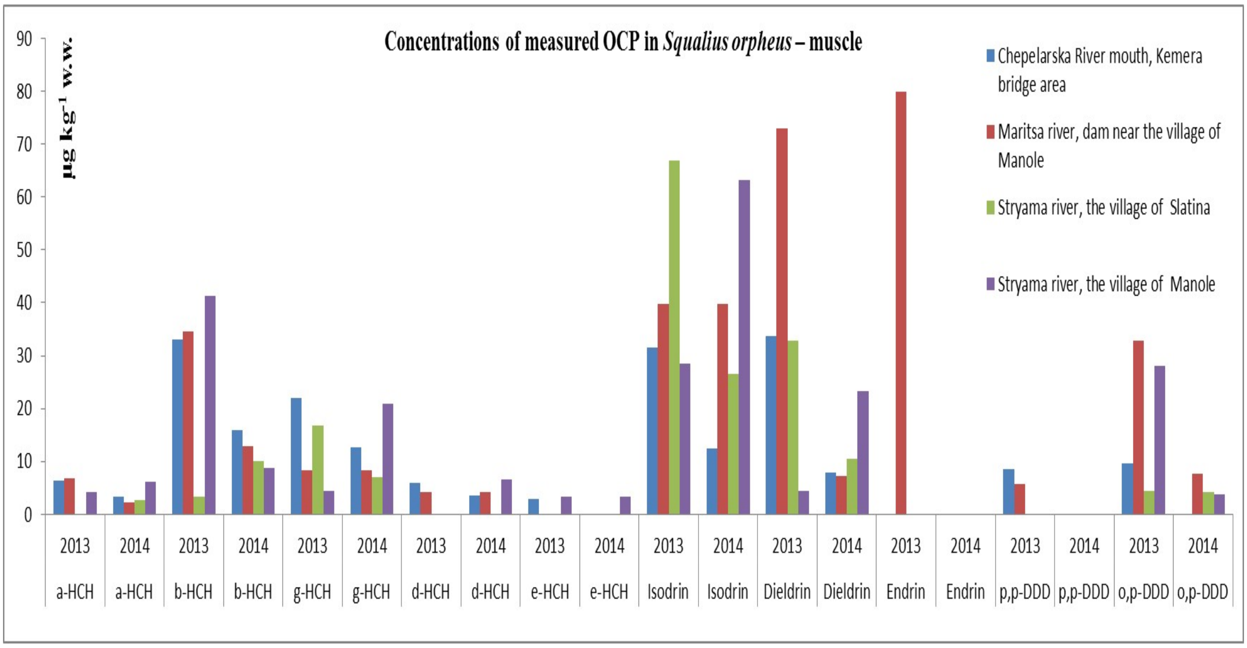

Fig. 5. Concentrations of measured OCP in *Squalius orpheus* - muscle.

Table 3. Minimum, average and maximum concentrations of 16 measured PAH in *Barbus cyclolepis* and *Squalius orpheus*, µg kg^{-1} w.w.

PAH	***Barbus cyclolepis*** (n=15)			***Squalius orpheus*** (n=15)		
	min	**max**	**average**	**min**	**max**	**average**
Naphthalene	23,88	30,64	27,33	25,48	34,65	30,11
Acenaphthene		<10			<10	
Acenaphthylene		<20			<20	
Fluoranthene		<20			<20	
Phenanthrene	10,18	34,75	16,11	3,73	32,54	17,92
Anthracene		<2			<2	
Flurene	3,11	4,56	3,78	3,59	4,10	3,85
Pyrene	8,91	20,60	18,64		<2	
Benzo(a)anthracene		<1			<1	
Chrysene		<1			<1	
Benzo(b)fluoranthene		<1			<1	
Benzo(k)fluoranthene		<1			<1	
Benzo(a)pyrene		<1			<1	
Indeno(1,2,3cd)pyrene		<3			<3	
Dibenz(a,h)anthracene		<3			<3	
Benzo(g,h,i)perylene		<3			<3	

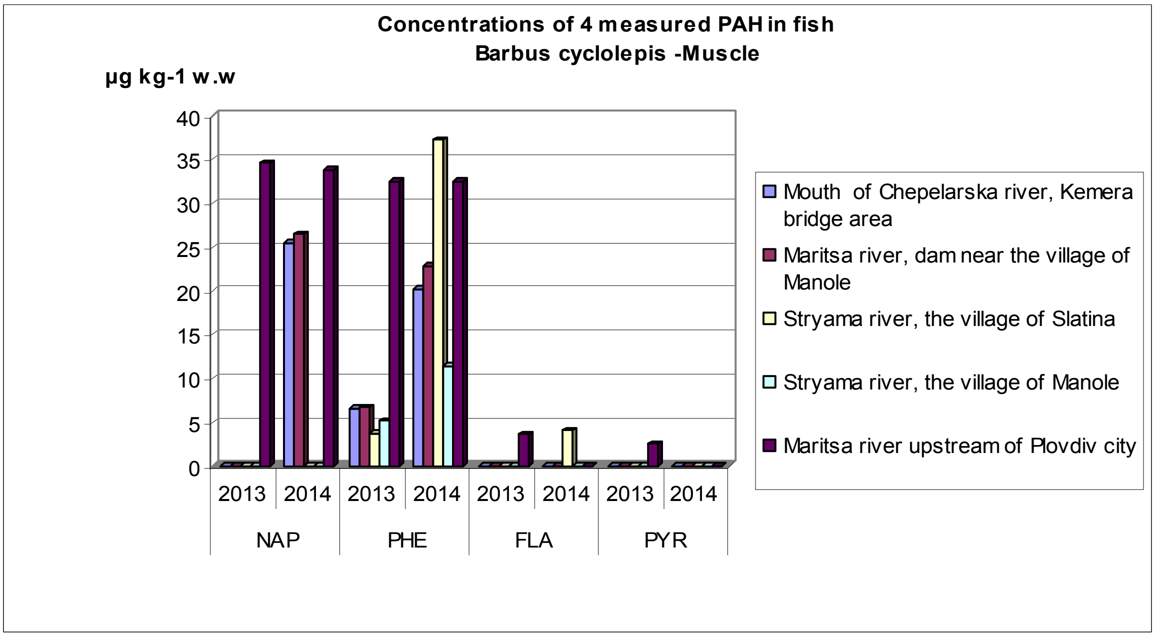

Fig. 6. Concentrations of measured PAH in *Barbus cyclolepis* - muscle.

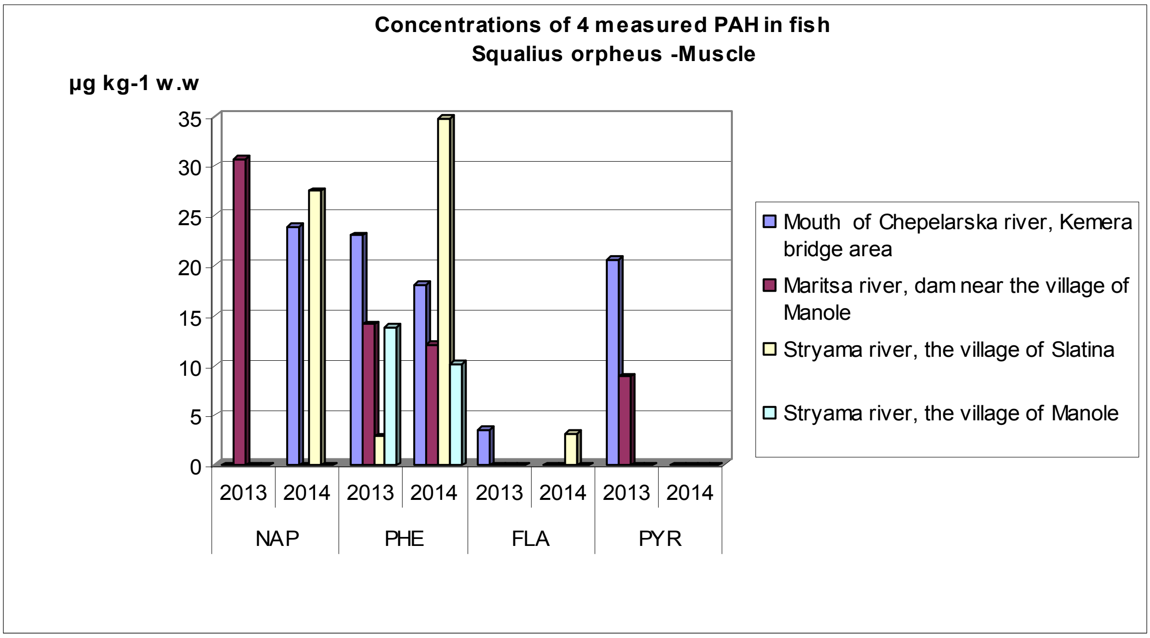

Fig. 7. Concentrations of measured PAH in *Squalius orpheus* – muscle.

The results for 24 OCPs in all fish showed concentrations exceeding the limit of quantification - α-HCH, β-HCH, γ-HCH, δ-HCH, Isodrin, Dieldrin, o,p-DDD (Table 2) and (Fig. 4, 5). The lowest concentrations were detected in the Stryama River, Slatina Village – a site slightly influenced by anthropogenic pressure.

Exceeding the limit of quantification for 16 PAHs analyzed was recorded for Naphthalene, Phenanthrene, Flurantene, Pyrene, (Table 3) and (Fig. 6, 7). The lowest concentrations were ascertained again in the Stryama River, Slatina Village, while the highest concentrations were in the Chepelarska River, Kemera Village – a site of significant anthropogenic pressure due to discharge of waste waters from settlements (Assenovgrad Town) and industrial enterprises (“KCM” Ltd. and “Agria” Ltd.).

Conclusions

At all monitoring stations PCB, OCPs and PAHs above the LOQ were detected, which was probably due to diffuse and point pollution in the area of the surveyed river sections. The most persistent PCB congener (PCB 138) were found only in *Barbus cyclolepis*, defined by WHO as important for evaluating the risk to human health. Ten substances from the group of OCPs were established. Moreover, six of them were found constantly in all five monitoring stations (α-HCH, β-HCH, γ-HCH, δ-HCH, Isodrin, Dieldrin, o,p-DDD). In the environment DDT metabolized slowly and the metabolite DDE is particularly persistent compound. The metabolite p,p′-DDE constituted more than 67% of the ΣDDTs for each species, followed by p,p′-DDD. In our study another metabolite of DDT, p,p′-DDD and o,p′-DDD, was also found, but in lower amounts. The p,p-DDT, o,p-DDT, p,p-DDE and o,p DDE in all analyzed fish species were found under limit of detection of the method.

The reported results could be a basis for initial examination of the level of pollution in the surveyed water bodies and identification of the sources of anthropogenic pressure. The data from the analysis conducted may be used for the purpose of monitoring the tendencies in the pollution of water bodies in terms of the examined indicators.

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