Fish biochemical markers as a tool for pollution assessment on the Svitava and Svratka rivers, Czech Republic

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Abstract

OBJECTIVES: The study was designed to assess the pollution of the Svitava and Svratka rivers in and around the industrial city of Brno (Czech Republic) by persistent organic pollutants using selected biochemical markers in chub.
DESIGN: Levels of selected biochemical markers were measured in liver and plasma samples of chub. The concentrations of persistent organic pollutants (POPs) were determined in bottom sediment, semi-permeable membrane devices (SPMDs) and muscle samples, and consequently used for correlation with biochemical markers.
RESULTS: Significant alterations ($p < 0.05$) in some biochemical markers were observed and associated with combined exposure to pollutants. The highest levels of pollutants were found at sites situated downstream from Brno. The most widespread changes were identified in the function of phase I detoxifying enzymes. Significant positive correlations were observed in cytochrome P450 content and DDT concentration in the semi-permeable membrane device ($p = 0.019$, $r_s = 0.886$), and between ethoxyresorufin-*O*-deethylase activity and content of DDT ($p = 0.041$, $r_s = 0.352$) and polychlorinated biphenyls ($p = 0.034$, $r_s = 0.365$) in muscle tissues of indicator fish.
CONCLUSION: The results presented in our study indicate the highest contamination of sites situated downstream from Brno, where the intensive industrial and agricultural activities as well as domestic waste and sewage most probably comprise the main impact sources of the enhanced level of pollutants and some biochemical markers in fish.

INTRODUCTION

The aquatic environment is continuously being contaminated with toxic chemicals, which originate from domestic, industrial, and agricultural activities that can disturb reproduction, growth and other physiological function of fish. Sensitive and reliable methods are therefore necessary to assess the influence of pollution to the aquatic environment (Guillete & Crain, 2000; Van der Oost *et al.* 2003).

ORIGINAL ARTICLE

Abbreviations

CYP450	cytochrome P450
EROD	ethoxyresorufin-*O*-deethylase
GSH	glutathione
GST	glutathione *S*-transferase
HCB	hexachlorobenzene
HCH	hexachlorocyclohexane
11-KT	11-ketotestosterone
PAHs	polycyclic aromatic hydrocarbons
PCBs	polychlorinated biphenyls
POPs	persistent organic pollutants
SPMDs	pemi-permeable membrane devices
VTG	vitellogenin
WWTP	waste water treatment plant

Modern analytical techniques allow to measure external levels of contaminants in abiotic (water, soil, sediment) or biotic (fish muscle) samples of aquatic ecosystem. However, this determination is regarded as insufficient when evaluating the negative effect on organisms. Besides, environmental pollutants are often present in mixtures, and not only separately, therefore the observed effect can be synergistic or antagonistic (Di Giulio & Hinton, 2008). Consequences of the exposure to some environmental chemical can be studied by means of the biochemical monitoring. Fish are useful experimental models for the evaluation of the health of aquatic ecosystems and biochemical changes; hence they have been used as biomarkers of environmental pollution. The biochemical marker is defined as a measurable change in a biological response, which can be related to the exposure to or toxic effects of environmental chemicals (Van der Oost *et al.* 2003). The advantages of using biochemical markers as pollution indicators were confirmed by many field (Blahova *et al.* 2008; Havelkova *et al.* 2008) and experimental studies (Modra *et al.* 2008).

Vitellogenin (VTG), a female specific protein and an egg yolk precursor, has been used as a biomarker for male fish whose endocrine systems were disrupted by endocrine disrupting chemicals (Nicolas, 1999; Mikula *et al.* 2006). It is now well established that some anthropogenic chemicals can disrupt the endocrine system of wildlife species. Therefore, the effects of xenobiotic on fish reproduction can be detected using plasma steroid hormone assays. Altered plasma sex steroid concentrations, including testosterone and 11-ketotestosterone (11-KT), were noted in different fish species living in variously contaminated sites (Hecker *et al.* 2002; Randak *et al.* 2006).

The liver plays a primary role in the metabolism of xenobiotic compounds with biochemical alterations occurring in some toxic conditions (Van Dyk *et al.* 2006), and it is a detoxification organ essential for the excretion of toxic substances in fish (Hinton & Lauren, 1990). Monitoring of aquatic pollution may also be conducted using liver biotransformation enzymes (phase I and II) (Van der Oost *et al.* 2003; Randak *et al.* 2006; Havelkova *et al.* 2008). The determination of cytochrome P450 (CYP450) content and ethoxyresorufin-*O*-deethylase (EROD) activity in fish liver is a well established biomarker of the exposure to xenobiotics such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and dioxins, and thus is a good indicator of contaminant exposure in fish (Whyte *et al.* 2000; Van der Oost *et al.* 2003). Changes of glutathione *S*-transferase (GST) activity in fish liver, which plays a key role in catalyzing the conjugation and potential excretion of different xenobiotics, were demonstrated in various fish species as the result of exposure to PCBs (Vigano *et al.* 1998; Krca *et al.* 2007) or PAHs (Vigano *et al.* 1998; Schreiber *et al.* 2006; Krca *et al.* 2007).

Objectives of the present study are to investigate the use of selected biochemical markers (VTG, 11-KT, CYP450, EROD, GST, glutathione (GSH)) in fish to evaluate the contamination levels in various location on the Svitava and Svratka rivers (Czech Republic). The concentrations of persistent organic pollutants (PAHs, PCBs, DDT and their metabolites, hexachlorobenzene (HCB) and hexachlorocyclohexane (HCH)) were measured in bottom sediment, semi-permeable membrane devices (SPMDs) and muscle samples, and consequently used to establish correlation with biochemical markers.

MATERIAL AND METHODS

Study area. The study was carried out upstream and downstream from Brno city (***Fig. 1***), which is the second largest city in the Czech Republic with approximately 370 000 inhabitants. Brno is located in the southeastern part of the country, at the confluence of the Svitava and Svratka rivers. It is an important industrial city with highly developed engineering as well as chemical, textile and food-processing industries. There were two research sites on the Svitava River. One was Bílovice nad Svitavou, which lies upstream from Brno, and one was downstream from Brno, just above the confluence with the Svratka River (Svitava before junction). The sites on the Svratka River were: a location upstream from Brno (Kníničky), a site downstream from Brno before the confluence with the Svitava River (Svratka before junction), and then Modřice, Rajhradice, and Židlochovice, all downstream from the confluence of the two rivers.

Sample collection. A total of 84 adult male chub (*Leuciscus cephalus* L.) were caught by electrofishing at the aforementioned 7 locations on the Svitava and Svratka rivers in June, July and September 2008. Blood samples were collected into heparinized tubes from the heart and/or caudal vein. The blood samples were stored at 4 °C and transported to the laboratory, where they were centrifuged (800 × g for 10 min). The plasma samples were stored at −85 °C until VTG and 11-KT analyses. Immediately after the blood collection, chub were killed, weigth recorded and their scales were collected for the age determination. The fish were dissected and the liver tissue was quickly removed, put in Eppendorf tubes and stored at −85 °C for later analyses of CYP450,

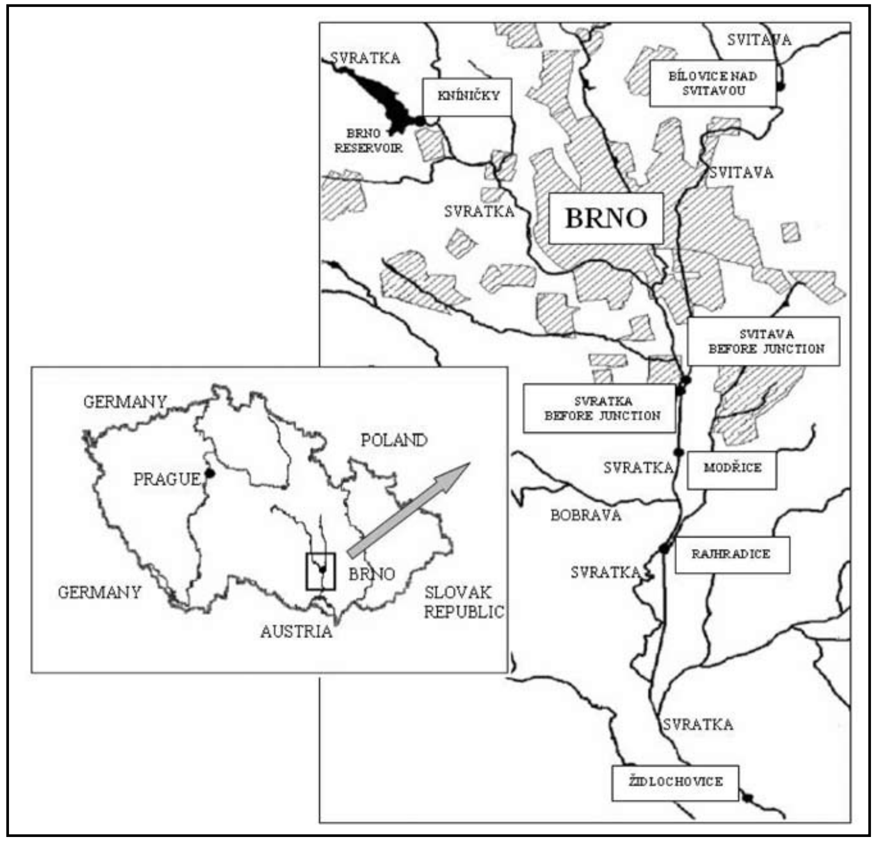

Fig. 1. Map of sampling sites around the Brno city (Czech Republic).

EROD, GST and GSH. Individual muscle samples were placed in polyethylene bags, labelled and stored at –85 °C for later chemical analyses. In every location, pooled bottom sediment was collected into dark glass bottle, stored at –20 °C and subsequently used for chemical analyses. One passive sampler, a semi-permeable membrane device, was placed at each site for one month (May 7th–June 4th 2008). Membranes were after the exposure briefly rinsed with distilled water and placed on ice for transport to the laboratory. They were stored in the laboratory at –20 °C until the chemical analyses were carried out.

Biochemical analyses. Vitellogenin and 11-ketotestosterone concentrations in plasma were measured using commercial enzyme-linked immunosorbent assay kits. Total content of CYP450 in liver samples was determined by visible light spectrophotometry (390 to 490 nm). Measurements were made following cytochrome reduction by sodium dithionite, after the complex with carbon monoxide was formed (Siroka *et al.* 2005). The catalytic activity concentration of EROD in liver samples was measured by spectrofluorometry (excitation: 535 nm, emission: 585 nm). In the presence of the enzyme, the substrate 7-ethoxyresorufin is transformed into resorufin in the presence of nicotinamide adenine dinucleotide phosphate (Siroka *et al.* 2005). The catalytic activity concentration of GST in liver samples was determined by measuring the conjugation of 1-chloro-2,4-dinitrobenzene with reduced glutathione (Habig *et al.* 1974), the specific activity was expressed as the nmol of the formed product per minute per mg of protein. Tripeptide glutathione was determined spectrophotometrically by Ellman's method (Ellman, 1959). The absorption of colored product was determined at 414 nm and its concentration (nmol.mg^{-1} protein) was calculated according to standard calibration.

Chemical analyses. Levels of selected POPs were determined in individual samples of muscle, sediment, and SPMDs. Hexachlorobenzene; α-, β-, γ-, δ-isomers of HCH; PCBs (indicator congeners – IUPAC number 28, 31, 52, 101, 118, 138, 153, 168, 170, 180), DDT, and its degradation products DDE and DDD, were determined by gas chromatography-mass spectrometry using an isotope dilution (Magnusson *et al.* 2006). Polycyclic aromatic hydrocarbons were analyzed by high performance liquid chromatography using a fluorescence detector with deuterated internal standards (Hosnedl *et al.* 2003).

Statistical analysis. The analysis was performed using Statistica software (StatSoft Inc. 2007). Biochemical markers as well as POPs in sediment, muscle tissue and SPMDs were tested for normal distribution using Shapiro–Wilk test. Since the non-normal distributions of parameters were identified, non-parametric tests were used. The Kruskal–Wallis test, followed by multiple comparison, was used to determine differences among groups. Non parametric Spearman rank correlation was applied to find out correlations among biochemical parameters and to prove the relationships between biomarkers and POPs. Significance was accepted at $p < 0.05$.

RESULTS

Biometric parameters. The main characteristics of the fish are summarized in Tab. 1. Significant differences ($p < 0.05$) among the groups were only observed in body weight. The highest mean body weight was observed in the locality of Modřice (293 ± 132 g) and the lowest in the locality of Bílovice nad Svitavou (141 ± 27 g). Fish age did not differ significantly among groups.

Biochemical analyses. Chub plasma VTG and 11-KT concentrations are summarized in ***Fig. 2***. Vittelogenin was only detected in some of the samples; in the cases

Table 1. Characteristics of chub captured at the individual localities. Significant differences (P < 0.05) are indicated by alphabetic superscript.

Locality (river km)	Fish (n)	Age (min – max) (years)	Weight ± SD (g)
Svitava River			
Bílovice nad Svitavou (18.0)	12	3.9 (2.5 – 5.5)^{a}	140.8 ± 27.3^{a}
Svitava before junction (0.6)	11	3.4 (2.5 – 5.5)^{a}	192.7 ± 79.3^{ab}
Svratka River			
Knínièky (56.2)	12	3.5 (2.5 – 5.5)^{a}	256.7 ± 103.1^{ab}
Svratka before junction (40.9)	14	4.1 (2.5 – 5.5)^{a}	290.0 ± 160.5^{b}
Modřice (38.7)	7	4.2 (3.5 – 5.5)^{a}	292.9 ± 132.0^{b}
Rajhradice (35.0)	15	3.6 (2.5 – 4.5)^{a}	291.3 ± 148.1^{b}
Židlochovice (30.0)	13	3.4 (2.5 – 4.5)^{a}	236.5 ± 94.3^{ab}

where VTG was not detected, half the detection limit (0.5 ng ml^{-1}) was applied for statistical analysis. In Fig. 2, there is also illustrated how many positive samples were found among the samples analyzed. The highest median VTG concentration values were found in the sites: Svratka before junction (503 ng ml^{-1}) and Bílovice nad Svitavou (498 ng ml^{-1}); and the lowest median levels were observed in the sites Židlochovice (0.5 ng ml^{-1}) and Modřice (0.5 ng ml^{-1}). Vitellogenin levels did not differ significantly among locations. The highest median level of 11-KT content was observed in samples from the location of Bílovice nad Svitavou (2878 pg ml^{-1}), and the lowest median level was found in Modřice (101 pg ml^{-1}). Significant differences ($p < 0.05$) in 11-KT concentration were discovered between the lowest median value and the content of 11-KT in samples from Bílovice nad Svitavou, Knínièky and Židlochovice.

The results of EROD activity and CYP450 content in chub liver are presented in ***Fig. 3.*** The highest median of EROD activity was found in liver samples from Židlochovice (491.9 pmol min^{-1} mg^{-1} protein), and the lowest median value in the Svratka location before junction (178.9 pmol min^{-1} mg^{-1} protein). Significant differences ($p < 0.05$) were found between Židlochovice and all the other locations. The highest median of CYP450 was, like in the case of EROD, obtained in the Židlochovice location (0.328 nmol mg^{-1} protein), and the lowest in the Knínièky site (0.084 nmol mg^{-1} protein). The highest median value was significantly ($p < 0.05$) higher than those obtained from Knínièky and Svratka before junction. Furthermore, positive correlation was found for EROD activity and CYP450 content ($p < 0.001$, $r_s = 0.401$).

The results of GST acitivity and GSH content in liver samples are shown in ***Fig. 4***. The highest median value of GST activity was found in the Modřice location (138.5 nmol min^{-1} mg^{-1} protein), and the lowest median value was obtained in the Rajhradice location (117.3 nmol min^{-1} mg^{-1} protein). The highest median value of GSH content was observed in the Knínièky location (4.4 nmol mg^{-1} protein) and the lowest median value in the location of Bílovice nad Svitavou (3.1 nmol mg^{-1} protein). However, significant differences were not confirmed in GST activity and GSH content between any two sites. Positive correlation was also found for GST activity and GST content ($p < 0.001$, $r_s = 0.583$).

Chemical analyses. Results of chemical analyses are presented in Tab. 2. Semi-permeable membrane device from the locality of Bílovice nad Svitavou did not produce samples due to a technical problem.

Concentration of HCH in muscle tissues were quite similar in all the monitored locations and ranged from 2.0 to 2.8 μg kg^{-1} d.w. Significant differences among the sites were observed regarding this pollutant in sediment samples as well as in SPMDs. The highest level of HCH in SPMDs was found in the Modřice locality (5821.2 pg l^{-1}); this value was several times higher than those obtained from the location with the lowest level of the monitored pollutant (Svratka before junction – 1276.9 pg l^{-1}). In case of sediment samples, the highest level of HCH was measured in the Knínièky location (1.5 μg kg^{-1} d.w.), and the lowest one in the Židlochovice location (0.3 μg kg^{-1} d.w.); y-isomer (lindane) was the most abundant, while δ-isomer of HCH was the least abundant.

The highest levels of HCB concentrations in muscle and sediment samples were identically found in the Svratka before junction site (3.2 μg kg^{-1} d.w. and 2.1 μg kg^{-1} d.w., respectively). The lowest levels were discovered in sediment samples from Svitava before junction (0.7 μg kg^{-1} d.w.), and in muscle samples from Bílovice nad Svitavou (1.0 μg kg^{-1} d.w.). Hexachlorobenzene content in SPMDs was similar in all the sites (ranged from 110.0–235.0 μg kg^{-1} d.w.) with the exception of the Knínièky location, where a lower value (70.0 μg kg^{-1} d.w.) was registered.

A very high concentration of DDT was detected in sediment samples from the Svratka before junction location (757.2 μg kg^{-1} d.w.). In other sites, the level of monitored pollutant in sediment ranged from 1.7 to 33.3 μg kg^{-1} d.w. Concentrations of DDT in SPMDs and muscle samples varied widely, and ranged from 187.8 to 641.4 pg l^{-1} and from 47.1 to 131.8 μg kg^{-1} d.w., respectively. The most common isomers were p,p'-DDE and

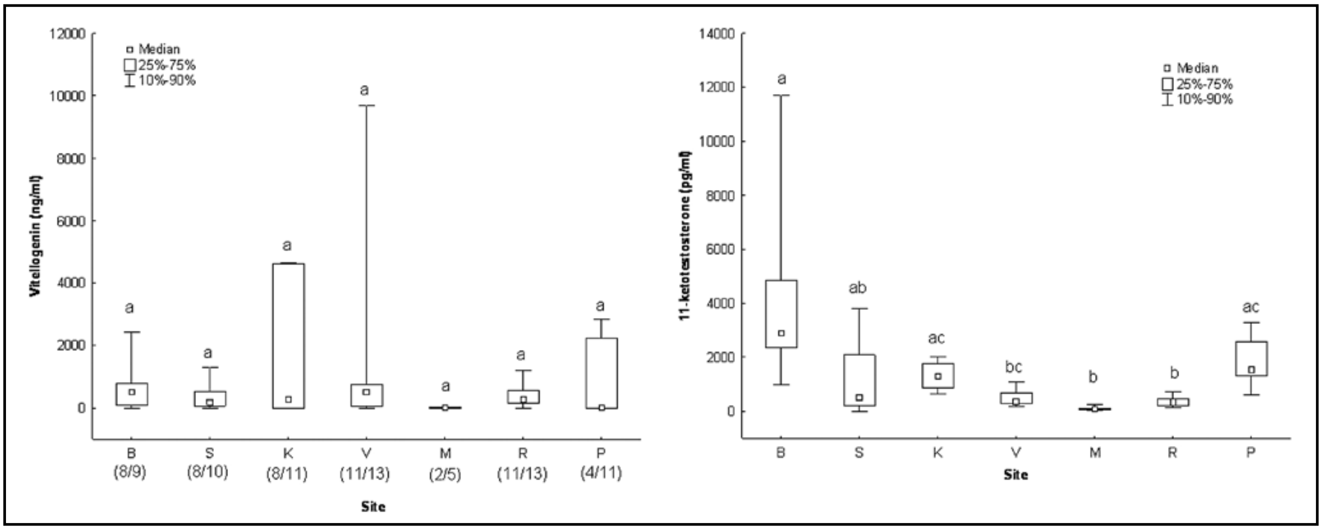

Fig. 2. Result of vitellogenin and 11-ketotestosterone contents in chub plasma from sample sites. Significant differences (P < 0.05) are indicated by alphabetic superscript. Numbers in the vitellogenin scheme represent the number of positive samples in the number of samples analyzed. (B – Bílovice nad Svitavou, S – Svitava before junction, K – Kníničky, V – Svratka before junction, M – Modřice, R – Rajhradice, P – Židlochovice)

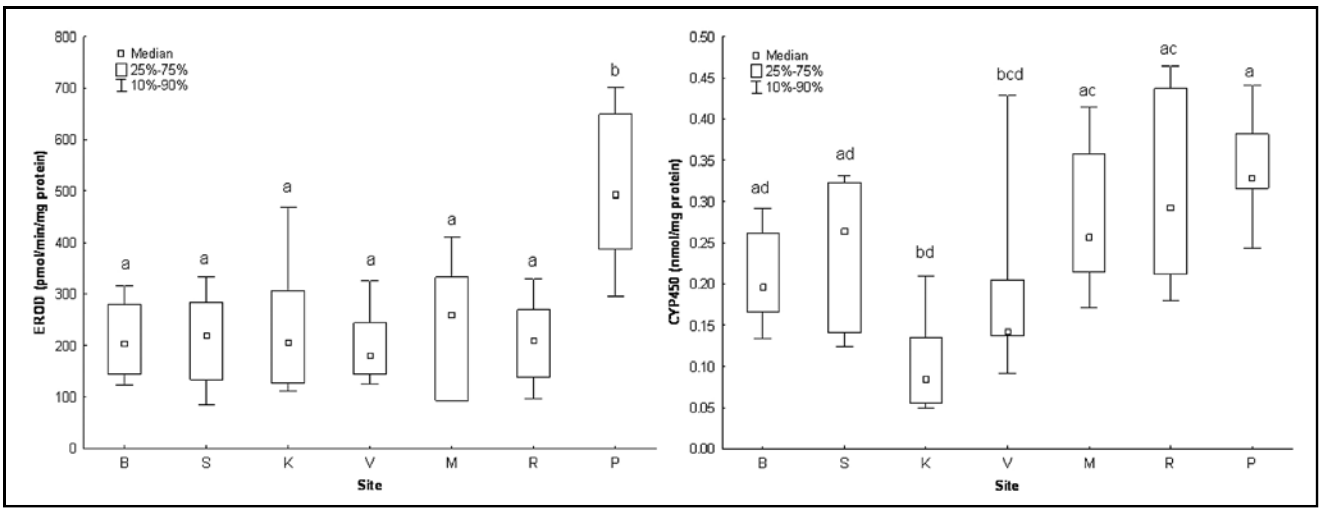

Fig. 3. Results of EROD activity and CYP450 content in chub liver from sample sites. Significant differences (P < 0.05) are indicated by alphabetic superscript. (B – Bílovice nad Svitavou, S – Svitava before junction, K – Kníničky, V – Svratka before junction, M – Modřice, R – Rajhradice, P – Židlochovice).

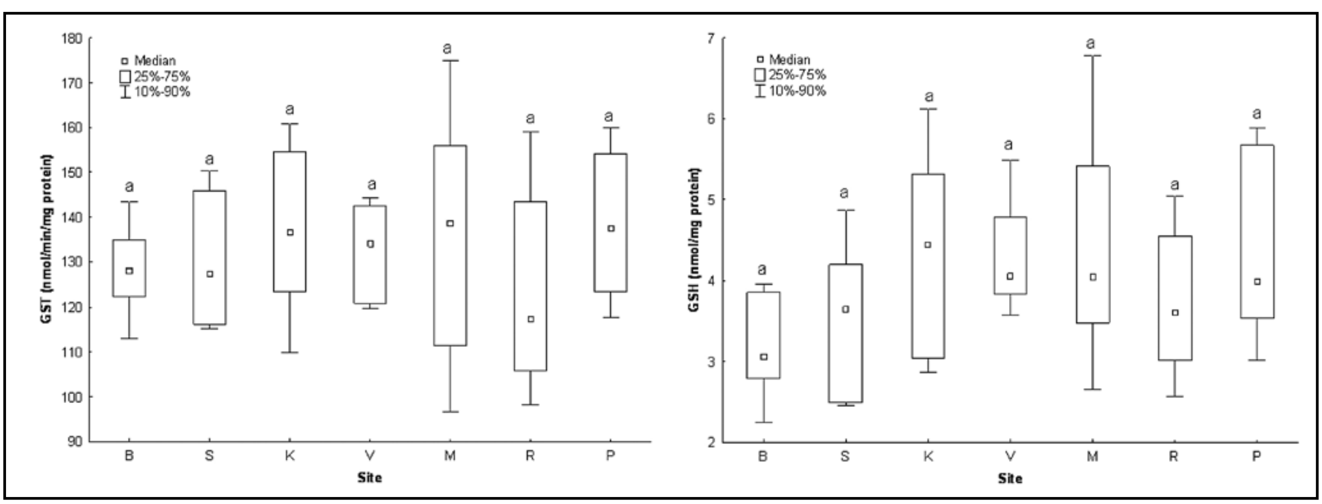

Fig. 4. Results of GST activity and GSH content in chub liver from sample sites. Significant differences (P < 0.05) are indicated by alphabetic superscript. (B – Bílovice nad Svitavou, S – Svitava before junction, K – Kníničky, V – Svratka before junction, M – Modřice, R – Rajhradice, P – Židlochovice)

Table 2. Content of persistent organic pollutants in SPMDs, sediment samples and muscle tissues (mean ± SD).

		Bílovice n. S.	Svitava b. j.	Knínièky	Svratka b. j.	Modřice	Rajhradice	Židlochovice
	SPMDs (pg l^{-1})	-	1356.6	1386.4	1276.9	5821.2	1567.8	1797.7
HCH	sediment (µg kg^{-1} d.w.)	0.9	0.5	1.5	0.5	0.8	0.4	0.3
	muscle (µg kg^{-1} d.w.)	2.5 ± 0.3	2.4 ± 0.6	2.8 ± 1.2	2.5 ± 0.6	2.4 ± 0.6	2.5 ± 0.7	2.0 ± 0.6
	SPMDs (pg l^{-1})	-	140.0	70.0	110.0	235.0	110.0	130.0
HCB	sediment (µg kg^{-1} d.w.)	1.1	0.7	0.9	2.1	2.0	1.7	1.7
	muscle (µg kg^{-1} d.w.)	1.0 ± 0.5	1.3 ± 0.5	1.8 ± 0.7	3.2 ± 2.0	2.5 ± 1.3	1.5 ± 0.5	1.4 ± 0.5
	SPMDs (pg l^{-1})	-	512.0	187.8	395.7	318.4	449.3	641.4
DDT	sediment (µg kg^{-1} d.w.)	5.6	12.1	1.7	757.2	20.6	29.7	33.3
	muscle (µg kg^{-1} d.w.)	47.1 ± 15.5	71.6 ± 21.7	131.8 ± 39.9	114.0 ± 29.9	63.4 ± 17.0	116.9 ± 34.5	117.3 ± 21.0
	SPMDs (pg l^{-1})	-	1670.9	417.6	298.5	1429.2	812.6	648.3
PCBs	sediment (µg kg^{-1} d.w.)	182.7	26.0	8.1	157.1	49.1	76.4	76.2
	muscle (µg kg^{-1} d.w.)	111.0 ± 40.4	194.3 ± 61.1	171.4 ± 64.4	268.5 ± 74.1	164.4 ± 57.2	232.8 ± 69.1	275.0 ± 48.7
PAHs	SPMDs (ng l^{-1})	-	65.4	19.4	28.0	39.1	38.6	42.9
	sediment (mg kg^{-1} d.w.)	15.0	19.0	2.3	15.0	12.0	26.0	13.0

p,p´-DDD. Furthermore, relatively high levels of *p,p´*-DDT were found in the sediment samples.

A sediment sample collected in the Bílovice nad Svitavou site contained the highest concentration of PCBs (182.7 µg kg^{-1} d.w.), which was approximately 23-fold higher than the lowest level obtained from Knínièky (8.1 µg kg^{-1} d.w.). In contrast, the levels of PCBs in SPMDs (417.6–1670.9 pg l^{-1}) and muscle samples (111.0–275.0 µg kg^{-1} d.w.) were similar in all the sites.

The lowest levels of PAHs both in SPMDs (19.4 ng l^{-1}) and sediment samples (2.3 mg kg^{-1} d.w.) were obtained in the Knínièky locality. In other localities, there were the levels in all matrices severalfold higher than in those from Knínièky; the highest levels of PAHs in SPMDs and sediment samples were found in the locations: Svitava before junction (65.4 ng l^{-1}) and Rajhradice (26.0 mg kg^{-1} d.w.), respectively.

Correlation between biomarkers and pollutants content. A Spearman correlation test was applied to find the relationship between individual biochemical markers in chub and POPs in several matrices. Significant positive correlation for CYP450 content and DDT concentration in SPMDs was observed ($p = 0.019$, $r_s = 0.886$). Positive correlations were also obtained for EROD activity and all the measured POPs in SPMDs (the correlation coefficients ranged from 0.54 to 0.73), but these correlations are not statistically significant. On the other hand, significant correlations were found between EROD activity, DDT content ($p = 0.041$, $r_s = 0.352$) and PCBs ($p = 0.034$, $r_s = 0.365$) in muscle tissues of indicator fish. No significant relationships were discovered between biomarkers and pollutants analyzed in sediment samples.

DISCUSSION

Several studies have demonstrated sensitive responses of selected biochemical markers in fish, when exposed to pollutants in many laboratory (Leon *et al.* 2007; Thorpe *et al.* 2009) or field experiments (Hecker *et al.* 2002; Lavado *et al.* 2006; Randak *et al.* 2009). Plasma VTG in male fish is one of the most often used biomarker for the evaluation of the occurrence of endocrine disruptors in aquatic ecosystem (Kime *et al.* 1999). Kujalova *et al.* (2007) indicate that synthetic hormones are the most common sources of estrogenic environmental endocrine disruptors. Furthermore, some of industry-made products and their metabolites (e.g. pesticides, alkylphenols, PCBs, PAHs and bisphenol A) can also act as weak estrogens. This is in agreement with the field study by Rodas-Ortiz *et al.* (2008), which confirmed that the exposure to HCB and benzo(*a*)pyrene caused VTG induction in male Nile tilapia (*Oreochromis niloticus*). Similarly, Havelkova *et al.* (2008) demonstrated that the highest VTG concentrations in male chub caught in the Tichá Orlice River (Czech Republic) were found in the Králíky site. Chemical analyses of sediment and muscle tissue samples verified higher levels of PCBs, HCB, DDT and PAHs at this site. Lavado *et al.* (2004) found that carp (*Cyprinus carpio*) collected near dicharges of sewage treatment plants and industrials wastes along Ebro River (Spain) had alterations on the endocrine system – high levels of plasma vitellogenin in male and depressed levels of sex hormones, and delay in maturation. On the other hand, Randak *et al.* (2009) demonstrated significant negative correlation ($p < 0.05$) between the VTG levels and the concentration of PCBs ($r = -0.426$) and octachlorostyrene ($r = -0.444$) in muscle. These non-standard results may have been caused by the presence of other pollutants with antiestrogenic activity. In our study we found the relatively high level of VTG concentration in the Bílovice nad Svitavou locality, which is situated upstream from Brno and was chosen as a control locality. The possible explanation for these results could be the effect of a waste water treatment plant (WWTP) in Bílovice nad Svitavou situated 150 m upstream from the target site. On the other hand, we obtained surprising results from the Modřice location, which is situated downstream from Brno, i.e. below the WWTP. This WWTP treats wastewater from the city of Brno and other municipal WWTPs. There was found the lowest VTG concentration, and only two samples out of five were positive for the VTG detection. The explanation of our results can be in a good effectiveness of this WWTP regarding the removal of estrogens contained in sewage (Nekvapil *et al.* 2009).

Some authors used 11-KT in male plasma as an indicator of the occurrence of endocrine disruptors with anti-/androgenic effect (Hecker *et al.* 2002; Randak *et al.* 2006). Hecker *et al.* (2002) documented that plasma 11-KT concentrations in males were distinctly lower in sites characterized by the elevated exposure to contaminants (PCBs, PAHs, HCH). We observed the highest concentration of plasma 11-KT in locations situated upstream from Brno (Bílovice nad Svitavou and Kníničky). By contrast, the lowest level was found in Modřice. Low level of both VTG and 11-KT in samples from the Modřice location indicates the presence of various chemical compounds, which can act as antiandrogens or antiestrogens.

Generally, alterations in levels and activities of biotransformation enzymes are sensitive biomarkers. In fish, the activity of these enzymes may be induced or inhibited upon exposure to xenobiotics (Van der Oost *et al.* 2003). The balance between phase I activation reactions and phase II conjugation pathways can underlie the toxicity of many organic xenobiotics (Havelkova *et al.* 2008). Although most studies did not demostrate any significant alterations in GST activity, an increase in hepatic GST activity after the exposure of fish to several pollutants has been reported in some studies (Van der Oost *et al.* 2003). Our results document enhanced levels of phase I and II enzymes of xenobiotics detoxification in the Židlochovice locality, which is situated downstream from Brno as the last monitored site on the Svratka River. In this location was observed the highest EROD activity, which was significantly ($p < 0.05$) higher than in all the other locations. Simultaneously, CYP450 content in liver samples from Židlochovice was the highest. Glutathione *S*-transferase activity was the second highest from all the monitored locations, however significant differences were not confirmed among any two sites. In correlation analyses we confirmed significant positive correlation for CYP450 content and DDT concentration (in SPMDs), and EROD activity and content of DDT and PCBs in muscle tissues. No significant correlations were obtained for the phase II enzymes. This complies with results obtained from other field studies, which are summarized in the study by Van der Oost *et al.* (2003). Randak *et al.* (2006) evaluated the contamination of the Elbe and Vltava rivers (Czech Republic) using biomarkers. They confirmed that the studied locations with the highest EROD activity were the most contaminated with xenobiotics, mainly with persistent organochlorine pollutants. In other studies on the Elbe River, there was also a observed significant correlation between the EROD activity and the concentration of PCBs, HCB, HCH and octachlorostyrene in musle samples (Randak *et al.* 2009). Similarly, Fernandes *et al.* (2008) documented positive correlations between EROD activity and PCBs bioaccumulated in muscle tissue of fish collected along the Northern Iberian coast (Spain). Van der Oost *et al.* (2003) pointed out that EROD activity appeared to be the most sensitive biomarker from the enzyme system for evaluation of aquatic environment contamination.

Organochlorine pesticide DDT belongs to the group of chemical insecticides, which have reproductive endocrine effects and also a major toxic effect on the adrenal glands (Keith 1997). Although DDT was in our country banned in 1974, this compound and its degradation analogs are still present in the soil and can consequently accumulate in aquatic organisms. Our results indicate that the dominant degradation product of DDT was isomer *p,p´*-DDE. High concentration of the *p,p´*-DDE metabolite indicates some old contamination because the DDT to DDE conversion is relatively slow (Kitamura *et al.* 1999). Similar results were also reported in the study by Havelkova *et al.* (2007), who found out that *p,p´*-DDE comprises up to 75–90 % of the total DDT amount in the muscle of fish caught in Czech rivers. The transformation of this pollutant takes place in the liver and the P450 cytochrome system plays a key role there (Kitamura *et al.* 1999; Ssebugere *et al.* 2009). Correlation analysis confirmed the impact of phase I detoxifying enzymes on the degradation of DDT and its metabolite. Significant positive correlation was found between CYP450 content and DDT concentration in SPMDs ($r_s = 0.886$) and EROD activity and DDT concentration in muscle tissues ($r_s = 0.352$).

The lowest levels of PAHs were observed in the Kníničky locality (the Svratka River), which is situated below the Brno Reservoir dam. The fact that downstream of the dam, there is a much lower content of these compounds than at the other sites indicates that PAHs are not usually present in the dissolved phase due to their low solubility. Instead, PAHs tend to bind to organic matter and small particles in the water column as well in sediments present in the reservoir above the dam (Blahova *et al.* 2008). Furthermore, low concentration of other POPs (HCB, PCBs) was found at this site. The only exception is the concentration of DDT in muscle and HCH in sediment and muscle samples, which was the highest of all the sites. The low level of some pollutants at this location can also be attributed to the gravelly and sandy characteristics of sediment samples with low proportion of organic compounds capable of binding compounds.

Overall, the present study provides further support for the use of persistent organic pollutants analysis with biochemical responses in fish as a tool to assess water contamination in aquatic ecosystem. The results obtained from chemical analyses and biochemical markers in fish show that the most contaminated location are situated downstream from Brno, especially in Modřice, Rajhradice and Židlochovice. In most cases, elevated biochemical marker levels were found together with the enhanced concentration of persistent organic pollutants. The main impact sources of elevated levels of pollutants are most probably intensive industrial and agricultural activities and last but not at least domestic waste and sewage.

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