ORGANOCHLORINES FROM THE SAVA RIVER (CROATIA), LEVELS IN FISH AND SPMD UPTAKE

Čalić V, Picer M, Picer N
Division for marine and environmental research, Rudjer Boskovic Institute, POB 180, Bijenicka 54, HR-10002 Zagreb, Croatia

Abstract
Monitoring of aquatic ecosystem pollution represents one of the major activities involved in measures aimed at environment protection. A lot of data on the ecological character of the Sava river basin are still lacking. One of the goals of FP6 EU project SARIB is collection and systematization of these data. This short paper came out as a result of this project.
European chub (*Leusiscus cephalus*) was used as monitoring specie. Levels of polychlorinated biphenyls (PCBs) and organochlorine insecticides (DDT) are determined in tissues samples, as well as in a semi-permeable membrane devices (SPMD) deployed at different locations downstream the Sava River. Levels of DDT are much lower in comparison with levels of PCBs in all (muscle, liver and SPMD) samples. Comparison with the available data about PCB levels in muscle tissue of chubs from European rivers leads to conclusion that the chubs from Sava River are moderately polluted with PCBs. Unfortunately data about levels of POPs in liver of European fishes are still sparse (none about E. chubs). For this reason determination of the levels of PCBs and DDT in the liver of the chubs from the Sava River is especially valuable.

Introduction
Because of the gradual diffusion and transport of persistent organic compounds from hot spots, as well as their cycling thereof among different environmental compartments, significant amounts of PCBs and DDT still exist, there are still in use and partially released into the environment.
Aquatic organisms can be used as biological indicators to monitor contamination of aquatic ecosystems with PCBs and DDT. The potential of fish as biomonitors was demonstrated by numerous monitoring studies.[1,2]
In recent years, semi-permeable membrane devices have increasingly attracted attention as passive sampling tools in both marine and freshwater environments. Because SPMD mimic the bioconcentration of trace waterborne lipophilic contaminants by fishes, are not subject to most stressors affecting the health of biomonitoring organisms, and are highly reproducible, these devices are being used worldwide for passive *in situ* monitoring of hydrophobic organic contaminants.[3,4,5] Results in this short paper are partly presented at DIOXIN 2006 conference.[6]

Materials and Methods
The sampling was carried out on the section of the Sava River from the Slovenian-Croatian border to the mouth of the Una River at Jasenovac.[6] This section was chosen due to the well-defined gradient of pollution ranging from low polluted sites before the city of Zagreb (1 mil. inhabitants, heavily industrialized) up to sites characterized by the pollution load from the Zagreb and Sisak city areas.
Altogether four fish catchments were carried out, two in 2005 and two in 2006, on different localities downstream the river (Otok Samoborski, Zagreb - Jarun, Oborovo, Lukavec Posavski, Jasenovac, Košutarica). During the four sampling campaigns chlorinated hydrocarbons were analyzed in 20 muscle and 18 liver composite samples, which represent an average concentration for all fish specimens caught at a give location. Unfortunately during some campaigns it was not possible to catch the fish due to bad weather conditions or there was not enough tissue for the chlorinated hydrocarbon analysis and data for these stations are missing.
Pooled tissue samples were homogenized after addition of Na_2SO_4 anh and extracted with n-hexane in a high revolution blender. In one third of the extract, extracted organic matter (EOM) was determined gravimetrically. In the other third of the extract, Mirex was added as an internal standard.
Passive samplers for organic pollutants (SPMDs) were deployed in order to define bioavailable concentration of pollutants. Deployment occurred over the extended period in autumn 2005 and spring 2006 on 5 different localities (Otok Samoborski, Zagreb-Jarun, Oborovo, Sisak, Lukavec Posavski). SPMD membranes were extracted with DCM in ultrasound bath, fallowed by GPC cleanup.
Both, tissue and SPMD extracts were cleaned on an alumina column. Separation of chlorinated insecticides from

polychlorinated biphenyls was made on a silica gel column. Quantification of chlorinated hydrocarbons was performed by high resolution EC gas chromatography.

Results and Discussion

Mass partition of organic pollutants is expressed on wet tissue mass (levels in ngg^{-1}) and respectively on EOM mass (levels in μgg^{-1}).

Determined ranges of PCB concentrations in E. chubs from the Sava River are:

- *expressed on wet tissue mass:* total PCBs as equivalents of the Aroclor 1254 in muscle tissue 4.5-60.5 ngg^{-1}, in liver 3.3-352.3 ngg^{-1}; the sum of the 7 PCB congeners in muscle tissue 2.4-19.8 ngg^{-1}, in liver 1.8-96.9 ngg^{-1};
- *expressed on EOM mass:* total PCBs as equivalents of Aroclor 1254 in muscle tissue 0.42-4.85 μgg^{-1}, in liver 0.10-2.93 μgg^{-1}; the sum of the 7 PCB congeners in muscle tissue 0.17-2.06 μgg^{-1}, in liver 0.06-1.53 μgg^{-1}.

Levels of DDTs are much lower in comparison with levels of PCBs in all analyzed fish samples. Determined ranges of the sum of DDTs (p, p'-DDE, -DDD and -DDT) are presented in Table 1.

We cannot say that the levels of PCBs in the pooled fish muscle and liver samples were completely in accordance with the expected pollution gradient. There are no definite spatial and/or seasonal patterns.

When expressed on wet mass basis levels of PCBs in fish liver are significantly higher than in the muscle tissue (Figure 1) but neither one of the analyzed samples reached high PCB amounts. As expected, the lowest PCB levels were determined at the most upstream location Otok Samoborski, in both muscle and liver tissue. PCB levels in muscle samples reflect the assumed pollution state of the river with one exception: Jarun in spring 2005, where surprisingly the highest PCB concentration is determined for that sampling campaign. PCB concentrations on liver wet mass are more variable and the compliancy with the expected pollution gradient is lesser.

In contrast, PCB concentrations on EOM mass basis are similar in both tissues, mostly below 1 μgg^{-1}, and show no significant variations on the more polluted locations downstream of Zagreb (Figure 2). The most probable explanation for the observed variations might be large differences in amounts of EOM in the muscle and liver tissues of the analyzed fish samples (muscle amounts 0.3 to 3.2% while in liver 1.9 to 33.8%), as well as species-specific differences probably caused by divergent evolutionary and life history and/or food and habitat preferences.

Comparing the available data about the levels of PCBs in the muscle tissue of chubs in the European rivers, with the levels of these pollutants determined in our monitoring, we were able to conclude that chubs from the Sava River are moderately polluted with PCBs.

Results of PCB levels in the SPMDs are presented in Figure 3. Sisak (autumn 2005) and Oborovo (summer 2005) are locations with the highest PCB membrane uptake. But again differences between "clean" and polluted locations are not clear as expected. Experimental problems we had with SPMD deployments (biofouling, high and low water flows) also make difficult the interpretation. These elevated PCB amounts, most probably, reflect the enhanced input of hydrocarbons by main municipal sewage vent at Oborovo, and the oil refinery and the ironworks situated in the city of Sisak, not far upstream of the sampling location.

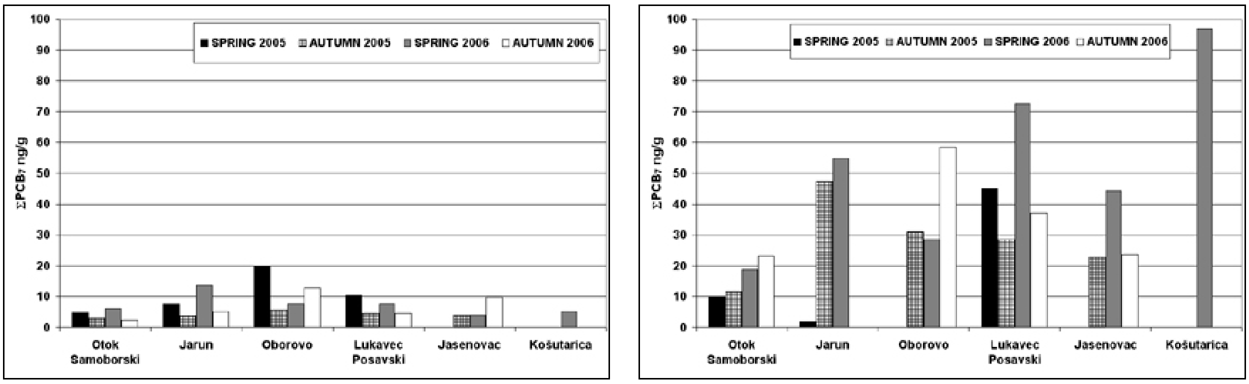
On Figure 4 we tried to make correlation between PCB levels in different E. chub tissues and SPMDs. The only significant correlation is in PCB levels in muscle and liver tissue when expressed on wet mass basis ($p=0.1$). No significance was found when correlating levels on EOM mass basis or SPMD and muscle ($R^2<<0.1$). SPMD - liver correlation also does not have satisfying significance ($0.5>p<0.1$).

For providing better understanding of the Sava River pollution with chlorinated hydrocarbons more measuring and data are needed.

Table 1**.** Ranges of the sum of DDT levels in European chubs and SPMDs from the Sava River (2005-2006)

STATION	**MUSCLE**		**LIVER**		**SPMDs**
	ng/g wet mass	**µg/g EOM mass**	**ng/g wet mass**	**µg/g EOM mass**	**ng/g triolein**
Otok Samoborski	0.2 - 1.5	0.03-0.24	1.2-10.6	0.05-0.56	1.3
Jarun – Zagreb	0.2-1.5	0.03-0.23	2.6-9.1	0.08-0.15	21.0-82.6
Oborovo	0.3-1.4	0.03-0.12	1.8-7.7	0.04-0.12	13.7-41.7
Sisak	n.a.	n.a.	n.a.	n.a.	20.1-22.0
Lukavec Posavski	0.2-1.2	0.03-0.08	1.1-12.8	0.03-0.07	2.9-30.1
Jasenovac	0.2-1	0.02-0.07	1.9-10.1	0.04-0.06	n.a.
Košutarica	1.2	0.04	17.2	0.05	n.a.

n.a. – *"not analyzed"*



a b

Figure 1. Levels of the sum of the 7PCB congeners in a) muscle and b) liver tissues (on wet mass basis) of E. chubs from the Sava River collected between March 2005 and October 2006

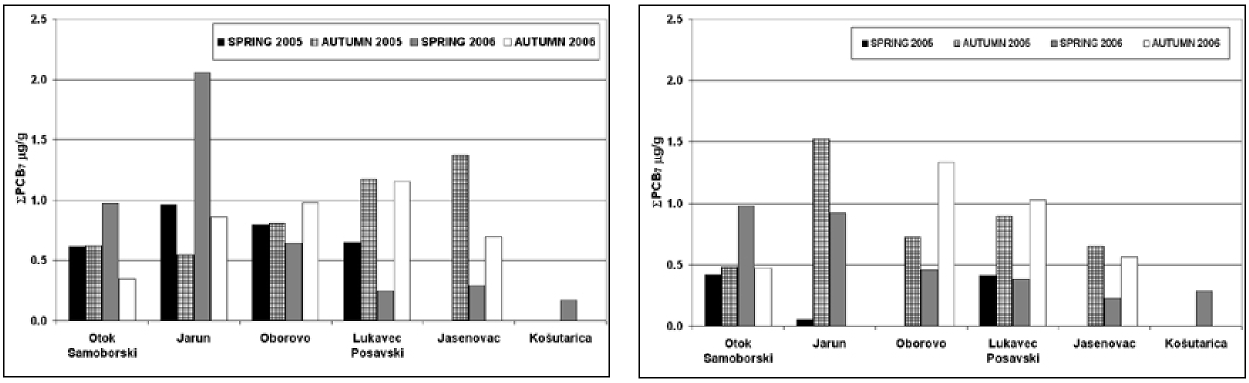


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Figure 2. Levels of the sum of the 7PCB congeners in a) muscle and b) liver tissues (on EOM mass basis) of E. chubs from Sava River collected between March 2005 and October 2006

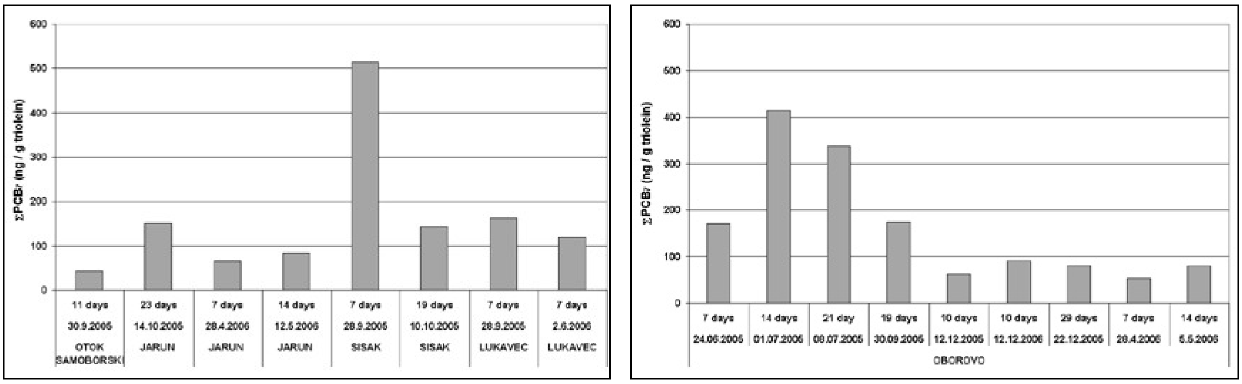

Figure 3. Levels of the sum of the 7PCB congeners in SPMDs deployed in Sava River in 2005 and 2006

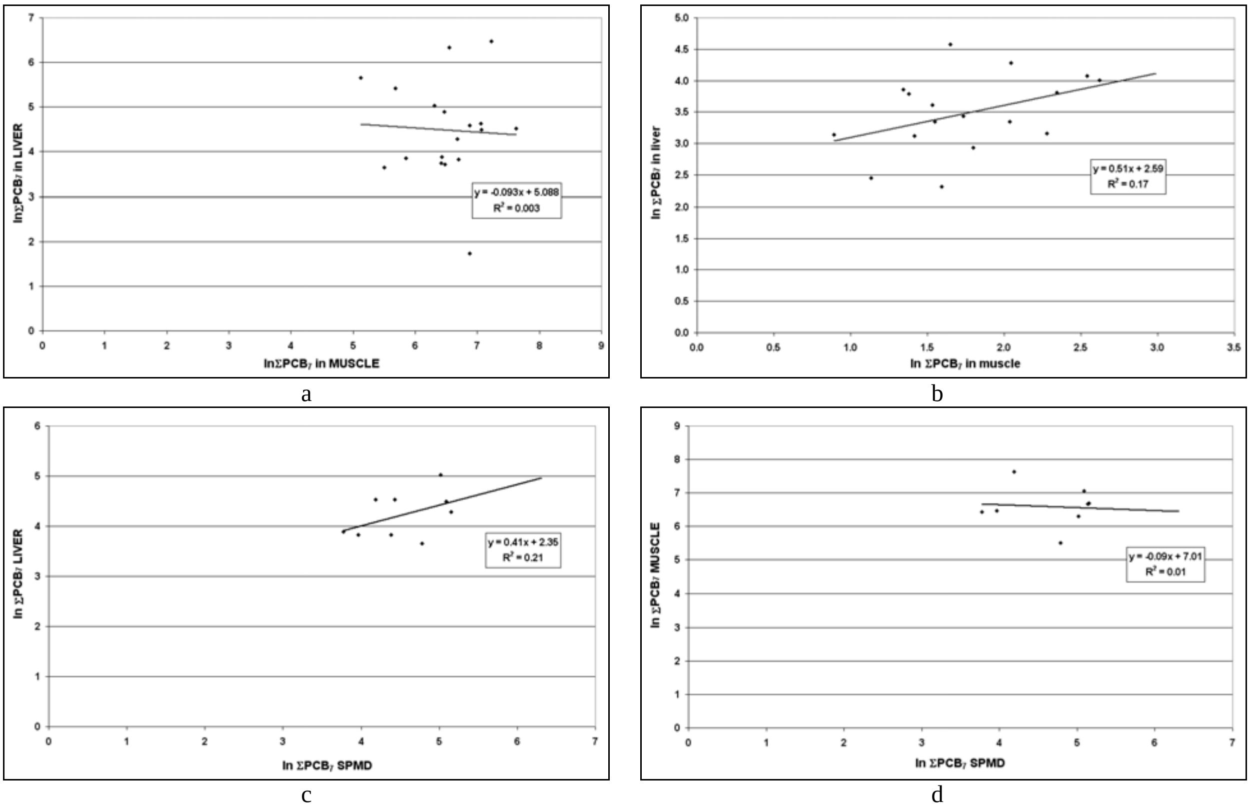

Figure 4. Correlations in PCB levels between fish tissue and SPMD samples from the Sava River: (a) muscle-liver on EOM mass; (b) muscle-liver on wet mass; (c) SPMD-liver; (d) SPMD-muscle

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