Cod: 4.2006

FIRST FINDINGS OF THE PRESENCE OF PCDD/FS AND PCBS IN FISH SPECIES FROM THE SAVA RIVER BASIN

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Introduction

Today, there is a common position about the adverse effects of the persistent organic pollutants (POPs) on the environment and exposed organisms because of their high toxicity. In some cases, a regulatory framework has also entered into force. The Stockholm Convention (SC) aware of the impact for the environment and humans listed a number of these substances as a target compounds to be eliminated, forbidden or reduced by means of Best Available Techniques (BATs). The European Directive regarding water policy also set Environmental Quality Standards (EQS) for some POPs in biota. The International Agency for Research of Cancer (IARC), in a report published in 2015, declared dioxins, furans (PCDD/Fs) and polychlorinated biphenyls (PCBs) as carcinogenic for humans. European Union (EU) set limits for dioxins and PCBs (both dioxin-like (DL-PCB) and non-dioxin-like (NDL-PCB)) content in feed and food.

Taking all this into account, in this study, dioxin-like substances (dioxins, furans and DL- PCBs) and NDL- PCBs were determined in different fish species collected along the Sava River Basin (SRB) during a sampling campaign performed in 2015. To the best of our knowledge, these are the first reported levels of this whole set of compounds in the SRB. The main goal was to quantify the levels of these families of POPs and to discuss the findings from a regulatory point of view. The impact on the environment due to the presence of these substances in fish from the SRB and implications in terms of food consumption will be discussed.

The Sava River Basin (SRB) covers a wide geographic area (Figure 1) with a total of 97,713 km2 and including population of about 8.5 million inhabitants. It is a macro region, an area that includes the territories of six countries - Slovenia, Croatia, Bosnia and Herzegovina, Serbia, Montenegro, with a minor part of the basin area also extending to Albania.

The SRB is one of the most significant sub basins of the Danube River Basin, with a share of 12%. The landscape within the SRB is diverse, the elevation varying between approx. 71 m above sea level (m a.s.l.) at the mouth of the Sava River in Belgrade (Serbia) and 2,864 m a.s.l. (Triglav, Slovenian Alps). Mean elevation of the basin is approximately 545 m a.s.l. In terms of land cover/land use, most of the basin is covered by forest and semi-natural areas (54.7%) and agricultural surfaces (42.4%), while the share of artificial surfaces is 2.2%.

Materials and Methods

A total of 10 fish pooled samples, collected in 2015, were processed from the Sava River for the analysis of PCDDs/Fs and PCBs, both dioxin like PCBs (DL-PCBs) and non dioxin-like PCBs (NDL-PCBs).

Due to heterogeneity of studied river stretches (>930 km of the river covered by the investigation), it was not possible to collect the same specie for the analyses in all the sampling sites. Thus, we tried to collect the species with a similar feeding behaviour and, if possible, taxonomically close. The data on processed individuals (species, length and weight) are presented in Table 1.

Once at the laboratory, samples were weighted and measured. Then, the samples were freeze-dried and homogenized as part of pre-treatment steps. Samples were extracted in a Soxhlet for ~24h with toluene:cyclohexane (1:1) after being spiked with known amounts of mixtures of $^{13}C_{12}$-PCDD/Fs (EPA-1613LCS, Wellington Lab., Guelp, Canada) and $^{13}C_{12}$-DL-PCBs (WP-LCS, Wellington Lab., Guelp, Canada). Next, the extracts were rotary evaporated and kept in the oven overnight (105 ºC) in order to eliminate the solvents prior to gravimetrical fat determination. Afterwards, fat residues were

dissolved again in n-hexane. Organic components, fat and other interfering substances were removed by treating the n-hexane extracts with silica gel modified with sulphuric acid (44%). Further sample purification and fractionation was carried out using multilayer silica, basic alumina and carbon columns. Instrumental analysis was based on high resolution gas chromatography coupled to high resolution mass spectrometry (GC-HRMS). All analyses were performed on a 6890N Network GC System Agilent gas chromatograph (Agilent Technologies Inc., Palo Alto, USA) fitted with a DB-5ms fused silica column (J&W Scientific, Folsom, USA) and connected through a heated transfer line kept at 280 ºC to an AutoSpec Ultima NT high resolution mass spectrometer with an EBE geometry (Waters, Manchester, UK). Electron ionization (EI+) mode was used, operating in the selected ion monitoring (SIM) mode at a resolving power of 10000 (10% valley definition). The two most abundant ions of the molecular cluster ions of each homologue group were monitored.
For NDL-PCB analysis, the extraction and purification methodology was similar to that previously described for PCDD/Fs and DL-PCBs. Briefly, freeze-dried samples were spiked with known amounts of $^{13}C_{12}$-PCBs (MBP-MXE, Wellington Lab., Guelp, Canada) and then extracted in a Soxhlet for ~24h using n-hexane: dichloromethane (1:1). After that, the extracts were rotary concentrated and transferred to n-hexane. Next, purification and fractionation of these extracts were carried out using a silica gel column modified with sulphuric acid (44%) and a basic alumina column. Chromatographic separations was performed using DB-XLB (60m x 0.25mm i.d. x 0.25µm film thickness) column from J&W Scientific (Folsom, USA). Instrumental conditions for NDL-PCB analysis by HRGC-HRMS were similar to those for PCDD/Fs and DL-PCBs.

Results and Discussion

Concentrations of individual PCDD/F and PCB congeners, as well as the total WHO-TEQs, are shown in Table 1. In addition, an example of the congener distribution of target compounds is given in Figure 2. For PCDD/Fs, the highest levels, expressed as total WHO-TEQ concentrations, were found in samples S4 and S5, with values higher than 1.0 pg WHO-TEQPCDD/F/g fresh weight (fw). Therefore, WHO-TEQ results for PCDD/Fs were below to the limit value of 3.5 pg WHO- TEQPCDD/F /g fw established by the EU Regulation. The isomer distribution of toxic congeners was usually characterized by the presence of the lowest chlorinated compounds, particularly 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDF, while congeners of a higher degree of chlorination were found in minor proportions.
A similar trend was observed for DL-PCBs, being S4 and S5 the samples that showed the highest concentration for these compounds, expressed as the total WHO-TEQ concentrations (10.6 and 7.0 pg WHO-TEQDL-PCB/g fw, respectively). In this two cases, the calculated WHO-TEQ levels for the sum of PCDD/Fs and DL-PCBs, (11.7 and 8.3 pg WHO-TEQPCDD/F+DL-PCB/g fw) were clearly higher than the limits established by the EU Regulation for this kind of food products (6.5 pg WHO-TEQPCDD/F+DL-PCB/g fw).
Total NDL-PCB concentrations (as the sum of the 6 congeners analysed) in the samples considered in this study also presented some high concentrations depending on the sample. Again the highest levels were found in sample S4 (168 ng/g fw) and ample S5 (132 ng/g fw), accounting levels above those established by the EU Regulation for freshwater fish species (125 ng/g fw).
It is remarkable that, as expected, the highest concentrations correlate with the length and weight for fish belonging to the same species. However, it should be pointed out that the analysis of dioxins and PCBs were performed not only on the muscle (edible part, as considered in EU regulation) but on the whole fish, including head, skin and other fatty tissues. This might explain part of the high levels observed.
In addition to that, in the Sava River, it is not possible to select a pristine site for sampling purposes, due to influence of agriculture, urban waste waters and heavy industry, including chemical industry, power plant stations, paper industry. Therefore, sampling was performed in so-called "Best Available Sites".
In summary, despite the limited number of samples analysed, data suggest that anthropogenic impact is observed in SRB in terms of the presence of dioxins and PCBs. Based on measured high concentrations of PCBs and dioxins in fish, there is obvious need to establish surveillance monitoring of these elements in fish tissues, especially having in mind fish consumption and potential risk for human health.

Acknowledgements

The research leading to these results has received funding from the European Comunities 7th Framework Programme under Grant Agreement No. 603629-ENV-2013-6.2.1-Globaqua and The SOLUTIONS

project, also supported by the European Union 7th Framework Programme (FP7-ENV-2013-two-stage Collaborative project) under Grant Agreement No. 603437.

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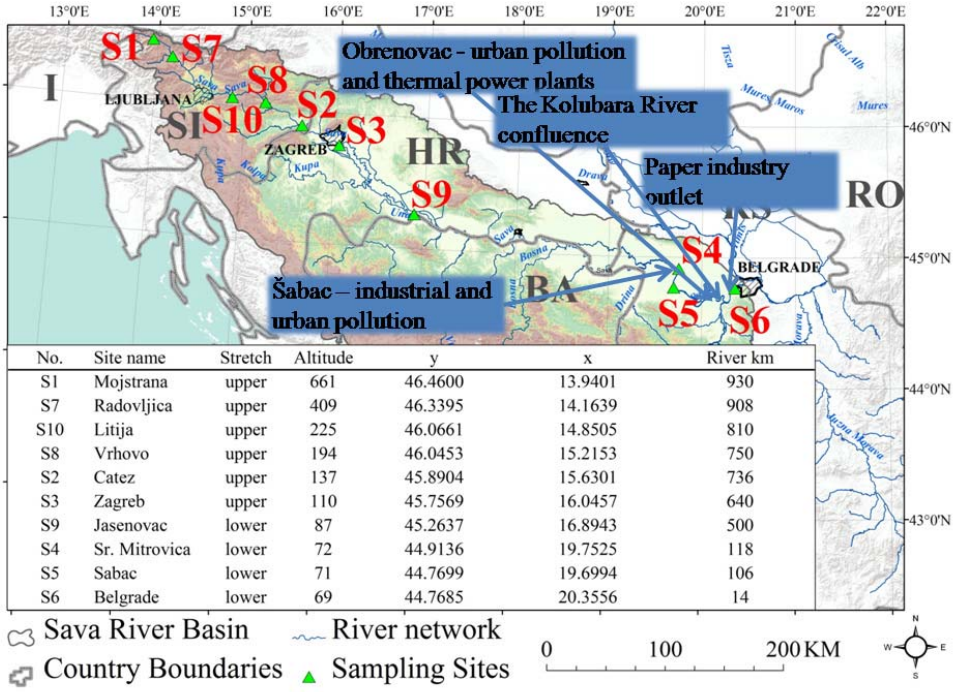

No.	Site name	Stretch	Altitude	y	x	River km
S1	Mojstrana	upper	661	46.4600	13.9401	930
S7	Radovljica	upper	409	46.3395	14.1639	908
S10	Litija	upper	225	46.0661	14.8505	810
S8	Vrhovo	upper	194	46.0453	15.2153	750
S2	Catez	upper	137	45.8904	15.6301	736
S3	Zagreb	upper	110	45.7569	16.0457	640
S9	Jasenovac	lower	87	45.2637	16.8943	500
S4	Sr. Mitrovica	lower	72	44.9136	19.7525	118
S5	Sabac	lower	71	44.7699	19.6994	106
S6	Belgrade	lower	69	44.7685	20.3556	14

Figure 1. Sava River Basin sampling sites.

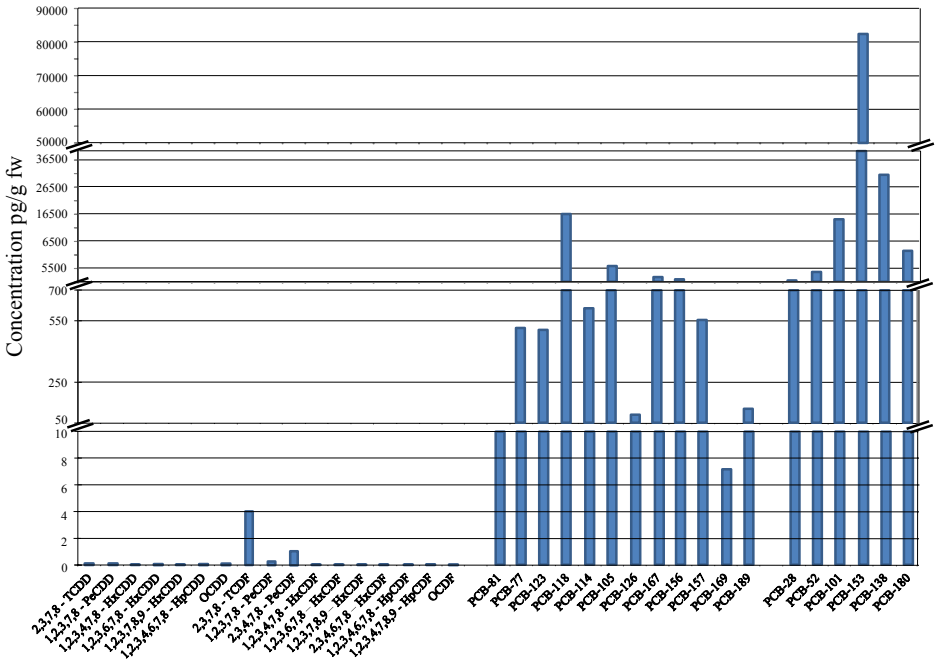

Figure 2. Congener distribution of PCDDs/PCDFs and PCBs for S6.

Table 1. Concentrations of individual PCDD/F and PCB congeners (pg/g fw), as well as WHO-TEQ values (upperbound) (pg WHO-TEQ/g fw) and ∑NDL-PCB (ng/g fw) in fish samples.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Code	MOJ	CAT	ZAG	SRM2	SAB1	BEO	RAD	VRH	JAS	LIT2
Species	*Onchorhyn-chus mykiss*	*Barbus barbus*	*Barbus barbus*	*Barbus barbus*	*Barbus barbus*	*Squalius cephalus*	*Onchorhyn-chus mykiss*	*Squalius cephalus*	*Squalius cephalus*	*Squalius cephalus*
Mean length (cm)	31	12	15	33	42	29	24	16	23	16
Mean weight (g)	320	28	55	530	568	555	154	52	145	58
Compounds										
2,3,7,8 - TCDD	<0.002	0.03	0.05	0.14	0.20	0.07	0.10	0.03	0.06	0.01
1,2,3,7,8 - PeCDD	0.01	0.04	0.06	0.16	0.19	0.09	0.04	0.02	0.06	0.01
1,2,3,4,7,8 - HxCDD	<0.003	0.02	0.02	0.03	0.03	0.03	0.01	<0.01	0.02	<0.004
1,2,3,6,7,8 - HxCDD	0.01	0.04	0.04	0.08	0.07	0.05	0.02	0.01	0.03	<0.004
1,2,3,7,8,9 - HxCDD	0.005	0.03	0.02	0.03	0.03	0.03	0.01	<0.01	0.02	<0.004
1,2,3,4,6,7,8 - HpCDD	0.01	0.12	0.12	0.07	0.07	0.07	0.04	0.04	0.05	0.01
OCDD	0.11	0.35	0.40	0.10	0.13	0.13	0.32	0.15	0.14	0.06
2,3,7,8 - TCDF	0.07	0.72	1.00	4.04	5.3	1.7	0.40	0.27	1.1	0.20
1,2,3,7,8 - PeCDF	0.01	0.05	0.08	0.26	0.33	0.16	0.04	0.01	0.06	0.01
2,3,4,7,8 - PeCDF	0.02	0.10	0.22	1.00	1.09	0.41	0.11	0.04	0.18	0.02
1,2,3,4,7,8 - HxCDF	<0.004	0.01	0.03	0.04	0.05	0.05	0.01	<0.01	0.01	0.003
1,2,3,6,7,8 – HxCDF	<0.004	0.02	0.02	0.05	0.06	0.04	0.01	0.01	0.02	0.003
1,2,3,7,8,9 – HxCDF	<0.004	0.02	0.03	0.03	0.04	0.05	0.01	<0.01	0.01	0.003
2,3,4,6,7,8 – HxCDF	<0.004	0.003	0.004	0.004	<0.007	0.005	<0.004	<0.01	<0.01	0.01
1,2,3,4,6,7,8 - HpCDF	0.006	0.02	0.04	0.02	0.02	0.03	0.01	0.03	0.04	0.01
1,2,3,4,7,8,9 - HpCDF	<0.004	<0.003	<0.004	<0.004	<0.003	<0.005	<0.01	<0.01	<0.01	<0.005
OCDF	0.009	0.02	0.01	0.004	0.01	0.01	0.01	0.01	<0.01	<0.003
$WHO\text{-}TEQ_{PCDD/F}$	0.03	0.19	0.30	1.0	1.3	0.48	0.22	0.09	0.31	0.04
PCB-81	<0.43	3.0	5.2	33	19	41	1.8	6.5	6.0	2.0
PCB-77	3.2	78	140	519	564	602	41	149	165	57
PCB-123	4.2	26.1	58	510	329	94	9.5	88	68	26
PCB-118	197	1601	3860	27197	16501	5128	735	5100	3980	1968
PCB-114	5.0	47	98	612	400	132	16	136	96	35
PCB-105	67	590	1493	7779	5481	1831	213	1727	1384	662
PCB-126	1.8	5.6	15	91	60	23	3.2	10.9	12	4.2
PCB-167	21	114	339	2962	1535	510	43	415	273	106
PCB-156	34	225	627	2654	1667	674	80	742	436	184
PCB-157	7.5	44	112	549	342	134	16	130	94	41
PCB-169	0.3	0.6	1.5	7.1	4.1	1.9	0.5	1.3	<0.96	0.4
PCB-189	3.3	16	62	113	78	55	6.6	57	36	11
$WHO\text{-}TEQ_{DL\text{-}PCB}$	0.19	0.66	1.8	10.6	7.0	2.7	0.37	1.40	1.46	0.53
$WHO\text{-}TEQ_{PCDD/F+DL\text{-}PCB}$	0.22	0.86	2.1	11.7	8.3	3.1	0.60	1.50	1.76	0.57
PCB-28	72	1644	2226	1980	5060	4418	906	2295	1228	456
PCB-52	110	1215	2211	4887	9690	8630	617	1358	2230	503
PCB-101	260	1812	5089	24290	24716	6456	773	3279	4979	1229
PCB-153	834	2373	8996	82482	52043	14464	1190	6248	6823	1711
PCB-138	512	1770	7099	41093	31336	9619	822	4744	4990	1326
PCB-180	259	849	3798	13108	9240	4024	474	2474	2145	411
∑NDL-PCB (ng/g fw)	2.0	9.7	29	168	132	48	4.8	20	22	5.6