POLYCHLORBIPHENILS IN WILD TROUTS (*Salmo trutta fario)*: PRELIMINARY RESULTS

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Introduction

Polychlorobiphenyls (PCB) are persistent chlorinated organic contaminants which use was suspended in the all west countries since the late seventies. As a result of their chemical and physical stability is possible to find them widespread in the environment. All the aquatic systems are of big interest since the PCB tend accumulate in aquatic organisms. While data are available on the contamination from PCB of bred trout of the Italian region Marche[1], since they are sampled and analysed in the frame of national monitoring plans, none or few information were found on wild species. To know the levels and pattern of contamination of wild trout can be extremely important both to control the fish safety as food and to use the same data to monitor the environment contamination The study of wild animals in general can give a big help in the assessment of the environment health, therefore is was decided to conduct a preliminary survey to try to understand the situation of the Marche area. At first only the fish coming from fresh water courses were taken into account and in August 2005 and January 2006 trout from the *Salmo trutta fario* species were sampled from rivers and torrents homogeneously distributed in the whole region.

The samples were analysed to determine the concentrations of PCBs 18-congeners (T_3CB-28, T_4CB-52, P_5CB-95, P_5CB-99, P_5CB-101, P_5CB-105, P_5CB-110, P_5CB-118, H_6CB-138, H_6CB-146, H_6CB-149, H_6CB-151, H_6CB-153, H_7CB-170, H_7CB-177, H_7CB-180, H_7CB-183, H_7CB-187)[2] together with their congener profiles, since both can give important information. The contamination levels can identify areas where to find point source of PCB due to industrial activities or uncontrolled waste damping, while the accumulation profile could help to understand the time of exposure (recent or remote) and, if recent, to trace the source of the contamination.

Materials and Methods

Sampling

The samples of trout pertaining to the *Salmo trutta fario* species were collected in rivers and torrents of the region Marche. The specimens were killed by the mean of electric shock. A preliminary survey took place in August 2005 and January 2006 involving the geographic areas identified in Figure 1.

At the arrival, the length an the weight of the single trout were measured and it was decided whether to analyse the single specimen or to form a pool of fishes comparable in size in order to have enough material to form a lab sample. The fish was open and the bones, organs and skin removed paying attention to collect all the subcutaneous fat layer. Roughly 100 g of sample were finely homogenized and stocked at –20 °C.

Analysis

The analytical method is detailed described elsewhere [3]. Brifely, 30 g of fishes muscle were weighted on a petri glass plate, frozen at –80 °C overnight and submitted to freeze drying (4 hrs). 5 g of Hydromatix (dispersing agent) were added to the dried minced material and the whole was loaded in the ASE (Accelerated Solvent Extractor) cell and submitted to pressurised extraction with a mixture of hexane/acetone (1:1 v/v). The fat obtained was spiked with two internal standards (25 ng for each standard: H_6CB-155 and O_8CB-198) and cleaned-up on Extrelut NT-3 column acidified with 3 mL of concentrated sulfuric acid connected on top of a silica cartridge. The analytes were then eluted with 13 mL *n*-Hexane and the solvent removed with nitrogen stream. The sample was re-dissolved in 0.5 mL *iso*-octane and injected in a GC-μECD. The results obtained were checked by analysing the extract using GC-MS.

Results and Discussion

Thirty one samples of *Salmo trutta fario* were analysed either singularly or in pools (two pools of 2 trouts each respectively) forming altogether twenty nine lab samples. Fifteen trouts were caught in the Pesaro district, seven in Ascoli Piceno and nine in Ancona.

Figure 1: Sampling Sites

District	City	Sampling Site	Number of sample
Ancona	Sassoferrato-S.Emiliano abbey	Sentino torrent	4
	Sassoferrato-Molino Malcotti	Sentino torrent	4
	Serra SanQuirico	Esino river	1
Pesaro	Casteldelci	Petroso torrent	1
	Carpegna	Mutino torrent	1
	Piobbico	Vitoschio torrent	5
	Cantiano	Burano river	4
	Cantiano	Bevano torrent	4
Ascoli Piceno	Amandola	Tenna river	3
	Acquasanta Terme	Tronto river	4

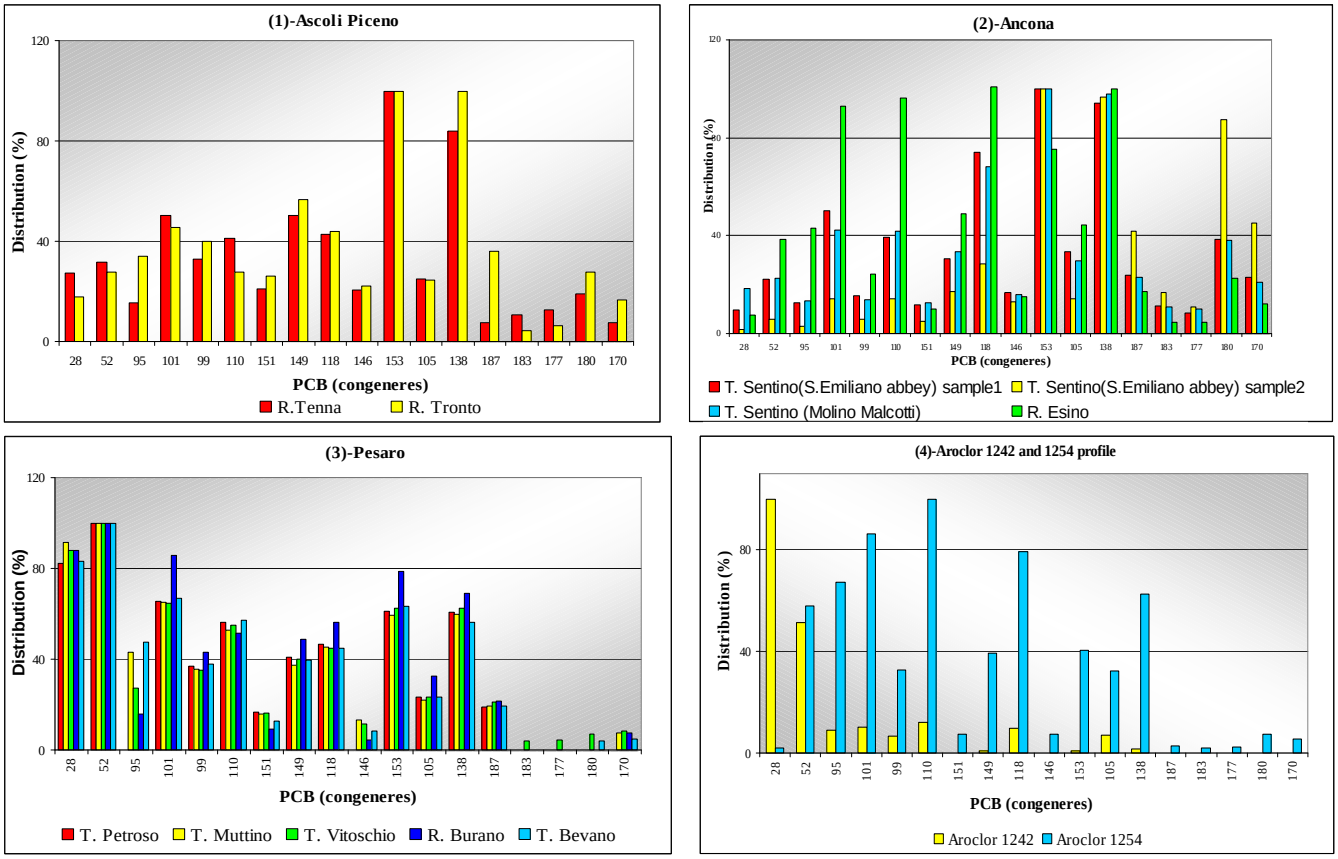
Table 1 shows the length, the weight and the lipid content of all the sample analysed together with the concentration (ng/g fresh weight) found for the PCB 18 congeners. For same rivers or torrents are reported the average values, since the analytical determinations performed on the single specimen gave comparable results.
Although the trouts coming from the Pesaro area are the smaller in size with weight between 109 - 158 g and length ranging from 18 to 23 cm, they have the higher lipid content (from 5.66% to 6.07%), while the samples from Ancona are larger (length: 27-38 cm ; weight 188-550 g) with a lower lipid content (0.74%-1.93%). Even if the *Salmo trutta* specimens caught in the Ascoli Piceno district have all similar length and weight (the average values of 28 cm for the length and weight of 254 and 261 g for Tronto and Tenna respectively), it is possible to highlight differences in contamination level (ΣPCB 2.08 *vs* 7.79 ng/g f.w. respectively) and fat content (2.29 *vs* 4.45 % respectively) between samples coming from the River Tronto and Tenna being the latter more contaminated.
The total PCB (ΣPCB) concentration of all the sample analysed was between 2.08 e 7.79 ng/g fresh weight (ng/g f.w.) not considering two very high values obtained in one sample of the Sentino Torrent and in an other trout caught in the Esino River for which 21.56 and 23.97 ng/g f.w. were obtained respectively. Nevertheless these two specimens were the larger in size with length of 32 cm for the first and 38 for the second and weight of 376 and 550 g respectively, eventually denoting a longer life. With the exception of the two latter samples, the results obtained are comparable both to the concentration found in S*almo trutta* species caught in European rivers[4] and to the contamination level of trout bred in Marche and Umbria Regions and analyzed in the frame of the National Residue Control Plan during 2001-2004 (average concentration level 7.63±9.46 ng/g f.w. for the ΣPCB)[1].
The bar graphs reported in Figure 2 describes the pattern distribution of the PCB 18-congeners in trout from the different sampling sites. In the ones coming from the Ascoli Piceno district the H_6CB-138 and H_6CB-153 take account of most of the contamination followed by P_5CB-101, H_6CB-149, P_5CB-118, according to patterns already reported for biological sample [4,5].
A similar pattern is found in sample from Ancona district caught in Sentino torrent, but not in trout from Esino river, where the abundance of P_5CB-118 is comparable with that H_6CB-138 and higher then H_6CB-153.
Despite the low concentration of total PCB, unusual is also the pattern distribution of the 18-congeners in trout caught in Pesaro district, where the more abundant are the T_3CB-28 and T_4CB-52 followed by P_5CB-101, H_6CB-138 and P_5CB-110. This particular pattern is not comparable with those of other samples and with patterns reported in literature while it is possible to find similarity with the distribution of the 18 congeners in some trade mixture of PCBs like Arochlor 1242, Arochlor 1254 showed in Figure 2-(4)[6].

Table 1: Length, weight, lipid content and PCB 18-congeners **c**oncentrations (ng/g fresh weight) in *Salmo trutta fario* specimens caught in rivers (R) and Torrents (T) of the Marche region.

District	Pesaro					Ascoli Piceno		Ancona			
Sampling site	T. Petroso	T. Muttino	T. Vitoschio	R. Burano	T. Bevano	R.Tenna	R.Tronto	T. Sentino (S.Emiliano abbey)		T. Sentino (MolinoMalcotti)	R. Esino
			Average (a.s.=3)[1]	Average (n=4)[2]	Average (n=4)[2]	Average (n=3)[2]	Average (n=4)[2]	SAMPLE 1 Average (n=3)[2]	SAMPLE 2	Average (n=4)[2]	
Fish weight (g)	127	109	124	158	155	261	254	188	376	200	550
Fish length (cm)	21	18	19	22	23	28	28	26	32	27	38
% fat	6.07	5.95	5.66	5.95	5.84	4.45	2.29	1.48	1.93	1.34	0.74
	ng/g fresh weight										
28	0.59	0.96	0.78	0.54	0.68	0.36	0.06	0.07	0.07	0.14	0.23
52	0.72	1.05	0.88	0.62	0.81	0.41	0.09	0.15	0.24	0.18	1.22
95	N.D.	0.45	0.24	0.10	0.39	0.20	0.11	0.09	0.13	0.10	1.36
101	0.47	0.69	0.57	0.53	0.54	0.65	0.14	0.34	0.59	0.33	2.94
99	0.26	0.38	0.31	0.27	0.31	0.43	0.13	0.11	0.25	0.11	0.77
110	0.40	0.56	0.49	0.32	0.46	0.54	0.09	0.27	0.58	0.33	3.04
151	0.12	0.16	0.14	0.06	0.10	0.27	0.08	0.08	0.21	0.10	0.31
149	0.29	0.39	0.35	0.30	0.32	0.65	0.18	0.21	0.70	0.26	1.55
118	0.33	0.48	0.39	0.35	0.36	0.55	0.14	0.51	1.17	0.53	3.19
146	N.D.	0.14	0.10	0.03	0.07	0.27	0.07	0.12	0.53	0.12	0.48
153	0.44	0.63	0.55	0.49	0.51	1.30	0.32	0.68	4.14	0.78	2.38
105	0.17	0.23	0.20	0.20	0.19	0.32	0.08	0.23	0.59	0.23	1.41
138	0.43	0.63	0.55	0.43	0.46	1.09	0.32	0.64	4.00	0.77	3.16
187	0.13	0.20	0.19	0.13	0.16	0.10	0.11	0.16	1.73	0.18	0.54
183	N.D.	N.D.	0.03	N.D.	N.D.	0.14	0.01	0.08	0.69	0.09	0.15
177	N.D.	N.D.	0.04	N.D.	N.D.	0.16	0.02	0.06	0.44	0.08	0.15
180	N.D.	N.D.	0.06	N.D.	0.03	0.25	0.09	0.26	3.62	0.30	0.71
170	N.D.	0.08	0.07	0,05	0.04	0.10	0.05	0.16	1.86	0.16	0.38
PCB tot S18 cong	4.36	7.03	5.96	4.40	5.44	7.79	2.08	4.21	21.56	4.79	23.,97

[1]: The results reported are the average of **3** analitycal sample (one with one single trout and the other two composed by 2 specimens each of comparable size).
[2]: The results reported are the average of **n** single determinations performed on **n** individual sample

Figure 2: Normalized bar graph of PCB 18-congeners in specimens from (1) Ascoli Piceno, (2) Ancona, (3) Pesaro. (4) Normalized bar graph of PCB 18-congeneres in Aroclor 1242 and 1254.



Conclusions

Preliminary results on the PCB (18–congeners) contamination levels and on patterns of trout from the specie S*almo trutta fario* caught in river and torrents of the Italian region Marche are reported. In most cases the levels of PCB residues found in the trout analysed are of the same order as those observed in other European fresh water courses. Generally the patterns are comparable with those reported in the literature with the H_6CB-138 and H_6CB-153 accounting for most of the contamination. Different are the PCB congener profile of all the specimens caught in the Pesaro district which looks a bit like the Arochlor 1242 and Arochlor 1254. Surely more data are needed to be able to ascertain the validity of this trend for the Pesaro fresh water courses.

References

1. Piersanti A, Fioroni L, Orletti R,. Paoloni A, Pecorelli I, Tavoloni T, Galarini R VI Congresso Nazionale S.I.Di.L.V., Conference proceedings 2004.
2. La Rocca C, Baldassarri LT, Quattrocchi W, Iacovella N. *Rapporti ISTISAN 02/38.*
3. Piersanti A, Fioroni L, Paoloni A, Tavoloni T, Pecorelli I, Galarini R. *Organohalogen Compouns* 2004; 66: 483.
4. Bordajandi L.R., Gòmez G., Fernàndez M.A., Abad E., Rivera J., Gonzàles M.J. *Chemosphere* 2003; 53: 163.
5. Bayarri S, Baldassarri L T, Iacovella N, Ferrara F, Di Domenico A. *Chemosphere* 2001; 43: 601.
6. http://www.epa.gov/toxteam/pcbid/aroclor_comp.htm