

POLYCHLOROBIPHENYL (18 CONGENERS) IN MUSSELS FROM MIDDLE ADRIATIC SEA

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Introduction

Humans can be exposed to persistent organic pollutants in several ways, dietary intake surely counting for more than 90% of the daily exposure¹. Among food, fish products in general and mussels in particular can be significant sources of dietary exposure to polychlorinated biphenyls (PCBs), in particular if coming from contaminated areas. Mussels can in fact concentrate chemicals reaching levels up to 10²-10⁵ times higher than the water they live in, as a result of their ability to filter large volumes of water and remove suspended particles with diameters between 1 and 250 µm².

Since Adriatic Sea is an area with a wide production of *Mytilus Galloprovincialis* (wild or farmed) and since they are stationary species, the investigation on the PCBs levels in these organisms could provide important information both on the status of the marine environment and on consumers' exposure to pollutants. Moreover, while several data are available on Persistent Organic Pollutants (POPs) in mussels coming from the Venice lagoon area, only a few papers report on the PCBs concentration in specimen from Middle Adriatic Sea³.

In this study, mussels collected for human consumption along the coast of the Marche region (Italy) and send to our laboratory to be controlled for microbiological, biotocological and chemical parameters in the frame of the D. Legs 530/1992 were analysed to determine the concentrations of PCBs 18-congeners (T₃CB-28, T₄CB-52, P₅CB-95, P₅CB-99, P₅CB-101, P₅CB-105, P₅CB-110, P₅CB-118, H₆CB-138, H₆CB-146, H₆CB-149, H₆CB-151, H₆CB-153, H₇CB-170, H₇CB-177, H₇CB-180, H₇CB-183, H₇CB-187)⁴. The samplings were carried out in different coastal sites either on wild or bred mussels during four different periods (spring, summer, autumn and winter 2003-2004).

Finally the analytical results were analysed in order to assess significant differences in contamination between sites, seasons and sources (wild/farmed).

Materials and Methods

Sampling

Sixteen areas were identified along the coast of Marche where to collected samples. They include 12 reefs where mussels grow naturally (wild sides) and 4 areas, roughly 1.5 - 2 miles away from the coast, where breeding plants (breeding sites) are located. In figure 1 is reported the map of Marche where the wild and the breeding sites are identified. The sampling campaign started in May 2003 and went on for one whole year until March 2004. The mussels were collected every three months from each site: 1) May-June 2003; 2) August-September 2003; 3) November-December 2003; 4) February-March 2004.

Within 24 hours from the arrival, the sample was prepared to be frozen (-20°C) in the following way: the bivalve mussels were opened, the sand and solid residues were removed under running water, the mussels removed from the shells and drained on a net. About 200 g of sample were finely homogenised and stoked at -20°C.

Analysis

The analytical method was described in detail elsewhere⁵. Briefly, 25g of sample were weighed on a petri glass plate, frozen at -80 °C overnight and submitted to freeze drying (13 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 after removing the solvents was cleaned-up after the addition of the two internal standards (25 ng for each standard: H₆CB-155 and O₈CB-198). The fat solution was loaded on an Extrelut NT-3 column acidified with 3 mL of concentrated sulfuric acid and connected on top of a silica cartridge. After ten minutes, the analytes were eluted

Levels in feed and food

with 13 mL *n*-Hexane. The solvent was removed with a nitrogen stream, the sample re-dissolved in 0.5 mL *iso*-octane and injected in a GC- μ ECD. The results obtained were checked by analysing the extract using GC-MS. Analysis of variance (ANOVA) was used to assess significant differences ($p < 0.05$) in contamination (sum of 18 congeners, Σ PCBs) among monitoring sites, seasons and sources.

Figure 1: Sampling Sites

	Sampling Sites		
	Breedings	Wild	
PESARO	1	Sotto la Croce	
		Baia Vallugola	
	2	Fiorenzuola sud	
		I palo da Pesaro	
ANCONA	3	Senigallia	
		Passetto	
	Pietrala croce		
	Vedova		
	Trave		
	Portonovo		
	Vela		
	Le Due sorelle		
San Benedetto del Tronto	4	Sassi Neri	

Results and Discussion

Fifty samples of *Mytilus Galloprovincialis* were analysed, 38 from wild sites and 12 from breeding ones. The wild and bred samples were, respectively, 12 and 3 in spring, 11 and 2 in summer, 6 and 3 in autumn, and 9 and 4 in winter.

Table 1 summarises the concentrations (ng/g fresh weight) found for the PCB 18 congeners in the mussel samples analysed. The analytical results are divided in two groups: wild and bred samples. For the first ones, results for each sampling period (spring, summer, autumn and winter) in each limited geographic area (Pesaro and Ancona) are reported as average values, while for the samples from breeding plants the results of the single determination are shown.

The PCB concentration varies between 3.17 and 16.48 ng/g fresh weight with an exceeding value of 50.15 ng/g obtained in one sample coming from an Ancona farming plant. The determined concentrations are comparable with the data reported in the literature for the Middle Adriatic Sea³. The pattern distribution of the PCB 18-congeners (Figure 2) shows that the H₆CB-138 and H₆CB-153 account for most of the contamination, followed by P₅CB-101, H₆CB-149, H₇CB-187.

The results reported in Table 2 show that there are no significant differences in the contaminant concentration levels between monitoring sites (Ancona, Pesaro and S. Benedetto del Tronto). Regarding the sampling period, the PCB concentration has the following trend: winter > summer > spring > autumn, with the samples collected during the winter significantly more contaminated than those collected in spring and autumn. Finally, the PCB concentrations determined in wild mussels are significantly higher than in the farmed ones.

Reasons for the lower contamination of the bred mussels as compared to the wild ones could be found mainly in the location of the breeding plants. These plants are further away from the antropogenic sources of PCBs than the wild reefs located on the coast. In open water, the breeding mussels are subjected to the main Adriatic stream moving from north to south, that guarantees a larger exchange of fresh water. Secondly, reasons can be found in collecting time. Bred mussels are in fact grown for a well defined and shorter period of time (the time necessary to become big enough to be commercialized: roughly six months) respect to the wild ones, thereby accumulating less contaminants.

Table 1: PCB 18-congeners concentrations (ng/g fresh weight) in mussel samples coming from wild and breeding sites. Mussels were collected in four sampling period (I spring, II summer, III autumn and IV winter) in three geographic areas (Pesaro, Ancona and San Benedetto del Tronto).

	WILD SITES							BREEDING SITES											
Sampling	I	II	III	IV	I	II	IV	I	III	IV	III	IV	I	II	III	IV	I	II	IV
	ANCONA				PESARO			PESARO					ANCONA				S. Benedetto del Tronto		
Sampling Sites	n=8*	n=7*	n=6*	n=5*	n=4*	n=4*	n=4*	1			2		3				4		
%FAT	1.0	1.0	1.1	1.6	0.7	1.0	2.0	1.8	2.1	2.9	1.9	1.1	1.5	1.6	1.7	2.5	1.6	1.4	1.2
	Average values (ng/g fresh weight)							ng/g fresh weight											
28	0.05	0.04	0.08	0.15	0.05	0.06	0.16	0.06	0.03	0.18	0.08	0.02	0.06	0.05	0.17	0.10	0.08	0.09	0.05
52	0.22	0.20	0.21	0.48	0.18	0.17	0.52	0.11	0.14	0.60	0.35	0.11	0.16	0.16	1.38	0.34	0.16	0.26	0.18
95	0.38	0.35	0.32	0.84	0.33	0.26	1.00	0.12	0.32	1.22	0.62	0.07	0.20	0.36	2.18	0.76	0.20	0.37	0.31
101	0.86	0.80	0.61	1.24	0.73	0.61	1.13	0.31	0.40	1.46	0.96	0.19	0.48	0.57	4.23	0.81	0.50	0.95	0.56
99	0.47	0.45	0.35	0.65	0.31	0.36	0.58	0.20	0.30	0.75	0.51	0.07	0.34	0.38	3.53	0.54	0.24	0.48	0.52
110	0.60	0.61	0.52	0.99	0.48	0.44	0.92	0.10	0.31	1.15	0.74	0.16	0.37	0.43	3.38	0.71	0.38	0.63	0.45
151	0.40	0.39	0.27	0.54	0.37	0.32	0.50	0.16	0.29	0.66	0.48	0.12	0.22	0.34	1.52	0.38	0.24	0.42	0.27
149	0.99	0.92	0.73	1.44	0.85	0.80	1.26	0.32	0.58	1.74	1.22	0.21	0.51	0.75	4.32	0.93	0.52	0.92	0.65
118	0.62	0.68	0.43	0.72	0.46	0.45	0.53	0.19	0.25	0.68	0.45	0.10	0.35	0.44	3.75	0.44	0.38	0.60	0.46
146	0.40	0.43	0.26	0.40	0.34	0.38	0.33	0.13	0.29	0.46	0.37	0.11	0.24	0.41	1.56	0.28	0.25	0.44	0.31
153	2.15	2.44	1.40	2.18	1.92	2.12	1.82	0.58	1.13	2.39	1.92	0.50	1.02	1.73	10.45	1.47	1.24	1.91	1.35
105	0.19	0.21	0.13	0.20	0.12	0.11	0.14	0.06	0.07	0.16	0.12	0.04	0.11	0.13	0.91	0.00	0.12	0.21	0.14
138	1.74	1.89	1.15	1.87	1.48	1.55	1.48	0.39	0.85	2.06	1.61	0.37	0.81	1.39	6.50	1.21	0.97	1.67	1.03
187	0.77	0.83	0.58	0.94	0.65	0.75	0.88	0.20	0.40	1.23	0.79	0.22	0.39	0.70	3.52	0.78	0.42	0.66	0.57
183	0.28	0.25	0.17	0.33	0.26	0.28	0.32	0.07	0.18	0.45	0.30	0.09	0.12	0.22	1.00	0.27	0.15	0.26	0.19
177	0.28	0.28	0.21	0.33	0.25	0.21	0.19	0.10	0.21	0.43	0.30	0.10	0.13	0.23	0.68	0.26	0.17	0.12	0.20
180	0.07	0.09	0.15	0.27	0.05	0.08	0.14	0.04	0.12	0.74	0.32	0.06	0.04	0.07	0.81	0.25	0.05	0.12	0.10
170	0.06	0.07	0.06	0.12	0.04	0.05	0.11	0.02	0.07	0.14	0.09	0.05	0.01	0.03	0.26	0.05	0.02	0.07	0.06
PCB tot Σ18 Cong	10.52	10.94	7.62	13.70	8.86	8.99	12.01	3.17	5.95	16.48	11.24	2.61	5.55	8.39	50.15	9.58	6.08	10.18	7.41

* The results reported are the averages of **n** single determinations performed on **n** individual samples collected in **n** different sites of the same geographic area.

Figure 2: Normalized bar graph of PCB 18-congeners measured in wild and farmed mussels collected along the Marche coast.

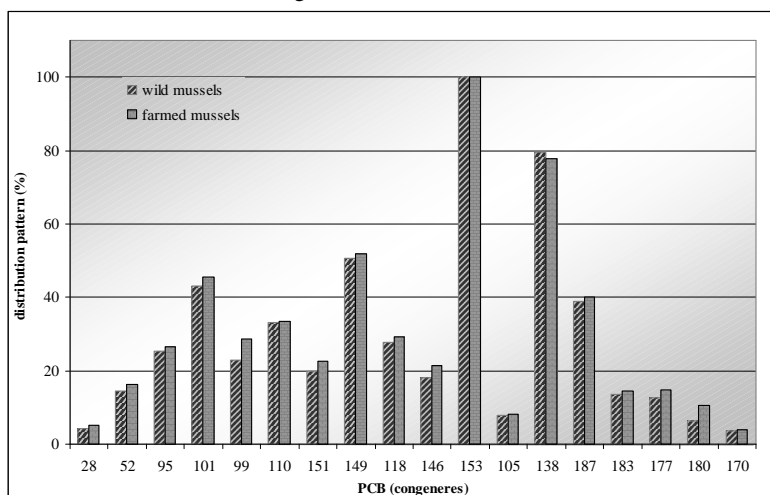


Table 2: Mean contents of PCBs in mussels sampled in different sites, seasons and source (lower and upper 95% confidence interval limits are given within brackets)

SITES	ΣPCBs (ng/g fresh weight)
PESARO	9.34 (7.98-10.71)
ANCONA	10.29 (9.24-11.33)
S.BENEDETTO	7.89 (4.64-11.14)
SEASONS	ΣPCBs (ng/g fresh weight) ¹
I (spring)	8.96 (7.63-10.29) b
II (summer)	10.08 (8.66-11.51) ba
III (autumn)	7.86 (6.04-9.68) b
IV (winter)	11.74 (10.31-13.16) a
SOURCE	ΣPCBs (ng/g fresh weight) ¹
WILD	10.38 (9.51-11.24) a
FARM	7.87 (6.27-9.48) b

¹ Different letters indicate significant differences between rows (p<0.05)

Conclusions

The present work surely contributes to extend the knowledge on PCB contamination of *Mytilus Galloprovincialis* along the Italian coast of the Middle Adriatic Sea and seems to show that bred mussels are less contaminated than the wild ones, collected on natural reefs. The latter results needs more investigations to be able to ascertain the general validity of this trend, at least in mussels bred on the Marche coast, also because of the eventual commercial implications.

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