ANALYSIS OF PBDEs, DL-PCBs AND PCCD/Ds IN CAGED MUSSELS IN THE WESTERN MEDITERRANEAN SEA. *MYTILOS PROJECT*

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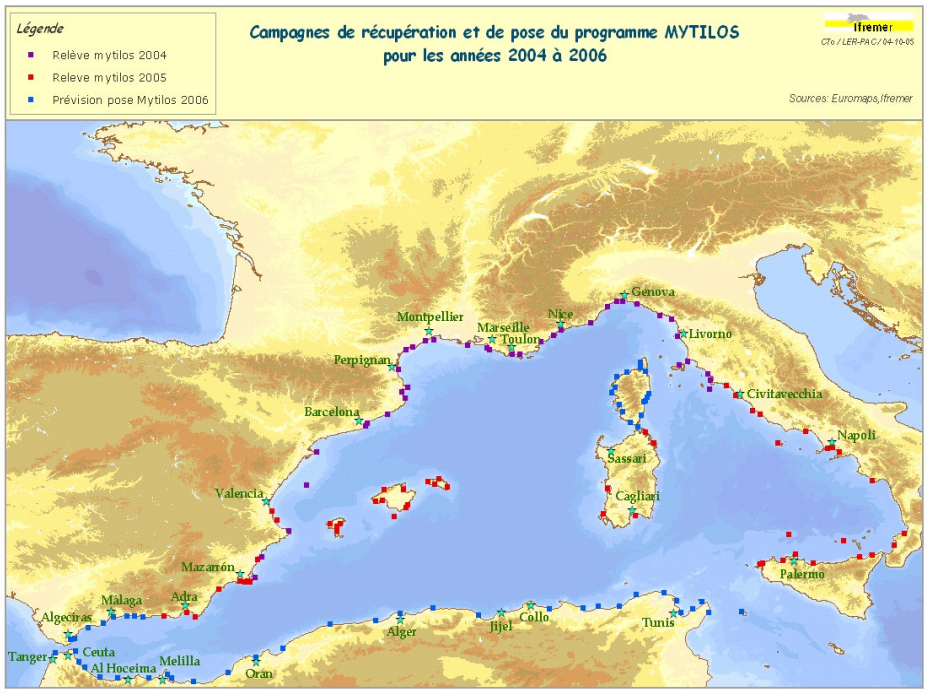
Abstract

In the frame of *Interreg IIIB, Medocc -Mytilos Project*, during 2004-2006, 33 stations of caged mussels were immersed, recovered and analysed, performing this way a network that covers all occidental Mediterranean coasts. The target pollutants were PBDEs, DL-PCBs, PCDD/Fs and others. These analyses were performed following EPA 1614, 1668 and 1613 methods using HRGC/HRMS techniques and isotope dilution. The results obtained were lowest than expected and more than 50 % of concentrations of pollutants obtained are low or below of detection limits. Then, total PBDEs levels were ranged between 0.051-3.15 ng/g (wet weight), DL-PCBs 0.014-2.53 pg/g WHO-TEQ and PCCD/Fs between 0.023-1.56 pg/g WHO-TEQ.

Introduction

The purpose of the Mytilos project is the development of an interregional costal water quality monitoring network through biological integrators (mussels), for the sustainable protection of the Western Mediterranean Sea. This 3-year project was launched in January 2004 and is an integral part of the INTERREG III B MEDOCC programme. Member countries associated with this project are France, Italy, Greece, Spain; and North African countries as Algeria, Morocco and Tunisia. The total Mytilos budget is €1,516,246.67, of which €800,000 are funded by the European Regional Development Fund.

Figure 1. Map of stations



The Mytilos project aims at obtaining an image of the chemical contamination in the Western Mediterranean Sea (Figure 1). To this end, caged mussels will be used as biological indicators of the contamination. The cages are

sampled and analysed by the project partners in order to search different types of contaminants such as polybrominated diphenyl ethers (PBDEs), *dioxin-like* polychlorinated biphenyls (DL-PCBs) and dioxins and furans (PCCD/Fs), and others (like PAHs, DDT, HCH, heavy metals, etc.).

This study shows the results of PBDEs, DL-PCBs and PCDD/Fs of 33 stations immersed in Spanish, France, Italy, Morocco, Algeria and Tunisia Coast obtained by our laboratory. In all countries the selected stations for analyse were one hot spot of region (in front of main populated cities or harbour areas) and one pristine area (like natural reserves).

Materials and Methods

The use of caged mussels

The bio-monitoring method is based on the ability of the mussel to concentrate chemical contaminants in its tissues (bio-accumulation) in relation to their presence in the environment. In addition, the *Mytilus galloprovincialis* family mussel has been selected because it meets several requirements as well documented physiology and bio-accumulation, large distribution area in the Mediterranean Sea, easy supply, and tolerance to a wide range of temperatures and salinity and to dehydration (ease of transportation).

An active biomonitoring caging method has been used in order to avoid the low natural stock of seashells in most of the Western Mediterranean sea line, as well as to avoid the possible cage depth differences. This method has been developed by Ifremer in the context of a biological integrators network[1]. This method involves the immersion of artificial cages containing a homogeneous quantity of mussels in the sites previously selected by the partners during 3 months. The immersion takes place when the mussels are sexually inactive. The selected sites have to be between 15 and 60 metres deep, and the cages are placed 7 metres deep. Thanks to an accurate positioning and sonnar, a diver can collect the cages.

Analytical methodology: Sample preparation, extraction and clean-up

The analyses of pollutants were carried out according different US-EPA methods. For the analysis of PBDEs was used US-EPA 1614 method, for dioxin-like PCBs was used US-EPA 1668 and for dioxins and furans US-EPA 1613, all based in the employ of High Resolution Gas Chromatography coupled to High Resolution Mass Spectrometry (HRGC/HRMS).

PBDEs and DL-PCBs. 10 grams of wet mussel were homogenized with anhydrous sodium sulphate (heated at 150ºC for 15 h), spiked with known mixture of $^{13}C_{12}$-PBDEs and $^{13}C_{12}$-PCBs and extracted by Soxhlet with hexane: dichloromethane (1:1) for 24 h. The extract obtained was concentrated with a nitrogen current to 4 ml. Clean up is performed with a chromatographic column packed with Florisil® (heated at 450ºC during 15 hours) and eluted with a mixture of hexane/ dichloromethane (85:15). The final extract was concentrated to 100 µl using current of nitrogen and spiked with $^{13}C_{12}$-PCBs as internal standards in order to calculate the recovery of labelled standards added at start of process.

PCCD/Fs. Samples were freeze-dried and homogenized prior to analysis. The different specimen pools and tissue samples were then spiked with known amounts of a mixture of 13C12-PCDD/Fs. They were then extracted for 24 h in a Soxhlet containing toluene:cyclohexane (1:1). Thereafter, the extracts were rotary evaporated and transferred to 100mL n-hexane. In order to remove any organic components, including fats and other unwanted substances, the n-hexane extracts were subsequently treated with sulphuric acid. Finally, the extracts were rotary concentrated and filtered prior to the cleaning process. An automated Power PrepTM system (FMS Inc.,MA, USA) was employed for cleaning. This system involves the use of multilayer silica, basic alumina and PX-21 carbon adsorbents, pre-packaged in columns made of Teflon and hermetically sealed.[2]

DL-PCBs. Instrumental analysis of DL-PCBs was carried out by HRGC-HRMS on GC 8000 series (Carlo Erba Instruments, Milan , Italy) coupled to an Autospec Ultima mass spectrometer (*Micromass*, Manchester, U.K.)

equipped with a CTC A200S autosampler. Acquisition mode SIM and minimum static resolution used were 10.000 (10% definition valley).[3]
The injection mode was split less and carrier gas used was helium. The chromatographic separation was achieved with capillary column DB-5 (J&W Scientific, Folsom, CA, USA) fused–silica (60 m x 0.25 mm ID, 0.25 µm film thicknesses). Quantification was carried out by isotopic dilution method and using OPUS QUAN software.

PBDEs. .The instrumental analysis of PBDEs was performed using the same instruments used in the case of DL-PCBs analysis. The chromatographic separation was achieved with a capillary column HT5 (SGE, Melbourne, Australia) 5 % phenyl -polycarbonare-siloxane (15 m 0.25 mm ID and 0.1 µm film thicknesses). The acquisition mode was SIR and electron ionization was 35 eV.

PCDD/Fs. Instrumental analysis was carried out by HRGCHRMS on a HP6890N Network GC System (Agilent Technologies, USA) coupled to an Autospec Ultima NT mass spectrometer double focussing magnet sector (Waters, UK), using a positive electron ionization (EI+) source and operating in SIM mode. Chromatographic separation was achieved with a DB-5MS (J&W Scientific, Folsom, CA, USA) fused–silica (60 m x 0.25 mm ID, 0.25 µm film thicknesses). Quantification was carried out by the isotopic dilution method.[3]

Results and Discussion

Table 1 summarizes all the results obtained. The total of PBDEs is the sum of BDEs nº IUPAC 47, 99, 100, 153, 154 and 209 and is expressed in ng/g of wet weight. For DL-PCBs (coplanars PCBs nº IUPAc 77, 81, 126 and 169 and monoorto PCBs nº IUPAC 105, 114, 118, 123, 156, 157, 167 and 169) and PCDD/Fs the results are expressed in pg of WHO-TEQ/g in wet weight basis, applying the TEFs factors.[4] All results are done in upper-bound.

The main PBDEs results are below of detection limits. Only in the stations from very populated areas the results of PBDEs are significant. These stations are Besos, Besos Depur (both in front of one of main waste water plant of Barcelona) and Napoli.

In all samples the presence of DL-PCBs and PCDD/Fs are up of detection limit, but the values obtained are considered low. The major concentrations are in the stations placed in front of big cities like Barcelona, Napoli or harbours like Lazaret. In all samples of North Africa the concentrations founded are low than these founded in the European Coast.

These results provide that the use of caged mussels is a good tool to measure the diffuse contamination of Mediterranean Sea.[5]

References

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Table 1. Stations analyzed and results obtained

Location	Country	Sampling Year	Σ PBDES[1]	[2]coplanars	Total [3]PCBs Monoorto	Total DL-PCBs[4]	Total PCDD/F	Total WHO-TEQ[5]
Cap de Creus	Spain	2004	1.289	1.649	0.058	1.706	0.063	1.770
Cap de Creus	Spain	2006	1.326	2.046	0.083	2.128	0.050	1.730
Badia de Ter	Spain	2004	0.972	1.649	0.037	1.686	N.A.	1.686
Golf de Roses	Spain	2004	0.972	1.655	0.121	1.776	N.A.	1.776
Besòs	Spain	2004	1.417	1.649	0.082	1.731	0.199	1.930
Besòs	Spain	2006	0.972	2.045	0.110	2.155	0.189	1.950
Besòs Depur	Spain	2006	0.972	2.043	0.086	2.129	0.133	1.868
BCN	Spain	2004	0.972	1.649	0.398	0.398	N.A.	0.398
Llobregat	Spain	2004	0.975	1.651	0.159	1.810	N.A.	1.810
Llobregat Depur	Spain	2006	1.256	2.048	0.274	2.322	0.325	2.252
Ebre Boca	Spain	2004	0.972	1.649	0.081	1.730	0.119	1.849
Columbrets Islands	Spain	2004	0.972	1.649	0.029	1.678	0.023	1.700
Javea	Spain	2004	0.972	1.649	0.031	1.679	N.A.	1.679
Tabarca	Spain	2004	0.972	1.649	0.027	1.675	N.A.	1.675
I. Hormigas	Spain	2004	0.972	1.649	0.024	1.672	0.062	1.735
El Portus	Spain	2005	0.990	1.649	0.010	1.659	0.540	2.199
Cabo de Gata	Spain	2005	0.787	1.649	0.014	0.014	0.041	0.055
Màlaga	Spain	2006	0.972	1.649	0.034	1.683	0.038	1.710
Port Palma	Spain	2005	1.282	1.649	0.019	1.668	0.075	1.743
Sta. Eulàlia	Spain	2005	0.805	1.649	0.024	1.672	0.180	1.852
Algeciras	Spain	2006	0.972	1.649	0.049	1.698	0.078	1.765
Ceuta	Spain	2006	0.972	1.649	0.079	1.727	0.053	1.727
Lazaret	France	2006	0.985	2.245	0.286	2.530	1.566	4.092
Tavolara	Italy	2005	0.990	1.649	0.024	1.672	0.080	1.753
Cagliari	Italy	2005	1.040	1.649	0.037	1.686	0.144	1.830
Palermo	Italy	2005	1.040	1.649	0.011	1.660	0.052	1.711
Napoli	Italy	2005	3.827	2.011	0.453	2.464	0.816	3.280
Civitavecchia	Italy	2005	1.295	1.649	0.026	1.675	0.246	1.921
Nador	Morocco	2006	0.972	1.649	0.084	1.732	0.051	1.731
Oran	Algeria	2006	0.972	1.649	0.075	1.724	0.214	1.933
Alger 1	Algeria	2006	0.972	1.649	0.074	1.722	0.235	1.953
La Galite	Tunisia	2006	0.972	1.649	0.117	1.766	0.034	1.706
Rades	Tunisia	2006	0.972	1.649	0.078	1.726	0.120	1.794
Promig	-	-	**0.897**	**1.627**	**0.092**	**1.718**	**0.212**	**1.835**
Desvest	-	-	**0.528**	**0.445**	**0.106**	**0.454**	**0.321**	**0.637**
Min	-	-	**0.051**	**1.649**	**0.010**	**0.014**	**0.023**	**0.055**
Max	-	-	**3.105**	**2.245**	**0.453**	**2.530**	**1.566**	**4.092**
Units (Wet weight)			**ng/g**	**pg/g (WHO-TEQ**	**pg/g (WHO-TEQ**	**pg/g (WHO-TEQ**	**pg/g (WHO-TEQ**	**pg/g (WHO-TEQ**

[1] Sum of BDEs nº IUPAC 47, 99, 100, 153, 154 and 209
[2] Sum of PCBs nº IUPAc 77, 81, 126 and 169
[3] Sumo of PCBs nº IUPAC 105, 114, 118, 123, 156, 157, 167 and 169
[4] Sumo of WHO-TEQs of coplanars and monoorto
[5] Sumo of WHO-TEQ of DL-PCBs and PCDD/Fs