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Experimental results and population response for selected chemicals

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1. Introduction

One of the objectives of S4 (Thresholds and drivers of contaminants) is to compare and assess the differences between contaminant thresholds (individual and mixtures) at several levels (molecular, individual, population and ecosystem). We have been studying four different levels responses to pollutant stress:

- *Molecular level*: The work on Real time PCR study of ecotoxicological effects of contaminants was already described in D4.4.1.
- *Individual level*: Bibliographic research was carried out for the organic contaminant families (PCBs, PAHs and PBDEs) and the two metals (Cd and Hg) . The results have been already describer in D.4.1.2
- *Population level*: Analysis and report of experimental results. Comparison with EQS and with other obtained levels
- *Ecosystem level*: Ecological risk assessment at ecosystem level will be performed and results compared with established levels. Emphasis will be placed on the evaluation of indirect effects.

A second objective of S4 is to study the effects of the speciation of contaminants and the influence of loading terms (pulse, seasonal, etc.) in their thresholds values.

In this report we present the experimental campaigns carried out to approach these objectives as well as the results obtained focusing on population level and the speciation of the two metals. Due to some delays in the experimental phase (shifting of campaigns) as well as in the analysis of samples, in some cases the results are still at a preliminary stage. Therefore, improvements in this Deliverable are expected as the analytical results will be available at the laboratories of the Thresholds' partners. It is expected that these new results will be included in the Synthesis deliverable (D4.1.5).

These experimental results also support the development of integrated fate and effects models that allows the estimation of the main fluxes between compartments, i.e. air/water/sediments as well as the simulation of scenarios that could produce reaching a threshold (see D2.6.2). Under this approach, the role of the air-water interface and the biota (phytoplankton and bacteria) is being examined. This will allow to determine levels of emissions that could produce a threshold point be reached.

2. Monitoring Campaigns

2.1. DISTRIBUTION OF MERCURY SPECIES IN THE WATERS OF THE THAU LAGOON

Knowledge of the mercury (Hg) speciation in water is required to predict bioavailability of this metal for the aquatic food webs. It has long been recognised that monomethylmercury (MMHg) is the most bioavailable, biomagnificable and toxic mercury molecule for aquatic ecosystems. In this context, we determined mercury speciation in the water column of the Thau Lagoon located along the seashore of the North-Western Mediterranean Sea. The analytical speciation scheme consisted of total mercury in unfiltered samples (HgT_{UNF}), dissolved total mercury (HgT_D), monomethylmercury ($MMHg_{UNF}$ and $MMMHg_D$), and dissolved gaseous mercury (DGHg) which mainly consists of dissolved element mercury (Hg^0).

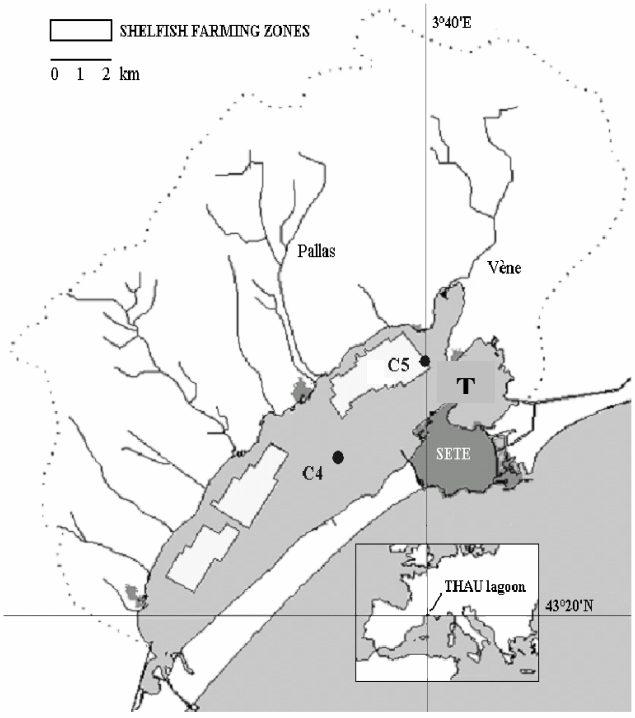

Figure 2.1. Sampling station locations.

2.1.1. Studied site and sampling collection

Located along the Gulf of Lions (North-Western Mediterranean) French coasts, the Thau Lagoon is a human impacted lagoon with an surface area of about 75 km², the mean depth 3.5 m, and the maximum depth of 11 m. The salinity varies between 31 to 39. Twenty percent of the surface is occupied by shellfish farming, mainly oysters *Crassostera gigas,* and mussels *Mytilus galloprovincialis*.

Three stations have been studied (C4, C5 and T12, Fig. 2.1). The sampling site C4 is centrally located in the lagoon (43°24.018^N, 03°36.703^E). At this point, the depth is about 8 m. Station C5 (43°25.994^N, 03°39.657^E) is located close to "mussel tables" (the mussel farming devices), where organic carbon fluxes are relatively high. At station C5 the water column is 9 m high. Station T12 (43°25.425^N; 03°

41.283^{E}) is located in the vicinity of the industrial and harbour activities of the city of Sête. The water depth at T12 station is 5 m. Three (3) campaigns were conducted since the beginning of the Thresholds program in order to indicate seasonal trends: the first was performed in February, the second in April 2006, and the third in September 2006. Four (4) to seven (7) depths within the water column depending the station. We present here the results obtain during the first two cruises.

2.1.2. Sampling and analytical techniques

The ultra clean sampling techniques and analytical methods applied for water analyses are those presented and discussed in detail by Cossa et al. (2003). In short, water column samples were collected by pneumatic pumping (an all Teflon double bellows ASTI pump) using acid-cleaned polyethylene tubing. Samples were stored in acid-clean Teflon (FEP) bottles. Filtrations were performed using Sterivex-HV cartridges (Millipore® 0.45 µm hydrophilic PVDF membranes) and polypropylene syringes. All water samples were acidified to 0.4 % (v/v) with Suprapur® HCl, double bagged and stored at +4°C in dark conditions until analyses were performed.

All mercury species in water samples were detected by cold vapour atomic fluorescence spectrometry (AFS, Tekran, model 2500). DGHg was measured by bubbling the water samples with Hg free Ar and concentrating the Hg^0 evolved on a gold sand trap. The trap was then heated and the Hg vapor quantified by AFS. Dissolved HgT_D was determined by the formation of volatile elemental Hg (released by $SnCl_2$ reduction, after acidic BrCl oxidation, and its preconcentration on a gold trap). The detection limits for HgT_D, defined as 3.3 times the standard deviation of the blanks, were 0.01 pM. The reproducibility (the coefficient of variation in percentage of five replicate samples) was lower than 10 %. The accuracy for HgT_D determinations was regularly checked using the reference material (ORMS-3) from the National Council of Canada as certified reference material (CRM). $MMHg_D$ was determined using the method initially proposed by Tseng et al. (1998) and modified by Cossa et al. (2003). Detection limit were 0.005 pM for a 60 mL water sample. Precision was better than 10 % for all analyses. No CRM is available for MMHg in water. The detailed procedure is given by Cossa et al. (2003). Ancillary parameters (temperature, salinity, and nutrients) were measured using standard methods (Grasshoff et al., 1983).

2.1.3. Results and discussion

Summary statistics for the first two campaigns are given in Table 2.1 and vertical profiles shown on Figure 2.2.

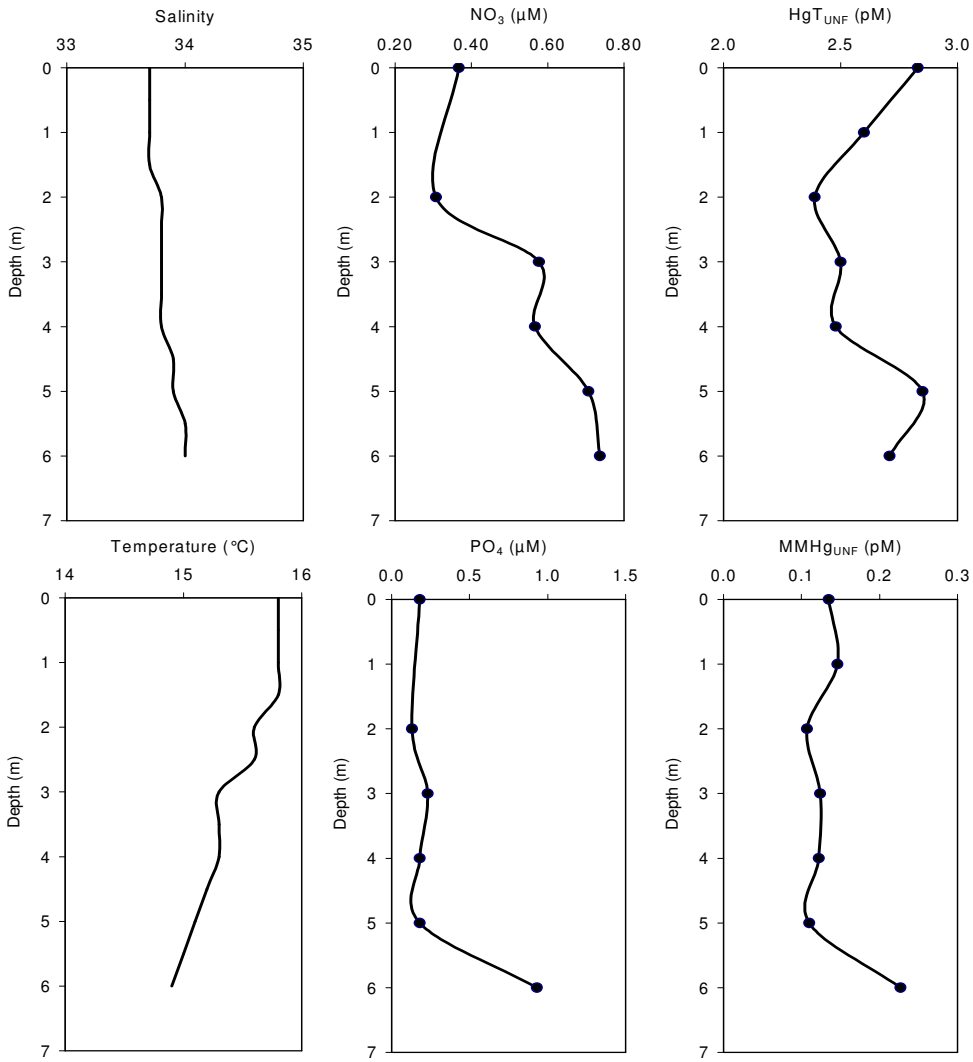

Figure 2.2. Vertical profiles for salinity, temperature, nitrate, phosphate, total mercury (HgT) and monomethylmercury (MMHg) in unfiltered samples of the water column at station C5 of the Thau Lagoon.

Table 2.1. Summary statistics on mercury species concentrations (pM) measured in the water column of the Thau Lagoon in February and April 2006. Mean ± standard deviation (number of determinations).

Station	Campaign	HgT_{UNF}	HgT_D	$MMHg_{UNF}$	$MMHg_D$	DGHg
C4	February	0.87± 0.27 (6)	0.57±0.45 (5)	0.14±0.16 (6)	0.12±0.14 (6)	0.20 (1)
	April	2.38± 0.29 (6)	1.91±0.10 (6)	0.16±0.04 (6)	0.08±0.02 (6)	0.17 (1)
C5	February	-	0.85±0.13 (6)	-	0.12±0.11 (6)	-
	April	2.62 ± 0.17 (7)	-	0.14±0.04 (7)	0.06±0.02 (7)	-
T12	February	-	2.14±2.34 (3)	-	0.04±0.02 (3)	-
	April	3.05 ± 0.41 (4)	2.36±0.29 (4)	0.16±0.03 (4)	0.10±0.03 (4)	-

- *Total mercury*:

The concentration range found for the HgT in the water of the Thau Lagoon (from 0.40 to 4.84 pM) are in the upper range of the Mediterranean open seawaters (Cossa and Coquery, 2005). These authors estimate that HgT concentrations in the Levantine Intermediate water and Western Mediterranean Deep waters range from 0.7 to 1.2 pM, while previous studies detected HgT concentrations up to 2.2

pM for the open North-Western Mediterranean sea (Cossa et al., 1997). For comparison with coastal waters, during a recent cruise in the Rhône plume area, 80 % of the HgT concentrations ranged from 0.61 to 3.50 pM, with mean ± standard deviation of 1.64 ± 0.78 pM (Bioprhofi cruise May 2006, unpublished results, http://www.insu.cnrs.fr/web/article/art.php?art=1783). The highest HgT concentrations in the Thau Lagoon were, as expected, found for unfiltered waters. The particulate mercury accounted for 18 to 34 % of the HgT_{UNF} depending on the station. Highest HgT was found at station T12, which is the station located near the industrial and harbour activity of the city of Sête. A tendency for higher HgT_{UNF} concentrations in the surface and bottom waters is visible on figure 2; this feature is probably related to the suspended particulate matter distribution.

- *Methylmercury*:

The methylated mercury concentrations range covered one order of magnitude, varying from 0.03 to 0.46 pM. However, MMg_{UNF} concentrations were quite stable regardless of the station or sampling period (Table 1). Methylated species accounted for 12 and 7 % of the HgT for the February and April cruise respectively. This proportion is within the range currently observed in marine surface waters. However, it is lower than the proportions calculated for the intermediate and deep waters in the North-Western Mediterranean Sea (Cossa and Coquery, 2005). Noteworthy are the similar vertical distribution patterns of PO_4 and $MMHg_{UNF}$ (Fig. 2). Such observation suggests a connection between methylmercury distribution or production and the organic matter mineralisation. This aspect has to be explored further on.

- *Air-sea exchange*:

The concentration of DGHg has been determined only in the surface waters. This volatile species represented 20 to 35 % of the HgT_D. (Table 1). These concentrations correspond to a supersaturation of the water when compared to the concentrations of mercury in the atmosphere. Indeed, total gaseous mercury was measured on the north shore of the Lagoon within the E.U. Mercyms project. Concentrations varied between 1.9 and 3.2 ng/m^3 (Amouroux et al., personal communication). Using a simple Henry's law diffusion model it can be inferred from these results that the surface waters of the Lagoon are a source for the mercury for the atmosphere.

- *Bioaccumulation factor within mussels*:

Mercury concentrations in the mussel (*Mytilus galloprovincialis*) are measured on a regular basis within the French Mussel Watch Program (Claisse, 1989). The median value for the last five years is 70 nmole kg^{-1} (wet weight). Claisse et al. (2001) determined that 64 % of the mercury present in the mussel soft tissues of the Lagoon consisted of MMHg. This, compared to the 7-12 % in the waters of the Lagoon, illustrates the favoured bioaccumulation of MMHg compared to inorganic mercury compounds. Combining the data for mussel soft tissues (expressed per unit of wet weight) with the

present concentrations found in unfiltered waters, we arrive with *in situ* BF of around 300 000 and 30 000 for MMHg and HgT respectively. These data are necessary for risk assessment studies.

2.2. CADMIUM SPECIATION RESULTS IN THE THAU LAGOON

It has long been recognized that dissolved and particulate elements do not present the same availability to marine living organisms. For example Borchardt (1985) showed that dissolved Cd was more available to mussels than particulate Cd.

Therefore, the total dissolved metal concentration is not pertinent information to assess the bioavailability and potential effects on the biomass of the presence of metals in the marine environment. Free hydrated ions, hydroxides, and inorganic complexes are the most readily available species for living organisms (Morel et al.1991; Morel and Hering,1993), besides neutral organic complexes (Phinney and Bruland, 1994)

In this study we have determined the total dissolved Cd concentrations, the particulate Cd, and the "electrochemically labile "Cd concentrations at three points of the lagoon and at 3 depths. Samples for this study were taken in February and September 2006. This is a partial report as some samples have not been analysed yet.

2.2.1. Sampling and conditioning of samples

Three sampling stations were visited twice (February 2006 and September 2006): C4 (43°24.018N, 03°36.703E), T12 (43°25.425N; 03° 41.283E) and C5 (43°25.994N, 03°39.657E), see fig. 2.1.On each station three depths were sampled; surface (50cm), mid depth , and bottom. (50cm from the floor).

Samples were collected using a pump (ASTI® Teflon pump, polyethylene tubing). In-line filtration was performed in February using a Nuclepore® polycarbonate membrane filter (47 mm in diameter, 0.4-µm pore size). In September the samples were taken to the laboratory and filtered within 4 hours under a clean bench. Samples devoted to total dissolved analysis were acidified under clean conditions, and put in two plastic bags.

Samples devoted to speciation studies were placed in two plastic bags and immediately frozen. Filters with SPM were put in polystyrene Petri dishes and frozen.

2.2.2. Analysis

Particles were dissolved in HCl, HNO_3 and HF. Cadmium was then analysed by spectrophotometric atomic absorption .

Total dissolved cadmium was analysed by atomic absorption spectrophotometry after liquid- liquid extraction in Freon s described by Danielsson et al (1982).

Cadmium speciation was studied using anodic stripping voltammetry. This method allows the determination of free ions and labile complexes that constitute most of the "bioavailable" cadmium.

Neutral organic complexes, which are directly available to phytoplanktonic cells are not accessible to ASV.

The raw filtered sample is divided into several parts; one is left untreated, the others are spiked with increasing quantities of cadmium and left overnight in the refrigerator to equilibrate. When enough cadmium has been added to saturate the ligands, the signal obtained in ASV increases linearly as a function of Cd spike augmentation (Morel and Hering 1993).Then the response is the same as if there was no ligand in the sample , and the increase rate of the peak VS spike may be used to calculate the initial concentration of cadmium which caused the peak obtained without any spike. This is the "electrochemically labile "or "electroactive " cadmium which we consider as representative of labile or available cadmium

Sampling and analytical methods have been described in detail in the Deliverable 4.2.3.

2.2.3. Results

- Total concentrations:

Particulate cadmium concentrations for the 2006 campaigns are not available yet. Results on samples obtained in May 2004 show relatively high values. This is in accordance with the probable phytoplanktonic nature of the SPM in late spring. Total dissolved Cadmium has been measured by AAS for the February 06 campaign (Table 2.2).

Table 2.2. Total dissolved cadmium in February 2006, concentrations in 10^{-9} mol L^{-1} (nM)

TH 02-06 C4surf	0.19
TH 02-06 C4 3m	0.19
TH 02-06 C4 bottom	0.17
TH 02-06 C5 surf	0.16
TH 02-06 C5 3m	0.19
TH 02-06 C5 6m	0.18
TH 02-06 T12 surf	0.19
TH 02-06 T12 2.5m	0.19
TH 02-06 T12 5m	0.18

Total dissolved concentrations are very homogenous (0.18±0.01 nM) and no clear spatial trend can be seen in total dissolved cadmium distribution. The concentrations are higher than those observed in May 2004 (mean=0.12nM, s;d;=0.02; n=20). This suggests that the winter rain and floods may be mainly responsible for cadmium inputs and higher concentrations in the lagoon.

- Speciation study:

At this time, three surface samples, C4 C5 and T12 , taken in February 2006, have been studied.

a/ Result of study for C4surface

Concentration augmentation (mol/L) (peak height, nA)	Asv signal
0	8.1
1.8 E-10	27.4
5.4 E-10	70
9E-10	93
1.8E-9	180
2.7E-9	302

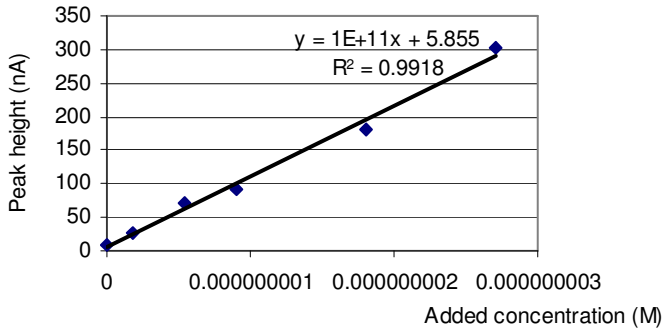

Table 2.3 and **Figure 2.3**.Labile Cd determination on station C4S.

Table 2.3 and Figure 2.3 show the response of Cd peaks in ASV upon growing spikes of ionic Cd. The quasi perfect linear regression line (r^2=0.99) shows no complexation of the spikes . This linear set of data allows calculation of the concentration by dividing the peak height for the natural sample 8.1 nA by the slope of the regression line 1 E11 nA/(M). This yields a concentration of 8.1 10^{-11} M, 0.08nM. This represents 40% of the total dissolved cadmium measured by AAS 0.16 nM, Table 2.1). This poses a question: the linearity of the relation between added Cd and the peak increments can be interpreted as a sign of absence of complexation? So, the ASV value of Cd concentration should be equal to the total AAS concentration, which is not. The answer to this question may be that all the ligands present in the sample are saturated by the Cd initially present in the sample, then allowing a linear response to the spikes.

b/ Station C5s

The same technique as for C4 S leads to a slight curvature of the graph in the low added concentrations part of the graph (Table 2.4, Figure 2.4). This is a mark of the partial complexation of the spikes, until 8.9 10^{-10} M Cd have been added. From this added concentration, higher spikes lead to a linear increase, the slope of which is used to calculate the initial electroactive cadmium as before.

This results in a labile concentration of 4.4 E-11M, which represent 27% of the total dissolved Cd (0.16nM ,tab.1).

Cd spike (M)	peak height (nA)
0	4
1,80E-10	7,3
3,60E-10	19,8
5,40E-10	30,7
7,10E-10	38,2
8,90E-10	63
1,30E-09	99
1,80E-09	116
2,20E-09	181
2,70E-09	218

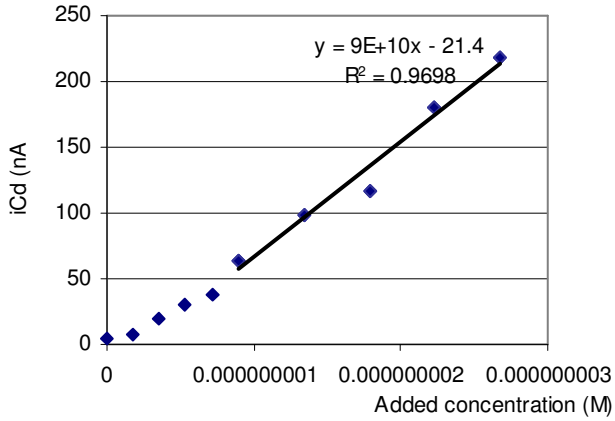

Table 2.4 – **Figure 2.4**. Labile Cd determination on station C5S

c/ StationT12S

Between the spikes 8.9 E-11 and1.1E-9 the ASV signal grows linearly (R2= 0.97)with the dissolved Cd concentration enhancement. (Table 2.5 – Figure 2. 5). This is an indication that complexing agents are saturated as soon as the first spike.

Cd spike (nM)	iCd (nA)
0	20
8,90E-11	26
3,60E-10	78
5,40E-10	138
7,10E-10	165
1,10E-09	238

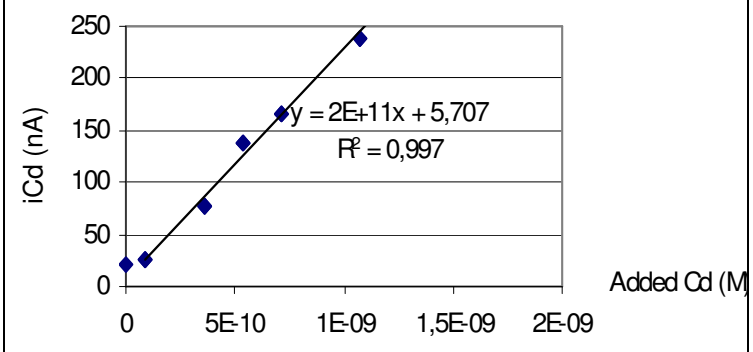

Table 2.5 – **Figure 2.5**. Electroactive Cd determination. Station T12.

Following the same method as before, we estimate the electro- labile Cd to be (20 / 2)E11 =1E-10 M. This represents 100/0.19 = 53% of the total dissolved cadmium.

Therefore on these three samples 25 to 50% of the total dissolved Cd appears to be in an electro active form; this means that 50 to 75% are in an organically complexed form. This is coherent with the results from Kozelka and Bruland (1998) who found slightly more complexed Cadmium in the Naragansett bay (73 to 83 %)

2.2.4. Preliminary conclusions

These first results give a coherent sight on the repartition of electroactive cadmium in the Thau lagoon. 25 to 50 % of total dissolved cadmium is in a labile form in the surface waters which constitute a non negligible stock of potentially bioavailable toxic metal. Next results will enable us to study the seasonality of the phenomenon and the influence of depth of the water column on the importance of the complexation.

2.3. AIR-WATER EXCHANGE OF POPs AND PHYTOPLANKTON ACCUMULATION ALONG THE "THRESHOLDS" MEDITERRANEAN CRUISE

The influence of drivers as pollutants on the changes in ecosystems, and points of no return, has not been assessed previously. The cause for the lack of this knowledge is the scarcity of comprehensive data sets that allow this kind of study. Within the framework of the Thresholds project, this has been tried to be solved by generating a data set that fills the gaps of previous studies. Indeed, a Mediterranean cruise between Barcelona and Istanbul and back to Barcelona took place from 2nd June to 6th July 2006. (Figure 2.6).

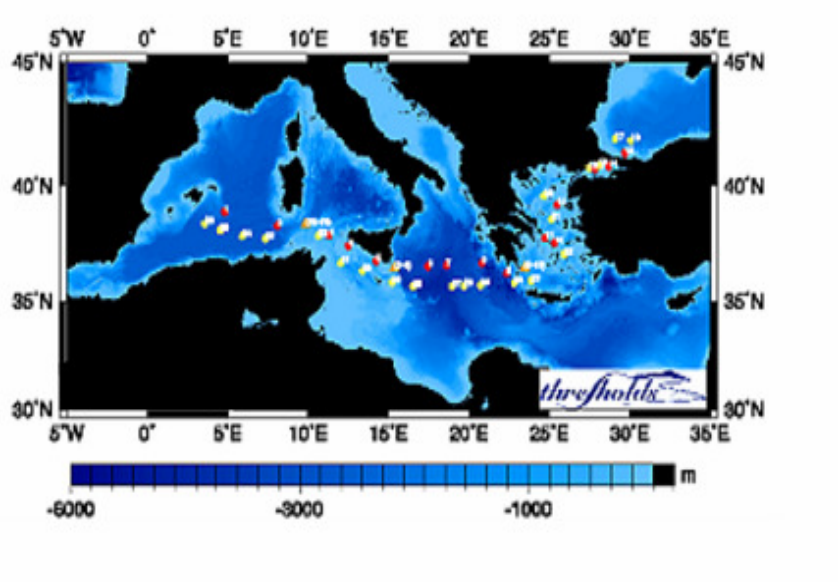

Figure 2.6. Sampling stations performed during the campaign. Red spots were station performed during the first lag (Barcelona-Istanbul) and yellow spots were stations performed during the second lag (Istanbul-Barcelona).

The objectives of this experimental campaign across the Mediterranean Sea were to study the spatial distribution of several POPs such us polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs) and polychlorinated dibenzo dioxins and furans (PCDD/Fs) in air, suspended particulate matter and water in order to understand the importance of long range atmospheric transport and deposition, and to assess the role of the phytoplankton, zooplankton and bacteria in the day-night and regional variability of POPs (Persistent Organic Pollutants), and to quantify the fluxes of POPs that after being deposited to surface waters enter into the planktonic food web. Moreover, data on spatial-temporal distribution and atmospheric input on open seas for some families of POPs has been obtained for the first time.

2.3.1. Preparatory work

In order to ensure the execution of the project, analytical methods able to detect these compounds at PPQ level were set up and developed. These compounds were expected even at a lower level in the sea water, far from the coast where emission sources are present. The ubiquitous character of these chemicals combined with the low level expected in the sample, required working in very clean

environment in order to avoid cross contamination. In addition to reach very low detection limits, large sampling volumes of water and air were required, 200/1000 L and 500/2000 m^3 respectively. Several sampling set ups were implemented in order to cover all aspects that wanted to be investigated.

A medium volume water sampling device was developed directly at JRC-IES laboratories. The system allows to filter suspended particulate matter (SPM) and to adsorb the compounds dissolved in the water phase on a polymeric adsorbent (XAD-2) column. This aspect is important for studying the partitioning particulate/dissolved phase of these compounds in marine water and consequently the possibility of a diffusive exchange between the dissolved and gas phase. A high volume sampling system was also employed. This system is particularly useful for sampling high volumes of water in few hours, which allows for determining diurnal cycles of POPs in waters, one of the objectives of the Thresholds campaign, since it is thought that there is a close coupling of the carbon cycle as driven by the planktonic communities and the POP dynamics. In addition, an in-situ low-volume system for analyzing the dissolve-particulate phase POPs was also applied by the group form IIQAB-CSIC. The combination of those methods allowed to take samples with different resolution; 6-12 hour samples for the diurnal cycle sites, and 12-24 hour sample for the regional variability study and to sample at different depth to study vertical profiles.

Regarding air samples, also complementary sampling was performed. In general thre king of sampling was performed. Day and night sampling to study POPs day-night cycling, weekly integrated sampling to study dioxins high resolution sampling (6h) to study POPs cycling during the day.

2.3.2. Sampling strategy and methods

The sampling cruise started in Barcelona on board of the Oceanographic vessel *B/O Garcia del Cid* from The Spanish Council for Scientific Research (CSIC) with duration of 35 days. A total number of 36 sampling stations of different duration (from 3h to 24h) were performed across the Mediterranean including the Tyrrhenian, Ionian, Aegean, and Marmara Seas. Moreover some of the station took place in the Black Sea. A distance of 3850 nautical miles (around 7100km) was covered in this sampling exercise.

Integrated air (particulate and gas phases) samples were collected (day and night cycles, high resolution sampling and 24h sampling) together with high volume water (surface and at the deep chlorophyll maximum) samples (particulate and dissolved phases) for the analyses of POPs. Sampling was performed during the stations and while cruising, covering transects between two stations. Water was taken on board by using a continuous pumping system, developed and installed on the boat by JRC-IES and CSIC technicians, consisting on a steel protected Teflon hose connected to a tow fish that navigated at around 4-5m depth and 2-3 m apart from the boat mainly while traveling. A membrane pump (operating ca. 25 L/min) was used to suck the sea water and fill in continuously a 50 L stainless

steel overflow container. Sampling lines were inserted in the overflow container so fresh water was always sampled. Two systems were used in parallel:

- A high volume sampling system, Infiltrex 300 (operating at around 1.5L/min).Samples of around 1000L were taken with this system.
- A low (or medium) volume sampling system (0.2 L/min) developed by us was for getting transects samples. Samples of around 300 L were taken with this system.

Figure 2.7: Tow-fish used during the Thresholds cruise for avoiding potential contamination of samples from the ship (left picture) and in-situ pump for sampling water at the deep chlorophyll maximum.

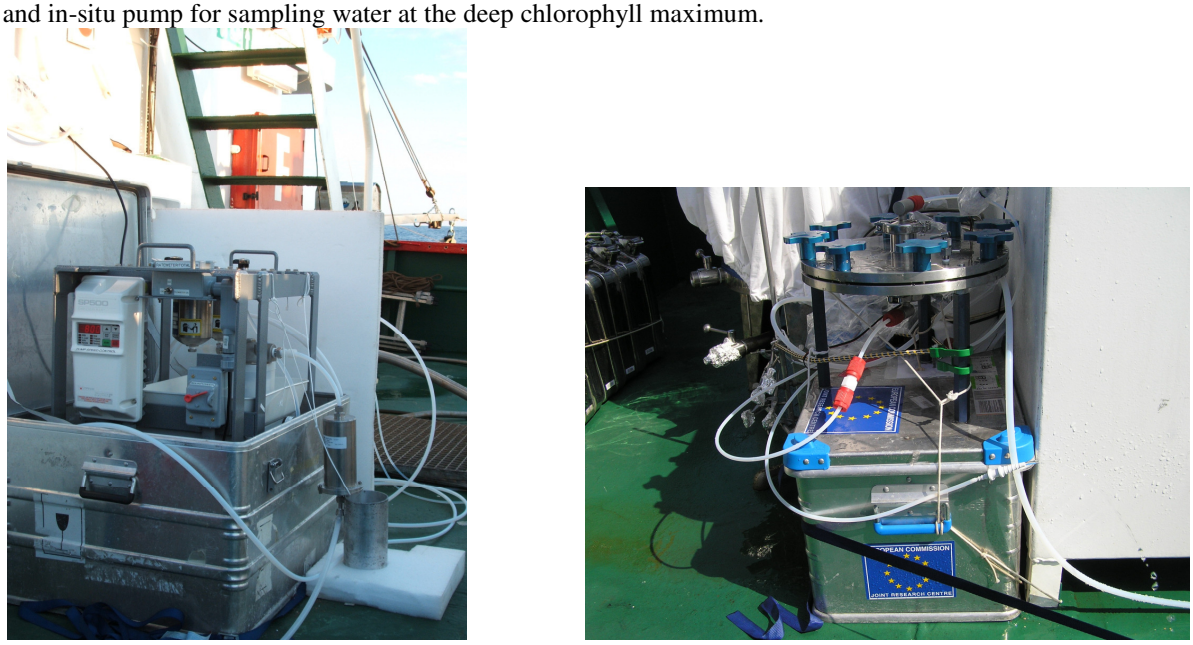

Figure 2.8: INFILTREX high volume sampler (left) and low volume device (right) on deck for water monitoring during Mediterranean campaign.

Water was preferably sampled while cruising but also in some of the stations. In the 24 h stations, samples were taken in the morning (6.00 to 14.00) in the afternoon (14.00 to 22.00) and during the night (22.00-6.00) in order to gather 3 samples within the 24h period. Air sampling occurred in parallel.

In addition, a limited number of samples, due to the requirements of ship times, were taken of water (dissolved + particulate) at the deep chlorophyll maximum. In addition, neston samples at all stations were taken using 200um nets.

2.3.3. Analytical work

In parallel, extremely sensitivity analytical methods have been developed, based on high resolution gas chromatography coupled with high resolution mass spectrometry (HRGC/HRMS) and isotopic dilution quantitative techniques. The use of labeled ^{13}C internal standards guarantees a high level of both accuracy and analytical precision. A great work has been performed in order to obtain suitable blanks and all sampling materials used during the campaign were previously cleaned and analyzed before handling.

More than 150 samples of gas phase, aerosol phase, dissolved phase, particulate matter and neston were collected during the campaign. An important dataset is being created that will be coupled with the biological data also taken during the campaign. It will constitute an essential tool for the development and validation of POPs models and for assessing the POPs status of the Mediterranean Sea.

2.3.4. Significance of the data set for the Thresholds objectives

This data set is currently being generated by IIQAB-CSIC and JRC-IES. The samples will be analyzed for organic pollutants with a wide range of physical-chemical properties, such as PCBs, PAHs, PBDEs, PCDD/Fs and some other fluorinated compounds. Furthermore, it is very remarkable that for the first time a data set with simultaneous samples of gas, aerosol, dissolved, particulate and neston is generated. This will allow estimating fluxes between environmental compartments and especially to elucidate the role of atmospheric deposition processes on the accumulation of POPs in aquatic planktonic food webs. The accumulation of POPs in phytoplankton and zooplankton is important because these organisms are the base of the food web and because they play an important role in the marine carbon cycle. Therefore, this data set will allow for example, to validate the model developed within the thresholds project that accounts for the interaction of water column and atmospheric deposition processes. Indeed, the model developed follows the schematics as shown in Figure 2.9, since the threshold cruise was performed at open sea, all the processes depicted for the atmosphere-photic water column will be evaluated and all fluxes estimated. Furthermore, it will be possible to elucidate up to which degree the atmospheric deposition processes are responsible for the levels of POPs in planktonic occurrence.

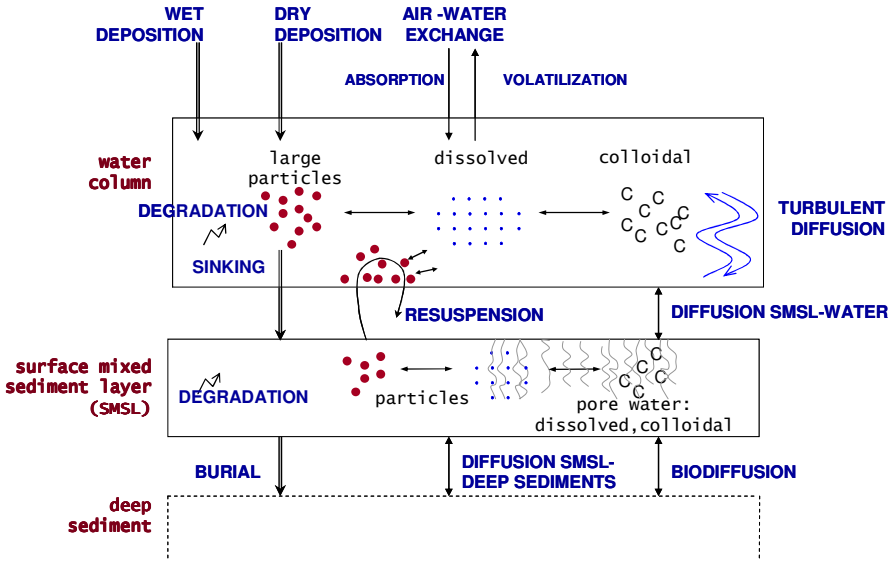

Figure 2.9: Schematics of the model developed within thresholds and that will be validated/applied using the data set generated during the thresholds sampling cruise in the Mediterranean.

The simultaneous determination and estimation of occurrence and transport fluxes of POPs with their effects in planktonic organisms (see following sections) has been done here for the first time and thus will provide a unique data set to study the effects of pollutants as drivers of toxic impacts in marine waters for the Mediterranean Sea.

2.4. LETHALITY OF DIFFERENT CONTAMINANTS TO MARINE PHYTOPLANKTON

Plankton abundance and community composition were studied during the oceanographic cruise THRESHOLD-1, on board the RV Garcia del Cid, along the Mediterranean and Black Seas from June 3 to July 5, 2006

Experiments to analyze the lethal threshold of PAH's and metals on natural populations of phytoplankton were performed with Mediterranean and Black Sea plankton, sampled during the oceanographic cruise THRESHOLD-1.

2.4.1. Plankton abundance

The abundance of bacteria and phytoplankton was quantified during the cruise in all the stations and depths sampled. Niskin bottles attached to a rosette-CTD system were used for sampling. Samples for bacteria and picophytoplankton were taken and analyzer on board using flow cytometric techniques using a Becton & Dickinson FACScalibur bench machine with a laser emitting at 488 nm. Samples for bacteria were stained for a few minutes with Syto13 (Molecular Probes) at 2.5 µM, and run through the flow cytometer at low speed, and data were acquired in log mode until around 10,000 events had been acquired. We added a small volume (10-20µL) per sample of a calibrated solution of yellow-green 1 µm Polysciences latex beads as an internal standard.

Bacteria were detected by their signature in a plot of side scatter (SSC) vs. green fluorescence (FL1) as suggested by del Giorgio et al. (1996). Picophytoplankton communities composed by *Synechoccoccus*, *Prochlrococcus* and eukaryotic phytoplankton was quantified in fresh samples by using the same flow cytometer. An aliquot of a calibrated solution of 1 µm diameter high-green fluorescent beads (Polysciences) was added to the samples as an internal standard for the quantification of cell concentration. Bead concentration in the standard solution was calculated by filtering replicated aliquots onto black nuclepore filters and counting the beads under an epifluorescence microscope. The red, green and orange fluorescence emissions, and the forward and side scattering of the cells and beads, were used to detect different cell populations and to differentiate them from the fluorescent beads.

Chlorophyll *a* concentration was measured in all the stations and depths sampled as a quantification of total phytoplankton abundance. 50-100 ml of water was filtered through Wahtman GF/F filters and the pigment extracted in 90% acetone for 24 hours. After extraction, filters were centrifuged and the fluorescence of the supernatant reader ina Shimadzu RF2400 espectrofluorimeter calibrated following Parsons et al. (1984).

2.4.2. Description of experiments

The experiments consisted on incubate replicated bottles on deck incubators, simulating seawater and air conditions (light, temperature, etc.). The experiments, 8 altogether, were carried out with PAH's (pyrene and phenantrene), metals (Cadmium and Lead) and methanol. For the PAH's and metal experiments we run duplicated bottles of 5-6 treatments + duplicated controls. Experiments began with surface water collection from sea in day 0 and inoculation of the contaminant, following daily the effects of these contaminants in the communities of picoplankton and microplankton during 4 days. The concentrations used in the treatments varied for Cadmium and Lead from 0.01 ppb to 112 ppb-1000 ppb, and for Pyrene and Phenantrene from 5 mg/l to 500 mg/l. Changes in total phytoplankton abundance during the experiments were followed by analyzing Chlorophyll *a* concentration, following the method described above. The effect of PAH's and metals on the picophytoplankton community was followed by analyzing changes in the abundance of *Prochlorococcus* sp, *Synechococcus* sp and Eukaryote picoplankton by using a flow cytometer, as described above. Samples are still under analysis and the results of the experiments are still provisional.

2.5. OCURRENCE OF POPs IN A MEDITERRANEAN COASTAL LAGOON (ETANG DE THAU, FRANCE)

As mentioned previously, Thau lagoon is one of the largest Mediterranean lagoons. Located on the French Mediterranean coast along the Gulf of Lion (Figure 2.1). The lagoon receives inputs from different human activities: urban activities, industries, agriculture and shell farming. The biggest town (Sète) and most of urban activities, like incineration, are located in the Eastern part of the lagoon. Thus, Thau lagoon appears to be under an intense anthropogenic pressure. For instance, the results of the French Monitoring Network (RNO Réseau National d'Observation) show high contamination of the Thau lagoon sediments by hydrophobic organic compounds such as, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). However, few data in relevant environmental compartments from Thau lagoon on other important groups POPs, like PCDD/Fs, PCBs or PBDEs have been reported to date. Moreover, the dynamics, long term impacts and ultimate fate of the contamination induced by these chemicals in the lagoon is not well known.

The aim of this work was to investigate the concentration and patterns in air (where no data are available yet), water, sediments and mussels from Thau Lagoon concerning the selected families in the Thresholds Project (PCBs, PAHs, PBDEs) and other important POPs such us PCDD/Fs, even though this last family was not selected in the project, (Fig. 2.10.). So far, a more detailed study has been performed with PCDD/Fs and it is presented in this report. In addition, preliminary results on PCBs are also presented in this document. Remaining analyses of more PCBs congeners, the other chemical families and of the other compartments are still under execution at Ifremer and JRC-IES laboratories.

Concerning the presented results, the influence of the atmosphere in the accumulation of these POPs in the aquatic system was studied. Two land air sampling sites were set up in the lagoon and sediments and mussels samples were collected from selected stations, see Fig. 2.11.

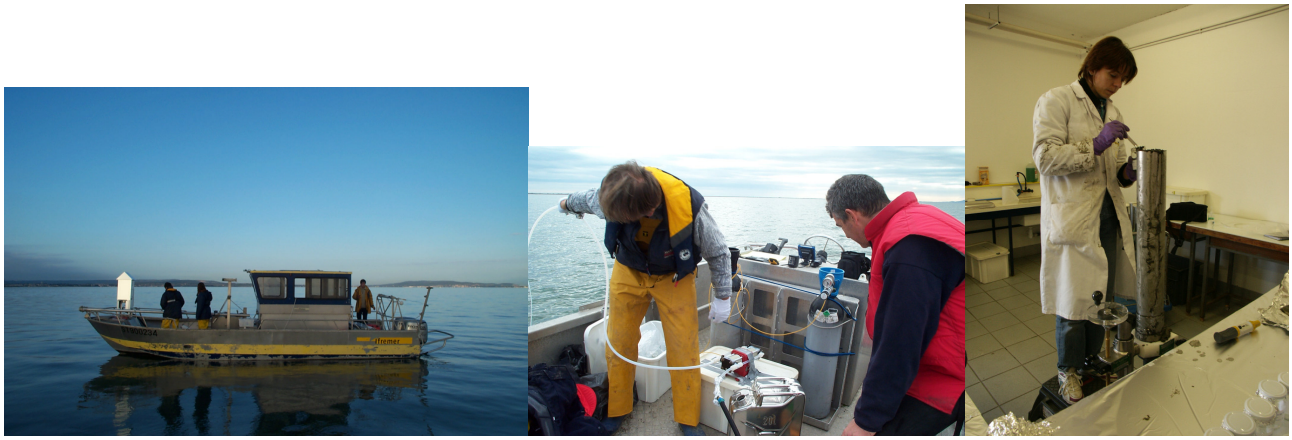

Figure 2.10. Air and water sampling, and sediments conditioning.

2.5.1. Materials and methods

- Sampling

Air samples were collected with two High Volume Samplers (TE-1000BL PUF sampler, Tisch Environmental, inc. USA) operating in a 24h basis at two locations (Ifremer Institute and at the other

side of the shore of the lagoon close to a small village, Bouzigues, (Figure 1). Sampling started on 14th November 2005 at IF site, on 15th November at BZ site and finished on 19th November 2005 in both sites. Thus, 5 samples were collected for IF site whereas 4 were obtained for BZ site. Air particle phase was retained by using a 102 mm diameter quartz fibre filter (QFF), whereas gas phase was trapped with a polyurethane foam (PUF) plug of 65 mm diameter and 60 mm length. An average volume of 400 m^3 was collected.

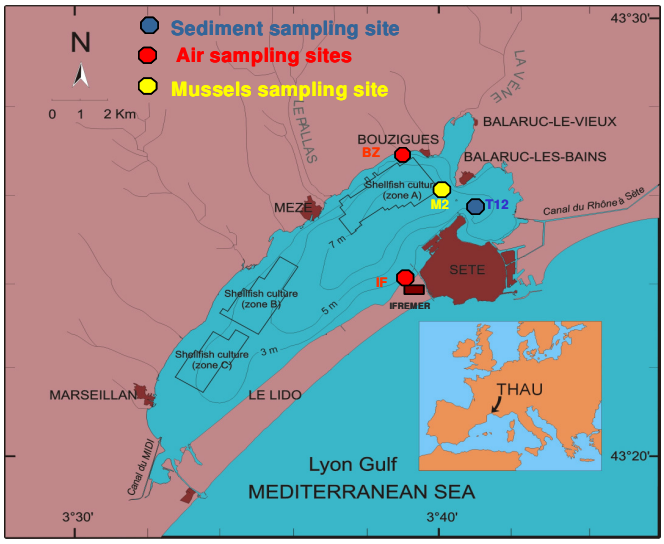

Figure 2.11. Location of Thau Lagoon (France) and air, sediments and mussels sampling sites.

Divers using PTFE sediment corers collected surface sediment samples. The results presented in this study were obtained from the analysis of the first centimeter of the sediment core. Mussels (*Mytilus galloprovincialis*) were collected by hand and depurated during 24 hours before further treatment. The mussels were shucked, homogenized, stored at –20°C before freeze-drying, and stored in the dark at room temperature until analysis. Surface sediments and mussels were collected in May 2004.

Analytical determinations

- Air samples

Samples were Soxhlet extracted with n-hexane/acetone (220/30) for 48 h after being spiked with internal standards (16 ^{13}C-labelled 2,3,7,8-chlorine-substituited congeners with 400 pg each, except OCDD with 800 pg each). Extract purification was executed with an automated clean-up system (Power-Prep P6, from Fluid Management Systems, Inc., Watertown, MA, USA) and PCDD/Fs and PCBs in the different fractions were quantified by isotope dilution using HRGC-HRMS.

-Mussels and sediment samples

The analytical protocols for extraction and cleanup have been described previously by Johansson et al , (2006) and Munschy et al.(2005).

2.5.2. Results and discussion

PCDD/Fs

- Concentration levels

Air

Total PCDD/Fs air concentrations (particle + gas phase) measured in both sampling sites are compiled in Table 1. PCDD/Fs WHO-TEQ concentrations found in IF site ranged from 15.8 to 25.7 WHO-TEQ fg m^{-3} whereas for BZ site varied from 6.9 to 22.6 WHO-TEQ fg m^{-3}. Concentrations observed in both locations were low, typical of those from rural areas (Lohmann, and Jones 1998; Castro-Jiménez et al., 2005; Castro-Jiménez et al., 2006).

Table 2.6. Concentrations of PCDD/Fs in air (fg m^{-3}), surface sediments, and mussels (pg g^{-1} dry weight) found in Thau lagoon.

Compounds	Air (gas+particle phase)									Sediment	Mussel
	Site IF (14-19 Nov 05)					Site BZ (15-19 Nov 05)				(May 04)	(May 04)
	IF-1	IF-2	IF-3	IF-4	IF-5	BZ-1	BZ-2	BZ-3	BZ-4	T12	M2
2,3,7,8-TCDD	<1.0*	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	1.4	1.15	n.d.
1,2,3,7,8-PeCDD	6.0	5.7	2.3	5.7	5.4	1.6	3.5	1.3	5.5	2.27	n.d.
1,2,3,4,7,8-HxCDD	8.5	8.3	2.8	4.9	6.8	3.5	6.1	1.7	6.8	2.08	n.d.
1,2,3,6,7,8-HxCDD	24.8	19.1	7.2	12.3	28.0	9.1	14.5	2.1	18.9	7.46	0.16
1,2,3,7,8,9-HxCDD	17.7	16.6	6.1	11.8	16.7	6.7	10.9	5.2	17.2	5.54	0.17
1,2,3,4,6,7,8-HpCDD	247.7	276.4	59.2	132.5	262.3	121.1	162.5	55.3	175.2	162.19	1.57
OCDD	379.2	917.9	132.0	273.2	576.7	310.2	340.8	95.4	406.1	1270.05	6.44
2,3,7,8-TCDF	6.7	7.1	2.4	4.9	6.0	2.5	1.6	1.2	2.3	12.61	4.00
1,2,3,7,8-PeCDF	5.7	6.5	5.9	4.5	4.5	1.5	1.7	1.6	6.1	5.05	0.27
2,3,4,7,8-PeCDF	7.0	11.1	10.9	8.0	7.7	4.6	7.1	4.1	8.5	6.21	0.58
1,2,3,4,7,8-HxCDF	8.9	11.1	10.0	6.9	7.9	5.3	18.2	3.2	11.8	6.57	0.11
1,2,3,6,7,8-HxCDF	5.5	6.6	9.9	6.3	4.8	4.0	7.6	n.d.	10.5	4.51	n.d.
2,3,4,6,7,8-HxCDF	8.9	19.6	13.6	9.5	9.1	5.9	18.9	n.d.	13.2	6.16	0.18
1,2,3,7,8,9-HxCDF	2.8	6.5	4.2	2.4	3.4	2.1	4.2	n.d.	7.9	1.35	n.d.
1,2,3,4,6,7,8-HpCDF	36.4	59.5	31.2	26.6	28.4	18.1	52.4	6.0	45.9	60.04	0.43
1,2,3,4,7,8,9-HpCDF	4.7	12.3	4.5	3.4	5.0	3.0	5.1	n.d.	4.8	3.99	n.d.
OCDF	14.9	57.0	14.0	15.0	20.9	18.5	21.6	4.0	70.7	98.88	0.36
∑PCDDs	*683.9*	*1244.1*	*209.6*	*440.5*	*895.9*	*452.1*	*538.4*	*161.1*	*631.1*	1450.73	8.34
∑PCDFs	*101.5*	*197.2*	*106.5*	*87.3*	*97.7*	*65.6*	*138.5*	*20.2*	*181.7*	205.37	5.92
∑PCDD/Fs	**785.4**	**1441.4**	**316.1**	**527.8**	**993.6**	**517.7**	**676.8**	**181.4**	**812.8**	**1656.1**	**14.3**
WHO-TEQ	***22.1***	***25.7***	***15.8***	***18.4***	***20.7***	***10.4***	***18.6***	***6.9***	***22.6***	***13.8***	***0.8***

* Limits of detection are (<) values

The whether conditions during the sampling week, where precipitation (14th and 15th Nov) and winds up to 9 m/s were registered (Meteo France, station from Sete), might have favored the low concentrations levels found some days. Wind blew with a predominant NW direction during the sampling period except for a short gap (in the beginning and the end of the period) that it blew from N-NE (Figure 2.12). The lowest concentration in IF site (IF-3) was found when the highest wind speeds were registered, and the maximum value (IF-2) when the lowest wind speeds were registered (Figure 2.10). Regarding BZ site, located at the other shore of the lagoon, the lowest PCDD/Fs concentrations were observed in BZ-3 corresponding with some wind speed peaks. The highest value was obtained in BZ-4 when N direction winds were predominant. However, although wind speed seems to affect the PCDD/Fs concentrations observed in different days, only some preliminary indications can be presented so far regarding dynamics of PCDD/Fs in air of Thau Lagoon. An atypical situation in the sampling week occurred, probably due to the meteorological local conditions, in which some important phenomena affecting the burden of POPs in the air masses were minimized such as, the sea

breeze. This phenomenon has been described as an important vector in modulating the transport of pollutants between terrestrial and marine ecosystems (Pérez et al., 2003).

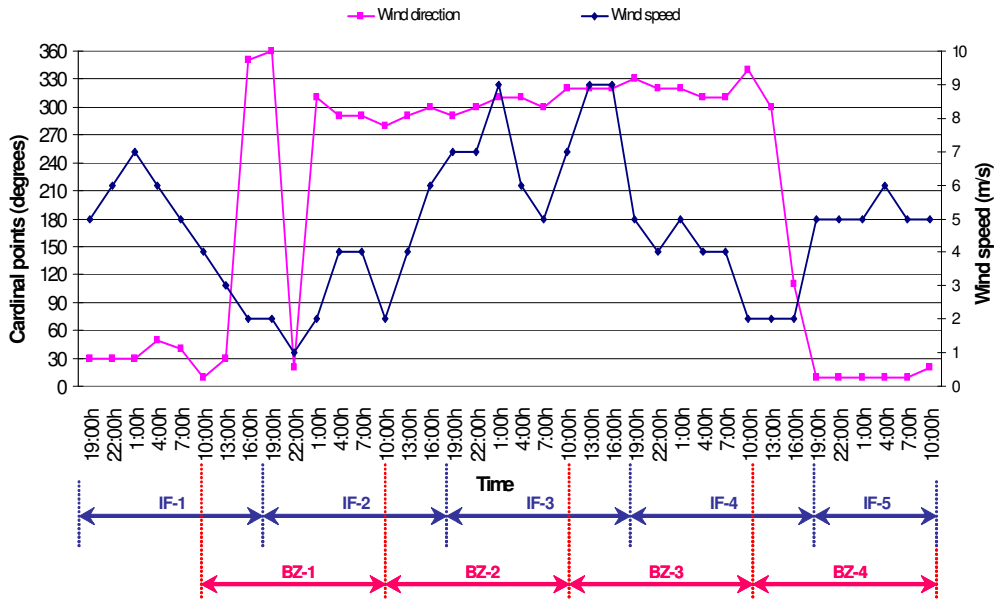

Figure 2.12: Wind directions and wind speeds registered during the sampling period (14-19 November 2005) together with the daily sampled intervals for both sites. Temperature varied from 5 to 16 ^{0}C during that period. Precipitation was registered on 14th and 15th Nov. Data are from Meteo France, station from Sete.

Sediments and mussels

A concentration of 13.8 WHO-TEQ pg g^{-1} was found for PCDD/Fs in surface sediment. Concentrations within the same range have been reported in the literature for surface sediments from several aquatic environments. PCDD/Fs concentrations in sediments from industrially influenced coastal areas in Southern and Easter Spain (Eljarrat et al., 2005) ranged from 0.1 to 48 WHO-TEQ pg g^{-1}. PCDD/Fs concentrations in sediments from an impacted estuarine system in Texas (USA) (Suarez et al., 2006) varied from 17.5 to 32.6 WHO-TEQ pg g^{-1}. Levels found in sediments from the Venice Lagoon (Dalla Valle et al., 2005) varied from 2.2 to 6.2 WHO-TEQ pg g^{-1}. In the latter study values from 20 to 11000 WHO-TEQ pg g^{-1} were also reported for the industrial canals of the lagoon. PCDD/Fs concentrations in lake sediments (located in a semi-rural area) in Northern Italy (Castro-Jiménez et al., 2005, 2007) varied from 0.13 to 16.9 WHO-TEQ pg g^{-1}.

A value of 0.8 WHO-TEQ pg g^{-1} was found for the analyzed mussels in the lagoon. Similar PCDD/Fs concentrations in mussels have been reported for the English Channel and the Atlantic French coast (Vilaine river bay) (Munschy et al., 2005). The observed value in Thau lagoon does not exceed the maximum level set by the European Community for marine products intended for human consumption (OJEC, 2002).

- Congener patterns

PCDD/Fs congener patterns observed in air samples for the different days of the sampled period in both sites were very similar suggesting an homogeneous situation during the sampled week in both

shores of the lagoon (Figure 2). Air and surface sediments patters were dominated for HpCDD and OCDD (Figure 3) and were in agreement with those usually reported in available literature (Hagenmeier et al., 1994; Lohmann and Jones, 1998; Castro-Jimenez et al., 2005; Castro-Jimenez et al., 2006), although a predominance of OCDF in sediments from Venice Lagoon has been reported (Dalla Valle et al., 2003).

The distribution pattern of PCDD/Fs in mussel samples are dominated by 2,3,7,8-TCDF and OCDD, these two congeners accounting for more than 70% of the quantified congeners (Figure 3) . This profile is similar to results previously described for mussels from other marine areas (Munschy et al., 2005; Abad et al., 2003).

When comparing the patterns from air, sediments and mussels (Figure 2.13), a very similar signal was observed in air and sediments. This finding suggests an influence of the atmosphere in the accumulation of PCDD/Fs in surface sediments. The signal observed in mussels was different to the one exhibited by the other two studied compartments suggesting a selective mechanism of accumulation. Thus, whereas the signal observed for HpCDD, OCDD, HpCDF and OCDF in the mussels it was very similar to the one in the sediment, the predominance of the low chlorinated PCDFs was not observed in any of the other compartments. This situation suggests a combined PCDD/Fs signal in mussels arriving in part from the sediment but also most probably from water column.

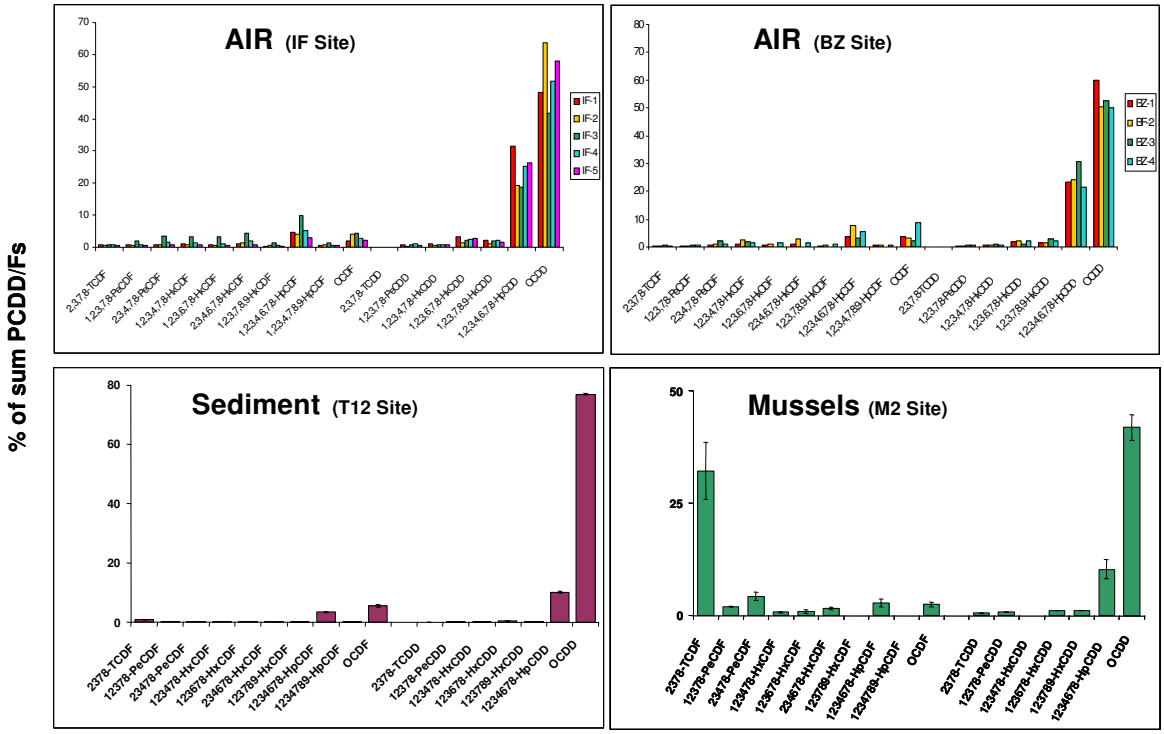

Figure 2.13: Distribution pattern of PCDD/F congeners in air (both air sampling sites), sediment (mean values and standard deviations, n=2) and mussels samples (mean values and standard deviations, n=3) from Thau lagoon.

In spite of some clear indications presented in this work, further research in needed in order to determine whether the atmosphere has a significant role in the accumulation of PCDD/Fs in surface sediments. Moreover, more data are needed to clarify the source of PCDD/Fs in mussels from Thau

lagoon. Thus, a wider spatial distribution of sediment sampling together with measurements of PCDD/Fs concentrations in the water columns is on going. Analyses of other relevant POPs such as, PCBs in air of Thau Lagoon is taking place in order to verify the presented hypotheses. Back trajectories of air masses arriving to the area will be also evaluated to better understand the occurrence of these pollutants in this coastal lagoon.

PCBs

Concentrations and levels

Concentration of PCBs in the environmental compartments from studied from Thay Lagoon are presented in Table 2.7.

An average ∑PCB air concentration of 38 ± 9 pg m^{-3} was obtained at I site whereas a value of 44± 19 pg m^{-3} was measured at BZ site. Concentration were writhing the same order of magnitude in both sites as happened for PCDD/Fs. Similar values were obtained in the less impacted area of another coastal lagoon, Venice Lagoon (Manodori et al., 2006). The observed concentrations are also within the range of those reported from rural and forested areas in Germany (25-58 pg m^{-3}) for the same sum of isomers except 118 congener (Mandalakis and Stephanou, 2006).

∑PCBs concentration in sediments from Thau Lagoon was 41.6 ng g^{-1}. Lower values were found in sediments from Venice Lagoon (Moret et al., 2001). Regarding mussels, 31ng g^{-1} was found for ∑PCBs.

Table 2.7. Concentrations of PCBs in air (pg m^{-3}), surface sediments and mussels (pg g^{-1} dry weight) found in Thau lagoon.

Compounds	Air (gas+particle phase)									Mussels	Sediment
	Site IF (14-18 Nov 05)					Site BZ (15-18 Nov 05)				M2 (May 04)	T12 (May 04)
	IF-1	IF-2	IF-3	IF-4	IF-5	BZ-1	BZ-2	BZ-3	BZ-4		
TrC28	7.6	5.6	3.6	4.1	3.7	9.2	5.4	4.4	1.9	173.4	914.0
TeCB-52	8.3	7.1	4.3	4.7	4.3	9.8	4.7	4.0	2.8	253.8	1332.6
PeCB-101	7.0	6.4	4.3	3.8	5.6	8.2	3.9	3.2	2.4	3542.5	4080.3
PeCB-118	0.8	n.d.	n.d.	2.7	6.0	1.3	2.4	26.1	60.6	3326.5	6140.6
HxCB-138	4.5	4.7	2.3	2.4	5.9	3.7	2.2	1.3	0.6	4388.9	4281.2
HxCB-153	7.3	7.6	4.5	3.8	8.4	7.8	3.6	2.2	1.7	18669.7	21148.7
HpCB-180	2.6	2.6	1.5	1.5	5.3	1.6	1.4	n.d.	n.d.	502.6	3756.4
∑PCBs	**38.1**	**34.2**	**20.4**	**22.9**	**39.1**	**41.6**	**23.6**	**41.3**	**70.0**	**30857.3**	**41653.8**

n.d = not detected .

Higher concentration observed at BZ-3 and BZ-4 for congener 118 were due to an analytical problem

3. Toxic effects at individual/population level

Laboratory experiments were performed with unispecific phytoplankton cultures to analyze the thresholds for the lethal concentration of some PAH's for populations of these organisms. A solution of PAHs dissolved in Acetonitrile, Pyrene dissolved in Methanol and Pyrene and Phenantrene dissolved in DMS were added to exponentially growing cultures of *Prochlorococcus marina* (CCMP1375), *Synechococcus sp* (CCMP833), *Chlorella marina*, *Dunaliella sp*, *Micromonas pusilla*, *Phaeodactyllum tricornutum* and *Thlassiossira pseudonana* (CCMP1335).

3.1. CULTURE AND EXPERIMENTAL CONDITIONS

The cultures grew in batch cultures under optimal temperature of 18ºC, but 21ºC for *Synechococcus* and *Prochlorococcus,*and under continuous light conditions, in a nutrient-rich medium (F/2 medium, except *Prochlorococcus marina*, which growth in Pro-99 medium), inside 5 litter glass bottles. Once populations entered in an exponentially growing stage, the culture was dispensed into different policarbonate bottles of 250 ml volume (2 replicates for each volume) where a gradient of increasing PAHs concentration, including single compounds and mixtures, were added to exponentially growing cultures. The lethality of a solution of a mixture of PAHs dissolved in acetonitrile, pyrene dissolved in methanol, and pyrene and phenantrene dissolved in DMS were tested. Two replicates without adding PAHs were run as controls, and two more replicates with added acetonitrile, methanol or DMSO at the final concentration equivalent to that of the highest PAHs concentration treatment were performed as a control of the effect of the solvents. The evolution of the population was followed from a total of 4 days (*Prochlorococcus marina, Synechococcus sp.*) to a maximum of 28 dyas (*Dunaliella sp*), depending on the population response.

The changes in the population abundance were followed by sampling the cultures daily or each two days, depending on the species growth rate. Fresh samples were analyzed by flow cytometric techniques using a a FACSCalibur flow cytometer. The proportion of living and death cells in the different populations were followed by applying a cell membrane permeability test, the cell digestion assay (Agustí and Sánchez 2002). The cell digestion assay was applied to replicate samples, by adding 200 µl of DNAse I solution (400 µg ml^{-1} in HBSS (Hanks' Balanced Salts)) to 1 ml sample of each treatment, followed by 15 minutes incubation at 35°C in a Digital Dry Bath. After this time, 200 µl of Trypsine solution (1% in HBSS) were added, followed by 30 minutes incubation at 35°C.

The cell death rate of the populations during exponential growth was calculated by using the abundance of dead and alive cells as indicated in Brussard et al. (1997), following the equation:

$$\delta_b = \frac{\ln x_t - \ln x_0}{t \cdot \left(\frac{(x+y)_t - (x+y)_0}{y_t - y_0} - 1 \right)}$$

where δ_b is the cell death rate (d^{-1}), x is the concentration of living cells, (x+y) is the total concentration and y is the concentration of dead cells. The total concentration of cells at time t is represented by $(x+y)_t$ and the concentration of dead cells at time t by y_t.

3.2. RESULTS

The experiments performed with acetonitrile and methanol, used as solvents to prepare the PAHs solutions, indicated that the amounts of the solvents used were toxic for phytoplankton, as indicated by the cell death induced in the solvent controls. However, Pyrene and Phenantrene dissolved in DMSO didn't induce lethality and the results presented are restricted to the experiments performed with Pyrene and Phenantrene dissolved in DMSO. The concentrations of Pyrene and Phenantrene analyzed varied from 5 to 1000 µg/l

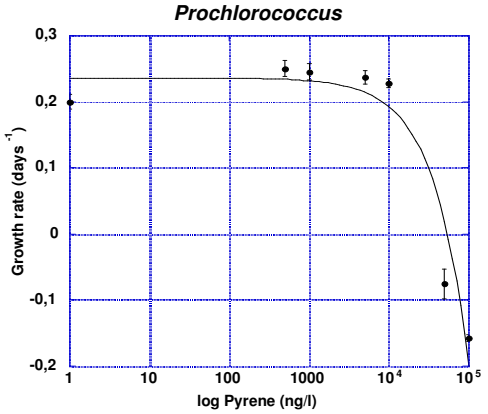

Figure 3.1: Variability of *Prochlorococcus marina* growth rate growing under a gradient of Pyrene concentrations dissolved in DMSO.

The results indicated that most species were quite resistant to Pyrene and Phenantrene, since there were not population mortality detected, except for the picosized species *Prochlorococcus marina* and *Synechococcus sp*., which showed strong lethality at high concentrations of Pyrene and Phenantrene.

The lethal threshold concentration of Pyrene was 80 µg/l for *Prochlorococcus marina*, whereas for Phenantrene it was between 100 µg/l (toxic), and 500 µg/l (lethal). On the other hand, *Synechococcus sp* appeared to be more resistant than *Prochclorococcus marina* for both Pyrene and Phenantrene.

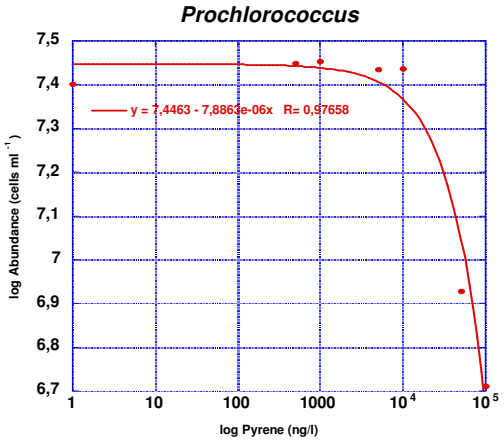

Figure 3.2: Variability of *Prochlorococcus marina* cell abundance (cells ml $^{-1}$) growing under a gradient of Pyrene dissolved in DMSO. The lethal Pyrene dose required to decimate the population of *Prochlorococcus marina* to the half, was 80,000 ng/l.

For Synechoccoccus sp., Pyrene appeared to be toxic above concentrations of 500 µg/l, and lethal at 1000 µg/l, while Phenantrene appeared to be toxic above concentrations of 100 µg/l and lethal at 500 µg/l.

On the other hand, *Chlorella marina*, *Dunaliella sp*, *Micromonas pusilla*, *Phaeodactyllum tricornutum* and *Thlassiossira pseudonana* (CCMP1335), didn't showed catastrophic mortality of their populations when growing at the concentrations of pyrene and phenantrene tested. However, we could detect some effect of Pyrene and Phenantrene toxicity as , for example, *Chlorella marina*, showed an important decrease in growth rate as the Pyrene concentration increased. Thus we are exploring now whether the lethality of the two PAHs tested could be detected when considering the changes in the proportion of dead cells within the populations, and in the cell mortality rates calculated during the exponential growth.

4. Experiments on Natural communities

Experiments to analyze the lethal threshold of PAH's and metals on natural communities of phytoplankton were performed with coastal Mediterranean plankton, sampled at the field station of Far Cap Ses Salines, Mallorca Island, and on oceanic plankton sampled during the oceanographic cruise THRESHOLD-1. The cruise THRESHOLD-1 was performed on board the RV Garcia del Cid, along the Mediterranean and Black Seas from June 3 to July 5, 2006.

A total of 8 experiments were carried out during the cruise THRESHOLD-1 with PAH's (Pyrene and Phenantrene), metals (Cadmium and Lead) and Methanol. The experiments performed with coastal Mediterranean plankton sampled at the Far Cap Ses Salines field station (Mallorca Island) were exploratory and were principally used to test the methodologies, and analyze the range of concentrations of pollutants to be tested during the cruise.

4.1. SAMPLING AND EXPERIMENTAL SET-UP

Surface water (5 m) of the Mediterranean and Black Sea used in the experiments was sampled by using Niskin bottles attached to a rosette-CTD system. Water from the Deep Chlorophyll Maximum (DCM), located at 50 m depth, was also sampled and used for the experiment with methanol.

The experiments, 8 altogether, were carried out with PAH's (Pyrene and Phenantrene), metals (Cadmium and Lead) and Methanol. Lethality of Pyrene, Phenantrene, Cadmium (two experiments), Lead and Methanol was tested for Mediterranean plankton, whereas Cadmium and Lead were also tested for Black Sea samples. Stock solutions of Pyrene and Phenantrene were dissolved in DMSO in a concentration of 1000 µg/ml. By the way, Cadmium and Lead were dissolved in distilled water in a concentration of 1 ppm.

Experiments began with the distribution of water sampled into 250 ml acid clean Pyrex bottles. After this gathering, contaminants were inoculated at different concentrations, and bottles were incubated on deck under natural solar radiation in a surface running system incubator to keep "in situ" temperature conditions. Bottles were covered with a neutral net to simulate 5 meters or DCM underwater light conditions.

Depending on the experiment, a gradient of concentration of PAHs and metals was tested in duplicated bottles. The following concentrations were used:

- For Cadmium and Lead: 0.01 ppb/ 0.02 ppb/ 0.11 ppb/ 1.12 ppb/ 12 ppb/ 112 ppb/ 1000 ppb.
- For Pyrene and Phenantrene: 5 µg/l – 10 µg/l – 50 µg/l – 100 µg/l – 500 µg/l – 1000 µg/l .
- For methanol: 10 µg/l – 50 µg/l – 250 µg/l – 1250 µg/l – 6000 µg/l.

The different concentrations were tested in duplicated bottles. Duplicated bottles without chemical additions were also run as controls. For the Pyrene and Phenantrene experiments, a control of the

effect of DMSO, used as solvent, was also run in duplicate, by adding DMSO at the equivalent concentration used in the 1000 µg/l treatment.

The effects of these contaminants in the communities of picoplankton and microplankton were followed daily for the following 4 days after inoculation.

4.2. ANALYSIS

The effect of PAH's and metals on the picophytoplankton community was followed by analyzing changes in the abundance and viability of Prochlorococcus sp, Synechococcus sp and Eukaryote picoplankton .

The changes in the abundance of picophytoplankton communities were quantified by using fresh samples counted in a FACSCalibur, Becton Dickinson, flow cytometer. The proportion of living and death cells in the picophytoplankton communities along the experiments were followed by applying a cell membrane permeability test, the cell digestion assay (Agusti & Sanchez 2002) which allow the counting and identification of living phytoplankton cells. The cell digestion assay was applied to replicate samples, by adding 200 µl of DNAse I solution (400 µg ml^{-1} in HBSS (Hanks' Balanced Salts)) to 1 ml sample of each treatment, followed by 15 minutes incubation at 35°C in a Digital Dry Bath. After this time, 200 µl of Trypsine solution (1% in HBSS) were added, followed by 30 minutes incubation at 35°C. At the end of this time, samples were kept in cold conditions in order to stop the cell digestion process.

After incubation, samples were counted using a FACSCalibur Flow Cytometer (Beckton Dickinson). Replicated fresh water samples without enzymes addition were counted and represented total population abundance (living + dying cells), whereas those samples exposed to the enzymes during the cell digestion assay, represented living cells. A calibrated solution of 1µm diameter fluorescent beads (Polysciences Inc.) was added to the 1 ml samples as a standard for the quantification of cell concentration. The red, green and orange fluorescence, and forward and side scattering signals of the cells and beads were used to detect different populations and to differentiate them from the fluorescent beads (Marie et al. 1999).

Apart of this, changes in total phytoplankton abundance during the experiments were followed by analyzing Chlorophyll a concentration. For this estimation, 50 ml samples were filtered from each bottle on the day 0, day 2 and last day (day 4). For this filtration, 25 mm diameter Whatmann GF/F filters were used and kept in tubes with 90% acetone for 24 hours. Then, Chlorophyll a was measured with an espectrofluorometer (Shimadzu RF-5301 PC), as described in Parsons et al. (1984).

4.3. RESULTS

Picophytoplankton populations composed by *Prochlorococcus sp, Synechococcus sp* and Eukaryote picoplankton, were important components of the phytoplanktonic community in the Mediterranean and

Black Sea waters examined. Preliminary results indicated that Pyrene and Phenantrene appeared to be toxic above 50 µg/l and lethal at 100 µg/l, for*Synechococcus sp* populations, as indicated by the decrease in the abundance of these populations during the experiments, observed at the highest concentrations added. The abundance of *Prochlorococcus* and eukaryotic picophytoplankton cells was low, and this unable to analyzed properly the effect of PAHs in theses populations.

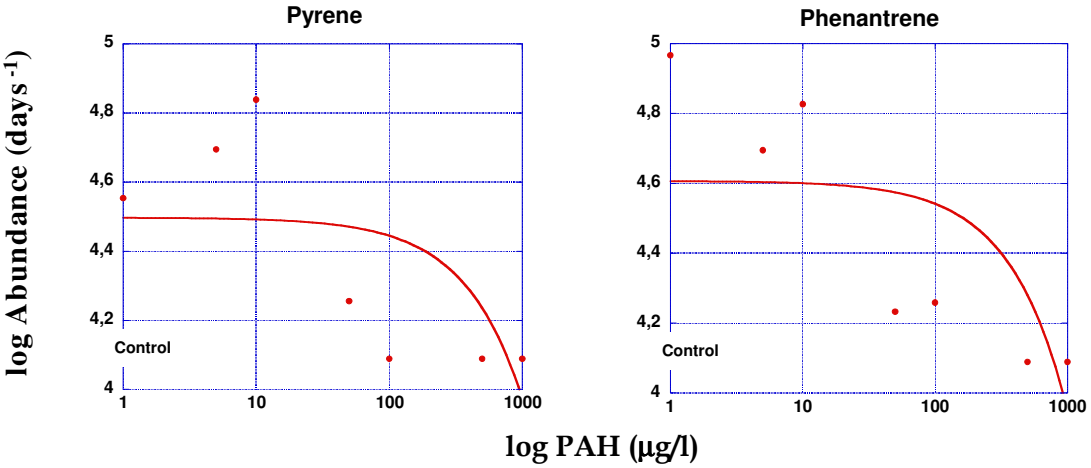

Figure 4.1. Variability of *Synechococcus sp* abundance (cells/ml) under a gradient of PAH concentrations dissolved in DMSO.

On the other hand, preliminary results showed that metals appeared to be toxic as well, at concentrations higher than 1.12 ppb for Cadmium and 12 ppb for Lead, and appeared to be lethal for concentrations at 12 ppb for Cadmium and 112 ppb for Lead, for both picophytoplankton populations. There was no effect of methanol in the picoplankton communities.

Finally, the variability in Chlorophyll a concentration was examined as an indicator of the effect of pollutants in all the phytoplankton community. Preliminary results showed that phytoplankton abundance, as Chla, decreased as the PAH concentration was increased.

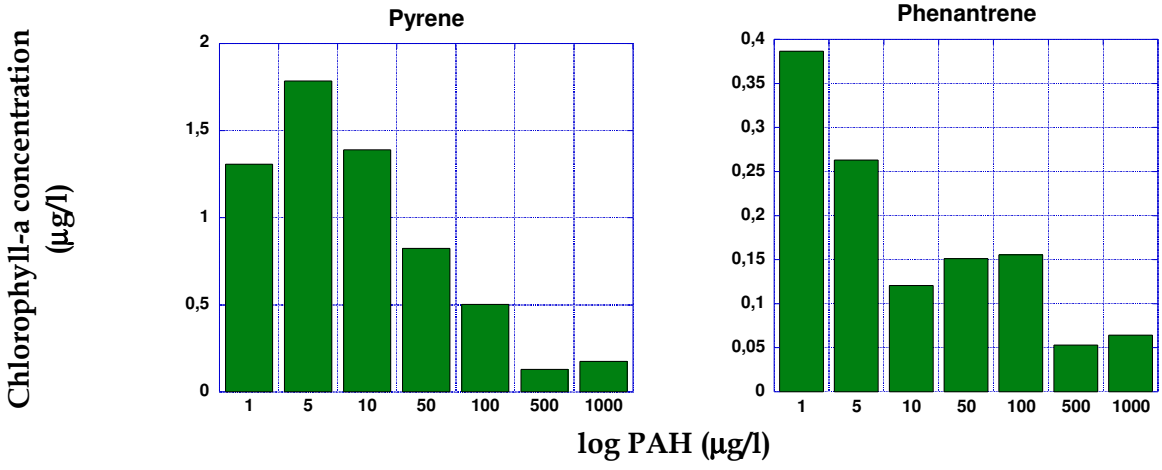

Figure 4.2. Chlorophyll-a concentration (µg/l) on day 4 under a gradient of PAH concentrations dissolved in DMSO.

5. Preliminary Conclusions

This deliverable shows the results and work done so far on the response of aquatic planktonic populations under the pressure of organic pollutants.

The experiments performed with incubations show that lethality to PAHs and methanol are only evident at high concentrations. However, other effects could also be found at lower concentrations due to the interactions with UV radiation, etc. In this respect, the realization of a sampling cruise in the Mediterranean will allow for the first time the generation of a comprehensive data set containing both fate and transport data for POPs and information of the status of phytoplankton cells and their variability at the Mediterranean regional scale. Furthermore, it will also be possible to apply the fate and transport model generated within the Thresholds project allowing determining under which conditions the systems goes to a point of no return in terms of pollutant effects in planktonic populations.

The topics covered in this work have received little attention so far and therefore the work being performed fills an important gap in the field of environmental toxicology and chemistry.

6. References

Abad E, Pérez F, Llerena J J, Caixach J, Rivera J. 2003. Evidence for a specific pattern of Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans in bivalves *Environmental Science and Technology* **37**: 5090-5096.

Agustí S. and M.C. Sánchez. 2002. Cell viability in natural phytoplankton communities quantified by a membrane permeability probe. *Limnology and Oceanography* **47**: 818-828.

Amouroux, D. LCABIE-CNRS, Hélioparc, Université de Pau, France

Borchardt, T.. 1983 .The influence of food quantity on the kinetics of Cd uptake and loss via food and sea water in *Mytilus edulis.Marine Biology* **76**: 67.

Brussard C.P.D., Noordeloos A.A.M., Riegman R. 1997. Autolysis kinetics of the marine diatom Ditylum brightwellii (Bacillariophyceae) under limitation and starvation of nitrogen and phosphorus. *J. Phycol.* **33**: 980-987.

Buffle, J..1988. *Complexation reactions in aquatic systems: An analytical approach*. J.Wiley and sons

Castro-Jiménez J, Canuti E, Christoph E H, Eisenreich S J, Hanke G, Mariani G, Skejo H, and Umlauf G.2005. Tracing atmospheric deposition of PCDD/Fs in Lake Maggiore(Italy): Preliminary results on settling material and sediments. *Organohalogen Compounds* **67**: 1209-1215.

Castro-Jiménez J, Mariani G, Eisenreich S J, Christoph E H, Hanke G, Canuti E, Skejo H, and Umlauf G. 2006, Atmospheric input of POPs into Lake Maggiore (Northern Italy): PCDD/Fs and dioxin-like PCBs profiles and fluxes in the atmosphere and aquatic system. *Chemosphere* (submitted).

Claisse, D. 1989. Chemical contamination of the French coasts: the results of a ten years Mussel Watch. *Mar. Pollut. Bull*. **20**: 523-528.

Claisse, D., D. Cossa et J. Bretaudeau-Sanjuan 2001. Methylmercury in molluscs along the French coast. *Mar. Pollut. Bull*. **42**: 329-332.

Cossa, D. and Coquery, M. 2005. The Mediterranean mercury anomaly, a geochemical or a biological issue. pp 177-208. In *The Mediterranean Sea*. Handbook of Environmental Chemistry, Vol 5. Saliot, A. editeur. Springer, 413 p. ISSN 1433-6863

Cossa, D., B. Averty, J.Bretaudeau et A.-S. Senard. 2003. Spéciation du mercure dissous dans les eaux marines. Méthodes d'analyse en milieu marin. Co-édition Ifremer et Ministère de l'Ecologie et du Développement Durable. 27 pp. ISBN 2-84433-125-4.

Cossa, D., J.M. Martin, K. Takayanagi et J. Sanjuan. 1997. The Distribution and Cycling of Mercury in the Western Mediterranean. *Deep Sea Res.* **44**: 721-740.

Dalla Valle M, Marcomini A, Sweetman A J, and Jones K C. 2005. Temporal trends in the sources of PCDD/Fs to and around the Venice Lagoon**.** *Environment International* **31**: 1040-1046.

Danielsson L.G., Magnusson B., Westerlund S. & Zhang K. 1982 Trace metal determination in estuarine waters by electrothermal atomic absorption spectrometry after extraction of dithiocarbamate complexes into freon. *Analytica Chimica Acta* **114**:183-188

del Giorgio, P.A., Gasol, J.M., Vaque , D., Mura, P., Agusti, S., Duarte, C.M., 1996. Bacterioplankton community structure: protists control net production and the proportion of active bacteria in a coastal marine community. *Limnology and Oceanography* **48**: 1169-1179.

Eljarrat E, De La Cal A, Larrazabal D, Fabrellas B, Fernandez-Alba AR, Borrull F, Marce RM, Barcelo D. 2005. Occurrence of polybrominated diphenylethers, polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls in coastal sediments from Spain. *Environmental Pollution* **136**: 493-501.

Grasshoff, K, M. Ehrardt and K. Kremling. 1983. *Methods of Sea water Analysis*. Verlag-Chemie

Hagenmeier H, Lindig C. and She J. 1994. Correlation of environmental occurrence of polychlorinated dibenzo-p-dioxins and dibenzofurans with possible sources.*Chemosphere* **29**: 2163-2174.

Johansson I, Moisan K, Guiot N, Truquet I, Munschy C, Tronczyński J. 2006. Polybrominated diphenyl ethers (PBDEs) in mussels from selected French coastal sites: 1981–2003. *Chemosphere* **64**: 296-305.

Kozelka, P.B. and Bruland K.W. 1998 Chemical speciation of dissolved Cu, Zn, Cd, Pb in Narragansett Bay, Rhode Island. *Marine Chemistry* **60**:267-282.

Lohmann R, and Jones KC. 1998. Dioxins and furans in air and deposition: a review of levels, behaviour and processes. *The Science of the Total Environment* **219**: 53-81.

Mandalakis, M and Stephanou G.E. 2006. Atmospheric concentration characteristics and gas-particle partitioning of PCBs in a rural area of eastern Germany. *Environmental Pollution* (in press)

Manodori L., Gambaro A., Moret I., Capodoglio G., Cairns W.R.L., Cescon P. 2006. Seasonal evolution of gas-phase PCB concentrations in Venice Lagoon Area. *Chemosphere* **62:** 449-458

Marie D., Partensky F., Simon N., Guillou L. And Vaulot D. (19999. Flor cytometry análisis of marine picoplankton. In R. A. Diamond and S. DeMaggio [ed.]. Living colors: Protocols in flor cytometry and cell sorting. Springer-Verlag.

Morel, F.M.M., Hudson, R.J.M. and Price, N.M. 1991. Limitation of productivity by trace metals in the sea. *Limnol. Oceanogr* **36**:1742-1755.

Morel, F.M.M.,& Hering J.G. 1993. Principles and applications of aquatic chemistry. Wiley Interscience . John wiley &sons Inc.

Moret I., Piazza R., Benedetti M., Gambero A., Barbante C., Cescon P. 2001. Determination of polychlorobiphenyls in Venice Lagoon sediments. *Chemosphere* 43: 559-565.

Munschy C, Johansson I, Guiot N, Héas-Moisan K, Tronczyński J. *Organohalogen Compounds* 2005; 67: 1243.

Official Journal of the European Communities (2002/201/EC). 2002. Annex I.

Parsons T.R., Maita Y. and Lalli C.M. 1984 *A manual of chemical and biological methods for seawater analysis*. Pergamon Press, Oxford

Pérez S, Dachs J, Barceló D. 2003. Sea Breeze Modulated Volatilization of Polycyclic Aromatic Hydrocarbons from the Masnou Harbor (NW Mediterranean Sea). *Environmental. Scence and. Technology* 37: 3794-3802.

Phinney J.T. & Bruland K.W. 1994 Uptake of lipophilic organic Cu, Cd, and Pb complexes in the coastal diatom Thalassiosira weissflogii. *Environmental Science and Technology* **28**: 1781-1790

Réseau National d'Observation de la qualité du milieu marin (http://www.ifremer.fr/envlit/surveillance/rno.htm).

Suarez MP, Rifai HS, Palachek R Dean K, and Koening L.2006. Distribution of polychlorinated dibenzo-p-dioxins and dibenzofurans in suspended sediments, dissolved phase and bottom sediment in the Houston ship Channel. *Chemosphere* **62**: 417-429.

Tseng, C.M., A. de Diego, H. Pinaly, D. Amouroux, O.F.X. Donard. 1998. Cryofocusing coupled to atomic absorption spectrometry for rapid and simple mercury speciation in environmental matrices. *J. Anal. Atom. Spectro*. **13**: 755-764.

U.S. EPA., 1994. Method 1613: Tetra-through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS.

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Abstract
In this report we present the experimental campaigns carried out to develop a comparison between contaminant thresholds at several levels focusing on population level, as well as the campaigns carried out to assess the speciation of a contaminant and hence on their on their bioavailability for the two selected metals in Thresholds: Cd and Hg. Due to some delays in the experimental phase (shifting of campaigns) as well as in the analysis of samples, in some cases the results are still at a preliminary stage. Therefore, improvements in this Deliverable are expected as the analytical results will be available at the laboratories of the Thresholds' partners. It is expected that these new results will be included in the Synthesis deliverable (D4.1.5).
These experimental results also support the development of integrated fate and an effects model that allows the estimation of the main fluxes between compartments, i.e. air/water/sediments as well as the simulation of scenarios that could produce reaching a threshold (see D2.6.2). Under this approach, the role of the air-water interface and the biota (phytoplankton and bacteria) is being examined. This will help to determine levels of emissions that could produce a threshold point be reached.

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