

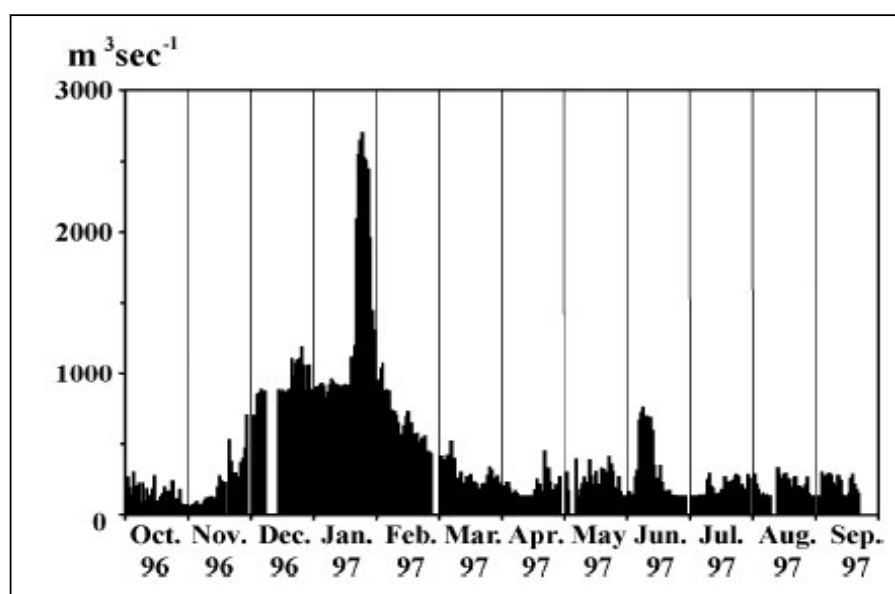
**DESENVOLUPAMENT DE METODOLOGIES ANALÍTIQUES PER  
A LA DETERMINACIÓ DE COMPOSTOS ORGÀNICS EN  
MATRIUS COMPLEXES.  
APLICACIÓ A L'ESTUARI DEL RIU EBRE**

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## 5. Estudi de cas: Estuari del riu Ebre

El riu Ebre és el més llarg de l'Estat Espanyol i drena una superfície d'aproximadament 84000 km<sup>2</sup> naixent a Cantàbria i desembocant al mar Mediterrani Occidental. Té un cabal mitjà de 9.2 km<sup>3</sup>/any però presenta una gran variabilitat podent així tenir cabals puntuals inferiors a 100 m<sup>3</sup>/s en època d'estiatge i superiors a 2500 m<sup>3</sup>/s en època d'avingudes (Figura 9). Malgrat això, la mitjana en el període 1960-1999 fou de 416 m<sup>3</sup>/s amb una tendència decreixent [UNEP/MAP, 2003]. És el segon riu més important del Mediterrani occidental tan sols superat pel Roine, que té un cabal de 53.9 km<sup>3</sup>/any.



**Figura 9.** Cabal de l'Ebre mesurat a Tortosa [CHE, 2005, Cruzado *et al.*, 2002].

Pel que fa referència a l'aport de sediments, es limitat pels més de 100 embassaments que es troben a la seva conca. Així doncs, pateix d'una forta reducció en el transport sedimentari: de les 14-25 Mt/any de l'època anterior als embassaments a les 0.12-0.15 Mt/any en l'actualitat [Guillén i Palanques, 1992].

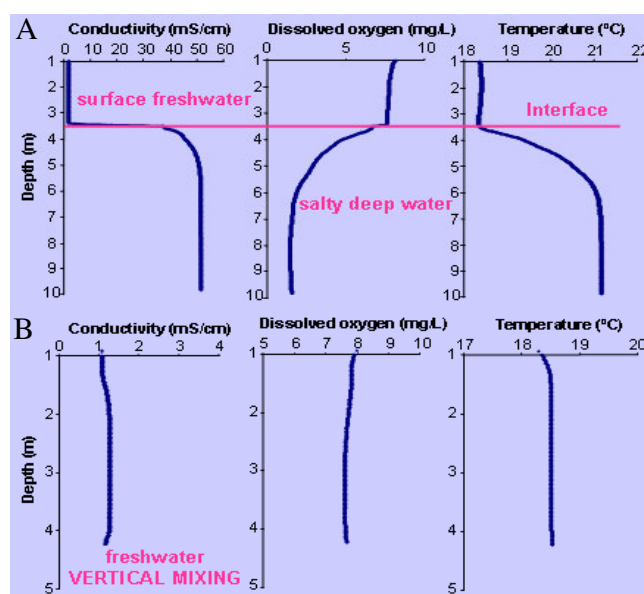
L'Ebre forma un delta a la desembocadura de 320 km<sup>2</sup> amb un 77% d'aquesta superfície utilitzada per l'agricultura [Sierra *et al.*, 2004]. Aquest Delta, també inclou habitats naturals, principalment aiguamolls, de gran riquesa ecològica que estan protegits per diferents regulacions. A la part superior de la Figura 10 s'observa perfectament la conca del riu Ebre que desemboca, com s'observa en la part inferior de la figura, al Mediterrani formant un delta.



**Figura 10.** Fotografies mostrant la conca del riu Ebre i un eixamplament de la zona del seu delta [NASA, 2006].

Si considerem les possibles fonts de contaminació que poden afectar a l'Ebre, hauríem de pensar en primer lloc amb les aigües residuals generades per les aproximadament 3 milions de persones que habiten a la seva conca. També, l'activitat agrícola tindrà com a conseqüència l'aplicació de plaguicides. En aquests sentit, cal considerar especialment la zona deltaica (77% superfície agrícola) ocupada en un 54% pel monocultiu de l'arròs [Prat i Ibañez, 1995]. Finalment, també s'esperen aportos d'origen industrial com a conseqüència dels diferents nuclis industrials existents a la conca (Saragossa, Monzó, Flix, etc.). Cal destacar especialment, la planta electroquímica situada a Flix i en la que han sintetitzat substàncies organoclorades durant més d'un segle.

Degut, a les característiques batimètriques del riu, al seu cabal i a que el Mediterrani és un mar de mareas febles; al delta de l'Ebre, s'observa el fenomen de la formació de la falca salina. Aquest fenomen correspon a la intrusió, terra endins, d'una llengua d'aigua salada d'origen marí que no es barreja amb l'aigua dolça del riu. Aquesta, menys densa, flueix pel damunt de l'aigua salada de mar, més densa, tot formant perfils verticals molt estratificats. Quan el cabal és inferior a 400 m<sup>3</sup>/s es començarà a formar arribant a 32 km terra endins (Barranc de la Galera, aigües amunt d'Amposta) amb cabals inferiors a 100 m<sup>3</sup>/s, en canvi la seva desaparició no tindrà lloc fins que s'arribin a cabals superiors a 400 m<sup>3</sup>/s.. A més cal comentar que no tan sols s'obtenen perfils verticals estratificats a la columna d'aigua per la conductivitat sinó també per la temperatura, l'oxigen dissolt, DOC, POC, PON, etc. (Figura 11).



**Figura 11.** Perfils verticals en condicions de falca salina (A) i en la seva absència (B).

En aquest apartat de la Tesi Doctoral, s'estudia amb detall l'estuari del riu Ebre i el fenomen de la falca salina. En primer lloc, s'estudien els marcadors moleculars lipídics que ens han de permetre d'identificar l'origen de la matèria orgànica, els diferents processos biogeoquímics que hi tenen lloc, avaluar l'ecosistema i finalment utilitzar alguns d'aquests compostos com a traçadors moleculars de la contaminació antropogènica. En un segon apartat, s'estudia la presència i el comportament de diferents contaminants orgànics en l'estuari de l'Ebre posant un especial èmfasi en l'estudi de la influència de la falca salina en la distribució d'aquests compostos i en el càlcul de la contribució de contaminants que el riu Ebre aporta al mar Mediterrani. Tant pels traçadors moleculars com pels contaminants orgànics s'estudia la seva distribució geogràfica, la seva variabilitat temporal i la influència de la falca salina en les seves concentracions.

Cal destacar que un estuari de falca salina és un sistema obert de gran complexitat espacio-temporal. En el cas dels contaminants orgànics presents a l'estuari existeixen diferents vies d'entrada (contaminació difosa, abocaments puntuals i variació estacional) i de sortida (emissió al mar, absorció en la matèria orgànica, evaporació, difusió a capes profundes) i també hi han diferents processos de degradació (reaccions fotoquímiques, biodegradació,...). A més s'ha de considerar que en el cas dels contaminants existeix una dificultat analítica inherent al treballar a nivells de traces. Així doncs, pel que fa als contaminants orgànics, s'ha desenvolupat un experiment de falca salina en microcosmos per a estudiar el comportament de les diferents famílies de contaminants orgànics identificats previament a l'estuari de l'Ebre.

## **5.1 Caracterització de traçadors moleculars lipídics en partícules en suspensió de l'estuari del riu Ebre**

### *5.1.1 Caracterització de lípids emprant GC×GC-ToF MS*

En aquest apartat es presenta l'article, "*Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection as a novel tool in environmental molecular marker characterization.*", que és el resultat de la col·laboració amb l'equip investigador del Dr. René Vreuls i del Dr. Udo U.A.Th Brinkman de la Vrije Universiteit d'Amsterdam. En aquest treball es presenta les potencialitats d'aquesta tècnica en temàtiques ambientals i més concretament en la caracterització de traçadors moleculars lipídics. De fet, l'article correspon a l'aplicació

de la metodologia desenvolupada en la secció 4.1 per la lanolina a la matèria en suspensió de l'estuari del riu Ebre.

***COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY WITH  
TIME-OF-FLIGHT MASS SPECTROMETRIC DETECTION AS A NOVEL TOOL  
IN ENVIRONMENTAL MOLECULAR MARKER CHARACTERIZATION.***

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Abstract

Lipids are ubiquitous in the environment and are constituted by a mixture of different chemical classes. Some of them have been used as molecular markers of different ecosystems, living organisms, anthropogenic influence or biogeochemical processes. Comprehensive two-dimensional gas chromatography coupled to Time-of-Flight Mass Spectrometry (GC×GC–ToF MS) has proven to be a powerful technique to characterize complex samples and has already been tested for complex lipid samples. In this work, this technique has been applied to the characterization of lipidic molecular markers in the Ebro River particulate matter, a vertically stratified estuary with a freshwater layer on top and a salty water layer in the bottom. The GC×GC-TOF technique was compared with conventional monodimensional GC-MS and GC×GC coupled to a flame ionization detector. The enhanced system resolution permitted to reduce coelutions and tedious sample pretreatment. Samples from the different water bodies were collected from the salt wedge stratified estuary, due to its characteristic behavior. One hundred fifteen potential molecular markers have been identified and some of them have been used to improve our knowledge on the estuary behavior. In this way, different organic matter origin has been found for the two water bodies and some of the occurring biogeochemical processes have been studied such as the photooxidation. Furthermore, this technique permitted to compare the plankton characteristics from the two water bodies finding a higher abundance of zooplankton in the bottom layer. For the phytoplankton, higher abundance of diatoms was found in the bottom water and the dinoflagellates were predominant in the top layer water. Finally, the anthropogenic influence was tracked by using some of the identified fecal sterols.

*Keywords*

Comprehensive two-dimensional gas chromatography, Time-of-Flight Mass Spectrometry, lipids, molecular markers, salt wedge.



## **Introduction**

Lipids are widespread compounds in living organisms and in the environment and their main abilities are related to structural properties, energy storage capacity and metabolic importance. The most important lipids are probably fatty acids (FA), which are essential parts of most living cells and cellular fluids. They can be present directly as free fatty acids or esterified. The diversity and abundance of FAs among living organisms are very useful for classifying and determining biological interactions [Zhukova *et al.*, 1999]. In this way, FAs, polyunsaturated fatty acids (PUFAs) but also other lipids, such as fatty alcohols (FAL) or sterols (ST) have been widely used in order to identify the planktonic contribution into the lipidic fraction of environmental samples. Molecular markers are often used in aquatic sediments or suspended particulate matter (SPM) in order to identify the organic matter (OM) origin and to describe the occurring biogeochemical processes. Estuarine and coastal zones are areas of particular interest, as OM can come from a continental or marine origin. Furthermore, they represent very active biogeochemical areas. A great effort has been done in order to identify useful lipidic molecular markers, but the lack of complete source specificity is often a problem leading to misinterpretations. Stable isotope determination of individual lipids can improve the source assessment specificity but the state of the art of IRMS limited its application to major components [Meier-Augenstein, 1999]. Moreover, more selective markers are often present in low concentrations within a complex environmental sample matrix, being, in this way, difficult to analyze. Therefore, in order to carry out an extensive molecular marker characterization, classical analytical methods, such as gas chromatography coupled to mass spectrometry (GC-MS), have to be improved in order to increase their chromatographic resolution and sensitivity.

Over the past decade, comprehensive two-dimensional gas chromatography (GC×GC) has emerged as a powerful technique especially suited for complex samples characterization [Dallüge *et al.*, 2003]. The three main achievements by GC×GC are the increase in chromatographic resolution; the decrease of detection limits due to the cryofocusing operated at the modulator and the presence of chemically ordered structures in the chromatograms [Dallüge *et al.*, 2003]. Furthermore, when coupled with a mass spectrometric system, four dimensions of information are available: retention times in two dimensions, mass spectra and intensity. Due to the fast scanning requirements of the GC×GC, the Time-of-Flight Mass Spectrometer (ToF MS) is the best fitted detection system. The potential of this coupling has already been reported in

several environmental applications such as the characterization of aerosol particulate [Welthagen *et al.*, 2003; Hamilton *et al.*, 2004], pesticides [Dalluge *et al.*, 2002b,] and polychlorinated biphenyls in seals [Harju *et al.*, 2003]. For lipid characterization, GC×GC has been used mainly coupled with a flame ionization detector (FID) in the analysis of FA in vegetal oils with different column sets [de Geus *et al.*, 2001; Western *et al.*, 2002; Mondello *et al.*, 2003 and 2004; Adahchour *et al.*, 2005] and in milk [Hyotylanen, 2004]. This technique has also been applied to the characterization of some fecal sterols in biological samples [Truong *et al.*, 2003]. Recently, an analytical method based on GC×GC–ToF MS has been developed as a tool for complex lipidic samples characterization [Jover *et al.*, 2005].

In this work, the potential of GC×GC–ToF MS as an analytical tool for lipidic molecular marker characterization in environmental samples has been evaluated. The technique has been applied to SPM samples from the Ebro River estuary (Spain). The Ebro River is one of the most important rivers flowing in the northwestern Mediterranean basin. The low-tidal regime and the hydrological river conditions gives special characteristics to the Ebro River estuary, forming a delta where the salt wedge phenomenon is developed. The salt- wedge effect consists in a strongly stratified vertical profiles where marine water is found in the bottom layer while continental fresh water flows on the upper layer. Salt-wedge position and residence time depend on the river freshwater flow and on the river bed topography. The strongly stratified profile is not only for salinity but also for other parameters such as temperature, nutrient and oxygen contents [Cruzado, 2002]. In this study, GC×GC–ToF MS will be used to carry out the SPM lipid molecular markers characterization including FAs, monounsaturated FAs (MUFA), PUFAs, FALs, monounsaturated fatty alcohols (MUFAL) and STs. Furthermore, differences in marker content between the top fresh water and the deep salty water will be highlighted trying to conclude on the OM origin, environmental conditions and the biogeochemical processes.

## **Experimental section**

### *Samples and reagents*

A standard solution of 37 fatty acid methyl esters (FAME), including different chain lengths and unsaturation degrees, was purchased from Supelco (Supelco Park, PA, USA). Sample analysis method was based on a sequential two derivatization steps;

first the methylation was carried out and then the silylation [Jover et al., 2005]. As methylation reagent, a 0.005 M solution of trimethylsulphonium hydroxide (THMS) from Fluka (Buchs, Switzerland) was prepared in methanol (Suprasolv®, Merck, Darmstadt, Germany). Reagent volume (1/1) was added to the sample and kept at room temperature during 30 min. Then, the extract was evaporated to dryness under a gentle flow of nitrogen in order to avoid the presence of methanol during the silylation step. For the silylation of the samples, BSTFA (bis-silyltrifluoroacetamide) from Merck (Darmstadt, Germany) was used as silylating reagent. Twenty microlitres of reagent were added to the sample and the derivatization was done at 70°C during 1h. Thereafter, the sample was evaporated to dryness under a gentle nitrogen flow. Then the extract was redissolved in the injection solvent (ethyl acetate, Merck), the internal standard was added (triphenylamine, Merck) and the sample was injected into the GC×GC–ToF MS system.

#### *Sample preparation*

Ebro River samples were taken at different points of the estuary in several sampling campaigns during 2003. Water samples (2L) were taken at different water depths and general parameters such as conductivity, temperature and oxygen content were monitored *in situ* by using an YSI 556 MPS (Yellow Spring, OH, USA) multi-parametric probe. Samples were then filtered through 0.7 µm GF/F filters from Whatman (Maidstone, U.K.). After drying (55°C), filters were extracted 3 times (15 mL) by sonication (15 min) using dichloromethane/methanol (1/1). Finally, after an alumina clean-up (3 g), samples were directly derivatised using ethyl acetate as eluting solvent, avoiding the classic fractionation step (i.e. open column chromatography with alumina).

In this work, FAs will be named using a shortcut notation A:BnXd, in which A represents the number of carbon atoms, B gives the number of double bonds, X gives the position of the closest double bond to the terminal methyl group and d=c for a *cis* compound and d=t for a *trans* compound. For sterols a similar notation will be applied A<sup>B,C</sup> where A represents the number of carbon atoms and B and C give the position of the double bonds.

#### *GC×GC–ToF MS apparatus*

The GC×GC–ToF MS system consisted of a HP 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph equipped with an Optic 2 programmable injector (ATAS, Veldhoven, the Netherlands) and working in the splitless mode at 300°C (2 min). The detector was a Pegasus II Time-of-Flight mass spectrometer (LECO, St Joseph, MI, USA) working at -70 eV, transfer line 280°C, ion source 250°C and scanning from 70 to 800 m/z at 50 Hz with a detector voltage of 1950 V.

Thermal modulation was performed with a home-made longitudinally modulated cryogenic system which has been described elsewhere [Jover *et al.*, 2005]. Ambient air (4 bars) was used to achieve the analyte modulation. The capillary column was run through a notch done on the two 1/8 inch steel tubes, separated by 3 cm, which contained the pressurized air. With this set-up the first FAME to be correctly modulated is the C<sub>14</sub> being C<sub>15</sub> the first FA of interest as molecular marker (*iso* and *anteiso* isomers are bacterial molecular markers).

An orthogonal system has been chosen as column set based on previous results [Jover *et al.*, 2005]. First dimension consisted on a 30 m ZB-5 (95% dimethyl-5% diphenylpolysiloxane) 0.25 mm internal diameter and 0.25 μm of phase thickness, which was obtained from Phenomenex (Torrance, CA). The second dimension was a 1 m BPX50 (50% phenyl-polysilphenylene-siloxane) 0.10 mm internal diameter and 0.10 μm of phase thickness from SGE (Ringwood, Victoria, Australia).

For data transformation and visualization two additional softwares were used. One to convert the raw data into a two-dimensional array (software provided by Ph. J. Marriott) and a program to generate contour plots from this array (“Transform”, part of Noesys software package; Research Systems International, Crowthorne, UK).

The chromatographic conditions for the oven temperature were 70°C (1 min), to 360°C at 5°C/min and keeping the final temperature for 20 min. The carrier, helium, was set-up at a constant pressure (20 psi). Finally, optimal ambient temperature air modulation time was 5 s. Specifically for FAME analysis the temperature program was 70°C (1 min), to 300°C at 2 rather than 5°C/min and keeping the final temperature for 4 min.

For quantification characteristic ions for each chemical class have been chosen (i.e. m/z 103 for FAL). For calculations, the area obtained for each compound was corrected by the internal standard. Moreover, when target analyte ratios are calculated

their response was normalized using their relative response, which has been calculated using standard solutions. When no standard was available, the compound response was extrapolated from similar compounds. For example, C16:3 was calculated based on C16 and the response shift existing between C18 and C18:3.

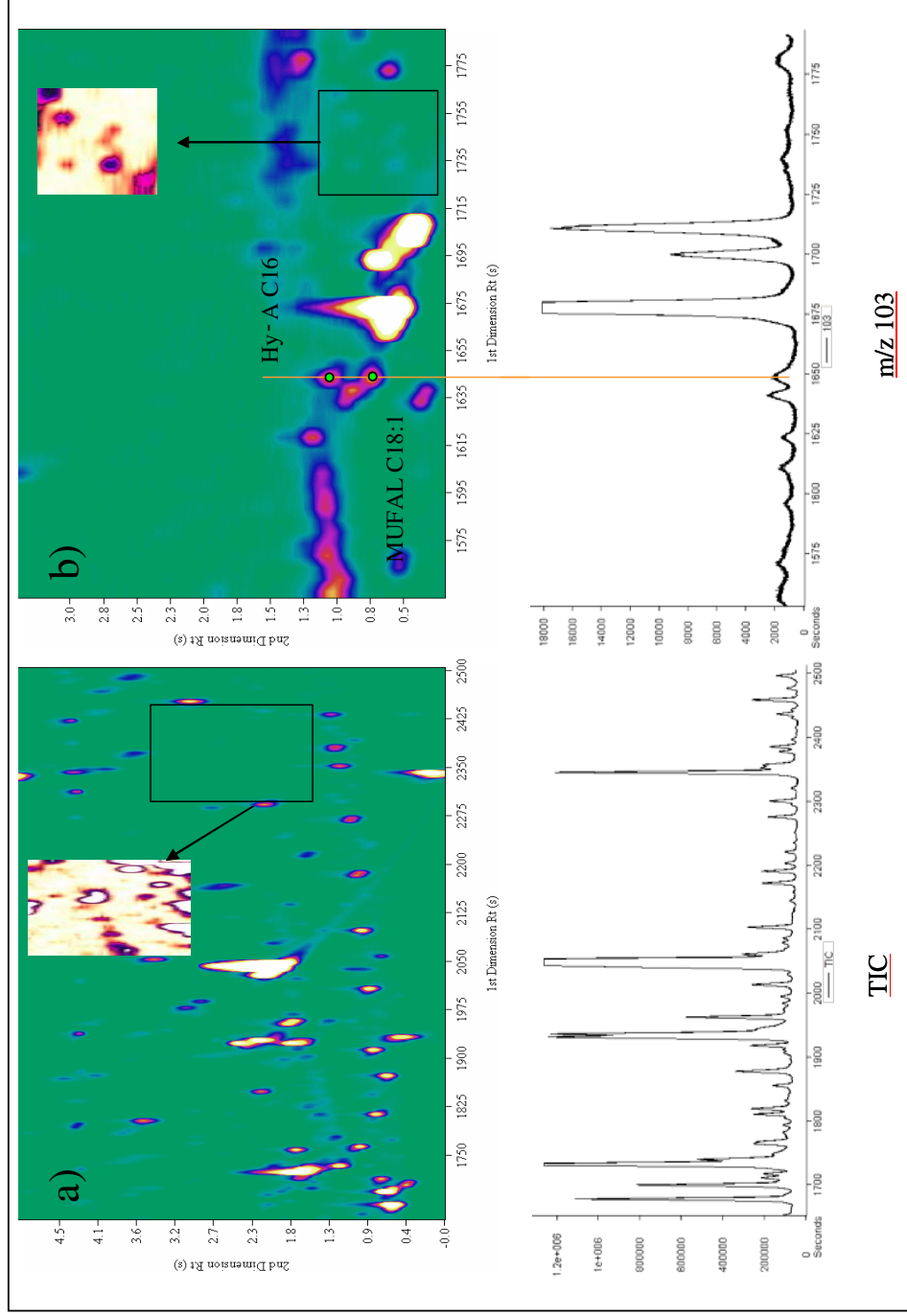
## Results and Discussion

### *GC×GC–ToF MS*

Co-elutions of target analytes with additional sample constituents cannot be avoided in a conventional GC-FID system due to the large variety of different lipids and to the complexity of environmental matrixes. Moreover, classical techniques for molecular markers analysis include time consuming sample preparation, which could go from a silica fractionation [Derieux *et al.*, 1998] to a saponification, sequential solvent extraction and silica fractionation [Canuel *et al.*, 2001]. All of these steps could be avoided by using GC×GC but need to be demonstrated. In fact, the real question would be if GC-MS has enough resolution to avoid these co-elutions and if not, whether the GC×GC–FID or the GC×GC–ToF MS is really needed. In fact, in Figure 1a the resolution obtained in a 1D GC-MS TIC chromatogram and in a GC×GC–ToF MS TIC contour plot are compared, which is similar to the contour plot expected with a GC×GC–FID system. The complexity of the analyzed sample 1D GC-MS is obvious with the presence of numerous coelutions between important molecular markers (i.e. FAME C18:3n3 and C18:1n9c). A big proportion of these coelutions can be solved in the second dimension of the GC×GC due to its increased chromatographic resolution. However, as we are working with an MS system, these TIC coelutions could be avoided by searching at the 1D GC-MS fragmentograms of characteristic ions. In Figure 1b, a fragmentogram for a characteristic FAL ion ( $[\text{CH}_2=\text{O}^+\text{Si}(\text{CH}_3)_3]$   $m/z$ 103) is compared with a GC×GC–ToF MS contour plot of the same characteristic ion. As can be seen, even if the coelutions are apparently solved in the 1D GC-MS, they can be observed when we have a look at the corresponding GC×GC fragmentogram. Therefore, the enhanced chromatographic resolution of the GC×GC is necessary in order to avoid coelutions for example between MUFAL C18:1 and the hydroxy acid C16 (Hy-A C16). Moreover, the detection of minor compounds, which can be relevant molecular markers, is possible thanks to the chromatographic peak cryofocusing and to the baseline improvement as it can be seen in the two zoom areas of Figure 1. In these complex

samples, coelutions in GC×GC–FID cannot be completely avoided and therefore, GC×GC–ToF MS, with its fourth information dimension, is needed. Furthermore, mass spectra information is very helpful when trying to identify unknown compounds. From these results, it is obvious that GC×GC–ToF MS is very useful in characterizing minor lipids in complex samples, in which, tedious sample pretreatment will be no more compulsory. In addition, the possibility to implement deconvolution algorithms can even improve the resolution of overlapping compounds.

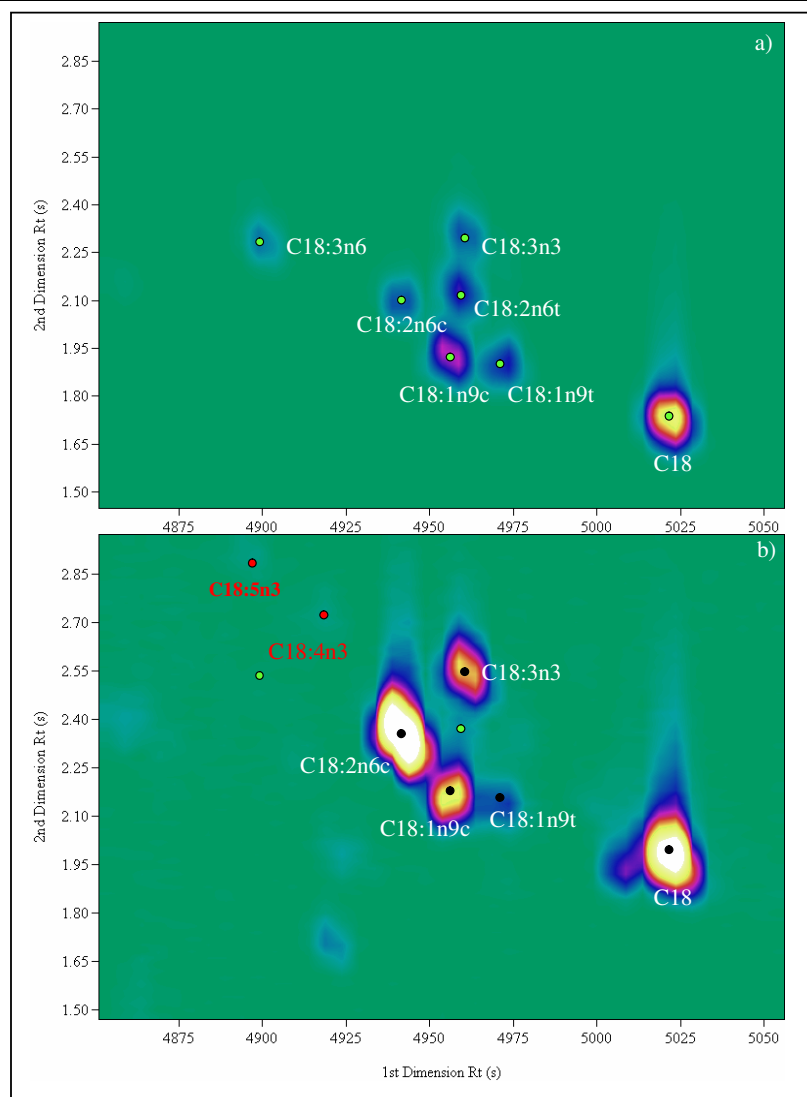
**Figure 1.** a) TIC of a 1D GC-MS and a GC×GC-ToF MS of an Ebro River sample. b) m/z 103 ((CH<sub>2</sub>=O<sup>+</sup>Si(CH<sub>3</sub>)<sub>3</sub>) fragmentograms of a 1D GC-MS and a GC×GC-ToF MS of an Ebro River sample.



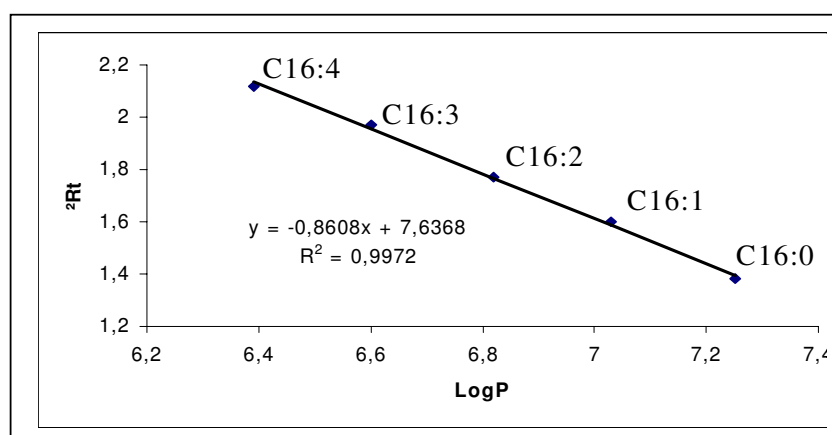
*Potential molecular markers identification*

As FAs are the most important lipids and are widely used as molecular markers, special attention has been devoted to their study. In this respect, we have demonstrated that the GC×GC chromatographic behavior is closely related to the compound chemical structure and that some of these parameters such as  $\log K_{OW}$  can be useful structural predictors [Jover *et al.*, 2005]. In this way, the second dimension retention time ( $^2R_t$ ) has been linearly correlated with the number of double bonds and with the compound chain length. Also, positional isomers (differing on the double bond position) behavior has been correlated with the fractional chain-length chromatographic index. Furthermore, when GC×GC is used, elution pattern that can be used to identify both target analytes and unknowns due to the chemically ordered elution. In Figure 2, the pattern technique is used in order to identify the different FA C18 compounds present in an Ebro river particulate matter by using characteristic FAs ions. Apart from the FAMES available in the authentic standard, two other compounds have been tentatively identified (based on the ordered structures) as being C18:4n3 and C18:5n3. Furthermore, their identification could be confirmed by their  $\log K_{OW}$   $^2R_t$  correlation. For the C18 group this correlation has already been reported in a previous work [Jover *et al.*, 2005]. But here and as an example, the identification confirmation for the C16:2, C16:3 and C16:4, not present in the standard, are shown in Figure 3 based on the  $K_{OW}$   $^2R_t$  correlation. It should be pointed out that the regression slope is very close to the one reported for the C18 group. The same approach has been followed for the identification of the other chemical classes.





**Figure 2.** a) Contour plot ( $m/z$  74+79x10) of the  $C_{18}$  region for the FAME standard mixture. b) Contour plot ( $m/z$  74+79x10) of the  $C_{18}$  region for the FAME for a SPM sample of the Ebro River. The compounds were tentatively identified due to their position on the chemically ordered structures are in red. Inter analysis  ${}^2R_t$  shifting were corrected using an internal standard or major compounds.



**Figure 3.** Correlation between second-dimension retention times of several  $C_{16}$  FAME and their  $\log K_{ow}$  values.

In the SPM of the Ebro River estuary, several lipidic chemical classes, which are especially relevant as molecular markers (FA, FAL, ST), have been studied. In summary, 115 potential lipidic molecular markers baseline resolved (66 FA, 34 FAL and 15 ST) and identified without tedious sample pretreatment steps, which may include a cleanup and chemical class fractionation. These compounds grouped by families are summarized in Table 1. They have to be considered as potential lipidic molecular markers in environmental studies. However, some of them have already been successfully applied in previous studies (Table 2). The usefulness of a molecular marker is usually related to its specificity. In other words, in its ability to differentiate between different ecosystem functioning, living organisms, depositional environment and/or biogeochemical processes. In this way, some lipidic compounds, such as FA C<sub>18</sub> or FA C<sub>16</sub>, are not useful as they are ubiquitous and, therefore, they are unspecific. It is important to point out that in this research area, the specificity of a molecular marker is under continuous re-evaluation. Therefore, organic geochemistry will need to avoid the single marker approach to move towards multivariate exploratory analysis (pattern recognition and principal component analysis) with the use of marker pool of different chemical classes. For this purpose, GC×GC–ToF MS will be highly useful.

**Table I.** Compounds identified in the Ebro River SPM.

Compound Group	Chemical class	Carbon number	Unsaturation degree	Number of compounds
Linear saturated fatty acids	FA	7-26	0	20
Iso-branched saturated fatty acids	FA	13-26	0	12
Anteiso-branched saturated fatty acids	FA	13-27 only odd	0	9
Cyclopropanoic fatty acids	FA	17,19	1	2
Monounsaturated fatty acids	MUFA	14,15,16,18,20	1	6
Polyunsaturated fatty acids	PUFA	16,18,20,22	2-6	17
Fatty alcohols (saturated)	FAL	12-30,32	0	20
Iso-branched saturated fatty alcohols	FAL	15,20,22,24	0	4
Anteiso-branched saturated fatty alcohols	FAL	17-23 only odd	0	4
Monounsaturated fatty alcohols	MUFAL	16,18	1	2
Isoprenoid fatty alcohols	FAL	16,18,20	0-1	4
Sterols	ST	27-29	0-2*	15

\*Excluding ring bonds

**Table 2.** Bibliographic study on the lipidic molecular markers used to identify the organic matter origin.

Common compound name	Chemical structure	Origin		References
		Terrestrial	Marine	
$27\Delta^{5,22}$	Cholesta-5,22E-dien-3 $\beta$ -ol	Algae, higher plants	Zooplankton, algae	Yunker <i>et al.</i> , 1995
Desmosterol $27\Delta^{5,24}$	Cholesta-5,24-dien-3 $\beta$ -ol		Zooplankton, algae, diatoms	Bayona <i>et al.</i> , 1989; Yunker <i>et al.</i> , 1995; Parrish <i>et al.</i> , 2000
Cholesterol $27\Delta^5$	Choleste-5-en-3 $\beta$ -ol		Zooplankton	Volkman, 1986; Yunker <i>et al.</i> , 1995; Mudge <i>et al.</i> , 1997
Cholestanol $27\Delta^0$	5 $\alpha$ -cholestan-3 $\beta$ -ol	Stenol reduction	Stenol reduction	Cranwell, 1981; Yunker <i>et al.</i> , 1995
Coprostanol $27\Delta^0$	5 $\beta$ -cholestan-3 $\beta$ -ol	Domestic wastewater		Grimalt <i>et al.</i> , 1990; Maldonado <i>et al.</i> , 1999; Hughes <i>et al.</i> , 2004
Brassicasterol $28\Delta^{5,22}$	24-methylcholesta-5,22E-dien-3 $\beta$ -ol	Algae, higher plants	Algae, diatoms	Volkman, 1986; Nichols <i>et al.</i> , 1990; Yunker <i>et al.</i> , 1995; Parrish <i>et al.</i> , 2000
Fucosterol $28\Delta^{5,24(28)}$	24-methylcholesta-5,24(28) -dien-3 $\beta$ -ol	Algae, diatoms	Algae, diatoms	Volkman, 1986; Nichols <i>et al.</i> , 1990; Yunker <i>et al.</i> , 1995
Campesterol $28\Delta^5$	24-methylcholest-5-en-3 $\beta$ -ol	Higher Plants	Algae, diatoms	Volkman, 1986; Yunker <i>et al.</i> , 1995; Parrish <i>et al.</i> , 2000
$29\Delta^{5,24(28)E}$	24-ethylcholesta-5,24(28)E-dien-3 $\beta$ -ol	Algae, higher plants	Algae	Volkman <i>et al.</i> , 1988; Yunker <i>et al.</i> , 1995
$29\Delta^{5,24(28)Z}$	24-ethylcholesta-5,24(28)Z-dien-3 $\beta$ -ol		Algae	Volkman <i>et al.</i> , 1988; Yunker <i>et al.</i> , 1995
Stigmasterol $29\Delta^{5,22}$	24-ethylcholest-5,22E-dien-3 $\beta$ -ol	Higher Plants	Algae	Volkman, 1986; Saliot <i>et al.</i> , 2002; Mudge <i>et al.</i> , 1997; Yunker <i>et al.</i> , 1995
$\beta$ -Sitosterol $29\Delta^5$	24-ethylcholest-5-en-3 $\beta$ -ol	Higher Plants	Algae	Volkman, 1986; Saliot <i>et al.</i> , 2002; Mudge <i>et al.</i> , 1997; Yunker <i>et al.</i> , 1995
24-ethylcoprostanol $29\Delta^0$	24-ethyl-5 $\beta$ -cholestan-3 $\beta$ -ol	Domestic wastewater		Parrish <i>et al.</i> , 2000
Dinosterol $30\Delta^{22}$	4 $\alpha$ -23,24-trimethylcholest-22-en-3 $\beta$ -ol		Dinoflagellates	Nichols <i>et al.</i> , 1984; Parrish <i>et al.</i> , 2000

5. *Estudi de cas: Estuari del riu Ebre*

Branched FAs	Iso and anteiso C <sub>15</sub> and C <sub>17</sub> carboxylic acids	Bacteria	Bacteria	Mudge <i>et al.</i> , 1997; Parrish <i>et al.</i> , 2000
Long-chain fatty acids	C <sub>24</sub> and C <sub>26</sub>	Higher plants		Mudge <i>et al.</i> , 1997; Parrish <i>et al.</i> , 2000; Saliot <i>et al.</i> , 2002
MUFA 16:1n7		Diatoms, Cyanobacteria	Diatoms, Cyanobacteria	Moreno <i>et al.</i> , 1979
PUFA 16:2		Plankton	Plankton	Bodineau <i>et al.</i> , 1998
PUFA 16:3		Plankton	Plankton	Bodineau <i>et al.</i> , 1998
PUFA 16:4n1			Diatoms	Parrish <i>et al.</i> , 2000
MUFA 18:1n9		Cyanobacteria	Cyanobacteria	Grimalt <i>et al.</i> , 1992
PUFA 18:2n6		Higher Plants		Ackman, 1986; Budge <i>et al.</i> , 1998; Parrish <i>et al.</i> , 2000
PUFA 18:3n3		Higher Plants		Ackman, 1986; Budge <i>et al.</i> , 1998; Parrish <i>et al.</i> , 2000
PUFA 18:5			Dinoflagellates	Colombo, 1996
MUFA 20:1			Zooplankton	Parrish <i>et al.</i> , 2000
PUFA 20:5n3			Diatoms, zooplankton	Colombo, 1996; Parrish <i>et al.</i> , 2000
MUFA 22:1			Zooplankton	Parrish <i>et al.</i> , 2000
PUFA 22:6n3			Dinoflagellates, zooplankton	Colombo, 1996; Parrish <i>et al.</i> , 2000
Alcohols	Even C <sub>14</sub> -C <sub>18</sub>	Bacteria, algae	Bacteria, zooplankton	Yunker <i>et al.</i> , 1995
Long chain alcohols	C <sub>20</sub>	Higher Plants		Cranwell, 1981; Yunker <i>et al.</i> , 1995; Mudge <i>et al.</i> , 1997; Saliot <i>et al.</i> , 2002
Alkenols	C20:1 and C22:1		zooplankton	Jeng <i>et al.</i> , 2004
Phytol	3,7,11,15-tetramethyl-2-hexadecen-1-ol	higher plants	phytoplankton, phototrophic organisms	Rontani <i>et al.</i> 2003
Dihydrophytol	3,7,11,15-tetramethyl-2-hexadecan-1-ol	zooplankton	zooplankton	Rontani <i>et al.</i> 2003

### *Organic matter origin*

Lipidic molecular markers of the Ebro River estuary SPM have been used in several ways. Due to the salt wedge effect, very stratified vertical profiles were obtained and characterized by fresh top-layer water (Tw) and bottom marine salty-water (Bw). The developed GC×GC technique has been applied to water samples in order to differentiate between the organic matter origin from the two water bodies. Several molecular markers are able to confirm the organic matter origin. In this way, higher plants lipids have been used as terrestrial molecular markers. C18:2n6 and C18:3n3 fatty acids have been suggested as higher plant markers (Ackman, 1986; Budge *et al.*, 1998 and Parrish *et al.*, 2000). Even if no clear result was obtained for C18:3n3 (0.45 vs 0.46 for Tw and Bw abundance respectively), C18:2n6 (1.91 vs 0.75) showed a clear terrestrial influence on Tw. It has to be pointed out that, with the used chromatographic system and selecting characteristic FAME ions, this compound would be coeluting on a 1D system with an unidentified compound which could represent, depending on the samples, an overestimation of 12% in the C18:2n6 quantification. Also high molecular weight FALs have been reported as being terrestrial molecular markers [Cranwell, 1981]. In our work, their content was slightly found higher (1.25 times more for FALs C 20 considering a RSD of 10% between different sample locations) in Tw than in Bw. Accordingly, significant differences were observed between the two water bodies. The occurrence of higher plants markers in the Bw could be attributable to diffusion processes from the Tw and from the detritic material present in the sediment.

### *Characterization of planktonic communities*

Several lipidic compounds have been used to assess if there is any difference in the living organisms found in the two water bodies. Molecular markers can be useful in the assessment of planktonic communities at several degrees. In a first step, if using unspecific planktonic markers such as 16:2 or 16:3 FAME [Bodineau *et al.*, 1998], the Bw is richer in these kind of lipids than Tw. It could be ascribed either to higher productivity or better preservation in the suboxic bottom waters.

In a second step, we can distinguish between phytoplankton and zooplankton. In this way, the ratios C18:1/C18:0 and C16:1/C16:0 FAME have been reported to be useful in order to discriminate between phytoplankton and zooplankton. When zooplankton is more abundant than phytoplankton, the ratios are higher and it is

predominant when C16:1/C16:0 ranges between 0.6 and 1 [Burns *et al.*, 2003]. In our case, zooplankton is predominant in Bw and phytoplankton in Tw (see Table 3). This could be explained by the vertical stratified profile limiting the light influence on Bw and therefore, limiting the phytoplankton growth. Even if the depth at the sampling point is only 8 m, an increase in the particulate matter in the salty water can enhance the light extinction with depth. It should be pointed out, that in our chromatographic conditions, FAME C18:0 is eluting at the same  $^1R_t$  than a compound tentatively identified as FAL C18:2. It could interfere in the FAME C18:0 quantification resulting in an overestimation depending on the samples, of 180%.

**Table 3.** Summary of the molecular markers used and their distribution in the Bw and Tw of the Ebro.

Molecular marker	References	Water bodies comparison*		Conclusion
		Tw	Bw	
<b>C18:2n6</b>	Parrish <i>et al.</i> , 2000	1.91	0.75	Terrestrial influence on Tw organic matter
<b>C16:2</b>	Bodineau <i>et al.</i> , 1998	ND	0.27	Higher planktonic presence in Bw
<b>C16:3</b>	Bodineau <i>et al.</i> , 1998	ND	0.66	Higher planktonic presence in Bw
<b>C16:1/C16:0</b>	Burns <i>et al.</i> , 2003	0.40	0.72	Zooplankton is more predominant in Bw than in Tw
<b>C18:1/C18:0</b>	Burns <i>et al.</i> , 2003	1.00	3.26	Zooplankton is predominant in Bw and secondary in Tw
<b>C16:4</b>	Budge <i>et al.</i> , 1998	ND	0.12	More diatoms in Bw
<b>C22:6n3/C20:5n3</b>	Budge <i>et al.</i> , 1998	1.56	ND	Dinoflagellates are the main constituent of the Tw phytoplankton
<b>Branched C15:0</b>	Volkman <i>et al.</i> , 1980	0.07	0.29	More Bacteria in Bw
<b>Coprostanol</b>	Hughes <i>et al.</i> , 2004	0.129	0.047	Higher domestic sewage influence on Tw
<b>Epicoprostanol</b>	Hughes <i>et al.</i> , 2004	0.013	0.007	Higher domestic sewage influence on Tw
<b>Photooxydation product/Phytol</b>		0.34	ND	Due to high vertical stratification, photooxydation does not take place in Bw

\*Calculation method is described in the experimental section

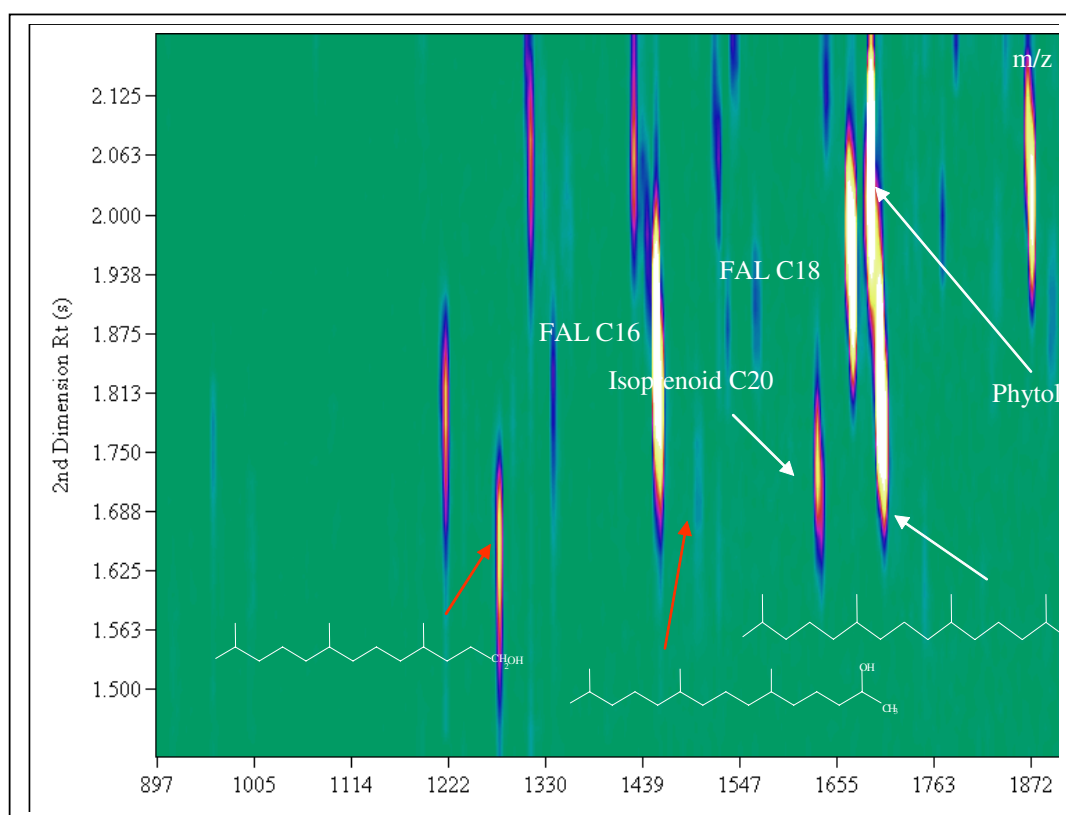
Finally, in a third step, the composition of phytoplankton communities can be investigated. Both water bodies are qualitatively different as has been confirmed by some specific molecular markers. FAME C16:4, characteristic for the occurrence of diatoms [Budge *et al.*, 1998] has only been identified in Bw. The ratio C22:6n3/C20:5n3 has also been reported for phytoplankton characterization, being dinoflagellates predominant if the ratio is higher than 1, and if not being diatoms predominant. Accordingly, dinoflagellates were the main phytoplanktonic organisms taking into account a 1.56 ratio for Tw. Conversely, diatoms may occur in the Bw with a low ratio (C22:6n3 not detected) [Budge *et al.*, 1998].

Branched isomers of C15:0, characteristic bacterial lipids [Volkman *et al.*, 1980], showed higher abundance in Bw, so higher bacterial biomass is expected, probably related to a higher Bw residence time and to the settling organic matter biodegradation.

#### *Biogeochemical processes*

Lipidic molecular markers can also be useful to track some physico chemical processes occurring in the aquatic environment. In the Ebro River estuary, the absence of vertical mixing should limit the influence of photo-oxidation on the bottom water layer. Phytol, a degradation product of the chlorophyll, has been identified in the two water bodies but when looking at its degradation products, more interesting results can be obtained. In this way, dihydrophytol obtained from the degradation of the phytol by the zooplankton grazing [Rontani *et al.*, 2003] has been found in the two layers. However, two photo-oxidation products of phytol [Rontani *et al.*, 2003] (Figure 4) were only identified in the fresh top layer water, indicating the limitation of light irradiation in the bottom water.





**Figure 4.** Contour plot ( $m/z$  103) showing the isoprenoids alcohols identified in Ebro River top layer water. Phytol photo-oxidation products are pointed by using red arrows.

#### *Anthropogenic influence*

Lipidic molecular marker can also be useful in order to assess the anthropogenic impact in the environment. Since one of the main anthropogenic inputs in rivers is the discharge of secondary wastewater effluents, fecal sterols have been widely used for this purpose [Grimalt *et al.*, 1990; Maldonado *et al.*, 1999; Hughes *et al.*, 2004]. In the Ebro river, Tw presents higher levels of coprostanol ( $27^0$ ) and epicoprostanol ( $27^0$ ) than Bw showing, as expected, a more important anthropogenic impact.

Phytol will be also an interesting molecular marker in order to detect eutrophication caused by nutrient transport (phosphates and/or nitrates) as it will sharply increase with an increase of the primary production (algal bloom).

Finally, the molecular markers, which have been used in this study are summarized in Table 3.

## Conclusions

In this work, GC×GC–ToF MS has been presented as a suitable analytical tool for lipidic molecular marker identification and determination in environmental samples. The four information dimensions obtained by this technique are necessary to avoid coelutions and to be able to detect and identify minor lipidic compounds with potential molecular markers. This analytical methodology has been compared to the more usual one dimension GC-MS and GC×GC–FID. Moreover, only a simplified cleanup step is compulsory due to high chromatographic resolution of the technique. The developed technique has been applied to Ebro River particulate matter samples in order to study the influence of the salt wedge on the water lipids constituents and on the general ecosystem behavior. From the 115 potential molecular markers identified some of them have been used to elucidate the organic matter origin, to characterize major planktonic communities and the biogeochemical processes involved and to track the anthropogenic impact on the river.

## Acknowledgements

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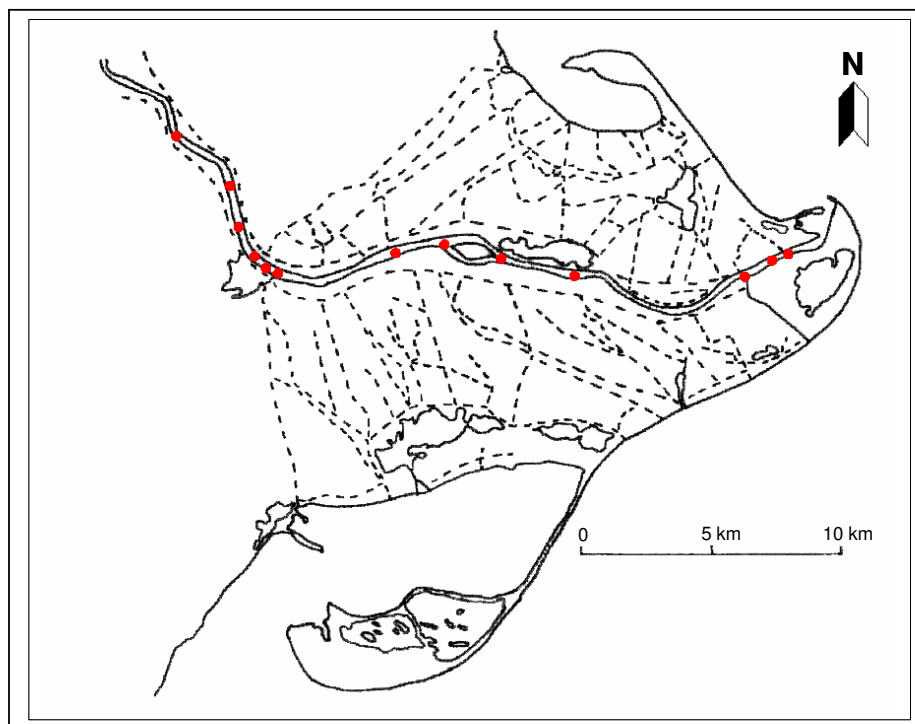
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### 5.1.2 Treball experimental adicional

En aquest apartat es presenten les dades experimentals addicionals disponibles referent a la caracterització lipídica de les mostres. Aquestes mostres són el resultat de 10 mostratges realitzats a la totalitat de l'estuari del riu Ebre entre el 2002 i el 2003  
Figura 12.



**Figura 12.** Distribució dels diferents punts de mostreig.

Durant els mostratges, gràcies a una sonda multiparamètrica s'avaluaven paràmetres generals de caracterització de les masses d'aigua (temperatura, oxigen i conductivitat) així com la posició exacta de la falca salina. Es van agafar mostres de l'aigua profunda, de l'aigua superficial i de l'interfase entre les dues capes.

#### *Distribució vertical*

A la Figura 13 es mostren dos perfils típics obtinguts pel FAL C<sub>20</sub> en presència de la falca salina. En el primer perfil no corregit pel material particulat en suspensió (SSM), s'ha observat en alguns casos un màxim en la zona de l'interfase però no ha estat reproducible per les diferents campanyes estudiades ni ha mostrat cap tendència clara. Així doncs, de moment no s'ha pogut extreure conclusions pel que fa un possible enriquiment de l'interfase com havia estat assenyalat qualitativament per un altre autor [Zutic i Legovic, 1987]. La capa inferior ha mostrat nivells inferiors o superiors a la

capa superficial dependent del compost. Quan corregim per la SSM, s'ha observat en tots els casos un enriquiment de la capa superficial (Figura 13). No s'ha observat cap discontinuïtat en l'interfase sinó que l'interfase és el intermediari entre els valors observats a les dues capes.

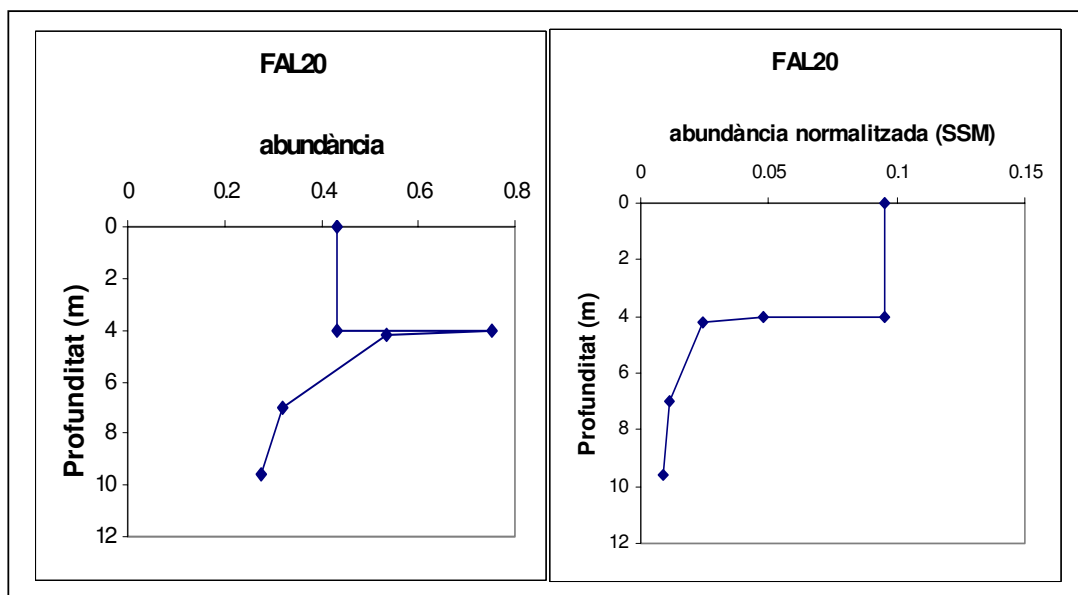


Figura 13. Perfils obtinguts pel FAL C<sub>20</sub> amb presència de falca salina.

Quan la falca salina no està formada no s'han observat diferències significatives entre les mostres d'aigua superficial i les mostres d'aigua profunda.

Per a realitzar més fàcilment un seguiment de les diferències entre traçadors lipídics a les dos masses d'aigua, s'ha definit un factor d'enriquiment  $EF$ :

$$EF = \frac{[X]_{Tw}}{[X]_{Bw}}$$

on  $[X]_{Tw}$  és la concentració del compost d'interès a l'aigua superficial i  $[X]_{Bw}$  la seva concentració a l'aigua profunda. A les taules següents (4, 5 i 6), es mostren els diferents valors trobats de  $EF$  en funció del compost estudiat i en presència o absència de falca salina. En el cas de no haver-hi falca salina, els valors de  $EF$  han estat sempre al voltant de 1, sense que s'observin diferències significatives. En canvi, quan hi ha falca salina els  $EF$ s són superiors a 1 arribant a valors de 32.9 pel FAL C<sub>12</sub> (aquest compost mostrava una desviació molt important), 22.9 pel FFA C<sub>12</sub> i 22.4 pel coprostanol. És interessant, destacar que els compostos de cada família que han mostrat el  $EF$  més elevat corresponen a compostos que poden relacionar-se de manera específica



(coprostanol) o no específica (FFA C<sub>12</sub> i FAL C<sub>12</sub>) a abocaments d'aigües residuals urbanes que, lògicament, van associats a la capa d'aigua dolça.





**Taula 5.** Distribució dels EF de diferents FFAs analitzats en presència i absència de falca salina corregits pel contingut de SSM.

EF	FFA16:1				FFA18:1				Monounsaturated FFAs								
	min	max	median	des. n	min	max	median	des. n	min	max	median	des. n					
SSM corr	0.4	14.2	8.6	6.8	5	0.2	12.5	7.9	5.8	5	7.9	8.6	8.2	0.5	2		
SaltW	1.0	5.2	1.4	1.8	5	0.9	4.4	1.4	1.7	5	1.4	1.4	1.4	0.0	2		
NoSaltW																	
SaltW	FFA12				FFA14				FFA15				FFA16				
	min	max	median	des. n	min	max	median	des. n	min	max	median	des. n	min	max	median	des. n	
SaltW	0.1	22.9	2.4	9.6	5	0.7	12.7	4.5	5.7	5	0.5	13.3	6.6	11.5	6.8	5.1	5
NoSaltW	0.6	4.0	2.0	1.4	5	1.3	4.1	1.6	1.3	5	1.3	4.2	1.4	3.5	1.6	1.1	5
SaltW	FFA17				FFA18				FFA20				Saturated FFAs				
	min	max	median	des. n	min	max	median	des. n	min	max	median	des. n	min	max	median	des. n	
SaltW	0.6	10.5	3.6	4.7	4	0.7	10.9	6.8	4.6	5	0.4	11.9	4.2	6.8	4.5	1.8	7
NoSaltW	1.0	4.0	1.6	1.3	5	0.9	4.2	1.4	1.5	5	0.8	9.0	2.4	2.4	1.6	0.4	7

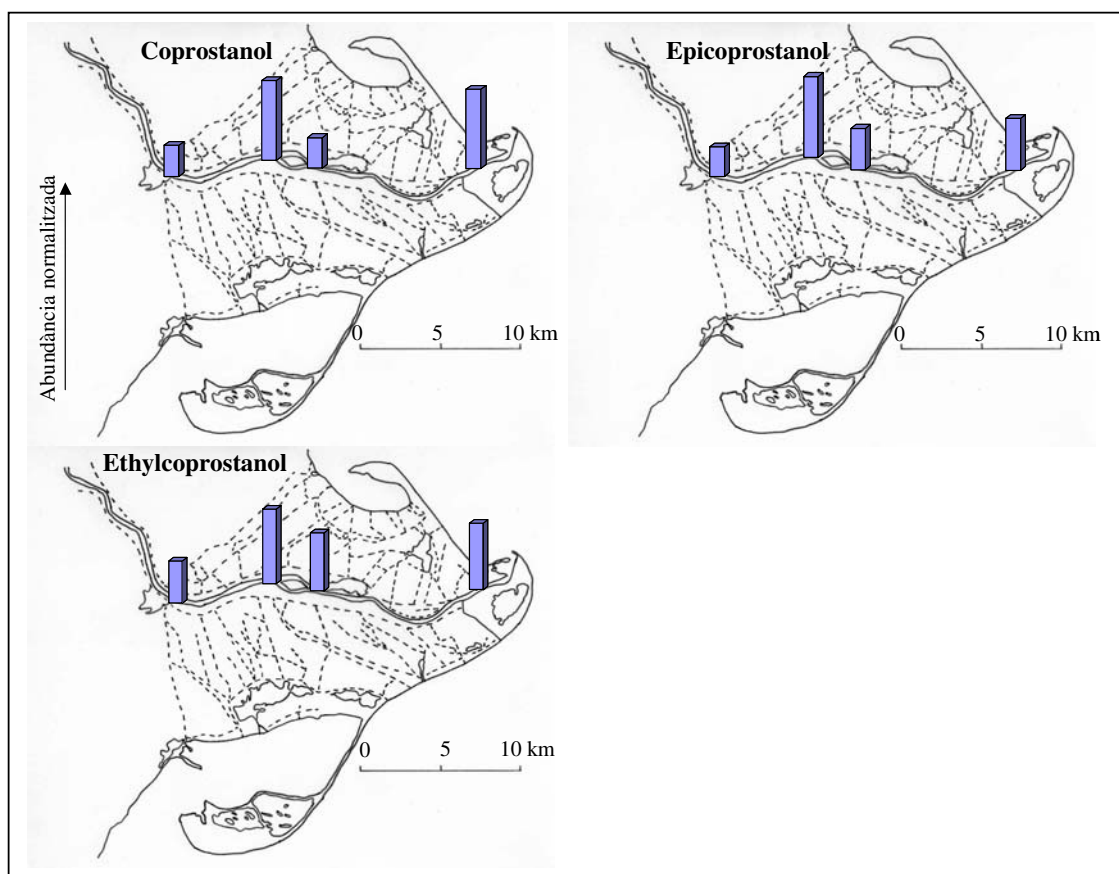
5. Estudi de cas: *Estuari del riu Ebre*

Taula 6. Distribució dels EF de diferents esterois analitzats en presència i absència de falca salina corregits pel contingut de SSM.

EF	Coprostanol			Epiprostanol			Ethylcoprostanol			Cholestanone											
	min	max	des. n	min	max	des. n	min	max	des. n	min	max	des. n									
SSM corr	5.3	22.4	11.9	6.8	5	3.0	7.8	6.9	2.0	5	4.4	2.7	5	2.6	6.8	3.9	2.2	3			
SaltW	0.5	1.5	1.4	0.5	5	0.7	1.4	1.0	0.3	5	0.8	1.1	1.0	0.1	5	1.0	6.4	1.4	2.6	4	
NoSaltW																					
	<b>C27:2</b>			<b>Cholesterol</b>			<b>C27:1</b>			<b>Cholestanol</b>											
	min	max	median	des. n	min	max	median	des. n	min	max	median	des. n	min	max	median	des. n	min	max	median	des. n	
SaltW	1.5	7.0	2.8	2.2	5	3.9	15.7	9.6	5.4	5	4.2	15.9	7.1	4.4	5	2.4	7.6	5.5	2.3	5	
NoSaltW	1.1	1.6	1.2	0.3	5	0.4	1.2	1.0	0.3	5	0.4	1.6	1.1	0.4	5	0.5	1.2	1.0	0.3	5	
	<b>C28:2a</b>			<b>C28:2b</b>			<b>C28:1</b>			<b>C28:0</b>											
	min	max	median	des. n	min	max	median	des. n	min	max	median	des. n	min	max	median	des. n	min	max	median	des. n	
SaltW	1.7	8.2	5.0	2.6	5	2.9	9.7	5.6	2.3	5	4.3	9.7	7.4	2.1	5	2.6	9.1	4.4	2.7	5	
NoSaltW	0.4	1.5	1.1	0.4	5	0.5	1.2	1.2	0.3	5	0.4	2.0	1.1	0.6	5	0.8	1.1	1.0	0.1	5	
	<b>Stigmasterol</b>			<b>Sitosterol</b>			<b>Stigmasterol</b>			<b>Stigmasterol</b>											
	min	max	median	des. n	min	max	median	des. n	min	max	median	des. n	min	max	median	des. n	min	max	median	des. n	
SaltW	5.6	16.7	9.0	4.4	5	5.9	9.4	8.8	1.4	5	2.1	10.8	5.3	3.3	5	0.7	5	0.7	5		
NoSalt W	0.5	9.8	1.1	4.0	5	0.4	14.1	1.1	5.9	5	0.4	2.3	1.1	0.7	5						

*Distribució geogràfica*

Quan s'ha estudiat la distribució geogràfica dels lípids en les aigües superficials de l'estuari de l'Ebre, no s'ha trobat un comportament únic per a les diferents famílies lipídiques. Malgrat això, si que, per alguns compostos s'ha observat un comportament diferenciatiu. D'aquesta manera, per exemple, els tres esterols fecals relacionats amb l'abocament d'aigües residuals urbanes mostren un mateix comportament amb un màxim al sud d'Amposta segurament degut a l'abocament de les aigües residuals d'aquesta ciutat i un lleuger augment en el punt pròxim a la desembocadura que pot provenir dels abocaments dels altres nuclis urbans del delta (Deltebre). Aquesta distribució geogràfica es mostra en la Figura 14.



**Figura 14.** Distribució dels esterols d'origen fecal en les aigües superficials de l'estuari de l'Ebre.

*Distribució temporal*

Es van realitzar 9 campanyes de mostreig de novembre del 2002 a l'octubre del 2003 per a integrar el comportament estacional del riu. Pel que fa als FALS, s'ha trobat un comportament diferent en funció de la longitud de cadena dels compostos. Pels compostos senars existeix una menor variabilitat temporal que pels compostos parells.

Pels compostos de cadena llarga  $C > 20$  tenim una distribució amb un màxim al mes de maig i un lleuger augment al mes d'agost que es correspon amb màxims en les SSM que no es correlacionen amb el cabal del riu i que s'atribueixen a l'activitat agrícola. Durant les campanyes realitzades, les SSM es corresponen, generalment, amb el cabal del riu. Així, en època d'avingudes (Febrer) és quan tenim un màxim en les SSM. No obstant, es van observar dos altres màxims en les SSM que no es relacionaven amb el cabal (Maig, Agost). Quan es van caracteritzar aquestes partícules es van trobar quocients atòmics de C/N elevats indicatius de matèria orgànica d'origen terrestre [Amon i Meon, 2004, Cauwet, 1991]. Es van associar aquestes partícules a l'activitat agrícola [Gómez-Gutiérrez *et al.*, 2006]. Així doncs, aquests FALs de cadena llarga, característics de les plantes superiors [Saliot *et al.*, 2002], estarien relacionats amb l'activitat agrícola. Pels compostos amb una longitud de cadena  $< C_{20}$  no s'han observat tendències clares.

Pels FFAs s'ha observat una tendència completament diferent amb una distribució bimodal per a tots els compostos estudiats amb el màxim a l'abril i un màxim local al juny. Pel que fa les característiques de les partícules, aquests mesos es corresponen amb màxims en el percentatge de carboni i nitrogen orgànic i amb un mínim del quocient atòmic C/N [Gómez-Gutiérrez *et al.*, 2006]. Aquests valors de C/N són indicatius d'una matèria orgànica d'origen biogènic del medi aquàtic [Hellings *et al.*, 1999]. Així doncs, els FFAs ens estarien indicant, majoritàriament, la evolució de la producció primària del riu.

Pel que fa els esterols, tots tenen un màxim local al mes de juliol que correspon al moment quan el cabal del riu és menor. No obstant, alguns compostos presenten un segon màxim local al febrer (estigmastanol,  $27\Delta:2$ ,  $30\Delta:1$ ) que correspon al moment de màxim cabal del riu.

*5.2 Estudi del comportament de contaminants orgànics en un estuari de falca salina*

En aquest apartat ens centrem a estudiar el comportament de diferents famílies de contaminants orgànics en sistemes estratificats i més específicament en l'estuari del riu Ebre. En aquest sentit, s'avalua el transport vertical dels contaminants orgànics en un sistema estratificat, la distribució geogràfica dels contaminants a l'estuari del riu Ebre, la seva variació temporal i finalment es quantifica l'aport contaminant del riu Ebre al mar Mediterrani Nordoccidental.

*5.2.1 Modelització del comportament dels contaminants orgànics en sistemes estratificats*

A l'article següent, "*Transport of organic contaminants through salinity stratified water masses. A microcosm experiment.*", es presenta un estudi en un microcosmos on s'avalua el transport vertical dels diferents contaminants orgànics en funció de les seves propietats fisicoquímiques. De la mateixa manera també s'estudia les possibles pèrdues del sistema correlacionant-les amb processos físics com ara la degradació o l'evaporació dels compostos.



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***TRANSPORT OF ORGANIC CONTAMINANTS THROUGH  
SALINITY STRATIFIED WATER MASSES. A MICROCOSM  
EXPERIMENT***

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<sup>2</sup>This paper corresponds to the Environmental Science PhD Programme from the Autonomous University of Barcelona

**Abstract**

A salt-wedge stratified microcosm, spiked with 31 target analytes, including PCBs, organochlorine and organophosphorous pesticides, triazines, organophosphate flame retardants and caffeine (an urban wastewater molecular marker), was set-up. Compound behaviour was monitored during a four week period, by sampling at six different levels of the water column, in order to understand the transport and loss processes of the different classes of chemical substances. Compounds transport from one water body to the other has been positively correlated with diffusion *via* their molar volume. Target analytes loss has been positively correlated with evaporation *via* their  $K_H$  and with degradation *via* their half-lives. From these two processes, evaporation was found to be predominant using a multiparametric regression.

*Keywords*

Salt-wedge, microcosm, organochlorine pesticides, polar pesticides, flame retardants

## 1. INTRODUCTION

Thermohaline stratified water bodies occur in the marine environment under a variety of conditions. Estuaries are particularly prone to water stratification because a strong gradient between freshwater and saltwater can be maintained, against the mixing tendency of tides and wind-induced turbulence (Geyer and Farmer, 1989). In these circumstances, a marked water salinity gradient can occur with depth between the upper (freshwater) and the lower (saltwater) layers. The friction between the two layers causes the interface to slope downwards in the upstream direction, forming a wedge-shaped saline intrusion.

Salt-wedge estuaries occur in rivers flowing into low tidal seas, where advective circulation is stronger than tidal circulation. The extent, advance and retreat of the salt-wedge in these estuaries are basically controlled by the river discharge. The topography of the estuary bed is also a determinant factor (Ibañez *et al.*, 1997). Examples include the Ebro (Spain), Rhone (France) and Po (Italy) estuaries. These systems can also be present in high tidal areas when the ratio of river discharge to width of the estuary is sufficiently high (Ibañez *et al.*, 1999), as it is the case of the Fraser River (Canada) (Kostaschuk *et al.*, 1992).

A general feature of the salt-wedge system is that mixing across the interface is reduced to a very low level. This is due to the strong vertical density gradient, which greatly hampers vertical turbulence (Lewis, 1997). However, instability can occur at the interface due to the velocity shear between the two layers, causing wave-like perturbations and originating an upward transfer of saltwater into the upper layer, with no corresponding downward movement of freshwater (entrainment process) (Dyer, 1991). To maintain the volume balance of the system, the water lost from the bottom layer is compensated by a slow movement of seawater towards inland.

Vertical mixing in stratified systems is normally considered to be a function of the gradient Richardson number,  $Ri$ . This depends on the density gradient in the water column and also on the vertical difference of horizontal velocity. Theoretically, mixing occurs (disappearance of the salt-wedge) when the  $Ri$  falls below a 0.25 value (Dyer, 1991). When the salt-wedge is formed, the movement of chemical compounds from one water body to the other is mainly due to diffusive phenomena, which can be described by the Fick's Law. However, in such complex systems the contaminants behaviour is difficult to predict because other factors influence their movement (particulate phase

partitioning, biological interactions, etc.). Therefore, a simplified experiment in a microcosm system would be an adequate approach for understanding the main processes taking place in the field.

In this work, 31 target analytes, encompassing a wide range of physicochemical properties, have been introduced in a container filled with two layers of fresh and salt waters and monitored during four weeks. These include polychlorobiphenyls (11 congeners), organochlorine pesticides (HCB,  $\alpha$ -HCH,  $\beta$ -HCH,  $\gamma$ -HCH, p,p'-DDD, p,p'-DDE, o,p'-DDT, p,p'-DDT, dieldrin,  $\alpha$ -endosulfan, heptachlor and methoxychlor), polar pesticides (atrazine, simazine, molinate, diazinon and fenitrothion), flame retardants (tributylphosphate and tris-chloroethylphosphate) and a molecular marker for urban wastewater (caffeine).

## **2. METHODOLOGY**

### **2.1. Experiment description**

The experiment was conducted in a 55 L glass tank (58 cm wide, 32 cm high and 30 cm of depth), where a vertical stratified system was created with 20 L of saltwater at the bottom and 20 L of freshwater at the surface. The water mixtures were prepared in previously cleaned glass amber bottles (20 L) and then transferred into the tank. The saltwater was prepared by adding sodium chloride (Carlo Erba, Milan, Italy) to distilled water in order to achieve a concentration of 37 g L<sup>-1</sup>. Aliquots of an initial solution of humic acid (Fluka, ash 10-15%, Mr 600-1000) in distilled water (342 mg L<sup>-1</sup>) were added to the fresh and salt waters to achieve a concentration of 2.5 and 1.5 mg L<sup>-1</sup> of dissolved organic carbon (DOC), respectively. After preparation, the saltwater was filtrated through kiln-fired glass fibre filters (400°C overnight) (Whatman, GF/F, 0.7  $\mu$ m, 47 mm).

The freshwater was spiked with a mixture in acetone (1  $\mu$ g L<sup>-1</sup>) of PCB congeners (PCB IUPAC # 18, 28, 31, 44, 101, 138, 153, 170, 180, 194 and 209), to achieve a final concentration of approximately 50 ng L<sup>-1</sup> for each compound. Some polar pesticides, namely molinate (500 ng L<sup>-1</sup>), diazinon (50 ng L<sup>-1</sup>) and atrazine (1  $\mu$ g L<sup>-1</sup>), as well as tributylphosphate (TBP, 500 ng L<sup>-1</sup>), were also added. On the other hand, the saltwater was spiked with a mixture in acetone of several organochlorine pesticides (HCB,  $\alpha$ -HCH,  $\beta$ -HCH,  $\gamma$ -HCH, p,p'-DDD, p,p'-DDE, o,p'-DDT, p,p'-DDT, dieldrin,  $\alpha$ -endosulfan, heptachlor (isomer A) and methoxychlor), achieving a final

water concentration of about 58 ng L<sup>-1</sup> for each compound. Simazine (1 µg L<sup>-1</sup>), fenitrothion (1 µg L<sup>-1</sup>), caffeine (750 ng L<sup>-1</sup>) and tris-chloro-ethylphosphate (TCIEtP, 500 ng L<sup>-1</sup>) were also added to the saltwater. All compounds were of analytical grade. Physicochemical properties of the target analytes are summarized in Table 1.

When the saltwater (about 56000 µS.cm<sup>-1</sup>) was placed at the bottom of the tank, the first 5 L of freshwater were carefully added by means of an HPLC pump with an average flow of 10 mL min<sup>-1</sup>. A piece of aluminium foil was placed in the tank where the freshwater drops fell in order to avoid the mixture of the two water bodies and to ensure a strong vertical stratification. The rest of the freshwater was then gently transferred. Vertical stratification was confirmed by means of conductivity measurements (<1000 µS cm<sup>-1</sup> and 55800 µS cm<sup>-1</sup> for fresh and saltwater respectively). The tank was kept in the dark and at ambient temperature.

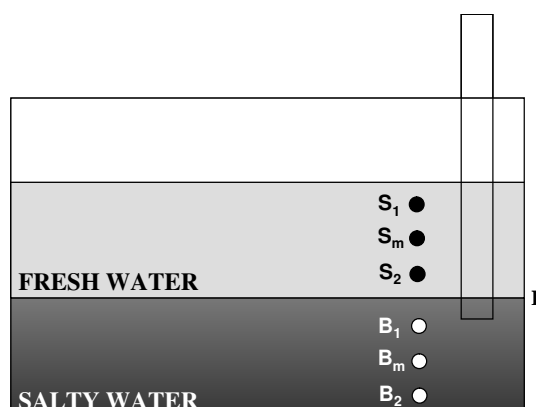
**Table 1.** Target analytes physicochemical properties.

Compound name	Chemical class	Molecular weight	log K <sub>OW</sub>	K <sub>H</sub>	Spiking phase
HCB	Organochlorine	285	5.5	5.07	B <sub>w</sub>
-HCH		291	3.81	1.10	
-HCH		291	3.8	0.07	
-HCH		291	4.14	0.1	
pp'DDE		318	5.7	34	
pp'DDD		320	5.5	9	
opDDT		355	6.2	6	
pp'DDT		355	6.2	6	
Heptachlor A		373	5.73	2.1	
-Endosulfan		407	3.62	10.23	
Dieldrin		381	5.2	1.12	
Methoxychlor		346	5.08	0.99	
PCB18		Polichlorobiphenyl	256	5.24	
PCB28	256		5.67	22.8	
PCB31	256		5.67	25.43	
PCB44	290		5.75	32.8	
PCB101	324		6.38	32.73	
PCB138	358		6.83	11.04	
PCB153	358		6.92	17.93	
PCB170	392		7.27	19.3	
PCB180	392		7.36	30.4	
PCB194	426		7.8	47.52	
PCB209	494	8.18	12.46		
TBP	Phosphate ester	266	3.82	0.152	B <sub>w</sub>
TCIEtP		284	1.63	0.0000329	
Atrazine	Triazine	215	2.82	0.000620	S <sub>w</sub>
Simazine		201	2.40	0.000084	B <sub>w</sub>
Diazinon	Organophosphorous	304	3.86	0.124	S <sub>w</sub>
Fenitrothion		276	3.30	0.0012	B <sub>w</sub>
Molinate	Thiocarbamate	187	3.3	0.159	S <sub>w</sub>
Caffeine		194	0.16	-	B <sub>w</sub>

## 2.2. Sampling strategy

The water tank was labelled as follows: the interface between the two water bodies (I), the middle point in the freshwater ( $S_m$ ) and saltwater ( $B_m$ ) and intermediate points ( $S_1$ ,  $S_2$ ,  $B_1$  and  $B_2$ ) (Figure 1). In order to keep undisturbed the vertical stratification during sampling, a cylinder was also fixed vertically in one side of the tank, prior to water filling. In this way, saltwater samples were taken through the cylinder. Samples of 50 mL were taken from the mid points of each water body when the experiment was being setup (0 h) and at 12, 22, 36, 46, 66, 90, 114, 162, 213, 276, 334 and 672 h. Moreover, the whole vertical profile was sampled five times during the experiment ( $t = 0, 46, 162, 334$  and 672 h).

Immediately after sampling, the water aliquots were spiked with a surrogate solution containing PCB IUPAC Nrs 65 and 166, and terbuthylazine (Dr. Ehrenstorfer GmbH, Augsburg, Germany) in methanol (Suprasolv®, Merck, Darmstadt, Germany) to achieve a final concentration of about  $60 \text{ ng L}^{-1}$  for each PCB and  $530 \text{ ng L}^{-1}$  for terbuthylazine.



**Figure 1.** Experimental design, showing the water sampling points.

## 2.3. Chemical analyses

Water samples were saturated with sodium chloride and liquid-liquid extracted with ethyl acetate (Merck) in three sequential extractions of 5 mL. The collected extracts were dried through anhydrous sodium sulfate (Merck), cleaned with 1g of neutral alumina (Merck) deactivated with 3% (w/w) of Milli-Q water and concentrated under a gentle nitrogen stream until a final volume of 100  $\mu\text{L}$ . Organochlorine compounds were analyzed by GC-ECD using a HP 5890 series II chromatograph

equipped with a HP 6890 autosampler. Samples were injected in the splitless mode (48 s) at 250°C. The detector was held at 310°C. Helium 4.6 (1.5 mL min<sup>-1</sup> at 70°C) and nitrogen 5.0 (50 mL min<sup>-1</sup>) was used as a carrier and make-up gases, respectively. Chromatographic separation was achieved by using a 50 m x 0.25 mm ID x 0.25 µm CPSil8 CB column (Chrompack, Netherlands) with a temperature program of 70 °C (1 min) to 150 °C at 15 °C min<sup>-1</sup> and then to 300 °C at 3 °C min<sup>-1</sup>, holding it for 8 min. The rest of the spiked compounds were analyzed using a GC-MS system working in the full scan acquisition mode and a BPX-5 column (30 m x 0.25 mm ID x 0.25 µm) from SGE (Ringwood, Australia) temperature programmed from 70 °C (1 min) to 300 °C at 10 °C min<sup>-1</sup> holding it for 20 min.

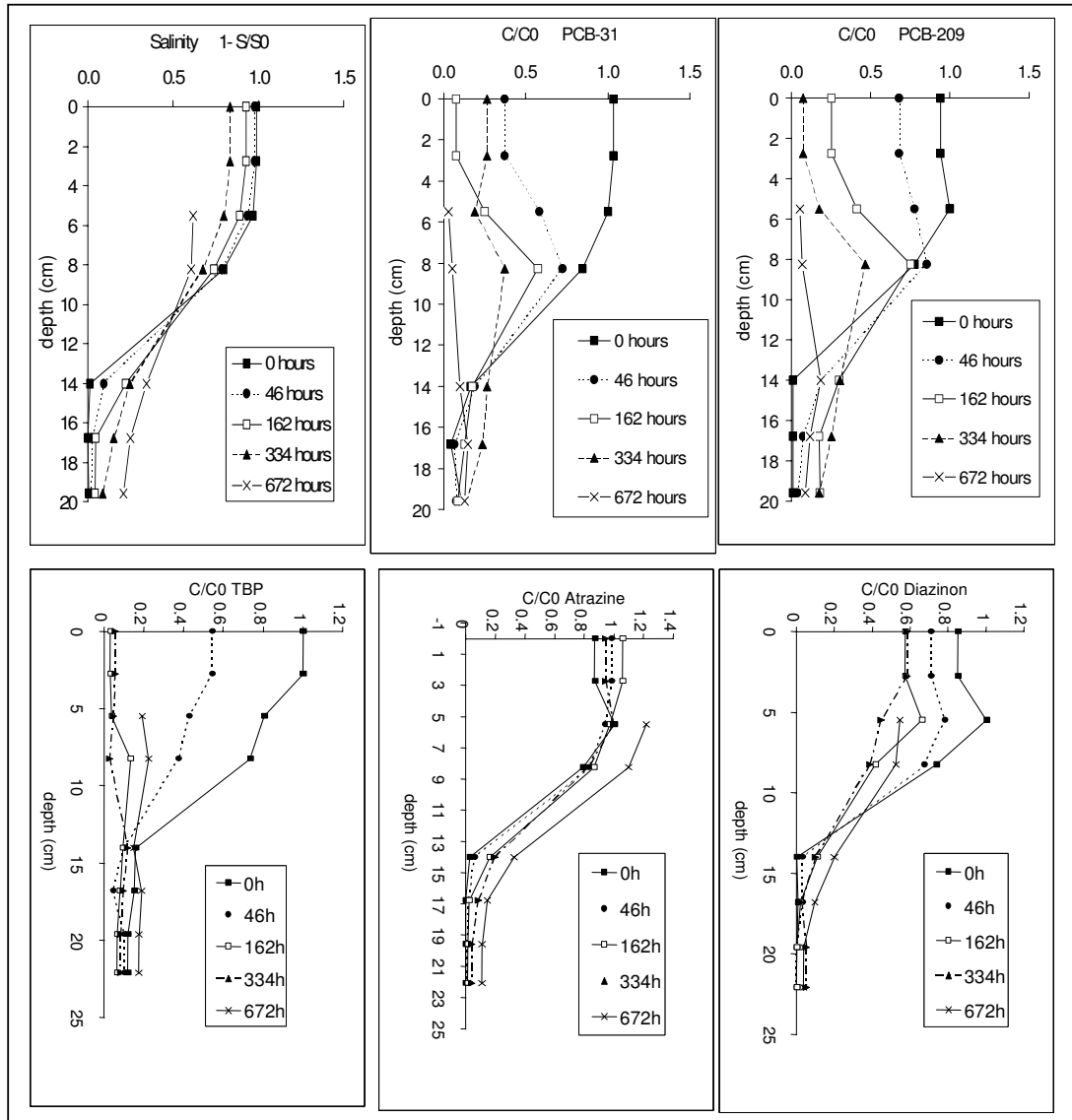
The internal standard (IS) procedure was used for quantification using 2,4,6-trichlorobiphenyl and 2,2',3,4,4',5,6,6'-octachlorobiphenyl, PCBs IUPAC # 30 and # 204 (Dr. Ehrenstorfer GmbH, Augsburg, Germany) for GC-ECD and triphenylamine for GC-MS. Quantification was based on external calibration mixtures containing all target and surrogates analytes. Mean recoveries found when using PCB-65 and PCB-166 as surrogates were 84% and 89%, respectively. For the polar pesticides recoveries were higher than 75%. Results were corrected for surrogate recoveries of the PCBs and organochlorine pesticides.

The statistical study was carried out using the SPSS 13.0 software (SPSS Inc., Chicago, IL, USA) setting the significance level at  $p < 0.05$ . Significant correlations were investigated using the Spearman non-parametric test.

### **3. RESULTS**

#### **3.1. Vertical profiles**

The vertical behaviour of target analytes was monitored during the whole experiment and compared to the sodium chloride behaviour. The evolution of the sodium chloride vertical profile is shown in Figure 2. As expected, at the beginning of the experiment, salt forms a sharply stratified vertical profile which smoothes out progressively with time. However, it has to be pointed out that equilibrium is not completely reached even after 672 h (4 weeks), showing the stability of the salt-wedge. If this profile is compared with the ones obtained for selected target analytes (Figure 2, only characteristic profiles shown), large differences can be seen among them. Therefore, turbulent mixing as the main compound transport motion (salt-wedge perturbation caused by sampling) can be excluded.



**Figure 2.** Vertical profiles showing the time trends for different target analytes.  $s_0$  and  $C_0$  correspond to the salinity of the salty water at the beginning of the experiment and to the initial concentration of the target analytes in the spiked water body, respectively.



The diagrams presented correspond to compounds which have been spiked on the top freshwater, but similar results were obtained for those spiked into the bottom saltwater. Looking at the PCB vertical profile, it can be seen that their concentration rapidly decreases at the top and that there is only a small increase in the bottom saltwater layer. Moreover, even if their behaviour is similar, it can be observed that the PCB-31 is dropping faster in the freshwater than PCB-209, at the 46h sampling. When looking at the more polar compounds, completely different trends are found. On one hand, atrazine and diazinon show more stable vertical profiles than those shown by the PCBs and salinity. On the other hand, the TBP decreases drastically after a short period of time.

Temporal trends could be better understood when looking at the evolution of the mid point of each water body as they were sampled at a higher frequency. In Figure 3, the temporal trends for four target analytes are presented, where the same behaviour observed in Figure 2 can be seen. Mainly, two kinds of trends have been identified depending on the evolution of the target analyte in the spiked water layer. Compounds such as PCB-170 and TBP, with a rapid exponential decrease, and compounds such as –endosulfan and simazine with a much slower and apparently linear decrease. Even if the two kinds of evolution are found in the two spiked water layers, the upper layer showed a predominant decrease. For all the studied compounds, a slight increase is observed in the non spiked layer due to compound diffusion from one phase to another.

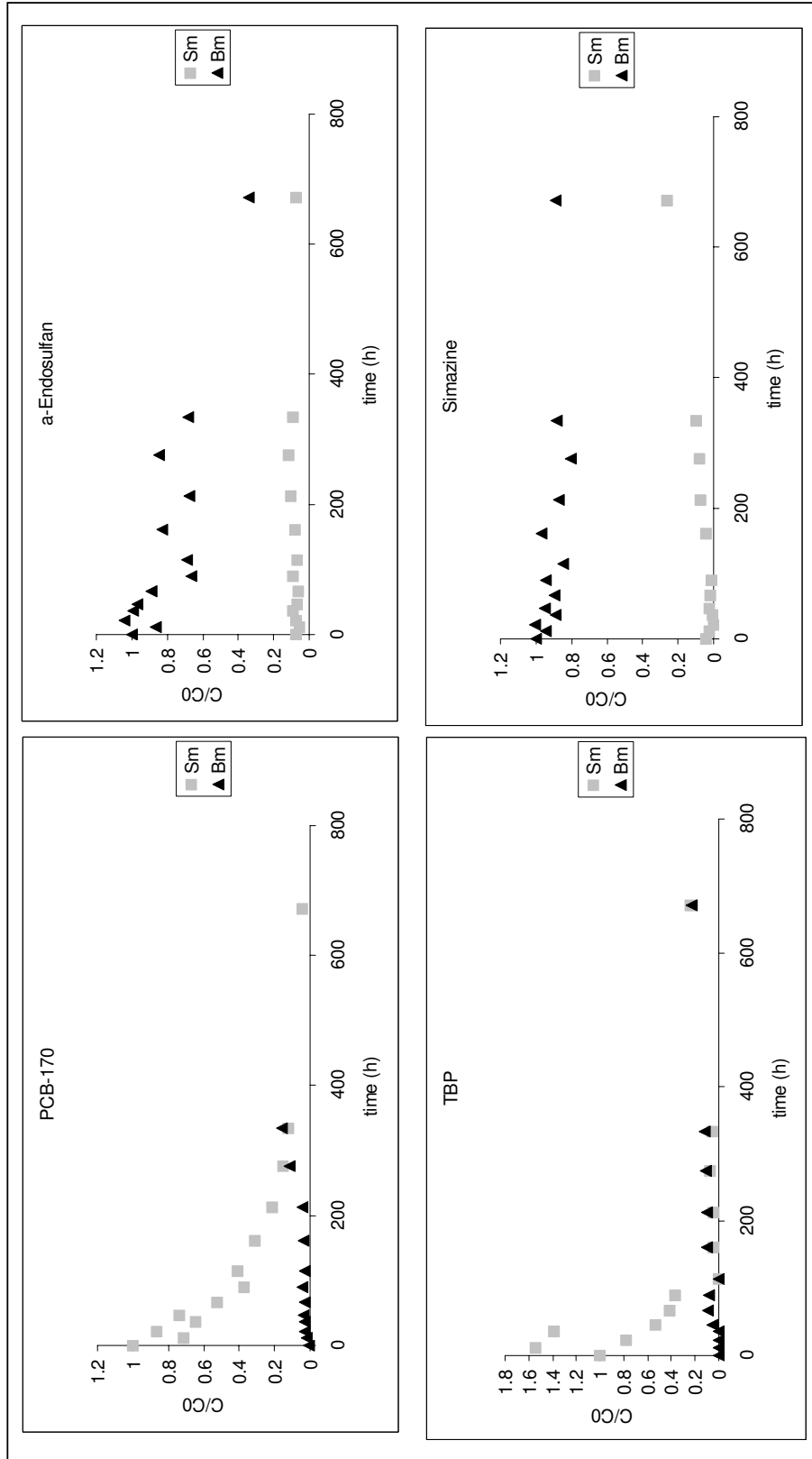


Figure 3. Temporal concentration trends of several target analytes, at  $S_m$  and  $B_m$ .

## 4. DISCUSSION

### 4.1. Diffusion

In the system of study, compound mixing will only be related to the diffusion from one water body to another as no mechanical mixing is taking place. The diffusive phenomenon can be described by the first Fick's law (Eq. 1):

$$J_A = -D_A \cdot \frac{\partial C_A}{\partial z} \quad (\text{Eq. 1})$$

where  $J_A$  is the flux of the compound A,  $D_A$  is its diffusion coefficient,  $C_A$  its concentration and  $z$  the diffusion direction. The diffusion coefficients can be estimated by the Wilke-Chang equation (Eq. 2) (Wilke *et al.*, 1955):

$$D_A = \frac{5.06 \times 10^{-7} \cdot T}{\mu \cdot V_A^{0.6}} \quad (\text{Eq. 2})$$

where  $T$  is the temperature (°K),  $\mu$  the media viscosity (cP) and  $V_A$  the molar volume of the compound A (cm<sup>3</sup>).

In the present experiment, since water viscosity is related to salinity,  $D_A$  will be different in the freshwater and in the saltwater for a same compound. Based on previously reported data (Boufadel *et al.*, 1999, and references therein) the empirical correlation between the viscosity and the salinity is defined as:

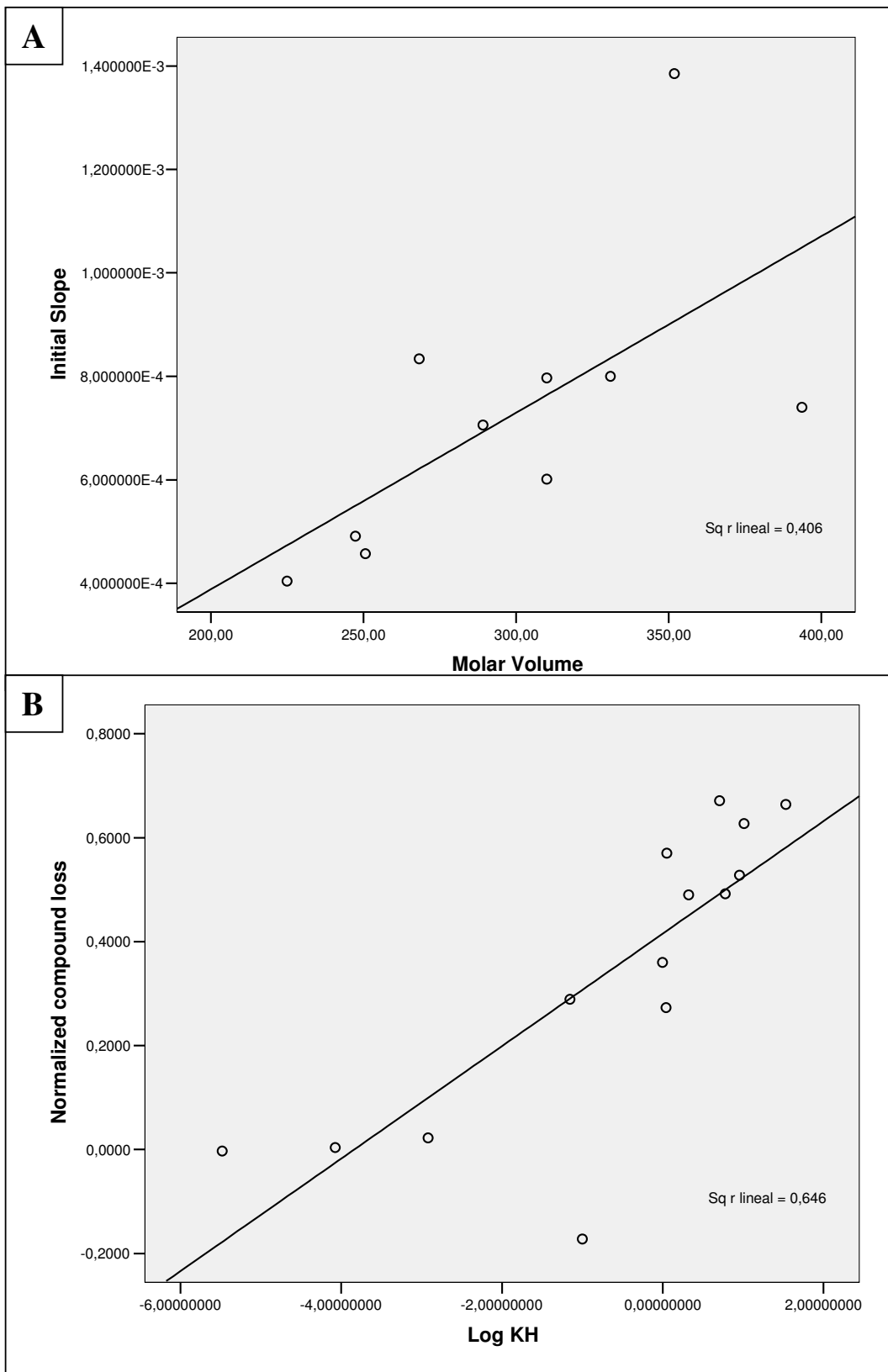
$$\frac{\mu_0}{\mu} = (1 - \xi \cdot s) \quad (\text{Eq. 3})$$

where  $\mu_0$  is the freshwater viscosity (0.001 kg m<sup>-1</sup> s<sup>-1</sup>),  $\xi = 1.566 \times 10^{-3}$  L g<sup>-1</sup> and  $s$  is the salinity (g L<sup>-1</sup>).

Despite the extreme salinity conditions of the experiment (freshwater, 0 g L<sup>-1</sup> and saltwater, 37 g L<sup>-1</sup>), the viscosity estimation showed a 6% variation between the two water layers ( $\mu_{\text{freshwater}} = 0.001000$  and  $\mu_{\text{saltwater}} = 0.001062$  kg m<sup>-1</sup> s<sup>-1</sup>), indicating the small influence of this parameter in the diffusion process, as it has already been reported for other transport models (Welty *et al.*, 1992). Therefore, in this work, water viscosity differences related to the salinity gradient will be considered as irrelevant.

In order to confirm that diffusion is the main transport mechanism from one water layer to another, the results obtained were correlated with the molar volumes of the target analytes estimated at the boiling point (Choy *et al.*, 1958), which determine their diffusion coefficients (Eq. 2). As the surface water is more influenced by evaporation, the bottom water was chosen to carry out these correlations. As expected, a

significant correlation (Spearman test) was found between the initial slope of the graph  $\frac{C}{C_0} = f(t)$  (0-96 h) and the molar volume of the studied compound ( $p=0.044$ ). In this statistical analysis, fenitrothion and TCIEtP were treated as outliers and not considered, since their behaviour is thought to be influenced by degradation. Moreover, if the increase in  $B_m$  of the compounds spiked in the top layer is considered, excluding diazinon and TBP, again a significant correlation between the initial slope of the graph and the molar volume of the studied compound ( $p=0.026$ ) is found (Figure 4a), thus confirming that diffusion is the driving process for compound transport from one water body to another.



**Figure 4.** Correlation between: **a.** initial concentration slopes  $[\frac{C}{C_0} = f(t)]$  and compound molar volumes (Compounds spiked in the salty water). **b.** normalized compound loss and log  $K_H$ .

## 4.2. Loss of compounds

Since the present microcosm setup was abiotic and protected from light, compound biodegradation and photolysis can be considered as negligible for the loss of compounds in the water column. In the same way, it is considered that no adsorption on the glass walls is taking place since the water tank was big enough to have a high volume to surface ratio. Therefore, two main processes will be considered, evaporation and hydrolysis.

### *Evaporation*

In order to study the influence of evaporation on the microcosm, the mass balance of the compounds spiked in the upper layer was studied. This mass balance can be defined by the following equation, considering that no degradation or adsorption on the tank walls took place.

$$m_{St} = m_0 - (m_{Dt} + m_{Et}) \quad (\text{Eq. 4})$$

where  $m_{St}$  is the target analyte mass in the surface water,  $m_{Dt}$  the diffused mass of the target analyte and  $m_{Et}$  the evaporation loss of the compound, at a time  $t$ , and  $m_0$  the initial target analyte spiked mass. In *Eq. 4*,  $m_{Dt}$  could be replaced by the mass of the target compound present in the bottom layer ( $m_{Bt}$ ), thus giving:

$$m_{Et} = m_0 - (m_{St} + m_{Bt}) \quad (\text{Eq. 5})$$

Therefore, the mass evaporated could be estimated from the chemical analysis of the two water layers and from the initial spiking conditions. If these assumptions are confirmed, the evaporated mass (*Eq. 5*) will be correlated with the Henry constant ( $K_H$ ) of each target analyte. In this way,  $\text{Log } K_H$  was found significantly correlated ( $p=0.001$ ) with the mass loss rate at the end of the experiment (4 weeks) (Figure 4b).

Furthermore, the same kind of calculations can be carried out for the compounds spiked in the saltwater. These compounds will diffuse to the upper layer and then will also be able to evaporate, even at a lower rate than those from the upper layer. Neglecting again adsorption to the tank walls and compound degradation, the mass loss was positively correlated with the  $\text{Log } K_H$  ( $p<0.001$ ).

From these results, evaporation seems to account for the main compounds loss process in the studied system. In this way, it should be pointed out that the loss rates by evaporation after 4 weeks from both water layers are significant (Table 2) and that this

process should not be neglected in the environment. Evaporation is more important for the less polar compounds (organochlorine and PCBs) but is not relevant for more polar compounds such as triazines, caffeine and  $\gamma$ -HCH. In fact, nearly 100% of the low chlorination degrees PCBs are lost by evaporation.

**Table 2.** Rates of evaporation for the two water layers after four weeks.

Compound name	Evaporation (%)	Median (%) <sup>*</sup>	Spiking phase	
HCB	81	53	Bw	
-HCH	32			
-HCH	34			
-HCH	n.s.			
pp'DDE	66			
pp'DDD	53			
opDDT	40			
pp'DDT	49			
Heptachlor A	57			
-Endosulfan	63			
Dieldrin	57			
Methoxychlor	36			
PCB18	97	95	Sw	
PCB28	95			
PCB31	84			
PCB44	89			89
PCB101	77			77
PCB138	86			88
PCB153	89			
PCB170	88			89
PCB180	90			
PCB194	84			84
PCB209	83	83		
TBP	n.c.	-	Sw	
TCIEtP	n.c.		Bw	
Atrazine	n.s.	-	Sw	
Simazine	n.s.		Bw	
Diazinon	n.c.	-	Sw	
Fenitrothion	n.c.		Bw	
Molinate	40	40	Sw	
Caffeine	n.s.	-	Bw	

n.s. Not significant

n.c. Not considered

<sup>\*</sup> PCBs were combined in accordance with their chlorination degree



*Degradation*

Compounds such as TBP, TCIEtP and some of the organophosphorous pesticides showed different loss patterns compared to the rest of the studied compounds. This behaviour could be tentatively explained by a certain degradation, as, for example, TBP is known to endure hydrolysis with a half-life in surface waters of roughly 1 week (Spectrum Laboratories, 2006). It is important to point out that half-life data show a large dispersion depending on the processes included in the degradation (photolysis, biodegradation, etc.) and the experimental conditions (laboratory or field). For B<sub>w</sub> spiked compounds no correlation was found between the compound loss and their halve-lives. However, for S<sub>w</sub> a slight correlation was found (Spearman test  $p=0.03$ ) between the compound loss at the end of the experiment and their halve-lives. These correlations would be better if more consistent half-life data would be available. Therefore, even if evaporation is the main elimination process for the two water bodies, degradation (hydrolysis) cannot be completely ruled out for these relatively long-term experiments. Table 3 summarises the compound loss correlations with the halve-lives and the  $K_H$ .

In order to confirm the prevalence of evaporation on degradation, a multi-parametric model, correlating the compounds elimination with evaporation ( $\log K_H$ ) and degradation (halve-lives) for the two water bodies (B<sub>w</sub> and S<sub>w</sub>), was tested. For the two water layers,  $\log K_H$  was found to be the only relevant parameter (Table 3), so that the hypothesis assumed in the *Eq. 4*, in the sense that degradation was negligible during the experiment, can be confirmed.

**Table 3.** Summary of the elimination descriptors. Significance degree is indicated between brackets.

	<b>Sw</b>	<b>Bw</b>
<b>Correlations</b>		
<b>Elimination vs <math>\log(K_H)</math><sup>1</sup></b>	0.741 (0.001)	0.859 (<0.001)
<b>Elimination vs halve-lives<sup>1</sup></b>	0.543 (0.030)	0.327 (0.234)
<b>Multiparametric regression</b>		
<b>Explained variance</b>	81% (<0.001)	66% (0.003)
<b><math>\log(K_H)</math> coefficient<sup>2</sup></b>	0.951 (<0.001)	0.755 (0.002)
<b>Halve-lives coefficient<sup>2</sup></b>	-0.067 (0.714)	0.128 (0.515)

<sup>1</sup>Spearman test

<sup>2</sup>Standardized coefficients

The microcosm experiment carried out in this work, in order to understand the

### **Conclusion**

behaviour of dissolved organic contaminants in a salt-wedge estuary, allowed to conclude that compounds transport from one water body to another may occur by diffusion as it has been correlated with their compound molar volume. Elimination processes were also observed, being evaporation largely predominant over degradation (hydrolysis) for the set of studied compounds.

The conclusions of this work could be useful in order to understand field results in stratified systems as salt wedge estuaries. Further research is in progress confronting the field results of the Ebro River estuary to the microcosm observed behaviour.

### **Acknowledgements**

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*5.2.2 Variació temporal de la concentració dels contaminants orgànics i càlcul de les seves emissions al mar Mediterrani*

En l'article següent, "*Organic contaminant loads into the Western Mediterranean Sea: Estimate of Ebro River inputs.*", s'estudia la variació temporal de diferents famílies de contaminants orgànics a la boca del riu Ebre. A més, s'han aprofitat aquests resultats per a avaluar l'aport contaminant del riu Ebre al Mediterrani integrant les dades obtingudes durant les diferents campanyes que tenen com objectiu integrar la variabilitat anyal del riu. Finalment s'han comparat aquests resultats amb les dades disponibles per a d'altres rius de la Mediterrània Nordoccidental.



## Organic contaminant loads into the Western Mediterranean Sea: Estimate of Ebro River inputs

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### Abstract

Annual input estimates for several organic contaminants from the Ebro River into the Northwestern Mediterranean Sea were carried out on the basis of monthly sampling from November 2002 to October 2003. Some organochlorine compounds (DDT and its degradation products, DDD and DDE, PCBs (9 congeners), HCB and  $\gamma$ -HCH) were selected due to their reported occurrence in the river. Furthermore, some polar pesticides used in the Ebro Delta were also determined (atrazine, simazine, diazinon, fenitrothion and molinate). Concentrations ranged from 0.4 to 19.5 ng l<sup>-1</sup> for the organochlorine compounds (sum of particulate and dissolved phases) and from not detected (ND) to 170 ng l<sup>-1</sup> for the more polar pesticides, which were only found in the dissolved phase. The sum of PCB congeners (mean 8.9 ng l<sup>-1</sup>) showed the highest concentrations among the organochlorine compounds and atrazine (mean 82 ng l<sup>-1</sup>) among the polar pesticides. Based on the contaminant concentrations and on hydrological data, contaminant discharges into the sea were estimated amounting in total to 167 and 1258 kg year<sup>-1</sup> of organochlorine compounds and polar pesticides, respectively. Furthermore, it was observed that PCBs, DDTs and HCB inputs were basically influenced by spate periods due to an increase in suspended particulate matter associated to runoff and sediment resuspension. Whereas for more water soluble contaminants, such as the agrochemicals, their seasonal use had a higher incidence in contaminant fluxes. Bulk chemical parameters such as SPM, DOC, POC, %OC, %ON and C/N ratio provided additional information on the organic matter sources. This provides a better understanding of the temporal variability of the contaminant concentrations.

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**Keywords:** Organochlorine compounds; Pesticides; River outflow; Contaminant transport processes; Contaminant loads; Temporal trends

### 1. Introduction

Large amounts of nutrients, organic matter, pathogens, heavy metals, organic contaminants and radioactive substances could reach the marine environment from land-based and diffuse sources. These include discharges of municipal, industrial and agricultural wastes, river and continental runoff and atmospheric deposition. In addition

to this, ground water entrances into coastal areas, affected by the seasonal exchange of freshwater and saltwater, have also been documented as a source of anthropogenic chemicals into the coastal waters (Michael et al., 2005). In fact, about 80% of the contamination load to the marine environment is said to have originated from inland human activities. In this context, river discharges have been recognized as a major pressure for the coastal waters. Thus, the assessment of contaminant loads transported by rivers is considered necessary for better coastal zone management.

Several studies have been carried out to estimate the transport of suspended sediments, nutrients, organic and inorganic contaminants by rivers into different regional

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seas (Webb et al., 1997; Quémerais et al., 1999; Steen et al., 2001; Meybeck et al., 2003; De Gieter et al., 2005; Warrick et al., 2005). However, this information is very limited in the Mediterranean, where about 80 rivers may contribute to its contamination. The large rivers such as the Po, Rhone and Ebro are contaminated by polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and organic solvents (Meybeck and Ragu, 1997). Moreover, rivers carrying agricultural runoff (e.g. Rhone, Ebro, Po, Axios, Loudias and Aliakmon) are also important point sources of pesticides discharged into the Mediterranean (UNEP/MAP, 2001).

An assessment of the regional contribution to land-based contamination load in the Mediterranean was done in the framework of MED POL Phase I (1975–1980). The assessment highlighted the importance of the North-western basin since it accounted for one-third of the total contaminant load, the Rhone and Ebro being the main rivers discharging their waters in this region (UNEP/MAP, 2004a). The discharges of organochlorine pesticides and PCBs were estimated for the Rhone River for the period of 1994–1995 (Tronczynski and Moisan, 1996). A survey of organochlorine contaminants was also carried out in the Ebro River during 1982–1983 (Cid Montañes et al., 1990). On the other hand, to the best of our knowledge, an estimate of contaminant fluxes are not available for polar pesticides in the NW Mediterranean Sea except for some organonitrogen herbicides that have been evaluated in the Ebro and Rhone Rivers (Tronczynski and Moisan, 1996; Gascón et al., 1998).

In this work, loads of organochlorine compounds (OCs) and polar pesticides from the Ebro River into the Mediterranean Sea have been estimated on the basis of nine sampling campaigns during the period of November 2002 to October 2003. PCBs, HCB, DDTs and  $\gamma$ -HCH were selected by their previously reported occurrence in the river (Grimalt et al., 1988; Cid Montañes et al., 1990;

Amaral et al., 1996; Fernández et al., 1999). Furthermore, two triazinic herbicides (atrazine and simazine), a thiocarbamate herbicide (molinate) and two organophosphate insecticides (diazinon and fenitrothion) were chosen since they are currently used in the agricultural activities undertaken in the river basin (Chiron et al., 1993; Readman et al., 1993; Oubiña et al., 1996; Pocurull et al., 1998; Aguilar et al., 1999; Santos et al., 2000). Finally, several bulk chemical parameters (e.g. SPM, POC, PON and DOC) were determined in order to better characterize the source of the aquatic dissolved and particulate organic matter.

## 2. Experimental methods

### 2.1. Environmental setting

The Ebro River is the longest (928 km) freshwater waterway in Spain with a watershed of about 84000 km<sup>2</sup> (Fig. 1). It flows through North Eastern Spain and reaches the NW Mediterranean forming a delta of 320 km<sup>2</sup>. Agricultural activities are developed (77% of the total area) (Sierra et al., 2004) around this delta. Furthermore, the Ebro Delta hosts a characteristic natural habitat, basically wetlands (Prat and Ibáñez, 1995).

The outflow of the Ebro into the Mediterranean Sea is about 9.2 km<sup>3</sup> year<sup>-1</sup>. It is one of the most important rivers in the Mediterranean, and the second in importance in the NW Mediterranean, after the Rhone (53.9 km<sup>3</sup> year<sup>-1</sup>). The mean annual discharge determined 38 km upstream the river mouth (city of Tortosa) was 416 m<sup>3</sup> s<sup>-1</sup> for the period of 1960–1999, showing a decreasing trend (UNEP/MAP, 2003). Furthermore, as a result of the river flow regulation during the last century, more than 100 reservoirs exist along its course. Sediment discharge estimates revealed a dramatic reduction from 14–25 Mt year<sup>-1</sup> (1880–1934, pre-damming) to 0.12–0.15 Mt year<sup>-1</sup> (1983–1990) (Guillén and Palanques, 1992). Even in years of high

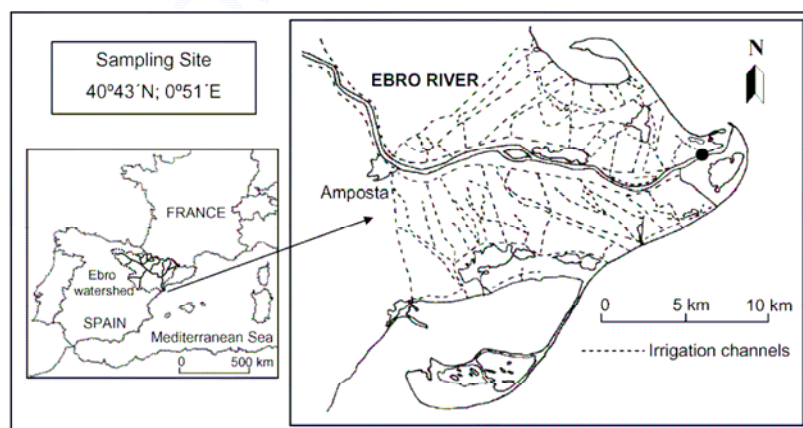


Fig. 1. Map of the study area showing the sampling site location (●).

precipitation, sediment discharge does not exceed the 5% of the pre-damming load (Palanques et al., 1990).

The main sources of anthropogenic compounds expected in the river are from urban wastewater (population in the basin is ca.  $3 \times 10^6$  inhabitants (1998), CHE, 2005) and pesticide inputs. These are derived from agricultural activities both in the basin and in the delta area, where the rice crop is predominant (54% of the total delta surface) (Prat and Ibáñez, 1995). Industrial inputs are also expected, mainly by the presence of a chlor-alkali plant (90 km upstream, Flix, Tarragona), with more than one hundred years of activity, and by several others chemical factories present in the basin.

## 2.2. Sampling

Surface water samples were collected once a month, from November 2002 to October 2003, except for December 2002, January 2003 and September 2003 (nine field campaigns). These were taken at a sampling point near the river mouth (40°43'N–0°51'E) (Fig. 1). Daily sampling was checked to be representative of the monthly river hydrodynamic conditions. The measured water conductivity values were verified to correspond to that of freshwater samples.

Precleaned 2.5 l glass amber bottles were deployed closed with a homemade device, at a 0.5 m depth, as described previously (IOC, 1984). This device consists in a stainless steel cage holding the sampling bottle, which is submerged sealed with a PTFE stopper that can be remotely opened at the desired sampling depth. The collected water samples were transported refrigerated to the laboratory where they were filtered through a previously kiln-fired (400 °C overnight) GF/F glass fiber filter (47 mm  $\times$  0.7  $\mu$ m; Whatman, Maidstone, UK). Filters (particulate phase) were kept in the dark at –20 °C until analysis. Dissolved phase refers to the fraction of contaminants passing through the filter. This includes the compounds that are both truly dissolved as well as those associated with colloidal organic matter. These filtrates were kept in the dark at 4 °C and extracted within the same day of sampling (3–6 h from sampling). Simultaneously to the sampling, dissolved oxygen, temperature and conductivity data were obtained with a multiparametric probe (YSI, Yellow Springs, OH, USA).

Prior to chemical analysis, three replicate aliquots of the filtered sample were acidified (pH = 2 with HCl) and then were determined for dissolved organic carbon (DOC). The dissolved phase was then spiked with a surrogate solution of PCB IUPAC # 65 (2,3,5,6-tetrachlorobiphenyl), PCB IUPAC # 166 (2,3,4,4',5,6-hexachlorobiphenyl) and terbutylazine (Dr. Ehrenstorfer GmbH, Augsburg, Germany) in methanol (Suprasolv®, Merck, Darmstadt, Germany). This achieved a final concentration in water of 4 ng l<sup>-1</sup> for each PCB and 700 ng l<sup>-1</sup> for terbutylazine. Filters were also spiked for recovery calculations with a solution of these three surrogates with 0.7 ng for each PCB and 0.1  $\mu$ g for terbutylazine.

## 2.3. Chemical analysis

### 2.3.1. Bulk chemical parameters

Suspended particulate matter (SPM) content was determined by gravimetry, after drying the filter in an air-heated oven (55 °C until constant weight) and equilibrated at room temperature in a desiccator. One quarter of the filter was used for POC and PON determination, which was done by dry combustion in an elemental analyzer (LECO CHNS 932, St. Louis, MO, USA). Prior to the measurement, inorganic carbon was removed by exposure to concentrated HCl fumes for 48 h. Following acid-fuming, the filters were dried under vacuum in a desiccator for 24 h and maintained at 45 °C for 24 h (Maldonado et al., 1999). DOC was measured in the filtrate using a TOC-5000 Shimadzu instrument, following the EPA Method 9060A.

### 2.3.2. Particulate phase

Spiked filters were extracted three times by sonication with 10 ml of dichloromethane-methanol (1:1) (Suprasolv®, Merck) for 15 min. The recovered extracts were combined and dried with anhydrous sodium sulfate (Merck). Then they were concentrated in a rotary evaporator to 0.5 ml and solvent-exchanged to hexane (Merck). Extract fractionation was carried out by open column chromatography (3 g of neutral alumina Merck, deactivated with 3% (w/w) Milli-Q water) and the OCl<sub>s</sub> were eluted with 5.5 ml of hexane (Merck) in fraction I (PCBs, HCB and *p,p'*-DDE) and 6 ml of hexane:ethyl acetate (9:1) (Merck) in fraction II ( $\gamma$ -HCH, *p,p'*-DDD and *p,p'*-DDT). Finally, the column was eluted with 12 ml of ethyl acetate (fraction III) containing more polar compounds. Cleaned extracts of fractions I and II were analyzed by GC-ECD using a HP 5890 series II chromatograph equipped with a HP 6890 autosampler. Samples were injected in the splitless mode (48 s) at 250 °C and the detector was held at 310 °C. Helium 4.6 (1.5 ml min<sup>-1</sup> at 70 °C) and nitrogen 5.0 (50 ml min<sup>-1</sup>) were used as carrier and make-up gases. Chromatographic separation was achieved by using a 50 m  $\times$  0.25 mm ID  $\times$  0.25  $\mu$ m CPSil8 CB column (Chrompack, Netherlands) with a temperature program of 70 °C (1 min) to 150 °C at 15 °C min<sup>-1</sup> and then to 300 °C at 3 °C min<sup>-1</sup>, holding it for 8 min. The presence of OCl<sub>s</sub> was confirmed by means of GC-MS using a Trace GC-MS (Thermo Quest, Austin, TX) working in the electron impact mode and operating at 70 eV. The transfer line and ion source temperatures were held at 240 and 200 °C, respectively. Confirmatory analyses were also performed by GC-MS in the negative ion chemical ionization mode (Agilent Technologies 6890A, Geneva, Switzerland) coupled with a 5973N MS detector. Ammonia was used as reagent gas and the ion source was kept at  $1.4 \times 10^{-4}$  Torr and 170 °C.

The concentrations were calculated from the calibration curves for 9 PCB congeners (IUPAC No. 28, 52, 99 + 101, 149 + 118, 153, 138 and 180) (Accustandard, New Haven, CT, USA), *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, HCB and

$\gamma$ -HCH (Dr. Ehrenstorfer GmbH, Augsburg, Germany). Herein, PCBs stands for the sum of the congeners described above and DDTs for the sum of *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD. Individual calibration plots were linear from 0.01 to 2 ng ( $r^2 > 0.996$ ). Recoveries were calculated from the surrogates. Quantification was performed by the internal standard method using 2,4,6-trichloro and 2,2',3,4,4',5,6,6'-octachlorobiphenyl, PCBs IUPAC # 30 and IUPAC # 204, respectively (Dr. Ehrenstorfer GmbH, Augsburg, Germany).

The presence of polar pesticides in the third fraction was determined by GC–MS working in the full scan acquisition mode and by using a BPX-5 column (30 m  $\times$  0.25 mm ID  $\times$  0.25  $\mu$ m) from SGE (Ringwood, Australia) temperature programmed from 70 °C (1 min) to 300 °C at 10 °C min<sup>-1</sup> holding it for 20 min. The polar pesticides studied (atrazine, simazine, diazinon, fenitrothion and molinate) were not detected in the particulate phase.

### 2.3.3. Dissolved phase

Two litres of previously filtered water were preconcentrated by solid-phase extraction (SPE) using a 100 mg polymeric phase cartridge Strata X<sup>TM</sup> from Phenomenex (Torrance, CA, USA). After eluting with 10 ml ethyl acetate–hexane (1:1), the extract was rotaevaporated to roughly 0.5 ml and dried through activated anhydrous sodium sulfate (Merck). The sample was fractionated using an alumina open column chromatography as indicated above for the particulate phase. OCIs were analyzed by GC-ECD and polar pesticides by GC–MS as explained for the particulate phase. Polar pesticides detected in the dissolved phase were quantified in the full scan mode using compound characteristic ions. Triphenylamine was used as internal standard to compensate for the sensitivity variation of the MS detector. Calibration curves were prepared with each injection of the samples in the GC–MS system.

### 2.3.4. Quality assurance and quality control

Repetitivity was evaluated by processing a triplicate of river water samples. An average RSD value was below 10% for polar pesticides and PCBs and below 20% in case of organochlorine pesticides.

Mean recoveries found when using PCB-65 and PCB-166 as surrogates were 82% and 102%, respectively, in the particulate phase and 86% and 83%, respectively, in the dissolved phase. For the polar pesticides recoveries were higher than 75%. Resulting data for PCBs and organochlorine pesticides were corrected for surrogate recoveries.

Procedural blanks were processed in the same manner as real samples and evaluated with each set of water samples. Blank values were ca. 0.4 ng for PCB congeners and 0.08 ng for organochlorine pesticides. Limits of detection (LODs) were calculated as the average blank value plus three times the standard deviation of the blanks and ranged from 0.003 to 0.04 ng l<sup>-1</sup> for individual PCBs and organochlorine pesticides in the particulate phase. In the dissolved

phase the values ranged from 0.002 to 0.09 ng l<sup>-1</sup>. For the polar pesticides, LODs ranged from 0.05 to 3 ng l<sup>-1</sup>.

### 2.4. Calculation of contaminant inputs

The method used to estimate the yearly contaminant discharges ( $F_{\text{annual}}$ ) was based on the UNEP guidelines (UNEP/MAP, 2004b) and has been widely accepted (Walling and Webb, 1985; HELCOM, 1993; Steen et al., 2001). A flow-averaged mean concentration ( $C_{\text{aw}}$ ) was calculated for the available data, which was corrected by the total water discharge in the sampled period. The equations used were:

$$C_{\text{aw}} = \frac{\sum_{i=1}^n C_i Q_i}{\sum_{i=1}^n Q_i} \quad (1)$$

$$F_{\text{annual}} = C_{\text{aw}} Q_T \quad (2)$$

where  $C_i$  and  $Q_i$  are the instantaneous concentration and water flow discharge, calculated by means of a daily averaged water flow, respectively for each sampling event.  $Q_T$  represents the total river discharge for the period considered (November 02–October 03), calculated by adding the monthly averaged water flow. River outflow data was obtained from the Confederación Hidrográfica del Ebro (C.H.E., Spanish Ministry for the Environment) from a continuous register.

Furthermore, to study the temporal contaminant discharge variation,  $C_i$  and  $Q_i$  were considered for each monthly period and expressed as g day<sup>-1</sup>.

## 3. Results

### 3.1. Bulk chemical parameters

Despite the high flow regulation, the hydrological regime of the Ebro River shows large variability between low and high water discharge periods (Fig. 2). In this regard, during the sampling period, the daily average flow ranged from less than 130 m<sup>3</sup> s<sup>-1</sup> (July and August) up to 1236 m<sup>3</sup> s<sup>-1</sup> (February). SPM correlated significantly with the flow rate (Pearson correlation,  $r = 0.678$ , significance value  $< 0.05$ ), reaching a maximum in February (19.6 mg l<sup>-1</sup>) (Fig. 2). However, other maximum concentrations in SPM were also evidenced in May and August, which cannot be related to an increase in the river flow. Values for POC and DOC did not show a definite trend, with the maximum and the minimum corresponding to different months of the sampling period. Organic carbon (OC) and organic nitrogen (ON) percentages in the particulate matter also presents monthly fluctuations with minima in February and May and a maximum in June. Considering the mass loads, similar patterns were observed for SPM, POC and DOC with a high mass emission period from February (maximum) to April and a low emission period from May to November. Although flooding events have been importantly buffered after the river damming, sediment transport to the Delta



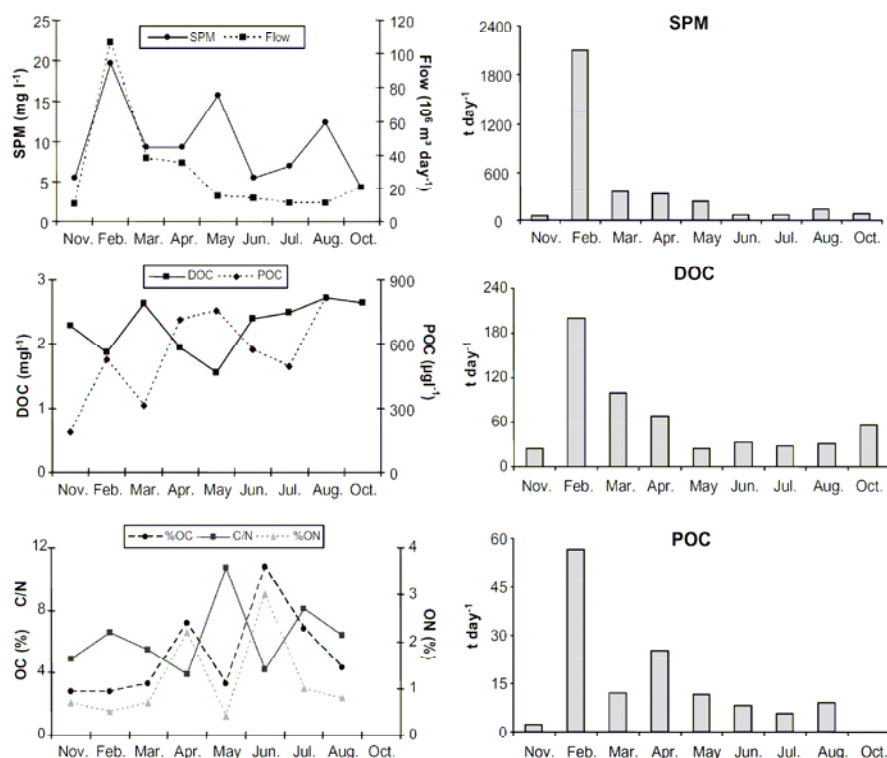


Fig. 2. The temporal trends and loads of some chemical bulk parameters. SPM: Suspended Particulate Matter ( $\text{mg l}^{-1}$ ); Flow refers to water discharge per day recorded at Tortosa (Tarragona) by the Confederación Hidrográfica del Ebro (Spanish Ministry for the Environment); DOC: Dissolved Organic Carbon ( $\text{mg l}^{-1}$ ); POC: Particulate Organic Carbon ( $\mu\text{g l}^{-1}$ ); %OC and %ON refers to the percentage of organic carbon and nitrogen in the SPM, respectively; C/N is an atomic ratio in the SPM.

is mainly related to spate periods (Ibáñez et al., 1996). Accordingly, the SPM daily load corresponding to February alone accounts for the 61% of sum of the total inputs along the sampling period.

### 3.2. Temporal variability of contaminant concentrations

Concentrations of OCIs in water are shown in Table 1 as the sum of the dissolved and the particulate phase contents. Their temporal variability on a monthly basis for both phases is plotted in Fig. 3.

PCBs in the particulate phase ranged from 0.3 to 2.5  $\text{ng l}^{-1}$  (78–249  $\text{ng g}^{-1}$ ) with a mean value of 1.2  $\text{ng l}^{-1}$  (140  $\text{ng g}^{-1}$ ). A maximum concentration expressed as  $\text{ng l}^{-1}$  was detected in February. This was concurrent with the maximum flow rate and the SPM concentration (Fig. 2). However, referring the PCB concentration to the SPM ( $\text{ng g}^{-1}$ ), higher values were found in the months with a lower flow rate and SPM (November 02, June and July 03). In the dissolved phase (2.2–18.1  $\text{ng l}^{-1}$ , mean 7.7  $\text{ng l}^{-1}$ ) higher values were observed during low water discharge conditions during the dry period from May to

August (daily mean discharges below 180  $\text{m}^3 \text{s}^{-1}$ ). However, in November, with a low daily discharge (121  $\text{m}^3 \text{s}^{-1}$ ), the concentration in the dissolved phase was in the lower range.

Concentrations of DDTs in the particulate phase (0.5–5.6  $\text{ng l}^{-1}$ , mean 2.2  $\text{ng l}^{-1}$ ) present the highest concentration in May, February and July samples. However, when concentrations are referred to particulate contents (62–634  $\text{ng g}^{-1}$ , 226  $\text{ng g}^{-1}$ ), the maximum value correspond to July. Individual compounds (*p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD) follow basically the same trend. DDTs in the dissolved phase (0.7–1.6  $\text{ng l}^{-1}$ , mean 1.3  $\text{ng l}^{-1}$ ) seem to be relatively constant in time, but a minimum was found in October.

The *p,p'*-DDT was predominant over its degradation products (*p,p'*-DDE and *p,p'*-DDD), representing a mean of 70% (ranging from 34% to 82%) of the DDTs in the particulate phase. In the dissolved phase, this percentage decreases to 45% (ranging from 17% to 63%). In this respect, the ratios between DDTs and *p,p'*-DDE and *p,p'*-DDD are lower than unity, being the DDE/DDTs ratio between 0.08 and 0.3 (mean 0.2) in the particulate phase

Table 1  
Comparison of contaminant concentrations ( $\text{ng l}^{-1}$ ) with data previously reported in the Ebro River (mean values in brackets)

Compound	This study	Other studies	Reference
PCBs <sup>a</sup>	3.0–19.5 (8.9)	2.04–3.30 (2.64) <sup>c</sup> 43.17–108.0 (76.29) <sup>d</sup>	Cid Montañes et al. (1990) Fernández et al. (1999)
DDTs <sup>a</sup>	1.3–7.2 (3.5)	0.98–1.31 (1.15) 1.97–6.77 (3.10) <sup>e</sup>	Cid Montañes et al. (1990) Fernández et al. (1999)
Lindane <sup>a</sup>	0.54–5.64 (2.29)	0.005–0.044 (0.026) <sup>f</sup> 2.1–5.5 (3.0) 0.22–20.58 (3.38) <sup>g</sup>	Cid Montañes et al. (1990) Amaral et al. (1996) Fernández et al. (1999)
HCB <sup>a</sup>	0.43–1.21 (0.78)	0.085–1.55 (0.66) 4.05–8.50 (6.27) <0.01–19	Grimalt et al. (1988) Cid Montañes et al. (1990) Amaral et al. (1996)
Atrazine <sup>b</sup>	12–170 (82)	17–190 100 60–240 (168)	Readman et al. (1993) Chiron et al. (1993) Aguilar et al. (1999)
Simazine <sup>b</sup>	9–59 (41)	87–440 150 50–1060 (345)	Readman et al. (1993) Chiron et al. (1993) Aguilar et al. (1999)
Molinate <sup>b</sup>	ND–26.3 (9.6)	254–1400 500 ND–290	Readman et al. (1993) Chiron et al. (1993) Aguilar et al. (1999)

<sup>a</sup> Sum of dissolved and particulate phase.

<sup>b</sup> Only detected in the dissolved phase.

<sup>c</sup> Quantified as Aroclor 1260.

<sup>d</sup> Sum of congeners #77, 101, 105, 118, 126, 138, 153, 156, 167, 169, 170, 180 and 194.

<sup>e</sup> Sum of dichlorobenzophenone, *p,p'*-DDE, *p,p'*-TDE and *p,p'*-DDT.

<sup>f</sup> Concentration only in the particulate phase.

<sup>g</sup> Sum of  $\gamma$ -HCH and  $\alpha$ -HCH.

and between 0.05–0.4 (mean 0.3) in the dissolved phase. The DDD/DDTs ratios range from 0.07 to 0.3 (mean value 0.1) and from 0.2 to 0.5 (mean value 0.3) in the particulate and the dissolved phases, respectively.

HCB concentrations in the particulate phase (0.01–0.67  $\text{ng l}^{-1}$ , mean 0.11  $\text{ng l}^{-1}$ ) show two maxima, one in February and a second in May. This is consistent with maxima values also reported for PCBs and DDTs. Furthermore, particulate HCB concentrations correlated with SPM (Pearson correlation,  $r = 0.815$ , significance value  $< 0.01$ ). When the concentration is normalized to the SPM content (0.3–36  $\text{ng g}^{-1}$ , mean 7.2  $\text{ng g}^{-1}$ ), the same trend appears. In the dissolved phase (0.34–1.19  $\text{ng l}^{-1}$ , mean 0.67  $\text{ng l}^{-1}$ ) the higher values were found in May–October with a maximum in June.

Lindane ( $\gamma$ -HCH) was detected in the particulate phase only in three out of the nine campaigns and concentrations were very low (not detected (ND)–0.04  $\text{ng l}^{-1}$ , mean 0.01  $\text{ng l}^{-1}$ ). In the dissolved phase, it was detected in all the samples at higher concentrations (0.53–5.63  $\text{ng l}^{-1}$ , mean 2.28  $\text{ng l}^{-1}$ ). The temporal concentrations of  $\gamma$ -HCH in the dissolved phase show two maxima in November and April.

For polar pesticides, the temporal variability encountered in concentrations was higher than that observed for organochlorine compounds. Nevertheless, for all the studied compounds the minimal concentration was found in the winter period, mainly in February (Fig. 4). The period

of maximum concentration is more dependent on the studied compounds, but it is generally found between April and July, apart from a local maximum for diazinon in November. The triazinic herbicides were the most abundant in all the sampling seasons, ranging from 12.2 to 170  $\text{ng l}^{-1}$  (mean 84.1  $\text{ng l}^{-1}$ ) and from 8.7 to 57.6  $\text{ng l}^{-1}$  (mean 42.4  $\text{ng l}^{-1}$ ) for atrazine and simazine, respectively. Molinate, a thiocarbamate, ranging from ND to 26.3  $\text{ng l}^{-1}$  (mean 9.6  $\text{ng l}^{-1}$ ), presents a more regular distribution with a high concentration period from May to August and a maximum in June. For the organophosphorous pesticides, diazinon ranged from 0.2 to 1.7  $\text{ng l}^{-1}$  (mean 0.8  $\text{ng l}^{-1}$ ) and fenitrothion from ND to 42.5  $\text{ng l}^{-1}$  (mean 13.8  $\text{ng l}^{-1}$ ).

### 3.3. Contaminants loads

Estimates of organochlorine discharges accounting for the Ebro River into the NW Mediterranean are shown in Table 2. PCBs are the chemical class released in the highest amount with a considerable importance of the discharge associated with the dissolved phase (60  $\text{kg year}^{-1}$ , 75% of the total); where the lower chlorinated congeners predominate. Moreover, a total of 50  $\text{kg year}^{-1}$  of DDTs is out flown by the river, being the *p,p'*-DDT the major constituent (33  $\text{kg}$ ), followed by *p,p'*-DDD (9  $\text{kg}$ ) and *p,p'*-DDE (8  $\text{kg}$ ). DDT discharge is predominantly associated to particles (66% for the DDTs), mainly due to the particulate

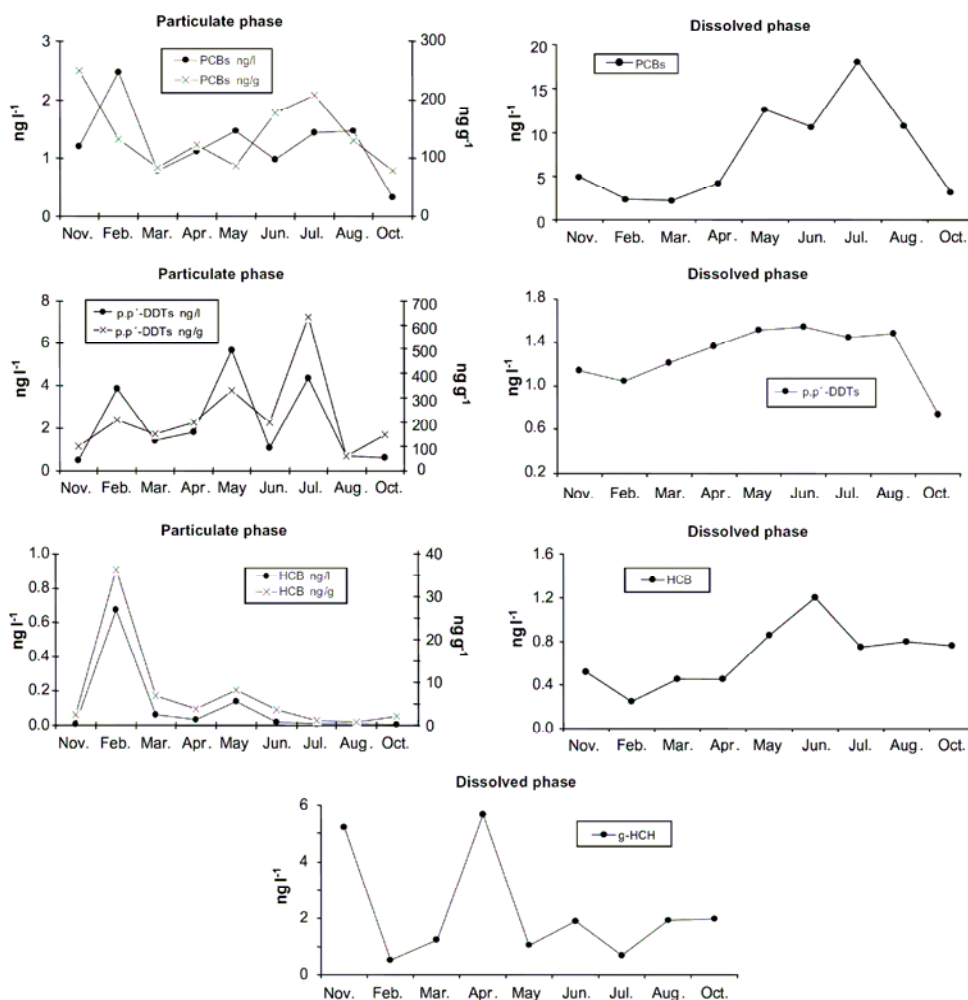


Fig. 3. Temporal trends of organochlorine concentrations in the particulate and the dissolved phases of Ebro River waters.

*p,p'*-DDT contribution, which represents 75% of the total discharge of this compound. The load of lindane was estimated at  $26 \text{ kg year}^{-1}$ , mostly in the dissolved form (more than 99%). About 11 kg of HCB are discharged per year to the sea, 4 kg within the particulate and 7 kg in the dissolved phase.

For a better understanding of the temporal fluctuation of these discharges, daily loads have been calculated. These are presented in Figs. 5 and 6. For PCBs, DDTs and HCB, a maximum is observed in February (Fig. 5), corresponding to a monthly mean flow ( $1086 \text{ m}^3 \text{ s}^{-1}$ , CHE, 2003) about three times higher than the monthly mean value for the studied period ( $398 \text{ m}^3 \text{ s}^{-1}$ , CHE, 2003). It is almost one order of magnitude higher than the typical values of the dry months (June–August 03). Furthermore, the increase

in the SPM transport during the overflow period leads to a substantial increase in the amount of PCBs, DDTs and HCB associated to the particles discharged into the sea. The trend for lindane is totally different since no maximum in discharge is observed in February, but an increase in loads was detected in April, which is concurrent with the high concentration found in the water ( $5.64 \text{ ng l}^{-1}$ ).

Annual loads estimated for the analyzed polar pesticides are summarized in Table 2, which are several orders of magnitude higher than those of the OCIs. The daily emission rates do not always parallel the concentration trends (Fig. 6). In this sense, even if in February the lowest concentrations were observed for all polar compounds, the emission was compensated for the high river flow during this period. In any case, triazinic compounds are still the

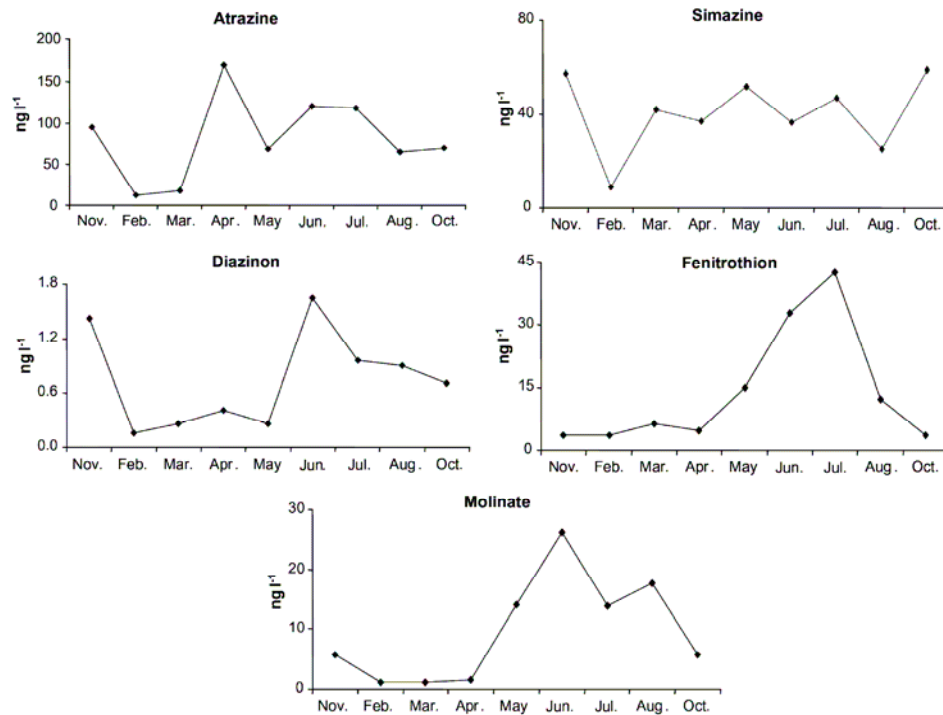


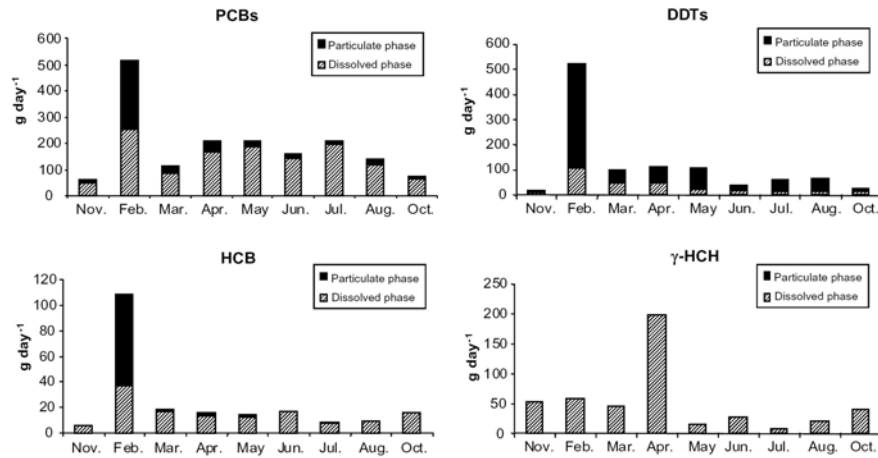
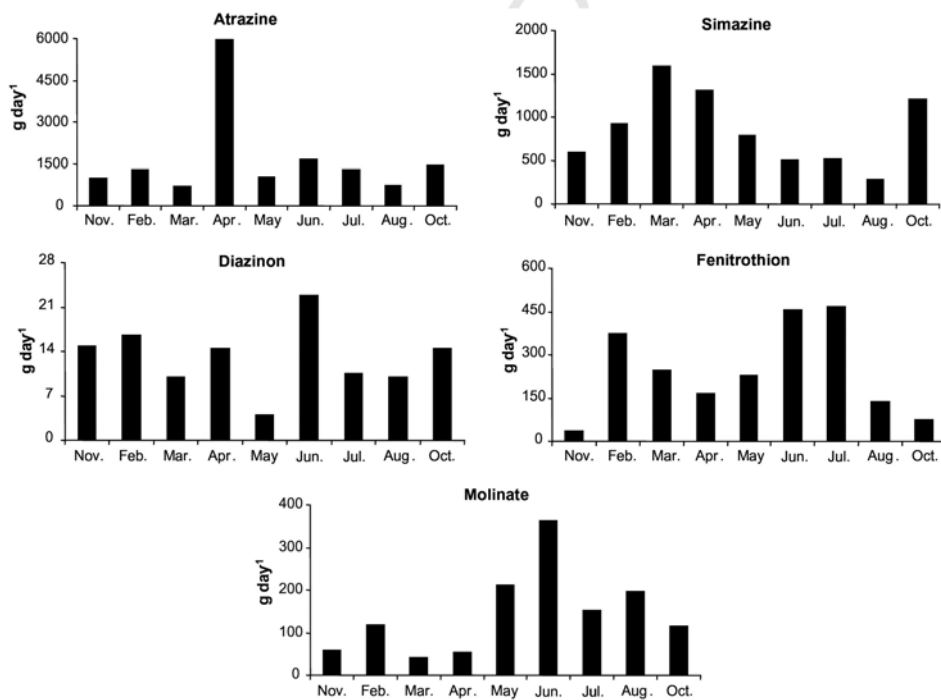
Fig. 4. Temporal trends of polar pesticide concentrations in the dissolved phase of the Ebro River waters.

Table 2

Riverine annual loads of chemical bulk parameters and organic contaminants (kg year<sup>-1</sup>) in the NW Mediterranean

Chemical component	River	This study	Other studies	Reference
SPM (Mt year <sup>-1</sup> )	Ebro	0.16	0.12	Guillén and Palanques (1992)
DOC (t year <sup>-1</sup> )	Ebro	26.5		This study
POC (t year <sup>-1</sup> )	Ebro	6.6		This study
PCBs <sup>a</sup>	Ebro	80	12	Cid Montanes et al. (1990)
	Rhone		304 <sup>b</sup>	Tronczynski and Moisan (1996) <sup>d</sup>
DDTs <sup>a</sup>	Ebro	50	8 <sup>c</sup>	Cid Montanes et al. (1990)
	Rhone		196 <sup>b</sup>	Tronczynski and Moisan (1996) <sup>d</sup>
Lindane <sup>a</sup>	Ebro	26		This study
	Rhone		403	Tronczynski and Moisan (1996) <sup>d</sup>
HCB <sup>a</sup>	Ebro	11	30	Cid Montañes et al. (1990)
	Rhone		172	Tronczynski and Moisan (1996) <sup>d</sup>
Atrazine	Ebro	719	869	Gascón et al. (1998)
	Rhone		5809	Tronczynski and Moisan (1996) <sup>d</sup>
Simazine	Ebro	367	497	Gascón et al. (1998)
	Rhone		2059	Tronczynski and Moisan (1996) <sup>d</sup>
Diazinon	Ebro	6		This study
Fenitrothion	Ebro	104		This study
Molinate	Ebro	62		This study

<sup>a</sup> Sum of dissolved and particulate phases.<sup>b</sup> Concentration only in the particulate phase.<sup>c</sup> Sum of *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDD, *p,p'*-DDT and *o,p'*-DDT.<sup>d</sup> Values reported refer to the estimations calculated with the same method used in this study.

Fig. 5. Temporal variation of organochlorine loads (g day<sup>-1</sup>) in the Ebro River.Fig. 6. Temporal variation of polar pesticides loads (g day<sup>-1</sup>) in the Ebro River.

predominant with atrazine fluxes ranging from nearly 6.0 kg day<sup>-1</sup> in April and 0.7 kg day<sup>-1</sup> in March and August, when there is the lowest water discharge. The

lower discharge was obtained for diazinon, which presents a maximum discharge of 23 g day<sup>-1</sup> in June and a minimum of 4 g day<sup>-1</sup> in May.

#### 4. Discussion

##### 4.1. Organic matter sources

The temporal profiles of the water SPM and the flow rate exhibit prominent maxima in February (Fig. 2). High water discharges are characterized by low %OC and %ON and high C/N values, indicative of the terrigenous origin of the SPM (Cauwet, 1991; Amon and Meon, 2004). On the other hand, the two local maxima in the SPM concentration found in May and August are not related with an increase in the water discharge. The high values of the ratio C/N observed for these months are more characteristic of a terrigenous SPM (Maksymowska et al., 2000). It suggests that these maxima could be attributed to the agricultural activity, which contributes to the soil remobilization. Conversely, in June the maximum in the %OC and %ON for the SPM corresponds to a minimum in the C/N ratio, which is more characteristic of biogenic aquatic production (Hellings et al., 1999).

##### 4.2. Data assessment

The concentrations of the present contaminants and those reported previously in the Ebro River are shown in Table 1. The total PCBs found in this study are higher than those reported by Cid Montañes et al. (1990) and lower than those found by Fernández et al. (1999). It is known that PCB data may exhibit a large variability due to different quantification methods. However, the lower concentrations (expressed as Aroclor 1260) reported by Cid Montañes et al. (1990), when PCB production was still active in the region, could be explained by a dilution effect since the water samples were collected out of the river mouth, where some mixing between river and marine waters could occur. On the other hand, the values reported by Fernández et al. (1999) are nearly one order of magnitude higher than those found in this study.

It is difficult to assess the variations among different studies because several reasons account for them, particularly the temporal trends of inputs, the location of the sampling stations, the hydrological regime of the river in the sampling period and the accuracy of the analytical methodologies. However, the present data are the most comprehensive in covering nearly an annual cycle in a station representative of the outflow of the river.

The mean concentrations of DDTs and lindane are in agreement with those reported previously (Table 1), but DDTs data are higher than those found by Cid Montañes et al. (1990). This could be attributed to the sampling location as for PCBs. In general, it has been found that *p,p'*-DDT is the predominant compound (maximum  $5.1 \text{ ng l}^{-1}$ ) and the calculated ratios between the degradation products and the parent *p,p'*-DDT are much lower than unity, indicating the possible presence of recent inputs in the area (Tolosa et al., 1995). The prevalence of *p,p'*-DDT over its degradation products has also been reported in a survey

carried out recently in lower course river sediments (ACA, 2005). It suggests the possibility that this pesticide could come from local applications, probably from agricultural use. However, remobilization from upstream contaminated sediments cannot be undervalued, due to the presence of an old factory 95 km upstream the sampling point (Flix, Tarragona).

The HCB concentrations are similar to those reported in 1985–1986 by Grimalt et al. (1988), but much lower than those reported in 1982–1983 by Cid Montañes et al. (1990), which reached  $8.50 \text{ ng l}^{-1}$ . This could possibly be attributed to the mobilization of residues from the production plant upstream the Ebro River (Flix), where the production of this compound reached about  $1 \text{ kt year}^{-1}$  in the period of 1980–1983. In this respect, concentrations as high as  $19 \text{ ng l}^{-1}$  were found in a sampling point close to the chlorinated organic solvent factory (Amaral et al., 1996).

The encountered concentrations for the triazinic compounds are of the same order of magnitude as those previously reported, taking into account that these studies usually refer to periods of usage of these pesticides, therefore, overestimating the annual range of concentrations. However, in the case of molinate a significant decrease seems to be occurring.

As far as the river discharges are concerned, the only data existing to the best of our knowledge are those reported by Cid Montañes et al. (1990) for OCIs and Gascón et al. (1998) for atrazine and simazine. The values reported for PCBs, DDTs and HCB (Table 2) are consistent with the concentration patterns described earlier, which may account for the differences observed. However, the monthly sampling adopted in this work, which encompassed months with elevated water discharge and a flood episode (flow rates reaching  $2420 \text{ m}^3 \text{ s}^{-1}$ ), may provide more consistent discharge values, in accordance with natural river fluctuations. The values obtained for triazinic compounds are slightly lower than those reported previously ( $\approx 30\%$ ), but still indicating a long standing input. No other estimates are available for molinate, diazinon fenitrothion and lindane.

Contaminant load estimates have a certain degree of uncertainty when a continuous record of water discharge is available whereas concentration data are obtained discontinuously, monthly in the present study. Errors in accuracy and precision in flux estimate may derive from the calculation procedures and several methods have been proposed and studied (Walling and Webb, 1985; Clarke, 1990; Littlewood, 1995; Moatar and Meybeck, 2005). The load estimates reported in this study could be subjected to the error sources described previously. However, the calculation of sediment transport based on the present data ( $0.16 \text{ Mt year}^{-1}$ ) has been successfully compared with a previous consistent estimate ( $0.12 \text{ Mt year}^{-1}$ ; Guillén and Palanques, 1992), which enhances the accuracy of the contaminant load values reported here.

Contaminant loads for the Rhone River (Table 2), also discharging the waters into the NW Mediterranean, are up to one order of magnitude higher than the values found for the Ebro (Tronczynski and Moisan, 1996). Apart from differences in water concentrations, it has to be taken into account that the Rhone River outflow is about 6 times higher than that of the Ebro (UNEP/MAP, 2003).

#### 4.3. *The fate of contaminants in the river water*

For PCBs, the dissolved concentrations represent 82% of the total as a mean of all samples, 88% for HCB and more than 99% for lindane. However, a higher fraction of DDTs was associated to the particulate phase (55% as a mean, ranging from 29% to 79%). In general, the particulate-dissolved partitioning of compounds is determined by their physicochemical properties, namely by the  $K_{ow}$  (Schwarzenbach et al., 2003), although the occurrence of a colloidal phase may enhance their solubility (Bergen et al., 1993). The apparent distribution coefficients ( $K_d$ ), defined as the ratio between the compound content in the SPM (compound mass per mass of particle) and the concentration in the dissolved phase (compound mass per volume), show differences among the studied compounds. In this way, DDTs seem to be relatively enriched in the particulate phase (with an average of distribution coefficient for the nine campaigns,  $\log K_d = 5.21 \text{ kg}^{-1}$ ) with respect to the sum of PCB congeners ( $\log K_d = 4.31 \text{ kg}^{-1}$ ) and HCB ( $\log K_d = 3.71 \text{ kg}^{-1}$ ). The fact that the *p,p'*-DDT predominance over its degradation products was higher in the particulate than in the dissolved phase (see Section 3.2) and that concentration maxima in particles for *p,p'*-DDT were found when the SPM was mainly terrigenous (higher C/N) may indicate the existence of recent inputs of this compound which have not reached equilibrium in the water body.

Maximum discharges were found for PCBs, DDTs and HCB in February in both the particulate and the dissolved phases (Fig. 5), which is attributable to the spate period. Since high OCl concentrations were also encountered in this month (Fig. 3), particularly for HCB, it appears that particles transported in this period are enriched in these kinds of OCl. In addition, the high value of the C/N ratio could indicate a higher contribution of terrigenous material (see Section 4.1). This suggests that part of these suspended materials could come from the remobilization of polluted sediments stored in dams upstream (i.e. Flix dam) (Swindler et al., 1995; ACA, 2005). An increase in SPM was also found in May, which is translated into higher organochlorine concentrations similar to what occurred in February. On the other hand, maximum concentrations observed in July (PCBs and DDTs) are not due to an increase in either SPM or in the river flow but probably due to a different source of organic matter (high C/N rate).

The trend for  $\gamma$ -HCH is different compared to the rest of OCl because it mainly occurs in the dissolved phase, behaving similar to polar pesticides. The fluctuations of its concentrations in the dissolved phase (Fig. 3) are not

attributable to hydrological variations. This temporal distribution could possibly reflect the recent use of this compound in agricultural activities, since lindane is one of the pesticides still used in most Mediterranean countries (UNEP/MAP, 2004a).

The emission patterns observed for the polar pesticides differ from those of OCl. Their presence and trends could be more associated to their agricultural application and weather conditions. This is in part due to the physicochemical properties because they are more associated with the dissolved than with the particulate phase and are less persistent in the environment. In this way, the leaching and runoff of these compounds from agricultural fields to the river due to rainfall cannot be neglected as it has already been reported in other studies (Southwick et al., 2003; Nakano et al., 2004). As a result, the behavior of these contaminants could be due to different processes and, therefore, the observed pattern is difficult to interpret. Finally, when considering the contribution of pesticides to the Mediterranean Sea, it has to be pointed out that the Ebro River is not the only source for these kinds of contaminants in this area; as it can be seen in Fig. 1, irrigation channels from the delta rice fields discharge directly into the sea.

#### 4.4. *Influence of hydrological conditions*

Organochlorine contaminant concentrations and loads in the particulate phase are related to the origin and abundance of suspended solids and are not highly influenced by water flow, with the exception of the February flooding event. The increase of the flow and of the SPM in February induces the mobilization of a major amount of organochlorine contaminants which were transported to the sea (Fig. 5). However, in the dissolved phase, concentrations seem to be more sensitive to water flow variations, with higher values during the dry period (May–August), possibly due to a lower dilution factor.

Polar pesticides are also affected by river flow variations, exhibiting lower concentrations in February when the river flow was at its maximum level. However, apart from hydrological conditions, the maximum discharges are associated with, as in the case of lindane, the higher water concentrations, in relation with other parameters like seasonal use and mobilization.

### 5. **Conclusions**

The emission rates of different contaminants (organochlorine compounds and polar pesticides) of the Ebro River into the NW Mediterranean have been assessed, polar pesticides being the most abundant. However, the data presented show that organochlorine contaminants continue to be of concern in this area, with a history of production. This highlights the need for their continued monitoring in the Ebro basin. Furthermore, DDT still predominates over its degradation products, indicating recent inputs of this banned product. This stresses the importance of the Ebro

River as a source of this compound into the Mediterranean Sea. Flooding events result in an increase of PCBs, DDTs and HCB inputs into the sea due to their influence on processes like soil leaching, runoff and sediment remobilization. In the case of lindane and the currently applied polar pesticides, it was found that the amount discharged into the sea was primarily affected by their seasonal use.

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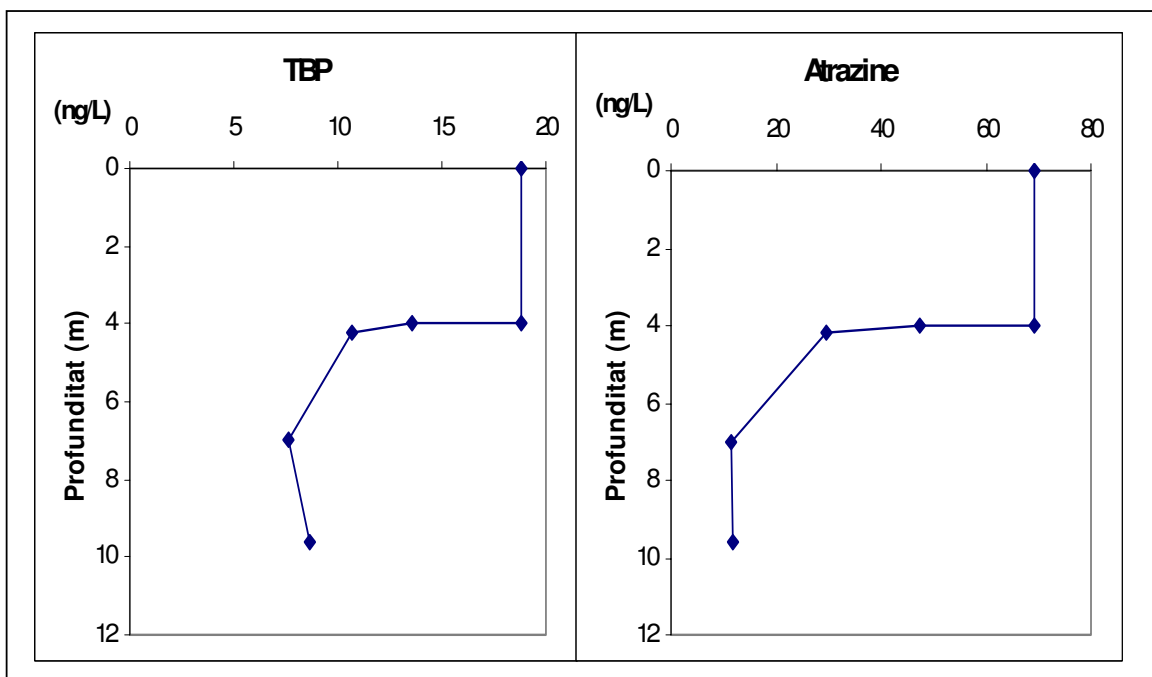
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## 5.2.3 Treball experimental addicional

A partir de dades experimentals addicionals i un cop observada la estratificació vertical en l'experiment de microcosmos, s'ha estudiat el comportament en l'estuari del riu Ebre centrant-nos en els contaminants emergents. Gràcies al sistema de mostratge descrit anteriorment a la secció 5.1.2 s'han aconseguit mostres a diferents profunditats caracteritzant així les dues masses d'aigua (aigua superficial dolça i aigua profunda salda) i l'interfase d'aquestes dues capes. A la Figura 15 es mostra el perfil vertical de dos contaminants en condicions de falca salina.



**Figura 15.** Perfil vertical de la concentració de TBP i de atrazina en condicions de falca salina.

Els resultats obtinguts mostren, per una gran majoria de compostos, una capa superficial amb concentracions de contaminants més elevades que la capa inferior. A la interfase, no s'ha observat cap tipus d'enriquiment, comportant-se tan sols com un punt intermediari entre les concentracions observades per la capa superior i les de la

capa inferior. La desisopropilatrazina, producte de degradació tant de l'atrazina com de la simazina [Gammon *et al.*, 2001], segueix una tendència diferent amb una capa profunda més contaminada. S'ha de destacar que, sorprenentment, la desetilatrazina que és també un producte de degradació de l'atrazina mostra un comportament més similar als dels altres compostos.

De la mateixa manera que pels lípids, també s'ha aplicat els factors d'enriquiment (EFs) per a avaluar millor el comportament d'aquests compostos. A la Taula 7, es presenten els EFs de diferents contaminants i traçadors moleculars en presència i absència de falca salina.

Per als compostos presentats (la desisopropilatrazina no s'ha inclòs al no haver estat detectada en prou campanyes) existeix en tots els casos un enriquiment de la capa superior ( $EF > 1$ ) quan la falca salina està formada, en canvi quan tenim un perfil d'aigua dolça no s'observen aquestes diferències ( $EF = 1$ ).

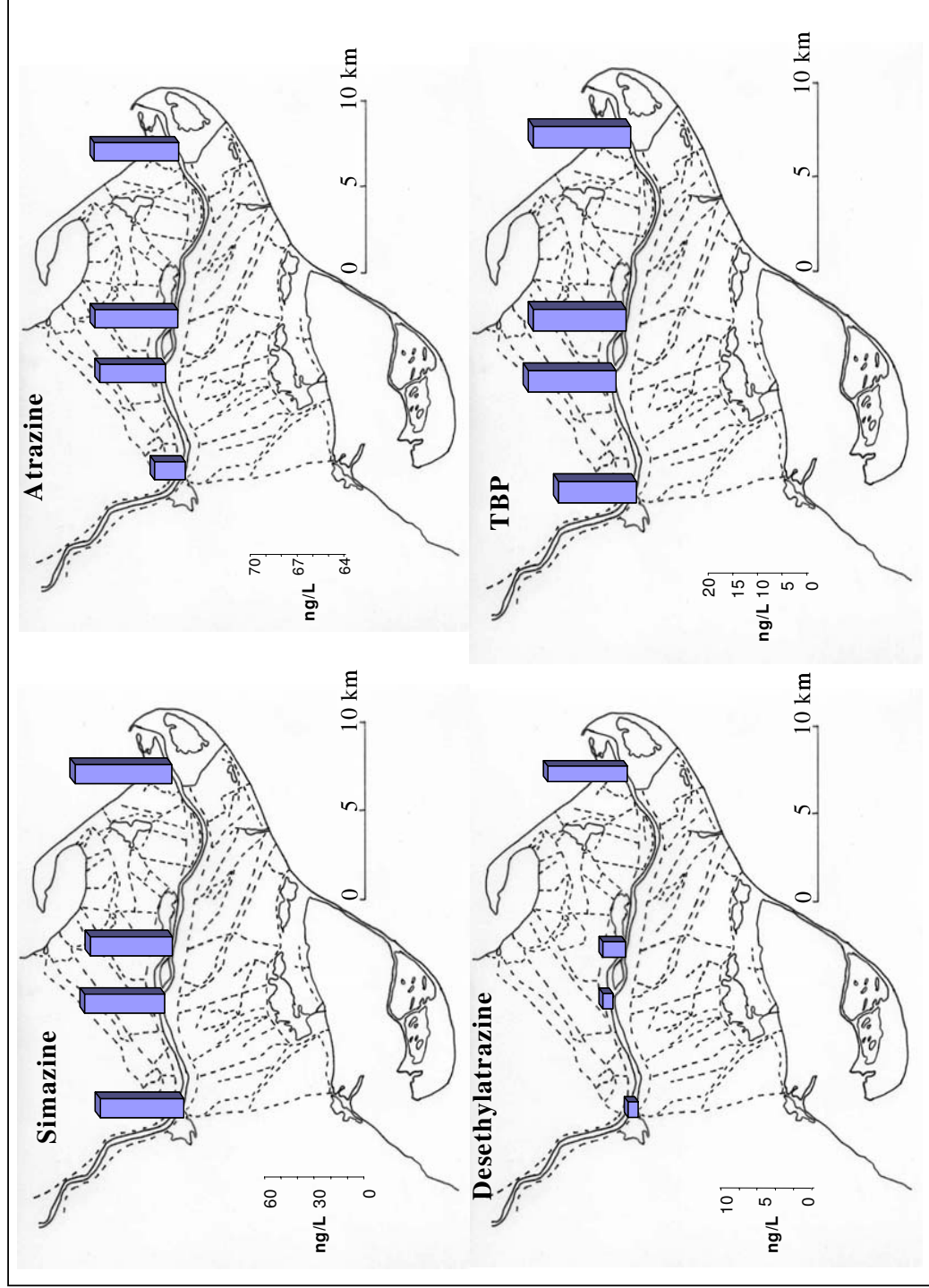


**Taula 7.** Factors d'enriquiment (EF) de diferents contaminants i traçadors moleculars en presència i absència de falca salina.

EF	Molinate			TBP			Trischloroethylphosphate		
	min	max	median des. n	min	max	median des. n	min	max	median des. n
SaltW	0.7	8.6	2.9 2.6 8	1.5	4.4	1.9 1.0 8	1.5	3.9	1.8 0.8 8
NoSaltW	0.6	1.4	1.0 0.2 11	0.9	2.8	1.1 0.7 11	0.7	1.3	1.0 0.2 11
	Simazine			Atrazine			Desethylatrazine		
	min	max	median des. n	min	max	median des. n	min	max	median des. n
SaltW	1.3	4.5	2.8 1.2 8	1.8	11.7	4.6 3.0 8	2.6	22.4	6.0 8.0 5
NoSaltW	0.9	1.7	1.1 0.2 11	0.9	1.9	1.0 0.3 11	0.3	3.7	1.1 1.0 10
	Diazinon			Fenitrothion			Caffeine		
	min	max	median des. n	min	max	median des. n	min	max	median des. n
SaltW	1.6	11.2	4.3 3.4 8	1.2	8.0	1.9 3.3 5	0.3	3.9	2.6 1.1 8
NoSaltW	0.5	1.6	0.9 1.4 11	1.0	2.2	1.1 0.5 9	0.6	5.4	0.9 1.4 11

### *Distribució geogràfica*

Encara que la zona estudiada és relativament petita geogràficament (últims 30 km del riu Ebre), s'han observat diferències significatives en la distribució geogràfica dels contaminants estudiats. En la Figura 16 es mostra el comportament d'alguns dels compostos més característics. S'observa que com per la majoria de contaminants la simazina i el TBP tenen concentracions molt similars en els diferents punts de l'estuari amb en alguns casos un lleuger augment en la zona de la desembocadura. No obstant, per l'atrazina s'observa un important increment aigües avall d'Amposta degut, segurament, a fonts locals d'aquesta zona. Per l'atrazina també s'observa un lleuger increment en la zona de la desembocadura. És interessant destacar que per la desetilatrazina, producte de degradació de l'atrazina, la tendència és molt diferent amb un augment molt marcat a la zona final. Aquest podria provenir tant de la degradació *in situ* de l'atrazina com per l'aportació del producte ja degradat per part de fonts puntuals i/o difuses. El fet de que no s'observi cap davallada del compost original, com la poca distància en la que s'observen aquests canvis de proporcions (anàlit/producte de degradació) fa pensar més aviat en que el compost entra ja sota la forma degradada al riu. En aquest sentit, cal recordar que el delta de l'Ebre és una important zona agrícola (77% de la superfície del delta) amb l'arròs com a cultiu majoritari. És possible que, fins i tot sense abocaments directes, la lixiviació dels contaminants dels camps d'arròs no sigui negligible [Nakano *et al.*, 2004, Southwick *et al.*, 2003]. Sobretot, si es consideren les elevades concentracions de plaguicides presents en els camps de conreu [Barcelo *et al.*, 1991].



**Figura 16.** Distribució geogràfica en el delta de l'Ebre de diferents contaminants orgànics en la capa d'aigua superficial.

### 5.3 Discussió de resultats

Pel que fa a la caracterització dels lípids de la matèria en suspensió de l'estuari del riu Ebre la utilització de la GC×GC-ToF MS ha demostrat ser una tècnica amb un gran potencial. En aquest sentit, s'han identificat 115 traçadors moleculars potencials incloent FAs (saturats, mono-insaturats, poli-insaturats, ramificats i ciclopropanoics), FALs (saturats, mono-insaturats, ramificats i isoprenoides) i esterols (amb diferent grau d'insaturació i de ramificació). D'aquests traçadors potencials, alguns d'ells ja han estat utilitzats per a determinar l'origen de la matèria orgànica, per a caracteritzar el ecosistema, com a traçador de les aigües residuals urbanes i per a realitzar un seguiment dels processos biogeoquímics. Els altres compostos, de moment no poden ser considerats com a traçadors moleculars, tot i que no es pot descartar la seva aplicació en un futur proper. No obstant això, cal destacar que per a efectuar estudis ambientals que incloguin, com en el nostre cas, un gran número de mostres, es continua preferint emprar la tècnica de GC-MS en comptes de la GC×GC-ToF MS. En aquest sentit, el cost i el volum de feina necessaris per a realitzar el tractament de dades tenen sovint un efecte dissuasiu. De totes maneres, s'ha de preveure que de la mateixa manera que el GC-MS ha esdevingut l'eina més emprada en estudis de Geoquímica Orgànica reemplaçant el GC-FID, que fins fa pocs anys era l'eina universal, la GC×GC-ToF MS també té el potencial d'ocupar el lloc de la GC-MS.

Els resultats obtinguts mostren una elevada estacionalitat tant en la composició lipídica de la matèria en suspensió com en el contingut en contaminants orgànics a l'estuari del riu Ebre. Aquesta variabilitat ve donada per la suma de diferents factors. En primer lloc, pel que fa als lípids, s'ha de considerar la variabilitat natural del riu, amb canvis substancials al llarg de l'any de la temperatura de l'aigua i la intensitat i durada de la insolació. Aquests paràmetres són importants a l'hora de regular els processos biogeoquímics que tenen lloc a l'estuari, com per exemple en el cas que hem presentat a la secció 5.1.1 de la foto-oxidació del fitol o també en el creixement biològic de certes espècies. En aquest punt, cal destacar la importància de les variacions de cabal en aquests processos ja que provoquen l'absència o presència del fenomen de la falca salina i impliquen un menor o major grau de dilució dels abocaments antropogènics que serà un factor determinant per les concentracions de contaminants orgànics a l'aigua. Però no es pot descuidar, tampoc, una estacionalitat "antropogènica", principalment marcada per l'agricultura que té com a conseqüència l'entrada al medi aquàtic de sòl i



material vegetal que influenciaran la composició lipídica de les aigües però sobretot, de compostos fitosanitaris, com a conseqüència de l'estacionalitat de la seva aplicació.

A aquesta variabilitat temporal s'hi afegeix una variabilitat geogràfica que encara que el tram de riu estudiat és relativament curt (30 km) no és negligible. Les diferències observades en les aigües superficials s'expliquen majoritàriament per aport puntuals o difusos en el tram de riu estudiat, sent menys importants els processos de transformació que poden donar-se en aquest tram. Cal destacar l'especial interès que presenta l'últim punt de mostreig, situat a proximitat de la desembocadura del riu, per a avaluar l'aport de contaminants i matèria en suspensió al mar. Gràcies a la localització d'aquest punt i a un mostratge periòdic que integra la variabilitat estacional es pot aproximar els fluxos d'emissió del riu Ebre en una base anyal i comparar-los amb d'altres rius. S'ha mostrat, en aquest sentit, que degut a un cabal inferior i a menors concentracions de contaminants l'aport del riu Ebre al Mediterrani és molt més petit que el del Roine que és el riu més important d'aquesta zona. Ens cal afegir que aquest fet no és sorprenent si es considera la pressió demogràfica que suporta la conca del Roine i de les nombroses activitats industrials que s'hi desenvolupen.

Si es considera la distribució vertical dels compostos d'interès, s'han observat grans diferències depenent de la presència o absència de la falca salina. En el cas de tenir un perfil estratificat de la salinitat, també s'han observat perfils estratificats tant pels compostos lipídics com pels contaminants orgànics. En canvi, si la falca salina no estava desenvolupada els perfils eren molt més homogenis. D'altra banda, encara que alguns autors han observat enriquiments en l'interfase en estuaris estratificats [Zutic i Legovic, 1987], en el nostre cas no s'han detectat diferències clares sent l'interfase tan sols un punt intermediari entre l'aigua dolça superficial i l'aigua profunda salada. Pel que fa als *EFs* calculats pels diferents compostos s'han observat importants diferències depenent dels compostos. Aquestes divergències es poden explicar o bé per una diferència de comportament dels compostos d'interès a la columna d'aigua o per un origen diferent. Així per exemple un compost d'origen marí es trobarà en concentracions més elevades a l'aigua salada presentant  $EFs < 1$  mentre que un compost d'origen terrestre mostrarà concentracions superiors a l'aigua superficial ( $EFs > 1$ ). Per a avaluar la importància de les diferències de comportament en el transport vertical s'haurà, en un pròxim treball, d'aplicar els coneixements teòrics desenvolupats amb l'experiment del microcosmos (secció 5.2.1) a l'estuari del riu Ebre intentant

correlacionar el comportament dels compostos d'interès amb les seves propietats fisicoquímiques.

#### **5.4 Conclusions**

En aquest capítol s'ha realitzat un estudi de cas aplicant els coneixements desenvolupats en les altres seccions a l'estuari del riu Ebre. En aquest sentit, s'han assolit els objectius següents:

- S'ha aplicat amb èxit la GC×GC-ToF MS a la caracterització lipídica de matèria en suspensió del estuari del riu Ebre, minimitzant el pretractament de la mostra.
  - S'han identificat 115 traçadors moleculars lipídics potencials.
  - S'ha identificat l'origen de la matèria orgànica en la falca salina trobant en l'aigua superficial un origen terrestre i marí per l'aigua profunda.
  - S'han caracteritzat les comunitats planctòniques presents identificant un aigua superficial més rica en fitoplàncton i més específicament en dinoflagelats. En canvi l'aigua profunda és més rica en plàncton sent predominant el zooplàncton i en el cas del fitoplàncton les diatomees.
  - S'han identificat processos biogeoquímics de foto-oxidació en l'aigua superficial utilitzant productes de degradació del fitol per a realitzar-ne el seguiment.
  - S'ha estudiat la influència antropogènica utilitzant esterols d'origen fecal.
- S'ha estudiat la variació temporal a l'estuari del riu Ebre dels compostos lipídics i dels contaminants orgànics d'interès trobant una gran variabilitat. Aquestes fluctuacions estarien associades a una estacionalitat natural i a una estacionalitat "antropogènica".
- S'han calculat els fluxos de matèria en suspensió i de contaminants orgànics al mar Mediterrani.
- S'ha avaluat la distribució geogràfica dels compostos d'interès atribuint les variacions observades a aportacions puntuals o difuses.
- S'ha estudiat la influència de la falca salina en el comportament dels compostos d'interès.
  - S'ha realitzat un experiment en un microcosmos avaluant el transport vertical i l'eliminació de contaminants orgànics en un sistema estratificat.

- El transport vertical està correlacionat amb la difusió que depèn del volum molar de les substàncies.
- El principal procés d'eliminació identificat és l'evaporació.
- S'han observat importants diferències entre els *EFs* dels diferents compostos d'interès que encara no s'han pogut explicar de manera detallada.