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Distribució i comportament de contaminants orgànics prioritaris a la conca hidrogràfica del riu Ebre

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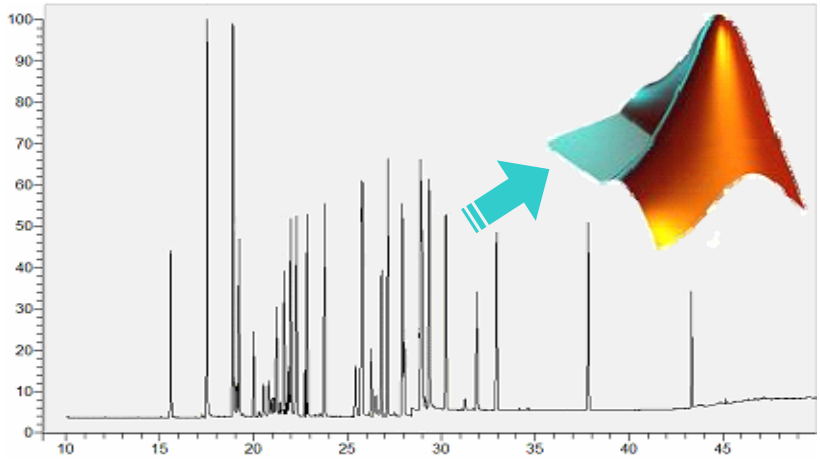
CAPÍTOL 4:

Avaluació de la contaminació de la conca de l'Ebre



4.1.- La quimiometria com a eina

Un cop obtinguts els valors analítics de qualsevol estudi cal fer-ne una bona interpretació. Tradicionalment aquesta s'ha fet utilitzant l'estadística univariable (màxim, mínim, mitjana, mediana, desviació estàndard...), que resulta molt útil per a una primera visió de les dades. Però quan el nombre de resultats és elevat, com en el cas d'extenses campanyes de presa de mostra, l'anàlisi es torna més complicada i laboriosa ja que es tracta d'una gran quantitat de valors difícils de comparar entre si. En aquests casos existeix la possibilitat de realitzar un tipus de tractament matemàtic multivariable, aplicant tècniques quimiomètriques.

La quimiometria és una eina extremadament útil i pràctica per a extreure el màxim d'informació d'una extensa sèrie de valors, ja que analitza simultàniament totes les variables enlloc de fer-ho de forma individual. La finalitat de les tècniques multivariables és el càlcul i representació gràfica de les tendències d'agrupament més importants que es presenten als resultats, buscant i identificant les possibles fonts de variació (en el nostre cas assimilables a les fonts de contaminació) i permetent deduir-ne la seva distribució geogràfica i temporal *(Peré-Trepat et al., 2007)*. La quimiometria engloba un conjunt de mètodes de complexitat variable entre els que destaca el PCA, que va ser un dels primers mètodes quimiomètrics aplicats a l'anàlisi de dades ambientals i és de fàcil enteniment i aplicació. Per altra banda, el PCA és habitualment el primer que s'utilitza per a l'anàlisi de dades ja que dóna una visió general dels resultats reduint considerablement les variables inicials i facilitant l'extracció d'informació útil *(Spate et al., 2006)*. Posteriorment es poden aplicar altres mètodes més complexes per a obtenir altra informació que no s'hagi reflectit en el PCA. En aquesta tesi es pretenia utilitzar la quimiometria com a eina per a millorar la interpretació de la informació obtinguda i complementar així l'aplicació de les tècniques clàssiques d'estadística univariable, i per això es va aplicar el PCA tal i com reflecteixen els articles científics 4, 5, 6 i 7.

Per a l'aplicació d'eines estadístiques de qualsevol naturalesa cal organitzar els valors en taules, també anomenades matrius. La creació de les matrius de dades i la seva anàlisi estadística univariable es va realitzar amb les funcions apropiades del full de càlcul Excel (Microsoft Corporation, *USA)*. Un cop realitzada aquesta anàlisi, les matrius es van exportar a l'entorn de programació, anàlisi numèrica i visualització MATLAB (The Mathworks, *Natick,*

USA). En aquest entorn es va utilitzar la PLS Toolbox v2.1 (Eigenvector Research, *Manson, USA*), actualment una de les eines quimiomètriques més desenvolupada i emprada.

4.1.1.- Diagrama de caixes i correlacions binàries

Per a conèixer millor les dades abans de realitzar el PCA és recomanable, a part de realitzar l'estadística descriptiva clàssica, investigar els diagrames de caixes. Aquesta eina estadística de representació gràfica de dades és molt útil i interessant i s'ha emprat força en aquest treball. Aquests diagrames són la representació semigràfica d'una distribució que permeten mostrar les seves característiques principals i senyalar els possibles valors atípics, és a dir, aquells valors que sobresurten de la resta (Figura 4.1). Per a cada variable es genera una caixa, on la línia horitzontal que la divideix mostra la localització de la mediana per a aquesta variable. El valor superior i inferior de la caixa indiquen la localització dels percentils al 25 i 75%, és a dir, dins de la caixa hi ha el 50% central de les mostres. Les línies exteriors a la caixa indiquen els límits admissibles superior i inferior (*whiskers*) *(Peña Sánchez de Rivera, 1993)*. El límit admissible superior assenyala el valor més gran dins de l'interval d'1,5 vegades el rang interquartil comptat a partir del percentil del 75% i el límit admissible inferior assenyala el valor més petit dins de l'interval d'1,5 vegades el rang interquartil comptat a partir del percentil del 25%. Els punts o valors per fora d'aquest rang es mostren com a creus (+) i són considerats valors atípics.

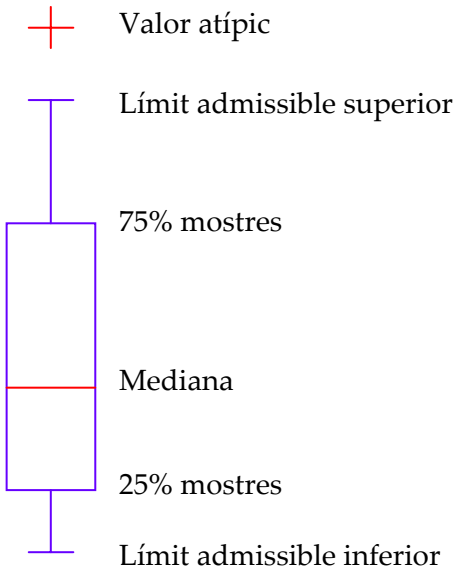


Figura 4.1: Esquema d'un diagrama de caixes

Com a pas preliminar de qualsevol estudi estadístic de matrius de dades ambientals també és convenient investigar les anomenades correlacions binàries entre totes les variables, per tal de conèixer les interrelacions entre elles. Això s'aconsegueix simplement a partir del càlcul dels coeficients de correlació entre totes les parelles de variables obtingudes a partir dels resultats experimentals. D'aquesta manera es poden trobar associacions entre les diverses variables.

4.1.2.- Processament preliminar de les matrius de dades ambientals

Des d'un punt de vista matemàtic, la taula de dades ambiental és una matriu bidimensional que conté les concentracions dels diferents compostos químics o altres paràmetres mesurats (variables, a les columnes de la taula) en les diferents mostres analitzades (mostres, a les files de la taula) per a un període de temps determinat. Quan diverses matrius de dades ambientals corresponents a períodes de temps diferents i amb informació correlacionada s'analitzen simultàniament, s'obté una nova estructura de dades tridimensional, un cub. Aquestes matrius es poden analitzar de forma individual o mitjançant la matriu augmentada *(Kiers, 1991; Massart et al., 1998)*. L'augmentació de matrius és un procés que transforma aquests conjunts de dades tridimensionals en una matriu bidimensional resultat d'apilar les diverses matrius individuals *(Tauler et al., 2008)*, que per tant han de tenir el mateix nombre de columnes, per a tractar-la com una única matriu *(Pardo et al., 2004)* (Figura 4.2).

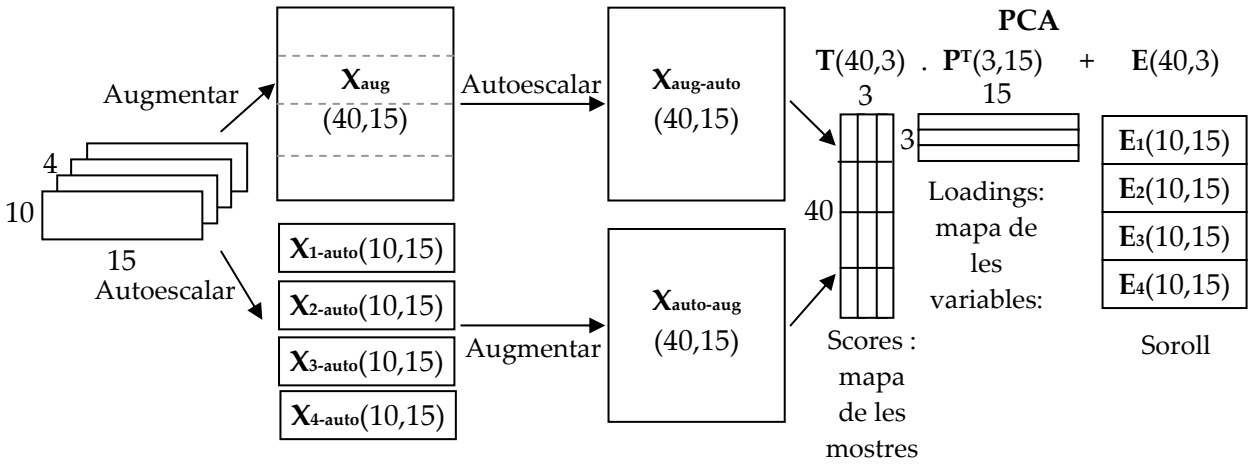


Figura 4.2: Esquema d'aplicació de l'autoescalat, l'augmentació i el PCA a les matrius de dades (exemple per a 3 PCs)

Per a l'aplicació del PCA és imprescindible que no hi hagi cap buit en les matriu de dades, és a dir, hi ha d'haver un valor per a cadascuna de les variables per a totes les mostres. Bàsicament es poden donar dues situacions:

- **Concentracions per sota del LD:** ja que és imprescindible treballar amb una matriu completa el millor és considerar els valors obtinguts directament a partir de les mesures instrumentals, encara que químicament no es puguin considerar. Quan no

es disposa d'un número real, el més habitual és considerar la meitat del LD de la variable en qüestió *(Farnham et al., 2002)*.

- **Dades no existents:** quan el nombre de buits a la matriu és petit i aquests es troben repartits de forma aleatòria es pot fer una estimació numèrica tot tenint en compte les variacions observades a la resta de variables i mostres. Utilitzant la funció adequada de la PLS Toolbox s'obtenen valors automàticament com a resultat de certes operacions matemàtiques. Un cop finalitzat el procés cal comprovar que els valors estimats tinguin sentit des d'un punt de vista químic, és a dir que els valors predits no siguin negatius o que la seva magnitud no surti del rang esperat *(Wise et al., 2004)*.

4.1.3.- Mètodes de pretractament de dades

Generalment, per extreure quimiomètricament de forma adequada la informació ambiental a partir dels resultats experimentals és necessària l'aplicació de tècniques de pretractament de dades. Aquest procés és especialment necessari quan les diverses variables d'una matriu tenen magnituds diferents que les fa incomparables, de forma que cal aplicar un pretractament que eviti que una sola variable domini a la resta. No existeix un únic mètode de pretractament de dades que serveixi per a tots i cadascun dels diversos tipus possibles de dades i problemes ambientals. Els pretractaments que s'han aplicat en aquest estudi són:

- **Transformació logarítmica:** transforma en logaritme els valors de la matriu de dades. Permet augmentar el pes dels valors baixos i disminuir el dels valors alts.
- **Centrat:** es resta a cada valor original la mitjana de la variable (columna de la matriu). Realitza un trasllat de l'origen que fa més fàcil visualitzar les variacions i diferències respecte a la mitjana.
- **Escalat:** es divideix cada valor original per la desviació estàndard de la variable (columna de la matriu). D'aquesta manera s'iguala la importància relativa de cada variable.

- **Autoescalat:** és l'aplicació simultània del centrat i l'escalat, restant a cada valor la mitjana de la variable i dividint-lo per la desviació estàndard. S'obté per tant un trasllat de l'origen a més a més d'igualar la importància de cada variable.

Qualsevol d'aquests pretractaments es pot aplicar a les dades abans o després del procés d'augment de matrius. Si primer es crea la matriu augmentada i després s'autoescala conjuntament, els resultats del PCA reflectiran més les variacions temporals, mentre que si les matrius individuals corresponents a cada campanya s'autoescalen per separat i després es crea la matriu augmentada, s'emfatitzaran les variacions geogràfiques (Figura 4.2).

4.1.4.- Anàlisi de components principals

El PCA *(Jolliffe, 1986; Wold et al., 1987)* és una tècnica matemàtica que permet simplificar i reduir el nombre de variables d'un sistema de dades conservant la màxima informació possible i les relacions existents entre els valors inicials *(Ying, 2005; Rigol et al., 2008)*. Aquest procés té lloc per la combinació lineal de les variables originals *(Tauler et al., 2000)* i genera un nombre més reduït de noves variables ortogonals no correlacionades entre si que s'anomenen components principals (PCs, de l'anglès *principal component*) *(Farnham et al., 2002)*. Degut a la manera com s'obtenen els PCs, la informació rellevant del conjunt de dades es troba concentrada en els primers i de forma decreixent, és a dir, el primer PC explica més variància que el segon i així successivament. La finalitat del PCA és precisament trobar aquest nou conjunt d'eixos ortogonals de coordenades (els PCs) sobre els que es poden projectar les dades originals.

Matemàticament aquest procés, en el que es treballa amb matrius de dades bidimensionals, es pot expressar amb la següent equació:

$$\mathbf{X = T \cdot P^T + E}$$

on X és la matriu de dades originals, que es descompon en el producte de la matriu T, anomenada d'*scores*, que descriu la composició de les mostres segons les noves variables i de la matriu P^T, anomenada de *loadings*, que descriu la contribució de les variables originals a cada nova variable o PC. Per altra banda E és la matriu de valors residuals (Figura 4.2).

Un cop descomposta la matriu X, cal triar quina és la quantitat de PCs òptima per a l'anàlisi, arribant a un compromís entre la variància total explicada i la senzillesa de l'estudi. Com més alt és el nombre de PCs més variància total explicaran però més difícil serà la seva interpretació. Generalment es considera com a últim PC vàlid aquell que explica més variància que la que podria explicar una de les variables originals. De totes maneres és molt comú examinar el total de PCs perquè en algun dels descartats és possible que es doni un resultat interessant. Cadascun dels PCs s'identifica amb una possible font de contaminació, ja que és una agrupació de variables que segueix el mateix patró en un conjunt de les mostres, que normalment no és deduïble de forma immediata a partir de les dades originals.

L'estudi de les mostres es fa construint un gràfic en el que els PCs són els nous eixos (2 ó 3 dimensions) i s'hi representa la matriu d'*scores*, que són la redefinició de les mostres originals segons les noves variables. Aquesta representació il·lustra els patrons dominants existents entre les mostres *(Farnham et al., 2002)* i permet localitzar aquelles que es comporten de forma semblant o diferent a les altres.

4.2.- Anàlisi de les dades històriques

Prèviament a la investigació sobre l'estat actual de la conca de l'Ebre (que en aquesta tesi es considera com l'estudi portat a terme dins del projecte AquaTerra durant els anys 2004-2006) es van fer dos estudis preliminars: per una banda es va portar a terme la campanya de presa de mostra de la RCSP corresponent a l'any 2003 per a la CHE i per altra es va fer una anàlisi dels resultats recopilats de la base de dades de la RCSP des de la seva creació fins la campanya que vam realitzar durant el 2003.

Pel que fa al primer estudi es va participar en la presa de mostra de sediments i peixos a la tardor de 2003 i la seva posterior anàlisi al laboratori. La realització d'aquesta campanya va suposar un primer contacte amb la presa de mostra, les metodologies analítiques de les dues matrius sòlides agafades així com el processament de les dades obtingudes. Un dels passos importants en el pretractament d'aquestes mostres va ser l'obtenció d'una mostra homogènia i representativa. Aquest no és un procés trivial, especialment en el cas dels peixos. Quan es tracta de grans exemplars es fa difícil triturar-los fins al punt desitjat i calen eines específiques de les

que la major part de laboratoris no disposen. Un altre aspecte a tenir en compte és que ambdues matrius, però també especialment els peixos, són molt complexes, fet que implica l'extracció de molts components intrínsecs a la matriu i que poden interferir en l'anàlisi dels compostos d'interès. En aquest estudi, recollit en l'article científic 3, van participar diferents experts en l'anàlisi de famílies de compostos específiques, com ara els TBTs, mentre que la part d'interès per a aquesta tesi es centra en els compostos organoclorats, els APs i els PAHs, objecte d'estudi de la resta de campanyes de mostra realitzades a la conca de l'Ebre.

El segon estudi, corresponent a les dades històriques de la RCSP en sediments, va suposar l'anàlisi d'una gran quantitat de valors, dels que era difícil extreure conclusions amb l'aplicació de l'estadística univariable. De forma que es va optar per fer un estudi quimiomètric d'aquestes dades i d'aquesta manera simplificar el contingut i fer-ne més fàcil la seva interpretació. Inicialment es va considerar l'anàlisi de les matrius d'aigua i sediments per a obtenir un estudi més complert de la conca, però en recopilar els resultats d'aigua existents es va veure que per a aquesta matriu únicament existien valors positius per als paràmetres fisicoquímics i no per contaminants orgànics. De forma que es va optar per fer només l'anàlisi de les dades de sediments. A mesura que es va avançar en aquest estudi es va observar que la informació era molt incompleta, tant pel que fa a compostos com a punts de presa de mostra. Això es deu a que des del 1992, any en que es va crear la xarxa, es van anar afegint punts i compostos a poc a poc. Per altra banda només s'analitzen tots els compostos de la xarxa a 9 dels 18 punts actuals, mentre que en els altres 9 únicament es considera la meitat dels compostos. Amb totes aquestes consideracions, i tenint en compte que per a fer un estudi quimiomètric cal que les matrius corresponents a cada any siguin iguals i completes, finalment es va reduir la matriu de sediments a 12 dels 30 compostos inicials a 9 dels 18 punts i per als anys de 1996 al 2003 amb l'excepció del 1999 per al que no existien dades. Tot i això la informació que es va obtenir d'aquest estudi és vàlida per a una primera aproximació de l'estat general de la conca de l'Ebre i es presenta a l'article científic 4.

Article científic 3

Pilot survey of a broad range of priority pollutants in sediment and fish from the Ebro river basin (NE Spain)

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Pilot survey of a broad range of priority pollutants in sediment and fish from the Ebro river basin (NE Spain)

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Organic pollutants were monitored in sediments and fish from the Ebro river basin (NE Spain).

Abstract

Priority organic pollutants were investigated in sediments and fish collected along the Ebro river basin (NE Spain) to evaluate their occurrence, transport and bioavailability. Sediments were collected in 18 sites and two species of fish were captured in nine sites according to the availability in each area. The sampling sites covered industrial, urban and agricultural areas. Four methods were used to detect 20 organochlorine compounds (OCs), 8 polycyclic aromatic hydrocarbons, 3 organotin compounds, 2 alkylphenols and 40 polybrominated diphenyl ethers (PBDEs) from purified extracts. The contamination pattern was site specific and no downstream increase in concentration of pollutants was observed but rather a generalized low level diffuse pollution. Target compounds were detected in sediments at 0.01 to 2331 μg/kg dry weight, and only OCs and PBDEs were accumulated in benthopelagic fish. Toxicological assessment was performed according to predicted environmental levels and revealed sites where adverse effects could occur.

Keywords: Monitoring; Priority compounds; Sediment; Fish; Transport; Bioavailability

1. Introduction

River pollution caused by the direct and indirect discharge of urban and industrial wastes and run-off has lead to the accumulation of toxic compounds such as pesticides (Zhou et al., 2001), surfactants (Ying et al., 2002), halogenated aromatics (Oberg, 2004) and hydrocarbons (Yunker et al., 1996) onto river sediments, being then a source to biota (Crane, 2003) and a risk for the environment (Carvalho et al., 2002). The discharge and presence of such compounds in the aquatic ecosystem has aroused questions on the risk they might cause to freshwater species and ecosystem as a whole. In Spain, there is evidence that the concentration of organic pollutants in the environment is enough to produce deleterious effects towards aquatic species (Fernandes et al., 2002). According to specific pollution episodes due to organochlorinated compounds (Fernández et al., 1999) or polybrominated diphenyl ethers (PBDEs) (Eljarrat et al., 2004), interest is now given to survey compounds with known toxicity in water, sediments or biota to evaluate their impact throughout the Ebro river basin. To date, most studies performed within the Ebro river have been focused on the monitoring of organochlorinated compounds (Fernández et al., 1999; López-Martín et al., 1995) or site specific studies involving a single chemical family but little is known about concentration and patterns of a wide spectrum of priority contaminants (DDTs, chlorobenzenes (CBz), organotins (OTs), alkylphenols (APs), and PBDEs in the Ebro aquatic ecosystem. The present pilot study employs river sediments and fish of two species which could vary depending on their availability in each area to evaluate the occurrence, transport, availability and toxic impact of a large number of

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organic compounds throughout the largest river basin in northeast Spain. The Ebro river basin is dominated by industrial activities concentrated close to the cities of Zaragoza, Vitoria, Pamplona, Logroño, Huesca and Lérida (Fig. 1), which to a greater or lesser extend have caused the release of organic compounds to the basin due to mining activities in the northern part of the river, historical Hg pollution coming from chloro-alkali industry, production and utilization of solvents and chlorinated pesticides, and finally, usage of flame retardants in car and electrical plants in the middle-lower course. On the other side, agriculture is spread in the area, with vines, fruit trees and maize.

This work was carried out using guidelines set by European Directives such as 76/464/CEE concerning the analysis of 132 toxic and persistent compounds in environmental matrices (Directive 76/464/CEE, 1976), the Water Framework Directive (Directive 60/2000/EC, 2000) aimed at improving the ecological quality of surface waters and Directive 2001/42/EC aimed at protecting the environment from toxic compounds (Directive 2001/42/EC, 2001). According to these Directives and to resolve the problem of sediment contamination and availability of hazardous compounds to biota, the present work contributes to the evaluation of the vulnerability of the Ebro river basin which has been impacted by multiple human activities.

2. Materials and methods

2.1. Site selection and sampling

The Ebro catchment (NE Spain) covers an area of 85,362 km^2 and is the largest river basin in Spain. The Ebro river, of 910 km, receives waters from several tributaries, which altogether represent 12,000 km of waterway network. The monitoring carried out in autumn 2003 included 18 sampling points covering the whole Ebro river basin (6 at the Ebro river and 12 at main tributaries) and fish of two species from the nine most vulnerable sites according to proximity to big cities or industries and knowledge on historical contamination episodes (www.che.es). Their specific location is shown in Fig. 1, and are numbered from 1 to 18 from north to south and "R" indicates a site on the Ebro River whereas "T" indicates a tributary site. Table 1 lists the locations of each sampling point, the corresponding river, its flow and flow contribution to the Ebro river, the conductivity, pH and dissolved oxygen. Among the 81 dams built throughout the Ebro basin, six reservoirs of a capacity between 2.5 and 1528 cubic hectometres located before or between two sampling stations, acted as physical barriers, retaining sediments and locking up fish within one emplacement (Fig. 1).

Surface sediments (0–2 cm) were collected from the middle river bed using a Van Veen drag. In sites with high flow, the middle bed mesh size was not adequate and samples were collected from the shore. Fine grain and clay (Table 2) was collected, although in R4, R6, T9 and T16, sediments were sandy. In sites R1 and T15, two sediments collected 100 m apart were analysed for comparison purposes. Sediments had a total organic carbon (TOC) from 0.58% to above 6% C (Table 2). Only T3 (Victoria) contained a high TOC (19.03% C) and reflects the sampled area, which differing from other sites, consists of a very small river receiving the direct input of industrial and urban waters.

The two fish species used as bioindicators in each station were selected by the Confederación Hidrográfica del Ebro (CHE) (Durán et al., 2001). Since the distribution of fish vary along the basin it was difficult to collect always the same species. Table 2 reflects the fish species sampled in each site. Fish captured were principally barbel (*Barbus graellsi*) and common carp (*Cyprinus carpio*), although when these species were not available, other species were collected and the order of preference was: red roach (*Rutilus arcasii*), toxostome (*Chondrostoma toxostoma*), bleak (*Alburnus alburnus*), goldfish (*Carassius auratus*) and rudd (*Scardinius erythrophtalmus*). In general, the second species captured was more variable according to the availability of fish in each area. All fish were benthopelagic and omnivorous. Adult fish were collected with electrical fishing. Fish were killed, weighed, and the body length was measured.

Samples were placed in glass containers and were stored in portable fridges and at the end of the day were sent to the laboratory by urgent courier. Upon receipt, samples were immediately processed to prevent degradation of target compounds.

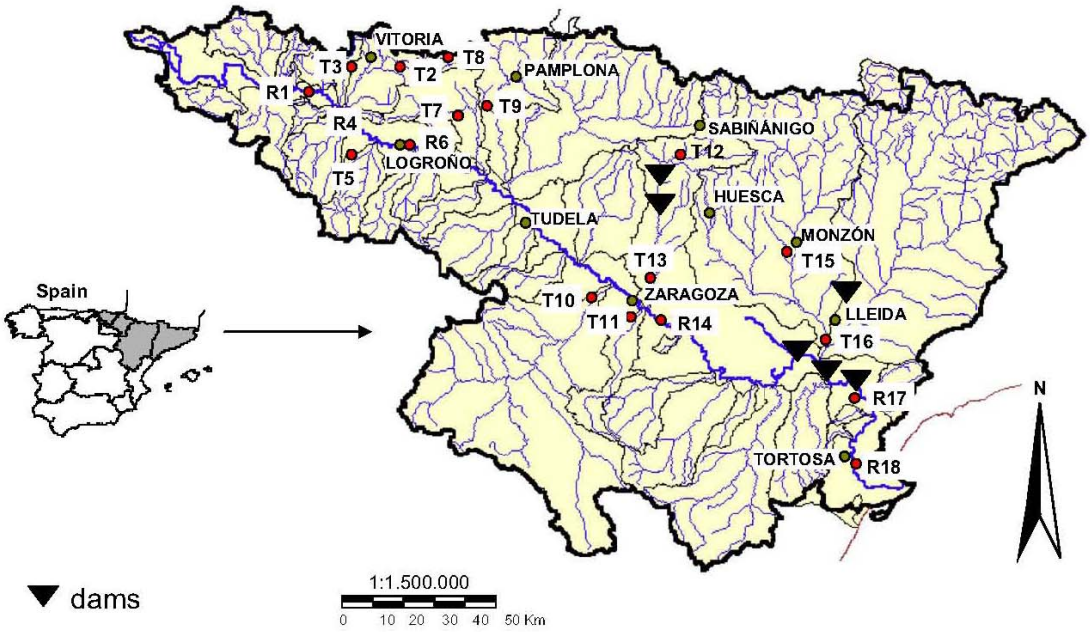

Fig. 1. Map of the Ebro river basin with sampling points.

Table 1
Description of the river basin sampling sites included in the study

Site	Sampling name	Basin	Sector	Surface (km^2)	Mean flow (m^3/s)	Influent to Ebro (cubic hectometres, %)	Conductivity (μS) at 20 °C	pH	O_2 (mg/L)
R1	Miranda de Ebro	Ebro	I	322	147.0		490	5.5	8.0
T2	Salvatierra	Zadorra	U, I	61	15.1	N.A.	620	7.6	8.0
T3	Gasteiz Trespuentes	Zadorra	U, I	734	145.6	592 (3.3%)	540	5.2	7.7
R4	Conchas de Haro	Ebro	I	7979	790.8		580	9.0	7.7
T5	Nájera	Najerilla	I	883	95.9	399 (2.2%)	460	8.5	7.6
R6	Logroño-Varea	Ebro	U, A, I	12470	984.3		600	7.0	8.0
T7	Ega a Arinzano	Ega	I	1100	167.8	492 (2.7%)	1030	8.5	7.9
T8	Alsasua-Urdiaín	Araquil	I	839	412.9	N.A.	340	9.2	8.2
T9	Puente la Reina	Arga	I, A	1349	356.4	1697 (9.3%)	450	8.1	8.2
T10	Grisén	Jalón	A	9844	61.9	551 (3.0%)	1550	8.1	7.5
T11	Fuente de la Junquera	Huerva	U, A	1022	17.2	N.A.	3410	0.5	7.7
T12	Jabarrella	Gállego	A	1437	56.0	1087 (6.0%)	310	10.1	8.1
T13	Villanueva	Gállego	A	N.A.	221.0	1087 (6.0%)	2670	6.2	7.8
R14	Presa de Pina	Ebro	U, A, I	46748	1838.7		2000	6.6	7.8
T15	Monzón	Cinca	I	4406.1	540.1	2915 (16%)	810	8.6	8.2
T16	Torres de Segre	Segre	A	12493.6	465.4	3441 (18.9%)	650	8.5	8.2
R17	Flix	Ebro	I	82914	1903.7		980	9.9	8.1
R18	Tortosa	Ebro	U, A, I	84718	1914.4		1000	8.3	8.3

N.A., not available; A, agricultural; I, industrial, U, urban.

2.2. Extraction and purification of sediments

Sediments were freeze dried for 48 h at 10^{-2} mbar vacuum then sieved through 500-μm and 120-μm sieves. One gram of the <120 μm fraction was spiked with surrogate standards (see below) and extracted by sonication (10 min) using 30 mL hexane/dichloromethane (1:1 v/v). The extract was centrifuged for 5 min at 2500 rpm. This was repeated twice more. Extracts were evaporated in a rotary evaporator to 0.5 mL and were purified using 5 g neutral alumina solid phase extraction cartridges (Waters, USA), which were pre-conditioned with 20 mL hexane/dichloromethane (2:1 v/v) and

Table 2
Description of sediment samples analysed and Ebro river fish included in the survey

Site	Sediment			Fish			
	Texture	<120 μm	TOC (% C)	Fish species	Length (cm)	Weight (g)	N
R1	Clay	36%	0.93	*Barbus graellsi*	34.5–43	523–1112	3
R1′	Clay and plastic	54%	1.84	*Cyprinus carpio*	34–37.5	748–870	3
T2	Clay	13%	2.62	Not studied			
T3	Clay	27%	19.09	*Barbus graellsi*	10.5–13.3	144–412	3
				Rutilus arcasii	22–31	21–30	3
R4	Clay and slightly sandy	22%	0.70	Not studied			
T5	Clay	18%	3.64	Not studied			
T6	Clay and slightly sandy	16%	1.01	Not studied			
T7	Clay	52%	3.46	Not studied			
T8	Clay	40%	3.94	Not studied			
T9	Sandy	6%	2.96	*Barbus graellsi*	35–44	468–853	3
				Chondrostoma toxostoma	10–13.5	9–25	3
T10	Clay	23%	3.15	Not studied			
T11	Clay	54%	0.58	Not studied			
T12	Clay and plastic	29%	0.71	*Barbus graellsi*	16.5–19.5	61–100	2
				Chondrostoma toxostoma	9–13	7–26	6
T13	Clay and plastic	41%	3.20	Not studied			
R14	Clay	8%	3.17	*Barbus graellsi*	25.5–48	218–1321	3
				Alburnus alburnus	9–11.8	9–16	6
T15	Clay	53%	1.54	*Barbus graellsi*	35–42.5	562–938	3
T15′	Clay	13%	1.13	*Alburnus alburnus*	9–13	13–20	3
T16	Sandy	2%	2.92	*Cyprinus carpio*	20–48	1680–1860	2
				Alburnus alburnus	11–13	11–24	3
R17	Clay	19%	0.82	*Cyprinus carpio*	42	1732	1
				Alburnus alburnus	11–13.5	18–24	3
R18	Clay	17%	5.83	*Carassius aurata*	26–28	432–449	3
				Scardinius erythrophtalmus	20.3–21.5	142–172	2

Species selected in order of preference: *Barbus* ssp. > *Cyprinus carpio* > *Rutilus arcasii* > *Chondrostoma toxostoma* > *Alburnus alburnus* > *Carassius aurata* > *Scardinius erythophtalmus*.

20 mL hexane/dichloromethane (10:1 v/v), and elution was carried out with 40 mL hexane/dichloromethane (10:1 v/v) and 40 mL hexane/dichloromethane (2:1 v/v). Using this combination of solvents, all target compounds eluted. The two fractions were combined, rotary evaporated and reconstituted in 250 μL hexane.

For organotins (OTs), 2 g of the <120 μm fraction was spiked with the surrogate standards tripropyltin chloride (TPrTCl) and tricyclohexyltin chloride (TCyTCl) (as tin) at 200 ng/L, sonicated with toluene/acetic acid (10:4 v/v) for 5 min and centrifuged at 2000 rpm for 3 min. The organic layer was pipetted out. This operation was repeated twice more. The toluene extract was percolated through activated Na_2SO_4 and rotary evaporated to 5 mL. The extract was derivatized with 2 mL of Grignard reagent (*n*-pentylmagnesium bromide 2.0 M in diethyl ether; Aldrich, Milwaukee, WI, USA) as reported elsewhere (Diez et al., 2002). The extract was evaporated to 1 mL and 200 ng/L of tetrabutyltin (TeBT) was added as internal standard.

2.3. Extraction and purification of fish

Composite samples of whole fish ($n = 1–6$) were pooled. Scales were removed from the fish. The whole fish (head to tail, bones included) was cut into pieces of 4–5 cm^2 with an electric knife and homogenized in a blender (Waring, Snijders Scientific, Tilburg, The Netherlands) to obtain a uniform paste, which was lyophilized at 10^{-2} mbar and homogenized again to a fine powder using a mortar and pestle. Zero point four grams of fish were spiked with the surrogate standards (see below), combined with pre-cleaned Hydromatrix (Varian, California, USA) and extracted with hexane/dichloromethane (1:1 v/v) using a Dionex 2000 accelerated solvent extractor (ASE) (Dionex, USA). The system pressure was set at 1500 psi and the temperature at 150 °C with a heat-up time of 5 min. Two cycles of extraction were performed during 10 min in static mode. The purge time was 90 s. The resulting extract was rotary evaporated to 1 mL and 2 mL sulphuric acid and 2 mL of hexane were added. After vigorous stirring in a Vortex-mixer (2 min) the mixture was centrifuged and the sulphuric acid layer was discarded. This clean up step was repeated five times. All hexane extracts were combined and concentrated by vacuum rotary evaporation and finally reconstituted in 250 μL of hexane.

For OTs, 0.5 g of fish was spiked with the surrogate standard at 200 ng/L and 5 mL of tetramethylammonium hydroxide (TMAH, 25% in water) were added. The mixture was stirred magnetically for 4 h at 60 °C, then 1.3 mL of acetic acid, 20 mL of acetate buffer (pH 5, 0.1 M), 1 mL of $NaBEt_4$ (0.6% w/v) and 5 mL of hexane were added and the mixture was sonicated for 5 min. Afterwards, the solution was centrifuged at 3500 rpm for 3 min and the organic layer was recovered. A clean-up step was performed on a neutral alumina column (3 g) with activated anhydrous Na_2SO_4, and elution was performed with hexane. The extract was concentrated to 1 mL.

2.4. Analytical procedures

A total of 73 organics were determined using four analytical protocols. Standards were purchased from Dr Ehrestorfer (Augsburg, Germany). Reagents and analytical grade solvents were from Merck (Darmstadt, Germany).

2.4.1. Method 1

This method permitted determination of the concentration of 20 organochlorine compounds in sediment and fish. The tailor-made pesticide mix standard solution Pesticide Mix 656 containing organochlorine pesticides *op′* DDD, *pp′* DDD, *op′* DDE, *pp′* DDE, *op′* DDT, *pp′* DDT, hexachlorocyclohexanes (α-HCH, β-HCH, γ-HCH, δ-HCH), hexachlorobenzene (HCB), pentachlorobenzene (PCB), aldrin, dieldrin, isodrin and endrin was purchased at 100 μg/mL (Dr Ehrenstorfer, Augsburg, Germany). Hexachlorobutadiene (HCBd), pentachlorophenol, 1,2,4-trichlorobenzene and 1,2,3-trichlorobenzene were from Supelco (Bellefonte, PA, USA). A working solution containing all these compounds was prepared at 10 μg/mL. The surrogate standard used for this mixture was decachlorobiphenyl. This mixture was analysed by gas chromatography (GC) (Carlo Erba GC 8000) coupled to a quadrupole mass spectrometer (Fisons MD 800) operated in electron ionization mode at 70 eV with temperature programme: 60 °C (1 min) to 175 °C (4 min) at 6 °C/min, to 235 °C at 3 °C/min and to 300 °C (5 min) at 8 °C/min. Injection was performed in the splitless mode (split purge valve closed for 48 s). Helium was the carrier gas (50 cm/s). Injector, transfer line and ion source temperatures were set at 280 °C, 250 °C and 200 °C, respectively.

2.4.2. Method 2

This method permitted determination of the concentration of eight PAHs and two APs in sediment. PAHs were not analysed in fish since they are metabolized rapidly and excreted (Broman et al., 1990), and they are not considered an accurate indicator of PAH exposure (Verweij et al., 2004). Naphthalene, anthracene, fluoranthene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*g,h,i*]perylene, benzo[*k*]fluoranthene and indene[1,2,3-*cd*]pyrene were purchased at 1000 μg/L in methanol (Supelco). Nonylphenol (NP) and octylphenol (OP) were from Aldrich. The surrogate standard contained naphthalene d-8, acenaphthene d-10, phenanthrene d-10, chrysene d-12 and perylene d-12 at 2000 μg/L in dichloromethane (Supelco). This mixture was analysed using the same equipment and conditions as in method 1, according to a previous described protocol (Martínez et al., 2004).

2.4.3. Method 3

This method permitted determination of the concentration of 40 PBDEs. The PBDE Solution EO-4980 was from Cambridge Isotope Laboratories Inc. (MA, USA) and contained 3 monoBDEs (BDE # 1, 2 and 3, following IUPAC nomenclature), 7 diBDEs (BDE # 7, 8, 10, 11, 12, 13 and 15), 8 triBDEs (BDE # 17, 25, 28, 30, 32, 33, 35 and 37), 6 tetraBDEs (BDE # 47, 49, 66, 71, 75 and 77), 7 pentaBDEs (BDE # 85, 99, 100, 105, 116, 119 and 126), 6 hexaBDEs (BDE # 138, 140, 153, 154, 155 and 166) and 3 heptaBDEs (BDE # 181, 183 and 190). Decachlorobiphenyl (Cromlab, Barcelona, Spain) was used as surrogate standard. PBDEs were analysed by gas chromatography coupled to mass spectrometry in negative chemical ionization mode (GC-NCI–MS) on a gas chromatograph Agilent 6890 coupled to a mass spectrometer Agilent 5973 Network according to a previous protocol (Lacorte et al., 2003).

2.4.4. Method 4

This method permitted determination of the concentration of three OTs. Monobutyltin (MBT, 95%) and tricyclohexyltin (TCyT, 90%) chlorides were from Aldrich. Dibutyltin (DBT, 96%), tributyltin (TBT, 96%) chlorides were from Aldrich Chemie (Steinheim, Germany). Tripropyltin chloride (TPrTCl, 98%) was from Merck–Schuchardt (Hohenbrunn, Germany) and tetrabutyltin (TeBT, 98%) and tetramethylammonium hydroxide (TMAH) were from Fluka AG (Buchs, Switzerland). Stock standards were prepared at 100 mg/L in toluene. Dimethyldioxirane (DMD) was synthesized in the laboratory and ammonium pyrrolidinedithiocarbamate (APDC, 97%) was from Aldrich. OTs were analysed by GC-FPD Carlo Erba HRGC 5300 equipped with an AS 200 autosampler and a flame photometric detector (FPD 700, Milan, Italy) equipped with a 610 nm bandpass filter. Acquisition and quantification was performed according to previous work (Diez et al., 2002).

2.5. Quality control and assurance

Quality control analysis to ensure unequivocal identification and quantification were carried out. For OTs, two certified reference materials were analysed, and consisted in marine harbour sediment PACS-2 from the National Research Council of Canada (Ottawa, Canada) and fish tissue NIES CRM 11 from the National Institute of Environmental Studies, Environmental Agency of Japan (Tsukuba, Japan). In both cases bias was below 10%. For organic compounds, freeze-dried sediments and fish were spiked with target compounds at 1.5–3.5 μg/kg dry weight (dw). Aldrin, isodrin, dieldrin and endrin were the least recovered both in sediments and fish (32–56%). Chlorobenzenes were recovered at 60–70% and DDTs and PBDEs at levels greater than 70%. Nonylphenol (NP) and octylphenol (OP) were recovered at percentages above 61% while all PAHs except naphthalene had recoveries > 50%. For all chemical families, the % RSD was higher in fish than in sediment, but was below 23%. For calculation of the concentrations in each sample, each compound was corrected with their relative recovery.

Procedural blanks (no matrix involved) were analysed using each method and contained traces (less than 5% of the individual target concentration) of

PAHs, NP and OP and PBDEs 47, 100 and 99. OC compounds and OTs were not detected. No blank correction was performed. The limit of detection (LOD) at a signal to noise ratio of 3 are reported in Table 3. Calibration curves were produced using concentrations of 0.05, 0.1, 0.5, 1 and 2 μg/mL.

All methods (except for OTs) involved the use of mass spectrometry (MS) and acquisition in time scheduled selected ion monitoring (SIM), following the generic acquisition protocol described elsewhere (Lacorte et al., 2000). Compound identification was performed by retention time match against a standard solution and confirmation of three characteristic ions. Quantification was performed using the base peak of the SIM chromatogram using external standard calibration (method 1 [OCs] and 3 [PBDEs]) and internal standard quantification (methods 2 [PAHs + APs] and 4 [OTs]).

3. Results and discussion

3.1. Summary data

In sediments, out of 73 target compounds included in the survey, hexachlorocyclohexane (4 isomers), pentachlorophenol, aldrin, dieldrin, endrin, isodrin, trichlorobenzenes and 27 PBDEs were never detected. In fish, hexachlorocyclohexane (4 isomers), pentachlorophenol, aldrin, dieldrin, endrin, isodrin, hexachlorobutadiene, trichlorobenzenes, OTs and 26 PBDEs were not detected. Considering only the detected compounds (indicated in Table 3), 60% of the data values were below the LOD. Geometric means were calculated for all compounds and the 75th percentile concentration was used to identify those sites with highest levels of contaminants. Concentration of organic pollutants were below the limit of detection in many cases (Table 3) and that precluded vigorous statistical analysis.

Summary data for all detected compounds in sediments and fish is given in Table 3, which indicates the detection threshold level as LOD, number of samples with levels above the LOD, number of samples with predicted effect levels, minimum, maximum concentration and geometrical mean and finally, toxicological data according to probable effect levels.

In sediments, the data encountered was characterized by overall low to medium concentration levels but most samples contained several organic compounds being PAHs, APs and PBDEs detected in more than 16 samples out of the 20 analysed and OTs and OCs (DDTs and CBz) detected in 7 and 6 samples, respectively (Fig. 2A). When considering total concentration loads, the abundance was APs > PAHs > OTs > DDTs > CBz > PBDEs (Fig. 2B). Persistent organic pollutants (DDTs, CBz and PBDEs) were detected at lowest total concentration.

Fish studied were considered from the same trophic level and more prone to bioaccumulate contaminants than nearshore benthic organisms (Kidd et al., 2001). The pattern encountered in fish was totally different to that of sediments. PBDEs were detected in all fish, followed by CBz, DDTs, and APs which were only identified at trace levels in three fish (Fig. 2C). Among them, DDTs, CBz and PBDEs were detected at higher concentration in fish than in sediment (Fig. 2D), indicating that they are available and bioaccumulated in biota although the specific isomer/congener composition was different. The concentration of these target analytes was analogous among the two species, suggesting that uptake and bioaccumulation of organic contaminants followed a similar pattern despite belonging to different species. Fish from T3 (*Barbus graellsi*) and from R18 (*Carassius aurata*) did not contain any traces of DDTs, CBz, AP or OTs, differing from the other species analysed from the same site. On the other hand, the *Barbus graellsi* collected from T3 was leaner than the median (see Table 1) and this might account for the lack of accumulation of any organic compound. *Carassius aurata* was only analysed in one site and its specific biology as regards to accumulation of organic compounds cannot be compared to other works nor other locations within the study sites.

3.2. Occurrence of organochlorine compounds

DDTs were detected in all sediments, except in T3 and T11, at levels from 1.55 to 168 μg/kg dw, whereas fish contained DDTs at levels between 0.91 and 1922 μg/kg dw. ∑DDTs (sum of isomers of DDT, DDE and DDD) had a geometric mean of 19.6 μg/kg dw in sediments while in fish was this was 143 μg/kg dw. *Cyprinus carpio* from R1, *Barbus graellsi* from T3 and *Carassius aurata* from R18 did not contain any DDT, differing from the other specie analysed from the same site.

Sediments with ∑DDT concentrations above the 75th percentile (35.6 μg/kg) were T8, T10, T13, T15 and R18, this last one containing 6 times the 75th percentile. Fish from T15 and R18 also had concentrations above the 75th percentile, indicating that the historical local use or release of DDTs has had an impact on sediment and fish in these areas. In these fish, bioaccumulation of DDT isomers was evidenced and especially, prevalence of DDE that was detected more regularly and at higher concentration than the parent compound.

pp′ DDT was the main isomer detected in sediments (Fig. 3); *op′* DDT was only detected in T8 at 5.44 μg/kg dw and in R18 at 167 μg/kg dw. Sediments from R1, T9, T13, T15, T15′, R17 and R18 contained DDE or DDD or both isomers at levels between 1.55 and 41.3 μg/kg dw. In these sites, except R18, the DDE + DDD/DDT ratio was >1, indicating that the source of DDT was not recent. In R18, the DDE + DDD/DDT ratio of 0.37 pointed toward fresh inputs of DDT to the river bed sediments, supported by the disproportional higher concentration of *op′* DDT than *pp′* DDT. *op′* DDE was not found in any of the sediments.

DDT levels in fish are in line with those previously reported from the Cinca and Gallego rivers (Raldúa et al., 1997) and in general, levels were similar between the two species collected from each site. The specific isomer distribution varied from sediments to fish. *pp′* DDE was the main isomer in fish, followed by *op′* DDE. Bioaccumulation and metabolism of DDTs can be explained by the high DDEs/DDTs ratio which was between 2.8 and 128. In fish, DDDs were only found in sites with higher DDE concentration. No correlation was found between the levels of ∑DDTs in fish and in sediment. Fish to sediment ratios for ∑DDTs ranged from 1.4 to 58.8 except in R1, which was the only site with a ratio of 0.17. Such spread values indicate the many environmental and geographical factors influencing bioavailability and accumulation.

Table 3
Compounds detected, LOD, number of positive samples out of 20 sediments and 18 fish (*N*), number of samples with concentration above probable effect level (PEL) (*n*) (Smith et. al., 1996), Minimum, Maximum and geometric mean (Mean), concentration >75th percentile, and PEL

Family	Compounds	Sediment							Fish						Toxicity
		LOD	*N* (*n*)	Min	Max	Mean	75%	PEL	LOD	*N* (*n*)	Min	Max	Mean	75%	
OCs	*pp′* DDT	2.34	18 (1)	4.36	36.7	10.6	15.7	62.9[a]	1.44	6 (3)	2.48	69.2	11.5	29.4	70 Total
	op′ DDT	2.24	2	5.44	167	30.1	127		1.44	9	3.67	97.9	22.6	46.6	
	pp′ DDE	5.32	5 (5)	11.9	26.9	19.1	22.5	6.57[a]	2.50	14	4.28	1922	112	528	
	op′ DDE	8.68	<LOD						0.70	14	0.90	565	21.1	34.3	
	pp′ DDD	1.14	6 (4)	1.55	41.2	4.14	3.74	8.51[a]	1.42	3	4.29	108	22.9	66.9	
	op′ DDD	2.26	4	4.62	9.96	6.51	7.57		1.42	7	6.89	127	33.9	78.1	
	Hexachlorobenzene	1.04	4	4.25	68.4	18.3	45.6	N.A.	0.60	16	2.08	165	15.8	28.8	N.A.
	Hexachlorobutadiene	4.02	2	6.11	8.71	7.30	8.06	N.A.	0.54	<LOD		N.A.			
	Pentachlorobenzene	0.48	5	0.58	2.57	1.17	2.31	N.A.	0.30	14	0.32	3.31	1.10	1.86	N.A.
APs	Nonylphenol	8.04	8	89.2	2331	542	1185	N.A.	11.48	3	74.5	146	113	140	N.A.
	Octylphenol	1.86	19	30.2	103	57.4	67.6	N.A.	6.66	1	6.95	6.95	6.95	N.A.	N.A.
PAHs	Naphthalene	1.00	19 (0)	9.32	39.6	18.1	27.3	561	Not analysed		N.A.				
	Anthracene	1.28	13 (0)	1.56	223.9	8.02	8.5	845	Not analysed		N.A.				
	Fluoranthene	0.72	19 (0)	1.07	156.0	14.9	29.1	2355	Not analysed		N.A.				
	Benzo[*a*]pyrene	0.96	17 (0)	1.43	57.3	8.59	15.3	782	Not analysed		N.A.				
	Benzo[*b*]fluoranthene	1	18	1.77	86.4	13.5	22.3	N.A.	Not analysed		N.A.				
	Benzo[*g,h,i*]perylene	0.16	13	2.66	32.6	6.46	8.52	N.A.	Not analysed		N.A.				
	Benzo[*k*]fluoranthene	0.94	17	1.73	186.3	8.97	14.7	N.A.	Not analysed		N.A.				
	Indeno(1,2,3-cd)pyrene	0.18	14	1.58	40.1	11.6	14.9	N.A.	Not analysed		N.A.				
PBDEs	Di (15/11)[b]	0.04	2	1.32	4.36	2.47		N.A.	0.04	2	0.96	1.42	1.17	1.31	N.A.
	Tri (30, 32/25, 28)[b]	0.02	2	0.14	1.49	0.26		N.A.	0.02	9	0.02	0.41	0.11	0.21	N.A.
	Tetra (71, 49, 47/75, 49, 47, 66)[b]	0.02	12	0.04	0.27	0.10		N.A.	0.02	32	0.06	8.34	0.80	1.71	N.A.
	Penta (100, 99, 116/100, 99, 85, 126)[b]	<0.01	20	0.01	0.79	0.08		N.A.	<0.01	28	0.02	1.47	0.14	0.22	N.A.
	Hexa (153, 154/153, 154)[b]	0.02	10	0.02	4.09	0.15		N.A.	0.02	16	0.03	97.9	0.64	9.46	N.A
	Hepta (183, 190/183)[b]	0.04	7	0.04	10.6	0.15		N.A.	0.04	6	0.04	17.5	0.32	2.58	N.A
OTs	MBT	5.24	5 (5)	18	156	63.9	92	N.A.	5.24	<LOD		N.A			
	DBT	5.28	7 (5)	13	512	124	416	N.A.	5.28	<LOD		N.A			
	TBT	3.82	5 (4)	25	202	44.9	38	N.A.	3.82	<LOD		N.A.			

Concentrations in μg/kg dw. Hexachlorocyclohexane, pentachloropohenol, aldrin, dieldrin, isodrin, endrin, trichlorobenzenes, mono BDEs were never detected. N.A., not available.

[a] Sum *pp′* and *op′* congeners.

[b] Congeners detected (sediment/fish).

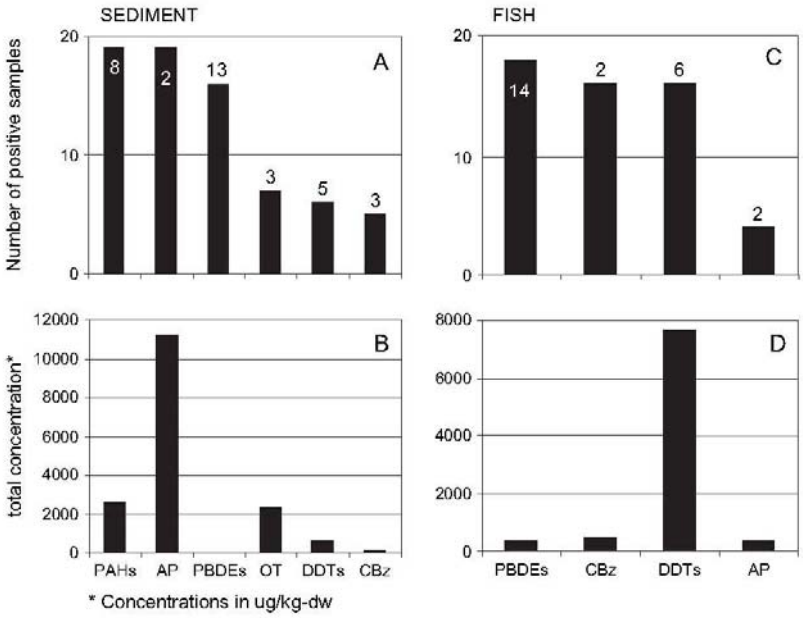

Fig. 2. Number of samples containing the different chemical families in sediment (A) and fish (B) and total concentration of the different chemical classes in sediment (C) and fish (D). Numbers on top of the columns indicate the number of detected compounds of each family. Acronyms: PAHs = polycyclic aromatic hydrocarbons; AP = alkylphenols; PBDEs = polybrominated diphenyl ethers; OTs = organotins; DDTs = sum of DDT, DDE and DDD; CBz = chlorobenzenes.

Highest ratios were found in the most polluted sites (T15′, T16 and R18) suggesting that DDT accumulated in sediment throughout the years is a source to fish which in the long term can suffer from deleterious effects.

In the Ebro river basin, DDT and DDT metabolites, present in sediment and fish, are derived basically from the historic use of DDT in this area. The overall levels and distribution of DDT isomers in sediment and fish are in accordance to previous studies carried out in the area (López-Martín et al., 1995; Fernández et al., 1999) or in other samples from Spain (Fernández et al., 2000; Bordajandi et al., 2003; Peris et al., 2005). This study demonstrates that throughout the years, DDT contamination has not yet been resolved in the Ebro river basin and might be an issue to consider in avoiding serious ecotoxicological effects.

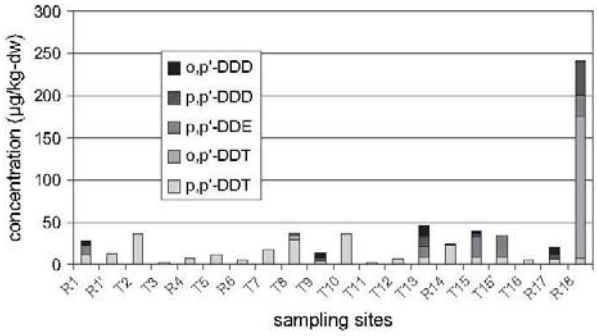

Fig. 3. DDT, DDE and DDD concentration in sediment from north to south of the Ebro river basin.

HCB, PCB and HCBd were found in 5–7 sediments at levels between 0.58 and 68.4 µg/kg dw, with the highest levels in R17 and R18, at the very end of the Ebro river. On the other hand, PCB and HCB were detected in 16 fish at levels up to 165 µg/kg dw. HCB content in fish was from 10 to 200 times higher than PCB, indicating its wider use especially at the lower course of the river, as reflected by the higher levels detected also in sediments. Summarizing, the OC levels in the Ebro basin reveal (i) diffuse contamination due to OC compounds in sediment and accumulation in fish and (ii) increase of the concentration of DDT and CBz at the lower course of the river attributed to local deployment of such compounds. Specifically, the source of DDT can be explained by pesticide industries located in the basin which produce 1500 t dicofol annually, and represent more than half of the worldwide consumption. Dicofol contains 0.1% of DDT as impurity. However, dicofol was not analysed in sediments.

3.3. Occurrence of alkylphenols

Octylphenol (OP) was detected in all sediments analysed at levels from 30.2 to 103 µg/kg dw with a geometric mean of 57.3 µg/kg dw whereas nonylphenol (NP) was detected in eight samples at concentrations from 89.2 to 2330 µg/kg dw, with a geometric mean of 542 µg/kg dw. These levels are similar to those detected in sediments of Portuguese rivers (Petrovic et al., 2002). Sites with concentration higher than the 75th

percentile were T2, T11, R14, T15 and R18. The higher concentration of NP in relation to OP points to nonylphenol ethoxylates (NPEOs) as the source of these compounds in river sediments. The technical mixtures containing NPEOs have OPEOs as impurities. Once released into the environment, the ethoxylated chain is degraded to form NP and OP to a lesser degree. Although these two compounds have a similar octanol–water partition coefficient (K_{ow}), OP has a lower vapour pressure, diminishing volatilization after use in relation to NP, thus enhancing deposition and sedimentation. In contrast to sediment, only 4 out of 18 fish analysed contained APs (Table 4). Accumulation of APs in fish was not observed, indicating that sediments were not a source of APs to fish or that fish may metabolize such compounds (Arukwe et al., 2000).

3.4. Occurrence of PAHs

In sediments, the concentration of PAHs ranged from 1.07 to 224 μg/kg dw, corresponding to geometric means ranging from 1.44 (benzo[*g*,*h*,*i*]perylene) to 15.7 μg/kg dw (naphthalene). Five to eight PAHs were detected in each sample, with naphthalene and fluoranthene the most frequently detected. The low recovery of naphthalene did not affect the detection of this compound since quantitation was performed using the deuterated naphthalene as surrogate. The overall levels are in the range of those found in sediments or soils from the study area (Nadal et al., 2004; Bartolomé et al., 2005; Olivella et al., in press). Sites with ∑PAH concentration above the 75th percentile (172 μg/kg dw) were R1, T8, T9 and R18 and in these samples anthracene and benzo[*k*]fluoranthene were the major contributors to ∑PAH. There are no limit concentrations for PAHs in sediment nor does the Oslo–Paris (OSPAR) convention indicate any background concentrations.

3.5. Occurrence of PBDEs

In sediments, PBDEs were found in 16 samples at 0.01–10.6 μg/kg dw and 78% of the data had values <LOD.

Table 4
Total concentration (μg/kg dw) of each chemical family in sediment and fish from the Ebro river and its tributaries

Sample point	Matrix	∑ DDTs	∑ CBz	∑ APs	∑ PAHs	∑ OTs	∑ PBDEs
R1	Sediment	28.8	<LOD	64.1	368	<LOD	0.63
R1′	Sediment	12.2	<LOD	75.5	326	<LOD	<LOD
	Barbus graellsi	5.18	2.08	<LOD	N.A.	<LOD	0.06
	Cyprinus carpio	<LOD	10.7	<LOD	N.A.	<LOD	0.53
T2	Sediment	35.7	<LOD	2433	11.2	<LOD	0.05
T3	Sediment	<LOD	<LOD	<LOD	<LOD	636	<LOD
	Barbus graellsi	<LOD	<LOD	<LOD	N.A.	<LOD	1.37
	Rutilus arcasii	64.1	33.4	<LOD	N.A.	<LOD	2.41
R4	Sediment	7.04	<LOD	58.7	126	<LOD	0.30
T5	Sediment	11.2	<LOD	45.8	49.7	<LOD	<LOD
R6	Sediment	5.40	<LOD	57.7	75.8	<LOD	0.55
T7	Sediment	16.8	<LOD	54.9	81.6	<LOD	0.52
T8	Sediment	37.8	0.74	50.1	219	129	1.39
T9	Sediment	14.2	<LOD	307	407	508	0.35
	Barbus graellsi	51.2	6.54	6.96	N.A.	<LOD	0.82
	Chondrostoma	105	16.0	<LOD	N.A.	<LOD	0.81
T10	Sediment	36.7	<LOD	57.9	25.8	156	1.29
T11	Sediment	<LOD	<LOD	1201	34.4	173	<LOD
T12	Sediment	5.74	<LOD	51.6	44.9	25.0	0.13
	Barbus graellsi	71.3	10.4	<LOD	N.A.	<LOD	0.79
	Chondrostoma	22.6	10.1	<LOD	N.A.	<LOD	0.14
T13	Sediment	20.9	<LOD	146	33.8	13.0	0.28
R14	Sediment	25.4	6.69	686	77.9	<LOD	0.11
	Barbus graellsi	35.9	8.78	<LOD	N.A.	<LOD	0.63
	Alburnus alburnus	71.9	14.5	146	N.A.	<LOD	1.09
T15	Sediment	40.3	10.3	1375	128	<LOD	2.16
T15′	Sediment	35.2	5.10	341	82.7	<LOD	20.9
	Barbus graellsi	2371	22.0	<LOD	N.A.	<LOD	113
	Alburnus alburnus	2356	30.3	<LOD	N.A.	<LOD	219
T16	Sediment	4.76	<LOD	30.3	96.9	<LOD	0.30
	Cyprinus carpio	91.3	9.17	<LOD	N.A.	<LOD	2.03
	Alburnus alburnus	165	8.95	<LOD	N.A.	<LOD	2.83
R17	Sediment	20.6	40.3	30.2	51.4	<LOD	0.04
	Cyprinus carpio	983	66.1	134	N.A.	<LOD	1.99
	Alburnus alburnus	487	169	74.5	N.A.	<LOD	1.23
R18	Sediment	241	79.6	621	338	683	0.86
	Carassius aurata	<LOD	<LOD	<LOD	N.A.	<LOD	1.09
	Scardinius	756	66.8	<LOD	N.A.	<LOD	0.77

<LOD, below limits of detection; N.A., not analysed.

The geometric mean was 0.12 μg/kg dw, so this type of contamination was not the most important in Ebro river sediments. Out of the 40 congeners analysed for, only 13 were detected (BDEs 15, 30, 32, 71, 49, 47, 100, 99, 116, 154, 153, 183 and 190). Other congeners were not detected even though the LOD of the method was relatively low (0.01–0.04 μg/kg dw, Table 3). One to seven congeners were identified per sample, BDE 47, 100, 99 and 183 being the most frequent congeners detected at 0.01–0.79 μg/kg dw, in agreement with results reported in sediments from Portugal (Lacorte et al., 2003). Other BDEs (15, 30, 32, 71 and 49) were detected in 1–6 samples at concentrations ranging from 0.08 to 4.63 μg/kg dw. No geographical nor congener specific distribution was observed in sediments taken from the different sites along the Ebro river. Only one site (T15′) contained ∑PBDEs at a higher concentration than the rest (up to 20.9 μg/kg dw). T15, T15′ together with T8 and T10 contained ∑PBDEs above the 75th percentile (0.97 μg/kg dw).

PBDEs were accumulated in fish and concentrations ranged from 0.02 to 97.9 μg/kg dw. In comparison to sediments, more congeners were detected per sample (1–11 congeners) and those were BDE 11, 25, 28 + 33, 75, 49, 47, 66, 100, 99, 85, 126 + 155, 154, 153 and 183. Table 3 summarizes the number of samples containing PBDEs and mean and maximum concentration levels. As for DDTs, a good agreement was found between the levels of individual PBDEs in the two fish species from the same emplacement, suggesting that the two species analysed in each area followed a similar accumulation rate. Among detected congeners, BDE 47 was identified in all 18 fish analysed, followed by BDE 100 and 154, detected in 13 and 10 samples, respectively. BDEs 11, 25, 28 + 33, 75 and 49 were detected in 2–7 samples at levels from 0.02 to 10.30 μg/kg dw, and appeared in conjunction with higher brominated ones and in samples with highest PBDE concentration.

Taking into consideration the six most abundant congeners detected, Fig. 4 shows the congener-specific profile in sediment and fish samples. Whereas in sediment BDEs 183 and 99 accounted for the main congeners detected, BDE 47 was the most abundant in fish, except both fish from T15, where the most abundant congener was BDE 153. This results corroborate the suggestion that BDEs can be metabolized in fish generating lower level brominated congeners (Vives et al., 2004) which might have longer half lives (Tomy et al., 2004). The ∑PBDEs fish to sediment ratio ranged from 0.4 to 49.75 in species a and from 0.6 to 30.75 in species b. Although these ratios are somewhat lower than for DDTs, it is confirmed that PBDEs are also bioavailable to benthopelagic fish. As in sediment, fish with highest PBDEs load was in T15. In this site, the ∑PBDE concentration in sediment was of 20.90 μg/kg dw and 113 and 219 μg/kg dw in fish a and b, respectively. Comparing the levels of PBDEs in fish and sediment from other locations, this site is considered a "hot spot" as regards DDT and PBDEs contamination.

There was not any correlation between the levels of ∑PBDEs and ∑DDTs nor with CBz in sediments and fish from the nine stations. Comparing the two families, the DDT/PBDE ratio was highly variable, ranging from 2 (T15) to 715 (T2) in sediment and from 11 (T15) to 869 (R18) in fish. Although DDTs have been banned in Spain for many years, the higher DDT load is attributed to the historical production and usage within the Ebro basin. On the other hand, the lower levels of PBDEs reflect their more recent use and time lag as regards to deposition and sedimentation.

3.6. Occurrence of organotin compounds

Organotin compounds (OTs) have been widely used during the last decades as biocides, polymer stabilizers, wood preservatives, and catalysts in industrial processes. Nevertheless, their main source in the aquatic ecosystem is related to their use as antifouling paint biocides. OTs were detected in samples located in the northern part of the river basin (T3, T7, T8 and T9). In these sites, tributhyltin (TBT) was detected from 25 to 38 μg/kg dw, whereas dibuthyltin (DBT) was detected from 40 to 512 μg/kg dw and monobuthyltin (MBT) at 18–156 μg/kg dw. Currently, in river and coastal zones, OT inputs from wastewater, sewage and leachates are the main sources. In such matrices, MBT and DBT are predominant, although they do not only occur as degradation products of TBT but also as direct inputs such as leaching and weathering of PVC materials containing these OTs (Hoch, 2001).

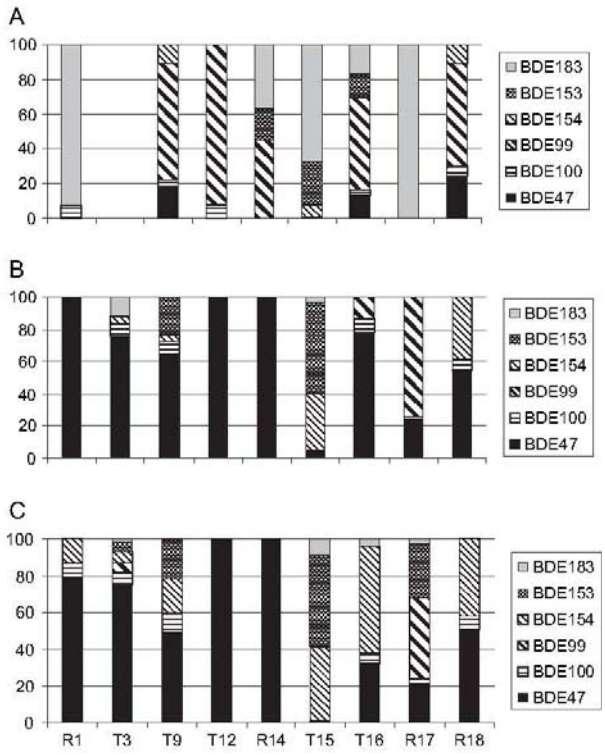

Fig. 4. Normalized composition profiles of the six main PBDE congeners identified in sediment (A) and fish from species a (B) and species b (C) of 9 sites along the Ebro river basin.

The site with higher loads was Tortosa in R18, with 202 μg/kg dw of TBT and 481 μg/kg DBT attibuted to local deployment of these compounds. As a comparison, sea harbour sediment of the Mediterranean Sea (Spain) presented TBT values of 400 μg/kg (Diez et al., 2002).

No OTs were found in fish probably because OTs are strongly sorbed to clays or humic acids and are less bioavailable to biota (Rüdel, 2003). Adsorption and concentration onto the clay fraction of particulate matter has been demonstrated as an important mechanism concerning distribution and fate of organotins in the environment (Hoch, 2001). Because TBT associates strongly with natural sorbents and because of its high persistence in anoxic sediments (Fent et al., 1991), TBT accumulates in sediments for a long period of time. As a consequence, high levels of TBT have been found in freshwater or coastal sediments with concentrations up to several mg/kg.

Bioavailability of OTs depends on the aqueous chemical speciation in ambient media. Thereby, environmental variables such as temperature, pH, oxidation/reduction potential, composition and concentration of other ions, particulate matter, and organic carbon content are relevant. The speciation of TBT shows a strong pH dependence. TBT is present as cation (TBT^+) at low pH and as hydroxide (TBTOH) at higher pH. The octanol–water distribution ratio (K_{ow}) of TBT is more than an order of magnitude higher at pH 8 than at pH 3. Moreover, dissolved organic matter such as humic substances led to a significant reduction on the bioavailability due to hydrophobic sorption of organotins (Fent et al., 1995; Looser et al., 2000). Experiments referred to in these articles clearly demonstrate that the bioavailability of organotins is a function of pH and concentration of dissolved organic matter.

3.7. Geographical distribution and toxicity implications

The sources, fate and availability of the different types of contaminants within the Ebro river basin were site specific and varied according to the input of contaminants from the different activities carried out. Therefore, specific contamination patterns were detected along the Ebro river basin. The concentration of each chemical family in sediment and fish is indicated in Table 4. Sites with highest concentration of priority contaminants were T2 (Zadorra at Salvatierra), T9 (Arga), T11 (Zaragoza area), T15′ (Cinca in Monzón) and finally, R18 (Tortosa). All these sites are near important industrial areas such as the automobile, metallurgic, pesticide and solvent synthesis. All of them had relevant levels (Table 4) of NP and OP although the sites T2 and T11 contained highest concentrations of APs and reflect the quality of the water downstream of urban areas. Another type of contamination detected in T9 and R18 was attributed to the presence of APs, OTs and PAHs at levels of around 500 μg/kg dw. Finally, sediment and fish from T15′ were highly loaded with both DDTs and PBDEs, which is explained by the discharge of effluents of a heavily industrialized town with a very important chemical industry (Eljarrat et al., 2004).

Sediment contamination and availability of contaminants depends on the specific physico-chemical properties of each contaminant as well as on multiple parameters such as the organic content and redox potential of the sediment, flow conditions, depth, eutrophication of waters, hydrologic, morphological and climatic conditions (Muir et al., 2003; Eggleton et al., 2004). Sediment contamination may account for toxic effects towards living organisms, as reported for DDTs effects on soil microflora (Megharaj et al., 2000), PAH on the immune function of *Limanda limanda* (Hutchinson et al., 2003) or phytotoxicity of dredged sediments (Chen et al., 2002). A question that arises is whether sediment contamination can produce toxic effects towards aquatic organisms living in the Ebro river basin. To assess the toxicological risk of Ebro river sediments, concentration values were compared to probable effect level (PEL), which is the concentration above which adverse effects are expected to occur frequently (Smith et al., 1996; MacDonald et al., 2000). Table 3 displays the PEL for each compound and indicates the number of samples that surpass this limit. DDT in R18 (Tortosa) exceeded the PEL value of 62.9 μg/kg dw while the PEL of DDE, considered of higher toxicity, was surpassed in R1, T13, T15 and R18. For DDD, stations T9, T13, R17 and R18 contained higher levels than PEL. Six sites had DDT, DDE or DDD concentrations above the PEL indicating potential deleterious effects and that remediation actions should be enforced in impacted locations.

OTs are extremely toxic to aquatic biota, as demonstrated in a variety of different organisms in vivo and in vitro (Fent, 2004). Many ecotoxicological studies on organisms of different evolutionary level have been reported (Alzieu, 2000; Nishikawa et al., 2004). Lowest toxic concentrations are in the range of 1–10 ng/L. Marine gastropods and oysters are among the most susceptible organisms, but fish are affected as well, although at concentrations of 1–10 μg/L. However, the long-term ecotoxicological effects of OTs on the structure and function of aquatic ecosystems are still not well understood, particularly with respect to biomagnification in food webs (Senthilkumar et al., 1999).

For PAHs, none of the samples exceeded the PEL concentration. For other chlorinated compounds, PBDEs, APs, no toxicity guideline was found for sediments nor fish.

To assess their impact on fish, the guideline used has been the maximum acceptable concentration in aquatic species used as food (Table 3). When considering $\sum$DDT maximum permissible concentration, fish from T15, R17 and R18 exceeded the value of 70 μg/kg dw. These data serve to evaluate whether the levels of contaminants pose a health risk for fish consumers. In addition, whole body concentrations are significant when considering the impact on other aquatic animals within the local food web.

The actual status as regards to organic pollution in the Ebro river basin is as follows: the area contains still important levels of compounds which in principle are out of use (DDTs) and even more important levels of new-era contaminants such as NP. The incipient but widespread contamination of PBDEs is a first warning, since PBDEs are bioavailable and can be accumulated by aquatic biota and be a future threat for living organisms. An increase of organic concentration in sediments

downward from river tributaries was not seen (e.g. river Cinca and Segre discharging to the Ebro), because in the case of the Ebro basin, mobilization of pollutants through sediment transport within the river or its tributaries was not an important process due to the presence of dams. These dams, in addition to natural barriers, impeded the mixing of fish populations, thus the levels of contaminants in fish from a given site were representative of this specific site. However, suspension and remobilization of contaminants might be the cause of trace pollution detected in most samples.

4. Conclusions

In this study we assessed the levels of contaminants in the Ebro river basin and their availability to fish, and evaluated the environmental risk. The type of contamination and its distribution throughout the Ebro basin reflects the human activities carried out, and it is possible to localize areas with high concentrations of DDTs, PBDEs and CBz in both sediment and fish. PAHs were spread throughout the river at levels lower than the predicted effect levels, whereas OT contribution was high in some heavily industrialized areas although these contaminants were not accumulated in fish. Our results show that within freshwater systems, sediments can act as drainage for pollutants and a source to biota. Also, sediment suspension might be the cause of the low-level diffuse contamination found in all sites. This type of contamination is more difficult to tackle, and future work will include the measurement of temporal tendencies so that action can be taken to avoid possible deleterious effects towards freshwater organisms of the Ebro river ecosystem.

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Article científic 4

Chemometrical investigation of presence and distribution of organochlorine and polyaromatic compounds in sediments of the Ebro River Basin

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Chemometrical investigation of the presence and distribution of organochlorine and polyaromatic compounds in sediments of the Ebro River Basin

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Abstract A multivariate statistical data analysis, using principal component analysis, of historical data from 1996 to 2003 concerning the concentration of different polycyclic aromatic hydrocarbons and organochlorine compounds in sediment samples from different sampling sites of the Ebro River Basin was performed under the UE funded project AQUATERRA. Three major contamination sources were identified and their composition and distribution profiles were resolved. The first contamination profile was mostly loaded by polycyclic aromatic hydrocarbons, the second contamination profile was loaded by some organochlorine compounds and the third contamination profile was more specifically loaded by naphthalene. Samples from the different geographical regions of the Ebro River Basin were grouped according to the contamination described by these three major profiles.

Keywords Polycyclic aromatic hydrocarbons · Organochlorine compounds · River sediments · Principal component analysis

Introduction

The Ebro River Basin (northeast Spain) (Fig. 1) drains an area of approximately 85,000 km^2, discharging into the Mediterranean Sea and forming a delta of more than 30,000 ha. In the whole basin there are more than 2,700,000 inhabitants distributed in a very heterogeneous way. One third of the population lives in small villages with fewer than 5,000 inhabitants and only five cities, next to the Ebro River or its tributaries, provide approximately 45% of the population. The region is basically agricultural and is the most important irrigated land in Spain, covering the areas of La Rioja (wine production), Lleida (fruit trees and corn production) and the Ebro Delta (rice production) [1]. But also the Ebro River Basin is dominated by industrial activities concentrated close to the cities of Saragossa, Vitoria, Pamplona, Logroño, Monzón, Flix and Lleida (Fig. 1).

The Confederación Hidrográfica del Ebro (CHE), which is the organization in charge of the management of the Ebro River Basin, has monitored the contamination in the whole river basin by establishing a control network with sampling sites in which different compounds are analyzed. Among industrial and agricultural pollutants, priority compounds according to Directive 2006/11/CE (follow-up of the recently abolished Directive 76/464/CE) were selected to determine their sources, transport and fate within the water sediment interface of the Ebro River Basin. This control network was introduced in 1992 with only four sampling sites and since then other sampling sites and compounds have been added to improve the water quality of the Ebro River Basin. Nowadays, the monitoring network consists of the analysis of different compounds in water, sediments and biota from 18 sampling sites once a year [2]. The results of this sample analysis performed by the CHE provide a preliminary data set for the investigation of the main temporal and geographical distribution patterns of the compounds analyzed in the Ebro River Basin [3].

Among all the compounds included in the CHE monitoring program, the ones selected for this study were seven polycyclic aromatic hydrocarbons (PAHs) and five organochlorine compounds (OCs). PAHs are an important group of organic micropollutants (xenobiotics) owing to their widespread distribution in the environment (atmosphere, water and soil) [4]. It is well known that some PAHs exhibit carcinogenic and/or mutagenic properties [5]. Moreover, PAHs have been discussed as potential synthetic estrogens on the basis of certain compounds having similar structures as natural molecules like estradiol. Owing to their physicochemical properties, PAHs, especially the higher molecular weight PAHs, are hardly degradable and tend to accumulate in different environmental compartments [6]. The contamination from OCs is of great concern because of the high distribution of their residues in the aquatic ecosystem and of their toxic and carcinogenic

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Fig. 1 Location of sampling sites in the Ebro River Basin analyzed in this work. Site numbers correspond to the codes given in Table 1

properties [7]. Owing to their high persistence, their hydrophobic nature and their low solubility in water, they are adsorbed on particulate matter and finally accumulate in sediments [8, 9]. Consequently, sediments, apart from being the final acceptors of these pollutants, act as their secondary contamination sources [10]. The contamination from OCs is very relevant in the Ebro River Basin since two chloroalkali plants are located in the lower part of the Ebro River Basin (Flix and Monzón).

These contaminants enter the environment through human activities (urban waste, deposits of waste-cleaning plants and agricultural cultivation) [11] and various other pathways (atmospheric and fluvial transport) [12]. Environmental pollution by these two groups of organic compounds has received considerable attention as a result of public awareness of environmental problems and expectations for good quality of life [13]. Within this framework, a long-term study was performed to evaluate the concentration of these priority pollutants in terms of temporal and geographical trends in sediments from the Ebro River Basin. Sediments were selected because river sediments are unique in providing historical contamination records and are good monitoring tools to evaluate general pollution episodes [1]. For this study, historical data sets in the public domain [14] from CHE were used.

The main goal of the present work is to contribute to the evaluation of the vulnerability of the Ebro River Basin using the historical sediments data from CHE. But obtaining significant environmental information from the individual analysis of each compound and distinguishing between their different sources is troublesome, specially when there is partial correlation between them [15]. For this reason, a deeper study of the presence and the correlations of different pollutants in selected sampling sites was proposed using multivariate exploratory data analysis techniques like principal component analysis (PCA) [16]. PCA is a frequently used multivariate technique that complements the application of classic univariate statistics techniques and provides a powerful tool for data compression, exploration and interpretation. Using this technique, the investigation of hidden environmental information and especially the identification of the main environmental sources causing the observed variation in the data set under study and the determination of the composition profiles of these sources and of their temporal and geographical contributions may be possible [3]. The present study is integrated in the European Union project AQUATERRA (contract no. 505428), which aims to provide the basis for improved river basin management, enhanced soil and groundwater monitoring programs and the early identification and forecasting of impacts on water quantity and quality [17].

Experimental data

The data set used for this study was downloaded from CHE Web page [14]. Among all the data sets available from the control network, sediment sample data sets covering the years from 1996 to 2003 were selected. Before 1996, the data sets were rather incomplete. Year 1999 was excluded from the data set under study because most of the compounds analyzed in the control network were not reported for that year. The sampling sites selected for this study were the most contaminated ones from the monitoring network in accordance with CHE criteria. Only those compounds having results for all these years and sampling sites were finally chosen for this study because we were also interested in the study of temporal trends.

The whole data set consisted of the concentrations in sediments (micrograms per kilogram, dried weight) of 12 different compounds, including seven PAHs (naphthalene, Naph; fluoranthene, Flu; benzo[*a*]pyrene, BaP; benzo[*b*] fluoranthene, BbF; benzo[*g*,*h*,*i*]perylene, BghiP; benzo[*k*] fluoranthene, BkF; indene[1,2,3-*cd*]pyrene), InP) and five OCs [hexachlorobenzene, HCB; hexachlorobutadiene, HCBU; sum of four hexachlorocyclohexane isomers (α-, β-, γ- and δ-hexachlorocyclohexane), HCHs; sum six

dichlorodiphenyltrichloroethanes (*o,p'*-DDT, *p,p'*-DDT *o, p'*-DDD, *p,p'*-DDD *o,p'*-DDE, *p,p'*-DDE), DDTs; sum of three trichlorobenzenes (1,3,5-trichlorobenzene, 1,2,4-trichlorobenzene, 1,2,3-trichlorobenzene), TCBs], in samples from nine sampling sites throughout the Ebro River Basin and over seven years (1996, 1997, 1998, 2000, 2001, 2002 and 2003). The whole data set had 756 entries or concentrations that were arranged as indicated later.

Figure 1 shows the location of the nine sampling sites within the Ebro River Basin and Table 1 reports the description of the main activity at these sites. Three of these sampling sites (R1, T3 and T9) are situated in the north part of the Ebro River Basin, which is an important industrial area also greatly influenced by the urban impact of the cities of Vitoria and Pamplona. T12, R14 and T15 are located in the central part of the Ebro River Basin: T12 correspond to the Gállego catchment, which receives the impact of agricultural activities, R14 is located downstream of the city of Saragossa, and receives industrial and urban wastewaters, and T15 is near Monzón, where there is a chloroalkali industrial complex which produces poly(vinyl chloride) and the pesticide Dicofol. The production of the latter generates DDT as an impurity [18]. The last three sampling sites (T16, R17 and R18) are in the lower part of the Ebro River Basin and are characterized by agricultural activities as well as by industry at R18 (Ebro Delta) and R17 (Flix). At Flix there is a chloroalkali industrial complex which generates hexachlorobenzene and other compounds related to chlorine [19].

Methods

Data arrangement

Before the application of univariate and multivariate methods, data were arranged in a table or a data matrix per year, with nine row samples (sampling sites) and 12 column variables (compounds analyzed). This gave seven data sets or data matrices (7 years). Such a multidata set can be analyzed by PCA using matrix augmentation [20, 21]. Matrix augmentation consists of setting the individual data matrices one on top of the other forming a columnwise augmented data matrix. In our case, the whole data set was arranged in a columnwise augmented data matrix of dimension 63×12, i.e., with 63 row samples and 12 column variables (Fig. 2).

Univariate descriptive statistics

Prior to multivariate data analysis, univariate descriptive statistics (minimum, maximum, mean, median, standard deviation and frequency of detection) of each of the measured variables were calculated at each sampling point. Pairwise correlations between two variables were investigated preliminarily to see the relationships between the compounds studied [22]. This was accomplished by calculating the correlation coefficients between them.

Data pretreatment

Two problems were considered before multivariate data analysis was started: (1) values below the detection limit and (2) missing values. Values below the detection limit were assumed to be equal to half the limit of detection [23]. Approximately half of the entries in the original data were less than the limit of detection and they were not distributed uniformly among variables. Detection limits were set equal to 0.5 μg/kg for all variables. HCHs, HCBU and TCBs were the variables with a higher percentage below their detection limit.

Missing values were handled using the PLS Toolbox (Eigenvector Research, Manson, WA, USA) appropriate functions under MATLAB (The Mathworks, Natick, MA, USA). Using this method, we obtained missing values automatically as a result of mathematically undefined operations [24].

After testing five different pretreatment methods, autoscaling was finally chosen since it gave a better understanding of the composition and distribution of the different contamination sources [26]. With this procedure, the mean of the column elements was subtracted from individual elements and divided by their column standard deviation. Consequently, each column has zero mean and unit variance [21, 25]. In Fig. 3, the box plot of the autoscaled measured variables is given.

Table 1 Description and location of network sampling stations (see Fig. 1)

Code	River	Location	Province	Section	Sector
R1	Ebro	Miranda de Ebro	Burgos (Castilla-Leon)	Upper course	I
T3	Zadorra	Villodas	Álaba (Euskadi)	Upper course	U, I
T9	Arga	Puente la Reina	Navarre	Upper course	I, A
T12	Gallego	Jabarrella	Huesca (Aragon)	Middle course	A
R14	Ebro	Presa de Pina	Saragossa (Aragon)	Middle course	U, A, I
T15	Cinca	Monzón	Huesca (Aragon)	Middle course	I
T16	Segre	Torres de Segre	Lleida (Catalonia)	Lower course	A
R17	Ebro	Flix	Tarragona (Catalonia)	Lower course	I
R18	Ebro	Tortosa	Tarragona (Catalonia)	Lower course	U, A, I

A agricultural, *I* industrial, *U* urban

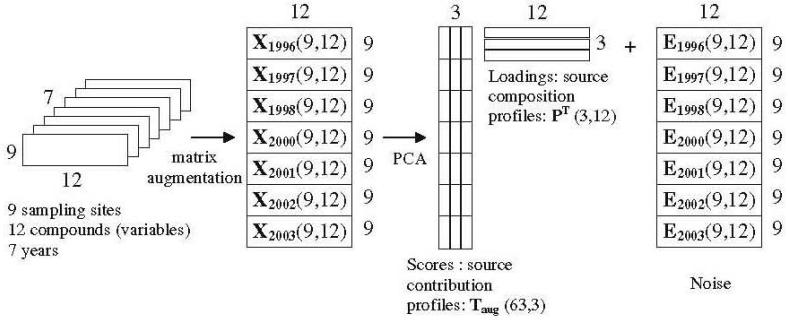

Fig. 2 Data matrix augmentation scheme and bilinear principal component analysis (*PCA*) model decomposition (Eq. 3)

In most of the cases, the median values were lower than the mean values and outliers were only located in the upper part of the box plot. These two considerations confirm that the concentrations of the compounds studied were rather skewed towards lower values. Naph was the compound with the greatest concentration variations but its values were distributed in a more homogeneous way, causing only the presence of three outliers (plus symbols away from whiskers in Fig. 3), meanwhile the other compounds had more (variable 6).

Principal component analysis

Multivariate methods analyze simultaneously for all the samples the whole set of variables instead of analyzing individual variables and samples one by one. This allows a better understanding of environmental processes [15] where diffuse instead of specific contamination prevails. PCA is a data-reduction technique that aims to explain most of the variance in the data, while transforming a set of correlated measured variables into a set of a few uncorrelated components (principal components, PCs) [23], while attempting to preserve at the same time the relationships present in the original data [27]. The main goal of this multivariate statistical technique is to extract useful information and provide an easier visualization of the existent relationships among objects and variables determined in large or complex data sets [28]. PCA can be easily extended to the simultaneous analysis of multiple correlated data sets.

Thus, this multivariate technique can be applied individually to the seven two-way data matrices that correspond to each of the years included in this study and

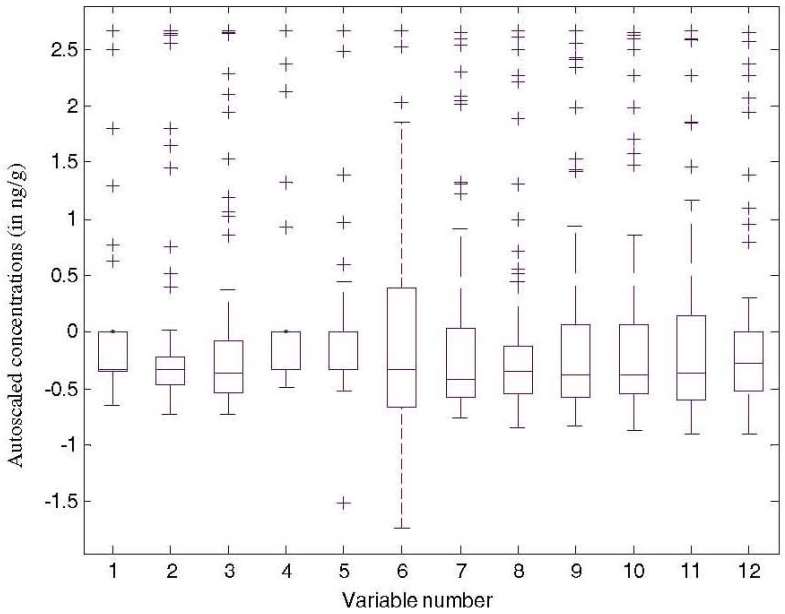

Fig. 3 Box plot of autoscaled measured variables. Each variable has 63 measured values. *HCHs* sum of four hexachlorocyclohexane isomers (*α*-, *β*-, *γ*- and *δ*-hexachlorocyclohexane); *DDTs* sum of six dichlorodiphenyltrichloroethanes (*o,p′*-DDT, *p,p′*-DDT *o,p′*-DDD, *p,p′*-DDD *o,p′*-DDE, *p,p′*-DDE); *HCB* hexachlorobenzene; *HCBU* hexachlorobutadiene; *TCBs* sum of three trichlorobenzenes (1,3,5-trichlorobenzene, 1,2,4-trichlorobenzene, 1,2,3-trichlorobenzene); *Naph* naphthalene; *Flu* fluoranthene; *BaP* benzo[*a*]pyrene; *BbF* benzo[*b*]fluoranthene; *BghiP* benzo[*g,h,i*]perylene; *BkF* benzo[*k*]fluoranthene; *InP* indene(1,2,3-*cd*)pyrene. For each variable, the box has lines at the lower quartile (25%), median (50%) and upper quartile (75%) values. The whiskers are the lines extending from each end of the box to show the extent of the data up to 1.5 times the interquartile range. Outliers are marked with + symbols

also to the augmented data matrix obtained with the reorganization of the multiple data matrices to obtain a single two-way augmented data matrix [28] (Fig. 2). The PCA bilinear model may be written using the following matrix decomposition equation:

$$X = TP^T + E, \tag{1}$$

where the data matrix, X, is decomposed to a scores matrix, T, and a loadings matrix, P^T [29]. This equation can be easily adapted to two situations:

$$X_i = T_i P^T + E_i, \quad i = 1, ..., 7 (analysis\ of\ individual\ matrices) \tag{2}$$

and

$$X_{aug} = T_{aug} P^T + E_{aug}, (analysis\ of\ augmented\ matrix), \tag{3}$$

where matrices X_i are each of the data matrices obtained when the 12 compounds (variables) were measured in the sediments from the nine sampling sites, one for every year (there are a total of seven X_i matrices of dimension 9×12). T_i is the scores matrix, which gives information about the source distribution profiles for the samples of year i. This matrix has a dimension of 9×N, where N is the number of sources detected during data analysis, which are called PCs and are weighted linear combinations of the original measured variables [3, 22]. PCs are extracted so that the maximum amount of variance is explained in the first PC and progressively less variance is explained for each subsequent component [23]. Matrix P^T gives information about the composition profiles of the N detected sources; in other words, this matrix gives information about the contribution of the original variables to each PC [29] (it has a dimension of N×12 and is called the loadings matrix). Finally, matrix E_i refers to the residual data variations not modeled by the N detected sources and it has the same dimension as X_i. In the ideal case, matrix E contains only noise and measurement errors [22], and it may still have a substantial percentage of unexplained data variance coming from multiple minor, unknown sources [3]. For the case of the augmented data matrix (Eq. 3), the only difference is that the original matrix, X_{aug}, has dimension 63×12 and consequently T_{aug} has dimension 63×N and P^T has dimension N×12. In Fig. 2, a detailed description of the data structure for the augmented matrix and of its PCA modeling by PCA are given for the case when the number of resolved components is 3, N=3.

A new row space is constructed with the PCs as new axes, on which the scores are plotted, which are the redefined original samples on the new axes. The plot of the scores in the space defined by the PCs illustrates the dominant patterns present within the samples [23].

PCA modeling was conducted using the PLS Toolbox appropriate functions under the MATLAB computer and visualization environment.

Results and discussion

Univariate descriptive statistics

In Table 2 the values of six different statistics for all the variables studied are given. These statistics were calculated for each sampling site separately but also for the whole data set. The frequency of detection of each compound varied from 7.9 to 92.1% considering the whole data set and from 14.3 to 100% considering the sampling sites individually. Considering the whole data set, the compounds with less frequency of detection were HCBU and HCHs, with 7.9 and 14.3%, respectively, and the compounds with higher frequency of detection were the family of PAHs, especially Flu, all with a frequency of detection between 52.4 and 92.1%. The sampling sites with more compounds with higher frequency of detection were R1, especially for PAHs, T9, T15 and R17, especially for OCs.

HCHs and HCBU had the lowest concentrations of all the compounds investigated, and also their frequency of detection was lower than for the other compounds. The sampling sites with the lowest concentrations of the compounds studied were T12 and R14 and also R18, but only for the family of PAHs. A high concentration of DDTs appears at R17, and also at T15 and R18, at the first two sampling sites owing to industrial activities and at the last site probably owing to agricultural activities. HCB was also high at R17 owing to industrial activities. TCB had its highest concentrations at T3 and T12, but was not detected at other sites. In general, the concentration of the family of PAHs was higher than for the other compounds; Flu had the highest concentrations, followed by BaP, BbF and BkF, and the sampling sites where these compounds had higher concentrations were R1, T3 and T9.

Comparison of the mean and median values of all the variables shows that in all cases the median was lower than or equal to the mean with very different values for DDTs, HCB and Flu. This points out that the data are skewed to lower values and that these are the compounds with more extreme values for some of the years considered in this study. In most cases, standard deviations had the same order of magnitude as the mean values or a higher order of magnitude. This points out that there was a great amount of data dispersion over the different years.

In Table 3, pairwise correlations between all the variables studied are given. Strong correlations were detected between Flu, BaP, BbF, BkF and BghiP, suggesting that these five PAHs may come from a common source [30]. Pairwise correlations between InP and the other compounds of the family of PAHs were moderate with BaP, BbF and BghiP and very low with Flu and BkF. Naph is the only compound of the family of PAHs giving very low correlations with the rest of the compounds of its family and only a moderate correlation with InP; one

Table 2 Descriptive statistics of data

Sampling point[a]	Parameters	Compounds (μg/kg)[b]											
		HCHs	DDTs	HCB	HCBU	TCBs	Naph	Flu	BaP	BbF	BghiP	BkF	InP
R1	FD		57.1			28.6	85.7	100.0	100.0	100.0	85.7	100.0	85.7
	Min		1.7			8.0	31.0	34.0	17.0	25.0	8.5	16.0	9.0
	Max		28.8			70.0	59.0	4461.0	941.0	994.0	412.0	1762.0	78.0
	Median	–	*2.9*	–	–	*39.0*	*40.0*	*156.1*	*50.0*	*260.0*	*35.0*	*60.0*	*28.5*
	Mean		9.1			39.0	41.9	808.0	239.9	400.1	92.8	359.8	32.9
	SD		13.2			43.8	10.3	1619.6	353.9	378.9	157.4	647.8	24.8
T3	FD		28.6	57.1		14.3	57.1	85.7	57.1	71.4	42.9	57.1	57.1
	Min		6.7	1.7		272.0	50.0	93.0	58.0	34.0	66.0	27.0	26.0
	Max		33.4	9.5		272.0	131.0	520.0	137.0	293.0	110.0	188.0	140.0
	Median	–	*2.9*	*6.2*	–	*272.0*	*61.5*	*218.5*	*120.0*	*117.0*	*90.0*	*70.0*	*74.0*
	Mean		20.1	5.9		272.0	76.0	238.3	108.8	144.8	88.7	88.8	78.5
	SD		13.2	3.3			37.7	153.9	35.7	97.8	22.0	69.7	47.1
T9	FD		85.7	57.1		14.3	57.1	100.0	71.4	85.7	100.0	85.7	85.7
	Min		3.6	0.7		35.0	24.8	102.0	57.3	86.4	32.7	40.2	40.2
	Max		190.4	17.9		35.0	90.0	590.0	365.0	559.0	328.0	453.0	335.0
	Median	–	*29.0*	*4.4*	–	*35.0*	*57.5*	*390.0*	*230.0*	*268.5*	*133.0*	*121.5*	*216.0*
	Mean		61.0	6.8		35.0	57.4	355.2	237.7	281.9	144.5	197.2	192.0
	SD		72.1	7.8			32.8	183.6	119.8	155.9	102.6	160.6	125.9
T12	FD	71.4	14.3	71.4		28.6	57.1	85.7	57.1	85.7	71.4	42.9	42.9
	Min	1.4	5.7	0.5		27.0	23.0	3.5	1.4	4.9	4.8	1.7	1.6
	Max	9.1	5.7	7.4		101.0	40.0	22.0	10.0	37.0	35.0	47.0	14.0
	Median	*4.0*	*5.7*	*1.0*	–	*64.0*	*27.0*	*20.0*	*4.3*	*15.0*	*12.0*	*10.0*	*7.0*
	Mean	4.7	5.7	2.4		64.0	29.3	15.6	5.0	16.5	16.4	19.6	7.5
	SD	3.3		2.9		52.3	7.4	8.1	3.6	11.9	11.8	24.1	6.2
R14	FD	28.6	71.4	42.9	14.3	14.3	57.1	100.0	85.7	71.4	42.9	57.1	28.6
	Min	0.3	1.3	0.7	6.1	47.0	17.0	5.0	5.4	8.2	20.0	4.6	11.0
	Max	3.5	25.4	15.0	6.1	47.0	39.6	123.0	57.0	72.0	36.0	63.0	30.0
	Median	*1.9*	*10.7*	*1.0*	*6.1*	*47.0*	*26.0*	*20.0*	*15.0*	*16.0*	*21.0*	*19.0*	*20.5*
	Mean	1.9	12.4	5.6	6.1	47.0	27.2	37.6	21.2	25.8	25.7	26.4	20.5
	SD	2.3	9.8	8.2			9.9	41.4	19.0	26.4	9.0	26.6	13.4
T15	FD		85.7	85.7	14.3	14.3	42.9	100.0	71.4	100.0	85.7	71.4	57.1
	Min		16.7	2.2	2.1	42.0	26.0	7.2	10.0	11.0	9.7	9.0	16.0
	Max		2081.0	68.9	2.1	42.0	43.0	130.0	40.0	110.0	70.0	40.0	60.0
	Median	–	*88.3*	*17.0*	*2.1*	*42.0*	*27.6*	*24.0*	*17.0*	*20.5*	*19.0*	*15.0*	*30.0*
	Mean		585.3	22.6	2.1	42.0	32.2	40.0	23.3	32.8	27.9	18.4	34.0
	SD		863.6	24.7			9.4	42.5	13.7	34.8	23.5	12.7	19.0
T16	FD		42.9	14.3		14.3	42.9	71.4	42.9	57.1	42.9	42.9	28.6
	Min		0.6	1.2		30.0	12.9	6.0	6.0	7.0	5.0	6.0	4.0
	Max		4.8	1.2		30.0	24.0	39.0	12.4	24.0	12.0	35.0	11.6
	Median	–	*1.4*	*1.2*	–	*30.0*	*13.0*	*17.0*	*12.0*	*13.2*	*5.7*	*10.1*	*7.8*
	Mean		2.3	1.2		30.0	16.6	18.1	10.1	14.4	7.6	17.0	7.8
	SD		2.2				6.4	12.9	3.6	7.5	3.9	15.7	5.4
R17	FD	14.3	100.0	100.0	28.6	14.3	57.1	85.7	71.4	85.7	71.4	57.1	57.1
	Min	5.0	11.0	6.3	3.0	47.1	14.0	10.6	4.3	8.7	3.8	3.8	3.3
	Max	5.0	79,287.0	4,532.0	4.0	47.1	46.0	840.0	270.0	310.0	190.0	150.0	240.0
	Median	*5.0*	*190.0*	*38.0*	*3.5*	*47.1*	*14.7*	*54.5*	*24.0*	*28.0*	*20.0*	*34.5*	*15.0*
	Mean	5.0	13,510.4	796.7	3.5	47.1	22.3	229.6	68.7	73.3	50.0	55.7	68.3
	SD		29,457.2	1,668.4	0.7		15.8	334.7	113.2	116.6	78.8	64.6	114.8

Table2 (continued)

Sampling point[a]	Parameters	Compounds (μg/kg)[b]											
		HCHs	DDTs	HCB	HCBU	TCBs	Naph	Flu	BaP	BbF	BghiP	BkF	InP
R18	FD	14.3	85.7	71.4	14.3	42.9	28.6	100.0	57.1	28.6	28.6	28.6	28.6
	Min	3.0	63.9	4.6	8.7	8.0	12.4	1.0	3.0	4.0	3.0	4.0	3.0
	Max	3.0	1,774.0	68.4	8.7	60.0	42.0	38.0	15.3	20.0	20.0	14.8	15.3
	Median	*3.0*	*224.0*	*26.0*	*8.7*	*14.0*	*27.2*	*20.0*	*8.5*	*12.0*	*11.5*	*9.4*	*9.1*
	Mean	3.0	474.9	33.1	8.7	27.3	27.2	18.0	8.8	12.0	11.5	9.4	9.1
	SD		646.0	24.4		28.4	20.9	11.3	5.2	11.3	12.0	7.6	8.7
Total	FD	14.3	63.5	55.6	7.9	20.6	54.0	92.1	68.3	76.2	63.5	60.3	52.4
	Min	0.3	0.6	0.5	2.1	8.0	12.4	1.0	1.4	4.0	3.0	1.7	1.6
	Max	9.1	79,287	4,532	8.7	272	131	4,461	941	994	412	1,762	335
	Median	*3.5*	*31.1*	*7.4*	*4.0*	*42.0*	*32.2*	*38.5*	*24.0*	*28.5*	*25.0*	*34.8*	*27.0*
	Mean	3.9	2,536	170	4.8	58.5	38.3	204	92.5	129.1	61.4	121.2	65.8
	SD	2.7	12,645	770	2.7	69.2	25.1	598	173	209	88.9	299.1	90.9

FD frequency of detection, *SD* standard deviation, *HCHs* sum of four hexachlorocyclohexane isomers (α-, β-, γ- and δ-hexachlorocyclohexane), *DDTs* sum of six dichlorodiphenyltrichloroethanes (*o,p'*-DDT, *p,p'*-DDT *o,p'*-DDD, *p,p'*-DDD *o,p'*-DDE, *p,p'*-DDE), *HCB* hexachlorobenzene, *HCBU* hexachlorobutadiene, *TCBs* sum of three trichlorobenzenes (1,3,5-trichlorobenzene, 1,2,4-trichlorobenzene, 1,2,3-trichlorobenzene), *Naph* naphthalene, *Flu* fluoranthene, *BaP* benzo[*a*]pyrene, *BbF* benzo[*b*]fluoranthene, *BghiP* benzo[*g,h,i*]perylene, *BkF* benzo[*k*]fluoranthene, *InP* indene(1,2,3-*cd*)pyrene

[a]Statistical analysis of the distribution of the variables (see legend to Fig. 3). Values given for individual sampling sites correspond to sampling campaigns of 7 years (1996 2003). Values given for *Total* correspond to nine sampling sites for sampling campaigns of 7 years (1996 2003).

[b]Concentration of compounds studied (see legend to Fig. 3)

reason could be that Naph was the only PAH studied that has only two aromatic rings, while the others have between four and six, and this characteristic confers a higher volatility to this compound than to the other PAHs. There were no significant correlations between the rest of the compounds analyzed, except between HCBU and HCB and between Naph and TCBs, which were moderately correlated. Better understanding about multivariate correlations among different variables is obtained by the application of PCA [15].

Principal component analysis results

Table 4 shows the results of PCA when it was applied to the augmented autoscaled data matrix X_{63x12} and also to the seven individually autoscaled data matrices X_{9x12}, corresponding to the seven different sampling campaigns, one per year.

Considering individually the seven data matrices, between 84.78 and 96.87% of the data variance for each year was explained using three PCs. The amount of variance explained for each PC was rather similar for the 7 years considered. These two facts suggested that the contamination sources were rather similar from one year to

Table 3 Pairwise correlation coefficients between the variables

	HCHs	DDTs	HCB	HCBU	TCBs	Naph	Flu	BaP	BbF	BghiP	BkF	InP
HCHs	1.00											
DDTs	−0.05	1.00										
HCB	−0.03	0.16	1.00									
HCBU	0.01	0.02	*0.32*	1.00								
TCBs	0.03	−0.02	0.10	−0.01	1.00							
Naph	−0.22	−0.01	0.10	0.02	*0.41*	1.00						
Flu	−0.09	−0.02	0.07	−0.04	0.17	−0.02	1.00					
BaP	−0.13	−0.04	−0.03	−0.10	0.06	0.09	*0.87*	1.00				
BbF	−0.13	−0.05	−0.05	−0.13	0.03	0.16	*0.75*	*0.84*	1.00			
BghiP	−0.09	−0.03	−0.03	−0.11	0.04	0.17	*0.78*	*0.86*	*0.77*	1.00		
BkF	−0.08	−0.03	−0.04	−0.07	0.16	−0.02	*0.95*	*0.89*	*0.79*	*0.76*	1.00	
InP	−0.14	−0.03	−0.01	−0.10	−0.15	0.27	0.18	*0.48*	*0.42*	*0.64*	0.13	1.00

The variables are the concentrations of the compounds studied (see legend to Fig. 3). Higher pairwise correlations (greater than 0.32) are marked in *italics* (significance 95%)

Table 4 Percentages of explained variances obtained by principal component analysis

Matrix	PC1	PC2	PC3
X_{1996}[a]	49.71	34.31 (84.02)	8.51 (92.53)
X_{1997}[a]	52.42	23.72 (76.14)	12.28 (88.43)
X_{1998}[a]	73.10	15.65 (88.75)	8.12 (96.87)
X_{2000}[a]	59.07	20.15 (79.22)	11.20 (90.42)
X_{2001}[a]	57.28	17.25 (74.53)	10.25 (84.78)
X_{2002}[a]	54.70	25.41 (80.10)	10.98 (91.08)
X_{2003}[a]	60.78	15.60 (76.38)	11.59 (87.97)
$X_{1996\ 2003}$[b]	52.29	16.81 (69.10)	9.41 (78.51)

The percentage of accumulated explained variance for that particular component is given in *parentheses*
PC principal component
[a]Individual data matrices for each year
[b]Augmented data matrix for all years

another. Considering the augmented data matrix, the total amount of variance explained with three PCs decreased only up to 78.51%, and also the amount of variance explained for each PC was similar to that for the individual data sets. This similar behavior in the results obtained with the application of PCA to all the data sets confirmed that the contamination sources were the same over the 7 years of the study. For this reason and considering also that the loadings and scores obtained in the analysis of individual data matrices were similar to those obtained in the analysis of the augmented data matrix, only the results of the analysis of the augmented matrix are presented here for brevity. Using the first three PCs from the PCA analysis, we reduced the data dimensionality from the 12 original variables to the three new PCs (75% reduction), with only 21.49% of the original information lost.

In Fig. 4, the loadings plot for the first three PCs of the augmented matrix is given. The first PC explained 52.29% of the total data variance and it had high positive loadings for all PAHs, more or less at the same level except for Naph, which is the most volatile compound of this group and has slightly different chemical behavior. In the analysis of the correlation coefficients, there was strong correlation between all these compounds with the exception of Naph, which is reflected here with a much lower loading. This can be attributed to the physicochemical properties of this compound, in accordance also with what Mackay fugacity models predict for PAHs [31]. Naph is found to partition primarily into the air because of its relatively high volatility and is expected to exhibit an equilibrium distribution of 73.5 and 17% in the air and soil, respectively. When the number of aromatic rings increases, the PAHs tend to be associated more with organic media in soils and sediments. The moderate correlation between InP and the rest of PAHs is also reflected with a slightly lower loading. This first PC identifies a general contamination profile by PAHs, showing a common origin of all these compounds, basically combustion processes [32]. In this first PC, OCs had very low loadings and correlated negatively with the PAH family of compounds, showing that in samples where PAH concentrations are high, concentrations of OCs are low. The second PC explained 16.81% of the total data variance and had very low loadings for PAHs and, in contrast, high positive loadings for three of the OCs studied (DDTs, HCB and HCBU). Pairwise correlation analysis only pointed out the correlation between HCB and HCBU but not with DDTs, so the analysis by PCA identifies more clearly the contamination profile of OCs. These compounds have mainly an agricultural origin because they are used as pesticides, but they also have some applications in

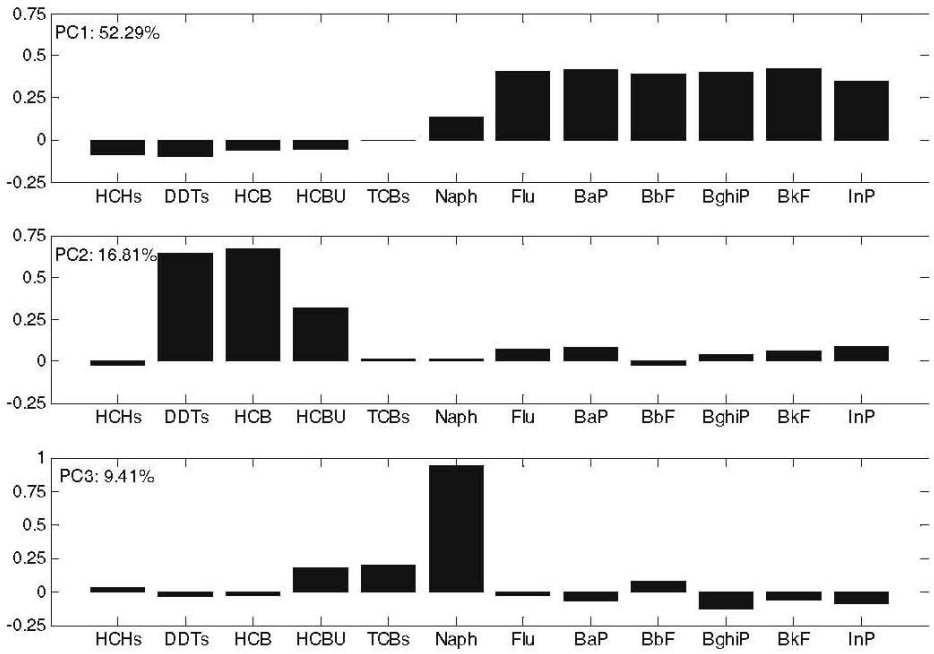

Fig. 4 Loadings for the three principal components (*PCs*; matrix P^T in Eq. 3 and Fig. 2)

industry. Finally, the third PC explained 9.41% of the total data variance and it had only a high loading for Naph and a much lower contribution for TCBs and HCBU. This PC shows a more specific profile of contamination of Naph, accompanied with TCBs and HCBU.

Scores plots of the augmented data matrix are given in Fig. 5.

In Fig. 5a, a PC1 vs. PC2 scores plot is shown. In this plot, four groups were distinguished, a first cluster of samples with different degrees of positive scores on the first PC and very low scores on the second PC. This sample cluster included sampling sites T9, R1 and T3. All these sampling sites were contaminated by PAHs to a greater of lesser degree depending on the year. The samples that are situated further from the origin of the coordinates were more contaminated by this contamination profile. No temporal trends were observed in the distribution of the three sampling sites along PC1, showing that the contamination by the first contamination profile is persistent over the years. These three sampling sites are located in the upper Ebro River Basin, close to Pamplona (Navarre) and Vitoria (Euskadi), which is an important industrial region. The second sample cluster was defined by positive scores on PC2 and low negative scores on PC1. Sampling sites

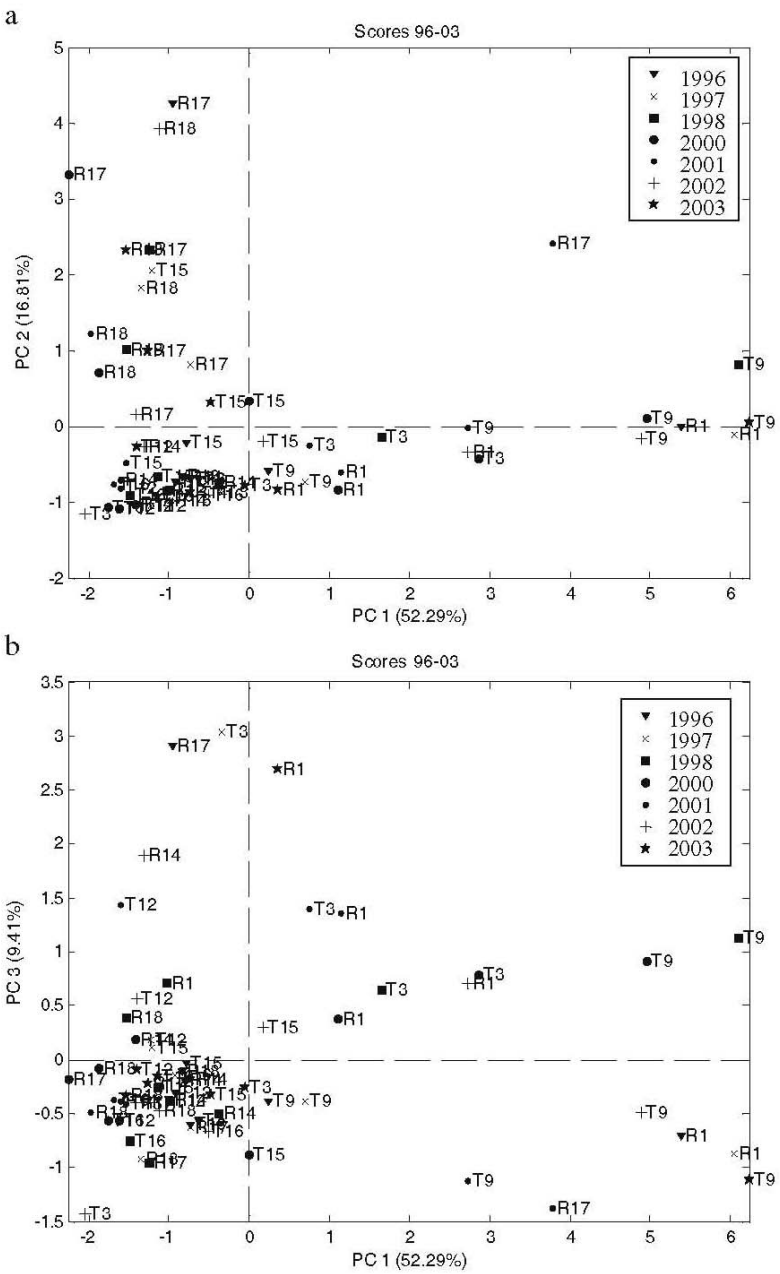

Fig. 5 Scores plots from PCA (matrix T_{aug} in Eq. 3 and Fig. 2). All sediment samples (all sampling sites identified in Table 1 and years given in the *top right*) were included in the analysis. **a** For the first two principal components; **b** for the first and the third principal components

included in this group were basically R17 and R18 and they had different levels of contamination by OCs. The level of contamination in the samples from this cluster was also irregular over the years considered and was without a clear diminution or augmentation trend. These two sampling sites are located at the lower Ebro River course, a region that is basically agricultural but that also has some industry. These two clusters showed two distinct sources of diffuse contamination either of PAHs or of OCs that were persistent over the years considered because for most of the years the samples from these sampling sites were included in the same cluster. A third cluster had only sampling site R17 from 2001 and was the only sample that showed a very high score for PC1 and PC2, showing a high concentration of PAHs and OCs at the same time. This was a specific contamination that was only present for sample R17 and for 2001. The rest of the sampling sites formed the fourth cluster and were concentrated near the origin of the coordinates, showing low concentrations for all the compounds analyzed. These sampling sites corresponded to the less contaminated samples in the center of the Ebro River Basin.

In Fig. 5b, a PC1 vs. PC3 scores plot is shown. The separation between the samples in the first cluster of the previous plot (T9, R1 and T3) was achieved according to the PC3 score. This means a difference in concentration of Naph in these samples. In this case there was no temporal trend in the concentration of Naph. A second cluster with high positive scores for PC3 was identified for samples from some specific years with no clear relation among them: R1 for 2003, T3 for 1997, R17 for 1996, R14 for 2002 and T12 for 2001. All these samples were in the cluster without contamination in the first scores plot, except R17. This means that these samples were specifically contaminated only by Naph. T12 and R14 are samples from the Gállego catchment. According to this plot all the other samples showed no specific contamination by Naph.

Conclusions

A multivariate statistical data analysis, using PCA, of historical data concerning the concentration of different PAHs and OCs in sediment samples from different sampling sites of the Ebro River Basin revealed three main contamination profiles. The first contamination profile was identified mostly loaded by PAHs. A group of samples which included sampling sites R1 (Ebro river in Miranda de Ebro, La Rioja), T3 (Zadorra river in Villodas, Alava) and T9 (Arga river in Puente la Reina, Navarre), all located in the upper Ebro River Basin and close to Pamplona and Vitoria, were mostly contaminated by this contamination profile. A second contamination profile was detected loaded mostly by OCs. A group of samples including R17 (Ebro river in Flix, Tarragona) and R18 (Ebro river in Tortosa, Tarragona), both located in the lower course of the Ebro River, were highly contaminated by this second contamination profile. And finally, a third contamination profile more specific for Naph was also detected. A group of samples with no clear relation among them (R1 for 2003, T3 for 1997, R17 for 1996, R14 for 2002 and T12 for 2001) was highly contaminated by this contamination profile, in which all the samples except R17 for 1996 were the only ones specifically contaminated by the contamination profile of Naph and not by the other contamination sources. In the group of samples mostly contaminated by the contamination profile of PAHs, the samples are also differentiated depending on the degree of contamination of this third contamination profile. The rest of the sampling sites, located in the center of the river basin, showed lower contributions of the three contamination profiles and therefore they were the less contaminated samples. The PAH and OC contamination profiles were diffuse and persistent over the years considered, while the Naph contamination profile was more specific. For these three resolved sources no temporal trends were observed in the sampling sites under study.

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4.3.- Anàlisi de l'estat actual

4.3.1.- Compostos prioritaris en aigües de la conca de l'Ebre

Tot i que inicialment a les aigües es van analitzar els 68 compostos seleccionats en aquesta tesi, l'article científic 5 es va centrar només en l'estudi dels grups d'APs, plastificants i pesticides anomenats anteriorment polars (triazines, organofosforats, carbamats i compostos amb base d'anilina), ja que tenen una tendència més hidrofílica i per tant una major probabilitat de trobar-se en aigües superficials (Taules 3.2, 3.3. i 3.4). En aquest article es pretenia avaluar l'impacte d'aquests pesticides en aigües de zones amb activitat agrícola de la conca del riu Ebre, on predominen clarament la vinya, el blat de moro i els arbres fruiters, i dels compostos d'origen industrial més comuns en aigües.

La legislació espanyola autoritza l'ús dels pesticides segons els cultius, especificant també el percentatge permès de principi actiu. D'aquesta manera, existeix la possibilitat que un pesticida pugui ser utilitzat en un tipus de cultiu i no en un altre. A efectes pràctics, tots els pesticides estan en venda a les cooperatives agrícoles i la responsabilitat última del seu ús recau en l'agricultor. Entre els herbicides permesos a l'inici d'aquest estudi, el 2004, trobàvem l'alaclor, l'atrazina, la simazina i la terbutilazina. Les cloroacetanilides i les triazines han estat probablement els herbicides més utilitzats a Espanya. Per a ambdues famílies existeix un compost de base, alaclor i atrazina respectivament, a partir dels quals es sintetitzen derivats d'eficàcia similar (metolaclor, acetoclor, simazina, terbutilazina, terbutrina...). Pel que fa als insecticides permesos per als cultius mencionats, l'any 2004 trobem clorpirifòs, malation, paration, fenitrotion, diazinon o dimetoat, tots ells insecticides organofosforats. També es van estudiar altres pesticides àmpliament utilitzats en el sector agrícola com ara el propanil, la trifluralina o el molinat.

Molts d'aquests pesticides s'han prohibit recentment, però a la restricció o prohibició d'un compost fitosanitari, com són els pesticides, li segueix la comercialització d'un compost de la mateixa família amb alguna variació de cadena o grup funcional. Com a exemple, la recent prohibició del metolaclor (isòmer R) i la seva substitució per S-metolaclor, on simplement s'ha modificat la estereoisomeria de la molècula, fet que permet al fabricant complir la legislació

sense necessitat d'una gran inversió en el desenvolupament d'un nou pesticida. Un cas similar s'ha donat en els últims anys amb l'ús de l'atrazina, que es va veure lentament reemplaçada per la simazina i recentment, davant la prohibició d'aquesta última, per la terbutilazina. D'aquesta manera tant l'administració com els fabricants entren en un joc convenient a les dues parts en el que l'administrador mostra el seu poder legislatiu i garanteix en certa manera la conservació del medi a canvi d'acotar les prohibicions a molècules molt específiques fàcilment reemplaçables pels fabricants.

A part de tots aquests pesticides i alguns dels seus productes de degradació també es van analitzar el BPA, el TBP, el NP i l'OP, que a més dels seus usos industrials també es troben a moltes formulacions de pesticides. Els dos primers són àmpliament utilitzats com a plastificants, una indústria molt estesa actualment. Pel que fa als APs, les propietats surfactants dels seus precursors, els APEOs, fan que s'utilitzin àmpliament com a detergents. Tot i que actualment estan prohibits en detergents d'ús domèstic, en els industrials encara estan permesos i a més són adequats per a qualsevol tipus d'indústria. Aquest fet pot explicar la seva àmplia distribució al medi, al no ser compostos específics d'un determinat tipus d'indústria.

Els resultats i conclusions de l'estudi dels pesticides polars, els plastificants i els APs en aigües es recullen a l'article científic 5, reproduït a continuació.

Article científic 5

Environmental influences on the Ebro Bsin inferred from statistical treatment of organic river water chemistry

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Environmental influences on the Ebro Basin inferred from statistical treatment of organic river water chemistry

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ABSTRACT

We report temporal and geographical variations of a set of 30 pesticides (including triazines, organophosphorus and acetanilides) and industrial compounds in surface waters along the Ebro River during the period 2004-2006. Using descriptive statistics (minimum, maximum, mean, median, standard deviation and frequency of detection) we found that the compounds with industrial origin (tributylphosphate, octylphenol and nonylphenol) appeared in over 60% of the samples analyzed and with very high concentrations, while pesticides had a point source origin in the Ebro Delta area and overall low-levels, between 0.005 and 2.575 μg L^{-1}. Correlations among pollutants and their distributions were studied using Principal Component Analysis (PCA), a multivariate exploratory data analysis technique which permitted us to discern between agricultural and industrial source contamination. Over a 3-year period, seasonal analyses revealed highest concentrations of pesticides over the spring-summer period following pesticide application.

Keywords: Monitoring; alkylphenols; pesticides; water; Principal Component Analysis (PCA)

1. Introduction

Europe has historically been a hotspot of environmental pressures due to the contamination caused by agricultural, municipal and industrial activities and high population densities (Barth et al., 2009; Hildebrandt et al., 2008). Such contamination has lead to poor water quality in many European river basins (Gunningham and Sinclair, 2005; Loos et al., 2009; Novotny, 1999; Palma et al., 2008; Spalding and Exner, 1993; Vryzas et al., 2009). In addition, this pollution can cause the accumulation into river sediments of toxic compounds such as pesticides (Zhou et al., 2001), surfactants (Ying et al., 2002), alkanes and alkyl polycyclic aromatic hydrocarbons (Yunker et al., 1999). These can in turn act as a source to biota (Crane, 2003) and as a potential risk for entire ecosystems (Carvalho et al., 2002) if the compounds bioaccumulate, entering in this way the food chain (Fernandez and Grimalt, 2003).

As a result, the European Union (EU) has published directives aimed at the protection of the river basins from a range of substances included in the priority lists (European Commission, 1998; European Commission, 2006). Some of the European legislation on environmental matrices includes directives such as 76/464/CEE concerning the analysis of 132 toxic and persistent compounds in environmental matrices, the Water Framework Directive (2006/11/CE) aimed at improving the ecological quality of surface waters and the Directive 2008/60/CE, that includes maximum environmental concentrations for water (European Council, 2008). Thus monitoring studies are needed to evaluate diffuse and point source pollution and identify historic pollution present in water and sediments for future remediation, if appropriate.

Until recently, most studies performed within the Ebro River have been site-specific or focused on a single chemical family (Amaral et al., 1996; Eljarrat et al., 2007; Gómez-Gutierrez et al., 2006; Pastor et al., 2004; Santos et al., 2000; Terrado et al., 2007). However, little is known about the concentration and patterns of a wide spectrum of priority contaminants in the whole Ebro aquatic ecosystem. In 2003, a more complete study concerning the entire river basin, various environmental matrices (sediments and two fish species) and five different chemical families (organochlorine compounds, polycyclic aromatic hydrocarbons, organotin compounds, alkylphenols and polybrominated diphenyl ethers) was carried out (Lacorte et al., 2006). In this study the authors assessed the levels of contaminants in the Ebro river basin and evaluated their environmental risk. They concluded that is necessary to extend this kind of monitorings for measuring temporal tendencies and avoid possible deleterious effects towards freshwater organisms of any river ecosystem.

The further inclusion of the Ebro river basin into the European Union project AquaTerra (contract no. 505428) led to a complete monitoring study of the basin that took into consideration the study of contamination over the years. The starting point of the Ebro study presented here was a chemometrical study of historical data (1996-2003) from Confederación Hidrografica del Ebro (CHE) (Navarro et al., 2006), the organization in charge of the management of the Ebro river basin. Based on this data set, a detailed monitoring program of

water and sediments including compounds such as PAHs, historical and current polar and chlorinated pesticides and alkylphenols was performed over the period 2004-2006. Environmental pollution by these groups of organic compounds has received considerable attention as a result of public awareness towards environmental problems such as bioaccumulation and the potential of endocrine disrupting capabilities. Such effects counter the expectations for good quality of ecosystem and human life (Zhou et al., 2001).

The results presented here are part of this complete monitoring, which included both water and sediments matrices. Organic compounds partitioning in water or sediment provide a different profile of detected contaminants, therefore each matrix has been considered separately. The main goal of the study presented here is to evaluate the vulnerability of the Ebro River basin using the water data obtained in the monitoring program. In addition to other studies mentioned above, contamination was evaluated in the entire basin and over a long period of time. Data was compared with legislated environmental levels to determine the impact of the studied compounds in the environment. A set of statistical techniques was applied to obtain temporal and geographical tendencies and to distinguish between households, industry and agriculture as the main sources of pollution. A deep study about the presence and correlations of different pollutants was done using Principal Component Analysis (PCA) as a multivariate exploratory data analysis technique. PCA is a frequently used multivariate statistical technique with the aim of data compression, exploration and interpretation (Smeyers-Verbeke et al., 1984).

2. Experimental data

2.1. Site description and selection of sampling sites

The Ebro River Basin (NE Spain) (Fig. 1) drains an area of approximately 85,000 km2, discharges into Mediterranean Sea and forms a delta of more than 300 km2. The Ebro River is 910 km long and receives waters from several tributaries, which altogether represent 12,000 km of waterway network. The entire basin hosts more than 2.7 million inhabitants, with 33% of the population living in small villages with less than 5,000 inhabitants, 22% in towns between 5,000 and 10,000 and only 5 cities located next to the Ebro River or its tributaries concentrate approximately 45% of the region population (Navarro et al., 2006). To its largest part, the Ebro Basin is agricultural land and counts among the most irrigated areas in Spain, but lately industry has been a growing sector (CHE, 2008). The most important plant types are grasses for grazing, wine production (La Rioja), fruit trees (Lleida) and rice (Ebro Delta) (Petrovic et al., 2002). Industrial activities are concentrated close to the most important cities, with Zaragoza being the most important industrial centre (automotive and chemical industry). The northern part of the basin with the cities Vitoria, Pamplona, Logroño and Miranda de Ebro (food industry) as well as the area around the cities of Lleida, Monzón and Flix (chemical industry), also host important industrial areas (Fig. 1).

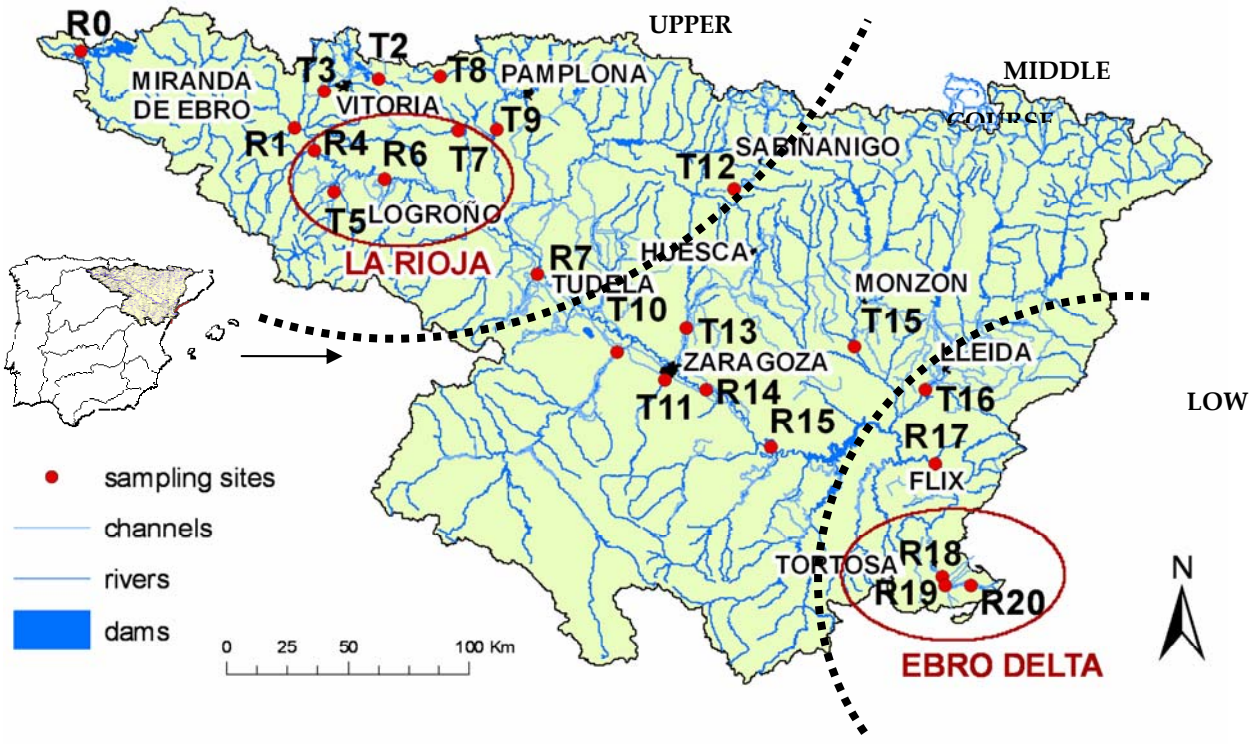

Fig. 1 Sampling sites location in the Ebro River Basin analyzed in this work. Site numbers correspond to Table 1

Table 1 Description and location of network sampling sites (see Fig. 1)

Code	River	Location	Province	Section	Sector[a]
R0	Ebro	Nestares	Cantabria	Upper course	Source
R1	Ebro	Miranda de Ebro	Burgos (Castilla Leon)	Upper course	I
T2	Zadorra	Audikana	Álaba (Euskadi)	Upper course	I
T3	Zadorra	Villodas	Álaba (Euskadi)	Upper course	U, I, A
R4	Ebro	Haro	La Rioja	Upper course	U, A
T5	Najerilla	Nájera	La Rioja	Upper course	A
R6	Ebro	Logroño	La Rioja	Upper course	I, U, A
T7	Ega	Estella	Navarra	Upper course	I
R7	Ebro	Tudela	Navarra	Upper course	U, A
T8	Araquil	Alsasua	Navarra	Upper course	I
T9	Arga	Puente la Reina	Navarra	Upper course	I
T10	Jalón	Grisén	Zaragoza (Aragón)	Middle course	I, A
T11	Huerva	Zaragoza	Zaragoza (Aragón)	Middle course	I, U
T12	Gállego	Jabarrella	Huesca (Aragón)	Upper course	A
T13	Gállego	San Mateo de Gállego	Zaragoza (Aragón)	Middle course	A, I
R14	Ebro	Presa de Pina	Zaragoza (Aragón)	Middle course	U, A, I
T15	Cinca	Monzón	Huesca (Aragón)	Middle course	I, A
R15	Ebro	Sástago	Zaragoza (Aragón)	Middle course	A
T16	Segre	Torres de Segre	Lleida (Catalunya)	Lower course	A
R17	Ebro	Flix	Tarragona (Catalunya)	Lower course	I, A
R18	Ebro	Tortosa	Tarragona (Catalunya)	Lower course	U, A, I
R19	Ebro	Amposta	Tarragona (Catalunya)	Lower course	A
R20	Ebro	Deltebre	Tarragona (Catalunya)	Lower course	A, mouth

[a] A: agricultural, I: industrial, U: urban

The monitoring undertaken in this study comprised six sampling campaigns during three years, between 2004 and 2006. Each year two sampling campaigns were carried out, the first one in June and the second one in October. This monitoring study included 23 sampling sites covering the whole Ebro river basin (11 on the Ebro river and 12 on main tributaries) from the most vulnerable sites, based on proximity to big cities, agricultural areas or industrial activities and knowledge of historical contamination episodes. Their specific locations are shown in Fig. 1, and are numbered consecutively following the Ebro river flow, from north-west to south-east. "R" indicates a site on the Ebro River whereas "T" indicates a tributary site. Among all the sampling sites, one 6 km downstream from the Ebro source (R0) and another one just before the sea (R20) are included. Table 1 lists the locations of each sampling site, the corresponding river, the section of the river basin and the main economical activities in the area. The 23 sampling sites are divided into three groups depending on their situation in the basin. From R0 to T9 and T12 are considered upper course, from T10 to T15 are part of the middle course and from T16 to R20, lower course. They receive different impact according to local activities.

At each site, water and air temperature, pH, dissolved oxygen and conductivity were measured in situ with an YSI 556 Multi Probe System/Data Logger from YSI Incorporated (Yellow Springs, OH, USA). At the same sites, water samples were collected using a stainless steel holder containing an amber glass bottle. The holder was lowered from a bridge in the middle of the water stream to a depth of 50 cm below the water surface in order to obtain a representative sample and to avoid the entrance of floating particles into the bottle. In few cases the stream was not deep enough or no bridge was available and the water was collected manually by submerging the bottle in the river a few meters from the shore. Samples were collected in 1 L single-use amber PET bottles and transported at 4 ºC in the dark to the laboratory. Analyses were performed within maximum one week. A fraction of each sample was used to measure the non-purgeable organic carbon (NPOC).

2.2. Standards and Chemicals used

Over 92.5% purity chemical compounds were purchased as a mixture from Dr. Ehrenstorfer (Augsburg, Germany) at 100 µg mL^{-1} in ethyl acetate: alachlor, atrazine, azinphos-ethyl, bisphenol A, bromophos-ethyl, bromophos-methyl, chlorfenvinfos, chlorpyrifos, desethyl-atrazine, diazinon, dichlofenthion, dimethoate, ethion, fenchlorfos, fenitrothion, malathion, metolachlor, molinate,

nonylphenol, octylphenol, omethoate, parathion-ethyl, parathion-methyl, propanil, propazine, simazine, terbutryn, terbutylazine, tributylphosphate and trifluralin. Single deuterium labeled surrogates (desethyl-atrazine-D6, atrazine-D5, alachlor-D13, parathion-ethyl-D10 and nonylphenol-D8) and an internal standard (terbutylazine-D5) were purchased from Dr. Ehrenstorfer at concentrations of 100 µg mL^{-1} in acetone. Standard working solutions were diluted from the commercial ones in hexane. SPE extraction cartridges Oasis HLB 60 mg (3 cm3) were from Waters (Milford, MA USA) and 0.45 µm nylon filters were from Whatman (Maidstone, UK). HPLC grade methanol and water and GC grade dichloromethane, ethyl acetate and hexane were purchased from Merck (Darmstadt, Germany). Nitrogen of 99.995% purity used as drying stream was from Air Liquide (Paris, France).

2.3. Sample extraction

Prior to extraction, samples were filtered through 0.45 µm nylon membrane filters to remove any particulate material. Desethyl-atrazine-D6, atrazine-D5, alachlor-D13, parathion-ethyl-D10 and nonylphenol-D8 were added as a pool of surrogate standards to the filtered water to obtain a final concentration of 0.2 µg L^{-1}. Next, the compounds were extracted from 200 mL of water sample using Oasis HLB SPE cartridges. Cartridges were firstly conditioned by purging with 5 mL of dichloromethane: ethyl acetate (1:1), 5 mL of hexane: dichloromethane (1:1), 1 mL of methanol and 1 mL of water. Then, samples were percolated using a Baker Vacuum system from J.T. Baker (Phillipsburg, NJ USA) at a flow rate of 6 mL min^{-1}. Afterwards, cartridges were rinsed with 2 mL of HPLC water to remove matrix interferences, then dried under vacuum for about 20 minutes to remove water. Immediately afterwards they were eluted with 5 mL hexane: dichloromethane (1:1) and 3 mL dichloromethane: ethyl acetate (1:1) followed by 2 mL of pushing air at a rate 1 mL min^{-1} using an automated ASPEC XL system from Gilson (Middleton, WI USA). These extracts were evaporated to almost dryness with a Reacti-Vap III from Pierce (Rockford, IL USA) operating under a laminar stream of nitrogen. Finally these purified extracts were taken up again in 250 µL of hexane in an amber glass vial. At this stage, the internal standard terbutylazine-D5 was added with an amount of 160 µg L^{-1}.

2.4. Instrumental analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was performed with a gas chromatograph Thermo Electron (San Jose, CA USA) model Trace 2000 coupled to a mass spectrometer from Thermo Electron. The mass spectrometer was operated in the electron impact ionization mode with an ionizing energy of 70 eV. Compound separation was achieved using a capillary column HP-5MS of 30 m x 0.25 mm i.d. with 0.25 µm film thickness from J&W Scientific (Folsom, CA USA) with the temperature program: from 60 ºC (holding time 1 min) to 130 ºC at 10 ºC min^{-1} to 220 ºC at 3 ºC min^{-1} and finally to 300 ºC at 10 ºC min^{-1} (holding time 5 min). Injection volume was of 2 µl in splitless mode keeping the split valve closed for 0.8 min before purging. Helium was used as carrier gas at a flow of 1.2 mL min^{-1}. The injector, transfer and ion source temperatures were held constant at 280 ºC, 250 ºC and 200 ºC respectively. Acquisition of data was achieved in time scheduled Selected Ion Monitoring (SIM) mode to increase sensitivity and selectivity.

2.5. Quality control and quality assurance

Two of the bottles used for monitor the water were filled with water at HPLC grade and carried during the sampling campaigns. This permitted the obtention of blanks under the same conditions as the samples. For the extraction step, samples were divided in two groups due to the use of a Baker Vacuum system that allowed to extract simultaneously up to 20 samples. For each group of samples, one sample blank was extracted. Considering the 12 blanks performed during the monitoring campaigns, only 4% of the analytes were detected. In the few cases where blanks produced positive detections of compounds, they were subtracted from the sample value. The compounds that gave positive results in more than one blank were tributylphosphate, octylphenol and nonylphenol, while the pesticides appeared only occasionally. In any case, the values obtained were very low (0.01 - 0.08 µg L^{-1}) with the exception of nonylphenol that presented blank contribution up to 0.32 µg L^{-1}. The limits of detection and the recoveries obtained during the method optimization are given in a previous study by Hildebrandt et al. (2007)

Identification and internal standard quantification were carried out automatically using the Xcalibur software fine-tuning. The expected time was introduced with a view width of 0.20 min and a maximum peak width of 18 s. The three more intense mass spectrometric ions of each native compound and their relative abundance were also verified in order to associate peaks to determined compounds. From these three identification ions, the base peak was used for quantification. Isotopically labeled standards were identified with two ions using the base peak for quantification purposes.

2.6. Univariate descriptive statistics

Out of the 30 compounds analyzed, 9 were never detected in the samples analyzed and were not considered in the subsequent analysis. These were omethoate, dichlofenthion, parathion-methyl, parathion-ethyl, chlorfenvinfos, fenchlorfos, azinphos-ethyl, trifluralin and bromophos-methyl. For the other 21 compounds data were arranged in a table or data matrix per monitoring campaign, with 23 row samples (sampling sites) and 21 column variables (positive

compounds). This gave 6 data sets or data matrices (6 sampling campaigns covering 3 years). The 21 detected compounds are listed in Table 2.

Univariate descriptive statistics (minimum, maximum, mean, median, standard deviation and frequency of detection) were calculated for each of the measured variables considering simultaneously the six sampling campaigns. Pairwise correlations between two variables were carried out to investigate relationships and interdependencies of variables. Before the application of pairwise correlations and multivariate methods, 6 physicochemical parameters (Temperature of air, temperature of water, pH, dissolved oxygen, conductivity and NPOC), measured during the sampling campaigns, were added to each of the data matrices to investigate their possible correlation with some of the other parameters included in the tables.

2.7. Chemometric methods

2.7.1. Matrix dimension and data pretreatment

For the chemometrical study, only those compounds detected in more than 10% of the samples above the limit of detection were finally considered, in order to ease the interpretation of the results with minimal loss of information. This led to 6 matrices with 23 row samples (sampling sites) and 18 columns (chemical variables). The latter included the 6 physicochemical properties from the field and concentrations of the 12 chemical compounds: simazine, terbutylazine, terbutryn, atrazine, desethyl-atrazine, alachlor, metolachlor, diazinon, dimethoate, tributylphosphate, octylphenol and nonylphenol.

Before multivariate data analysis, values below the detection limit and missing values had to be treated. Values below the detection limit of the 12 compounds included in the PCAs were assumed to be equal to half the limit of detection (Farnham et al., 2002). This criteria was met by about 50 % of the entries in the original data set. Note that they were also not distributed uniformly among variables. Detection limits for each variable are given in Table 2. Out of the 138 samples, 5 were not collected because of a snow storm during October 2005 sampling campaign. To have a consistent set of values in the data matrices (same number of rows and columns), the values of these five samples for chemometric analysis were considered to be the mean of the rest of the existing values of the corresponding sampling site.

The whole data set from the six sampling campaigns was analyzed by Principal Component Analysis (PCA) using a column-wise matrix augmentation strategy (Navarro et al., 2006; Tauler and Goupy, 2008). Column-wise matrix augmentation consists of setting the individual data matrices one on top of the other forming a new column-wise augmented long data matrix. In our case, the whole data set was arranged in an augmented data matrix with 138 row samples (23 samples x 6 sampling campaigns) and 18 column variables (see Fig. 2).

Autoscaling was the pretreatment chosen for data normalization because it gave a better understanding and interpretation of the composition and distribution of the different contamination sources (Hendriks et al., 2005) operating over the study area. In a previous study using historical data on Ebro river basin, five different pretreatments were tested and autoscaling gave the best results for these types of data (Navarro et al., 2006). With this autoscaling procedure, the mean of the column elements was subtracted from individual elements and divided by their column standard deviation. Consequently, each column has zero mean and unit variance (Brodnjak-Voncina et al., 2002; Massart et al., 1998).

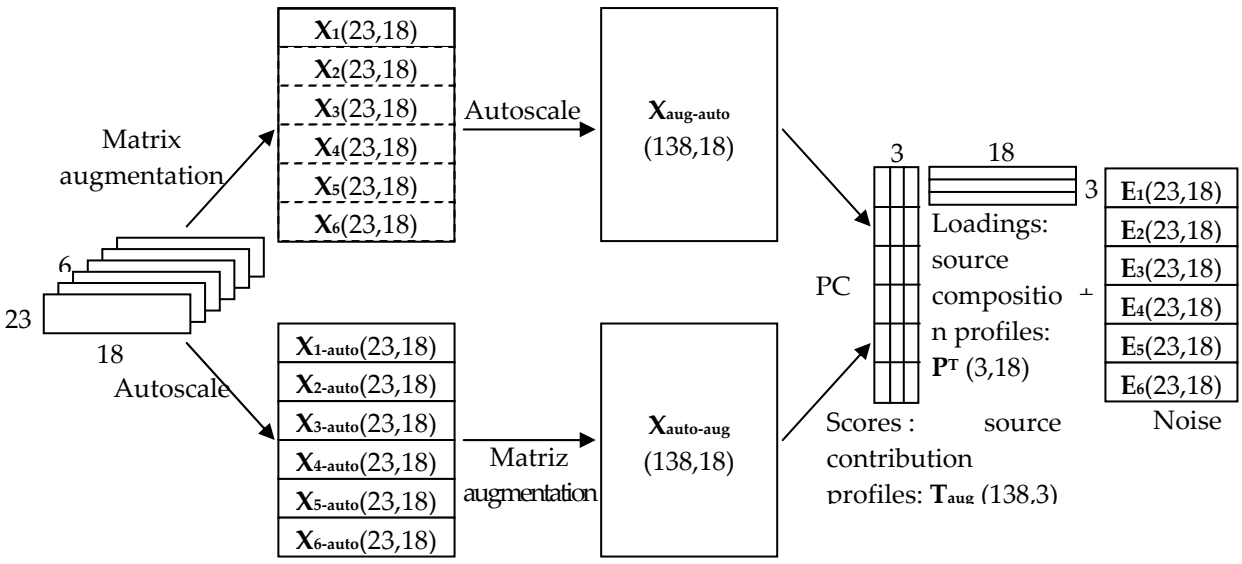

Fig. 2 Data matrix augmentation, pre-treatment scheme and bilinear principal component analysis (PCA) model decomposition (Equation 1). aug: augmented; auto: autoscaled; 1-6: number of sampling campaign.

Autoscaling, can be applied to the individual matrices corresponding to each sampling campaign before matrix augmentation or once they have been arranged in the column-wise augmented data matrix. The results obtained with these two procedures emphasize different aspects of the data. When the augmented matrix is first built up and then autoscaled, the results of PCA will show more clearly the temporal variations (differences between the 6 sampling campaigns). On the contrary, when the matrices are first autoscaled separately and then they are used to build the column-wise augmented data matrix, the results of the PCA will emphasize the differences among sampling sites (geographical variations). This is because the intensity of the differences among campaigns (temporal variations) has been eliminated by the autoscaling procedure in the first step. In Fig. 2 these two combined procedures of pretreatment and matrix augmentation are illustrated.

Before autoscaling, the physicochemical variables completely dominated the data matrix and conductivity in particular had much larger values than the rest of variables, confirming the necessity of applying a pre-treatment before using PCA. When the pre-treatment was applied before the augmentation the distribution of the values was more homogeneous than when autoscaling was applied after matrix augmentation. In both cases, extreme values only occurred for very high concentration samples, confirming that data was generally skewed towards lower values.

2.7.2. Principal component analysis

PCA is a data compression technique that aims to explain most of the variance in the data, while transforming the set of correlated measured variables into a smaller set of new uncorrelated variables or principal components (PCs) (Farnham et al., 2002). It attempts at the same time to preserve and emphasize the relevant relationships present in the original data (Ying, 2005). In this study, PCA was applied to the augmented matrices obtained from the two different ways previously explained. PCA decomposes the original data matrix into the scores matrix and the loadings matrix as it is developed in Navarro et al. (2006). PCA modeling was conducted using the PLS Toolbox (Eigenvector Research, Manson WA, USA) appropriate functions under the MATLAB computer and visualization environment (The Mathworks, Natick, MA, USA).

3. Results and discussion

3.1. Univariate descriptive statistics

Table 2 summarizes statistical data for all the detected compounds. The frequency of detection varied from 1.5 % to 72.0 % considering the results from the six monitoring campaigns. The compounds with the lowest frequency of detection were malathion, propazine, fenitrothion and chlorpyrifos, with detection frequencies between 1.5 and 2.3 %. Apart from these compounds there was another group of five compounds with less than 10% frequency of detection that included propanil, ethion, molinate, bisphenol A and bromophos-ethyl. The compounds with highest frequency of detection were tributylphosphate, atrazine, nonylphenol and octylphenol, which were detected in more than 60 % of the samples analyzed. Except for atrazine, the other three compounds have an industrial origin. Organophosphorus pesticides are the largest family of analyzed compounds. However they were detected less frequently than the triazine and chloroacetanilide pesticides.

The compounds that presented highest maximum concentrations were the same as the ones with highest frequencies of detection, with the exception of bisphenol A, which was detected in a few samples but at high concentrations, indicating a point source contamination of this plasticide. The compounds with highest median concentrations were again bisphenol A and nonylphenol, but also desethyl-atrazine and propazine. Comparison of mean and median values of all the variables showed that in most of the cases the mean values were between 1.5 and 2 times higher than the median values. This large difference between mean and median values shows that concentration data were skewed towards low values due to the existence of punctual high values. Compounds that fulfilled this criterium were propanil, chlorpyrifos, alachlor, tributylphosphate, octylphenol and nonylphenol. They were the ones with more extreme values among the samples. In most cases, standard deviations had the same order of magnitude as mean values. One third of the compounds showed larger values for the standard deviations than for the mean, especially octylphenol and nonylphenol, indicating a large variability of the concentration of these compounds.

Summarizing the information obtained from descriptive statistics, we concluded that the compounds with industrial origin (tributylphosphate, octylphenol and nonylphenol) appeared in a high percentage of the analyzed samples, in some cases at very high concentrations. Industrial compounds show a more widespread distribution than agricultural ones. They also show some high peaks from point source contamination. For most of the investigated pesticides, with intermediate frequencies of detection, their concentrations were not particularly high, between 0.05 and 0.825 μg L^{-1}. This behavior can be a consequence of the regular use of these pesticides, which produces a widespread distribution of low-level concentration of these compounds spread over a large area of the Ebro Basin, thus indicating the presence of diffuse contamination sources.

Existent legislation has been used to compare the results obtained in the Ebro Basin, the Canadian water quality guidelines for protection of aquatic life (Canadian Council of Ministers of the Environment, 2007) and the recently approved Directive 2008/105/EC (European Council, 2008), that includes

water environmental quality standards for the priority substances included in the Water Framework Directive (2000/60/EC). The substances that have some samples above these limits are also the three compounds with highest frequency of detection (atrazine, nonylphenol and octylphenol), with the exception of tributylphosphate that does not appear in any of the considered legislations. In particular, for nonylphenol, 75% of the samples were contaminated with concentrations higher than the limit proposed by the European Directive (0.3 µg L^{-1}). This compound was also encountered in 26.2% of the samples above the Canadian guidelines limit. Most of these samples are around the city of Zaragoza. This indicated that one important contamination problem in the Ebro Basin is due to industrial compounds. Another finding is that pesticide contamination in the Ebro River is presently below the legislated values.

Table 2 - Descriptive statistics of data

Compounds	Parameters[a]										
	LD (µg L^{-1})	Min. (µg L^{-1})	Max. (µg L^{-1})	Median (µg L^{-1})	Mean (µg L^{-1})	SD (µg L^{-1})	FD (%)	European limit (µg L^{-1})	Canadian limit (µg L^{-1})	% > Europ. limit	% > Canad. limit
Tributylphosphate	0.004	0.005	1.068	0.062	0.147	0.191	72.0	-	-		
Atrazine	0.004	0.006	0.825	0.037	0.062	0.101	65.2	0.6	1.8	1.2	
Nonylphenol	0.098	0.152	24.288	0.517	1.189	2.320	63.6	0.3	1.0	75	26.2
Octylphenol	0.006	0.007	1.542	0.027	0.067	0.146	61.4	0.1	-	12.3	
Simazine	0.008	0.008	0.109	0.018	0.026	0.021	41.7	1.0	10		
Desethyl-atrazine	0.010	0.016	0.377	0.074	0.113	0.095	36.4	-	-		
Terbutylazine	0.001	0.006	0.267	0.034	0.053	0.052	36.4	-	-		
Diazinon	0.003	0.005	0.256	0.012	0.022	0.037	34.8	-	-		
Metolachlor	0.002	0.005	0.093	0.009	0.013	0.015	26.5	-	7.8		
Terbutryn	0.005	0.005	0.184	0.041	0.053	0.048	18.2	-	-		
Alachlor	0.002	0.005	0.272	0.013	0.032	0.051	16.7	0.3	-		
Dimethoate	0.015	0.016	0.259	0.055	0.115	0.088	10.6	-	6.2		
Propanil	0.006	0.007	0.156	0.010	0.034	0.042	9.1	-	-		
Ethion	0.007	0.007	0.011	0.009	0.009	0.005	6.8	-	-		
Molinate	0.003	0.037	0.344	0.048	0.107	0.105	3.8	-	-		
Bisphenol A	0.282	0.327	2.575	0.945	1.198	0.624	3.0	-	-		
Bromophos-ethyl	0.006	0.007	0.017	0.009	0.010	0.006	3.0	-	-		
Chlorpyrifos	0.007	0.010	0.071	0.010	0.031	0.014	2.3	0.03	0.0035		
Fenitrothion	0.005	0.013	0.029	0.024	0.022	0.012	2.3	-	-		
Propazine	0.003	0.009	0.182	0.096	0.096	0.057	1.5	-	-		
Malathion	0.003	0.046	0.060	0.053	0.053	0.014	1.5	-	-		

[a] LD: limit of detection; Min: minimum; Max: maximum; Median; Mean; SD: standard deviation; FD: frequency of detection; % > European limit: percentage of samples above the limit considered in the Common Position (EC) No 3/2008; % > Canadian limit: percentage of samples above the limit considered in the Canadian water quality guidelines for protection of aquatic life

For the sake of a better comparison and interpretation of the achieved results, bar plots for the different families of analyzed compounds were drawn. Fig. 3a shows the bar plot of pesticides total concentration for the six sampling campaigns in each of the sampling sites ordered following the Ebro direction, from north-west to south-east. The bars in grey correspond to the June sampling campaigns and the ones in black correspond to the October sampling campaigns. In general, the total concentrations found in the June sampling campaigns were higher than in the October campaigns for almost all the sampling sites. This is most likely due to the field application of pesticides, which takes place in May/June, and the short response of these compounds once in the environment. An increasing tendency of the total concentration of the pesticides is also observed while approaching to the middle and lower sections of the river. This may also represent an cumulative effect due to an increasing use of the land for agricultural purposes as we go down the river and add up influences from the stations above.

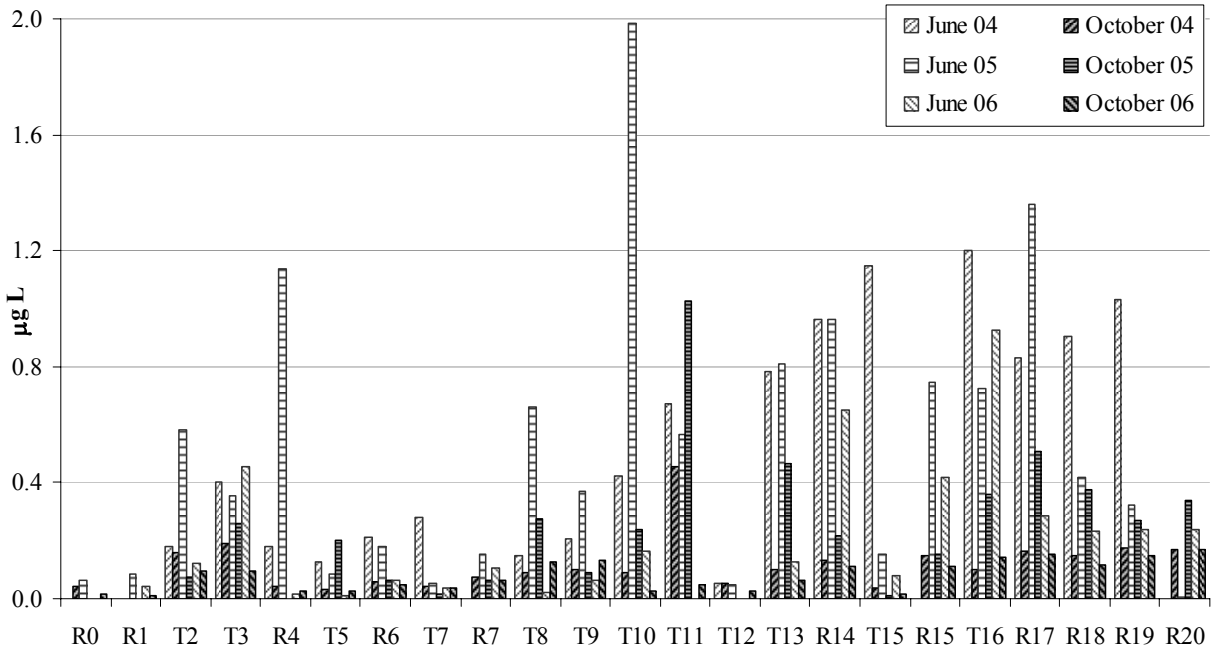

Fig. 3 Total concentration of pesticides observed in the 23 sampling sites along the Ebro during the six sampling campaigns.

Table 4 Pairwise correlation coefficients between the variables[a]

	T a.	T w.	pH	O_2	Cond	NPOC	Sim	Terb	Ttryn	Atra	DEA	Ala	Met	Diaz	Dim	Trib	OP	NP
T a.	1																	
T w.	**0.91**	1																
pH	-0.05	-0.12	1															
O_2	-0.55	-0.61	0.19	1														
Cond	0.26	0.21	0.09	0.05	1													
NPOC	0.09	0.18	-0.01	-0.22	0.11	1												
Sim	0.10	0.22	-0.10	-0.12	0.14	-0.09	1											
Terb	0.13	0.18	**0.54**	-0.24	0.00	0.21	0.13	1										
Ttryn	0.14	0.10	-0.01	-0.15	-0.20	0.19	-0.15	**0.27**	1									
Atra	0.19	**0.26**	0.17	-0.13	**0.26**	0.06	**0.26**	0.18	-0.09	1								
DEA	0.24	**0.30**	0.04	0.04	**0.33**	-0.02	**0.35**	-0.04	-0.16	**0.45**	1							
Ala	0.09	0.16	0.14	-0.07	0.17	0.04	0.16	0.10	-0.08	**0.85**	**0.44**	1						
Met	0.07	0.12	**0.31**	-0.01	**0.26**	0.02	0.06	0.14	-0.06	**0.55**	**0.53**	**0.59**	1					
Diaz	0.13	0.18	0.01	-0.02	-0.04	-0.02	**0.34**	0.06	0.11	**0.37**	0.21	0.13	0.05	1				
Dim	0.19	**0.27**	-0.10	-0.09	0.10	-0.01	**0.29**	-0.11	-0.07	0.13	**0.69**	**0.28**	**0.36**	0.05	1			
Trib	0.03	0.03	**0.31**	0.17	0.18	0.09	-0.07	0.12	0.00	0.20	0.23	**0.25**	0.17	-0.05	0.09	1		
OP	0.02	0.03	-0.06	-0.04	0.04	0.12	0.01	-0.04	-0.05	0.14	-0.04	0.18	0.06	-0.01	-0.04	-0.01	1	
NP	0.11	0.06	0.21	-0.05	**0.31**	0.17	-0.08	-0.04	-0.05	0.05	0.05	0.03	0.07	-0.03	0.05	**0.27**	0.06	1

[a] Identification of variables: T a.: Temperature of air; T w.: Temperature of water; pH; O_2: Dissolved O_2; Cond: Conductivity; NPOC: non purgable organic carbon; Sim: Simazine; Terb: Terbutylazine; Ttryn: Terbutryn; Atra: Atrazine; DEA: Desethyl-atrazine; Ala: Alachlor; Met: Metolachlor; Diaz: Diazinon; Dim: Dimethoate; Trib: Tributylphosphate; OP: Octylphenol; NP: Nonylphenol.
Higher pairwise correlations (>0.25) are marked in bold (significance 95%)

In Table 3, pairwise correlations between all the studied variables are given. Considering the physicochemical variables, there was an extremely strong correlation between both water and air temperatures, as expected (0.91). On the contrary, a strong negative correlation between both temperatures and dissolved oxygen was found, illustrating the natural behavior of oxygen that dissolves more in cold waters. The rest of physicochemical variables revealed small correlations. Between these variables and the compounds measured, temperature of water, pH and conductivity had moderate correlations between some of the compounds. NPOC had very low correlations with all compounds under study demonstrating that this parameter is not as significant for the contamination load as in other matrices (Yang et al., 2008). In general, the correlations obtained between physicochemical parameters and the compounds analyzed are not important enough to derive that these parameters might control the appearance of contamination in the water samples. Regarding the target compounds, atrazine, desethyl-atrazine, alachlor, metolachlor and dimethoate showed higher positive correlations among each other (most of them between 0.44 and 0.85) than with the other variables. The first four belong to two different herbicide families, triazines and acetanilides, and dimethoate is an organophosphorus insecticide. These correlations indicate that these five compounds may come from a common source (Scheytt et al., 2005), suggesting that they are applied together in the fields. Other compounds show moderate correlations. They include nonylphenol and tributylphosphate, that both have industrial origins. Further increased understanding about arrays and common factors of influence among different variables is obtained by the application of PCA with multivariate correlations (Boruvka et al., 2005).

3.2. Principal Component Analysis results

Here the results from the PCA applied to the organic compounds and physicochemical parameters simultaneously are shown. Table 4 shows the results of PCA both when it was applied to the augmented and autoscaled data matrix ($X_{aug\text{-}auto}$) and also to the autoscaled and augmented data matrix ($X_{auto\text{-}aug}$).

Table 5 Percentages of explained variances obtained by PCAs applied to $X_{aug\text{-}auto}$ and $X_{auto\text{-}aug}$ matrices

Matrix	**$X_{aug\text{-}auto}$**	**$X_{auto\text{-}aug}$**
PC1	21.29	24.78
PC2	13.30 (34.59)	11.51 (36.29)
PC3	10.80 (45.39)	9.00 (45.29)
PC4	8.62 (54.01)	8.45 (53.74)
PC5	7.03 (61.04)	7.19 (60.93)
PC6	6.23 (67.27)	6.37 (67.29)
PC10	3.98 (86.19)	3.51 (85.01)

[a] The percentage of accumulated explained variance for that particular component is given in parentheses

In both cases the explained variances were rather similar. They increased very slowly and they did not reach more than 85 % of the total variance until PC10, indicating the presence of multiple independent contamination sources The results using the first three PCs are presented here because they can explain almost 50 % of the total variance. In Fig. 4, the loadings plots for the first three PCs of both augmented data matrices ($X_{aug\text{-}auto}$ and $X_{auto\text{-}aug}$) are given.

In the case of Xaug-auto matrix, the first PC explained 21.29% of the total variance. It had moderate positive loadings for both temperatures and slightly smaller correlation for conductivity, while dissolved oxygen was negatively correlated with these variables and pH and NPOC had a very small contribution of the physicochemical variables. Herbicides atrazine, desethyl-atrazine, alachlor and metolachlor presented high positive loadings for this first PC, more or less at the same level, and dimethoate showed a moderate positive correlation with these herbicides. The rest of compounds had small loading contributions for this first PC. This first PC identifies a contamination source of agricultural origin, correlated with temperature or in other words season of application. Usually pesticides are applied during the peak of the growth season (May/June) and our results show that they occur with a short response in close vicinity of the river. In this PC we can also appreciate that the only physicochemical variables correlated with the compounds analyzed are both temperatures while the influence of conductivity, pH, dissolved oxygen and NPOC is minimal.

The second PC explained 13.3% of the total variance and it gave a slightly similar scheme for the physicochemical variables but with a negative correlation with pH. In this case none of the compounds gave a significative positive loading. Overall, this second PC mainly describes the temporal physicochemical properties of the samples, mainly dominated by their temperatures and consequently by the season of the year but with no correlation with the chemical contamination. Consequently 13.3% of the variability found in the samples is only due to physicochemical parameters. Temperature, dissolved oxygen and pH in water samples are important parameters to evaluate the chemical and ecological status of a water body. In our case it was found that waters were always oxygenated and had neutral pH, thus, biota can not be affected by these parameters.

The third PC, with 10.80% of explained variance, gave high positive loadings for terbutylazine, terbutryn and two of the industrial compounds, tributylphosphate and nonylphenol, as well as for pH and NPOC. The moderate correlation coefficient previously found between nonylphenol and tributylphosphate is also reflected in this third PC with higher loadings of these compounds. Simazine, desethyl-atrazine and dimethoate showed negative loadings in this PC. This third PC therefore, helps to distinguish a more specific industrial contamination, which is negatively correlated with some

of the pesticides. This industrial contamination source had and influence on the pH and NPOC of the river water, as they are positively correlated.

In the case of the PCA of $X_{auto\text{-}aug}$, the explained variation and the profile of the three PCs considered were similar to the ones obtained for the PCA of Xaug-auto. For that reason the contamination sources that can be distinguished when emphasizing temporal variations or geographical variations are very similar. The main difference was found in second PC, where all physicochemical variables were positively correlated with compounds of industrial origin, while the pesticides had negative loadings in this PC. Therefore, this second PC distinguishes the two major different patterns of contamination present in the geographical area of study, industrial and agricultural, that appeared in different PCs for the PCA of Xaug-auto.

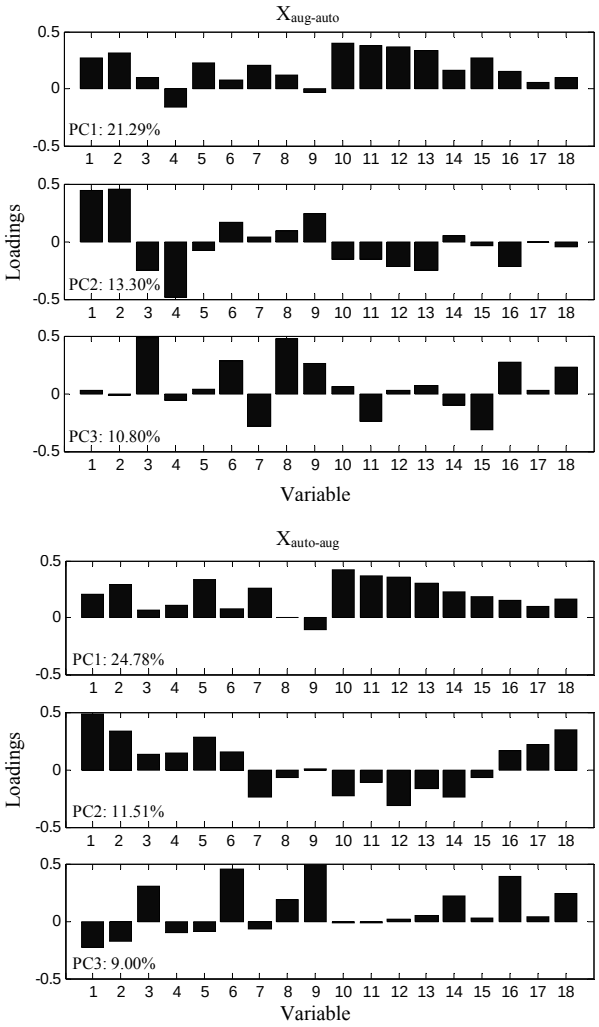

Fig. 4 Loadings for the three principal components (matrix P^T in Equation 1 and Fig. 2). (a) for $X_{auto\text{-}aug}$, (b) for $X_{aug\text{-}auto}$. Identification of variables: 1: Temperature of air; 2: Temperature of water; 3: pH; 4: Dissolved O_2; 5: Conductivity; 6: NPOC; 7: Simazine; 8: Terbutylazine; 9: Terbutryn; 10: Atrazine; 11: Desethyl-atrazine; 12: Alachlor; 13: Metolachlor; 14: Diazinon; 15: Dimethoate; 16: Tributylphosphate; 17: Octylphenol; 18: Nonylphenol.

Scores plots of Xaug-auto data matrix are given in Fig. 5. Score plots permit to evaluate the distribution of samples according to PCA. For a better interpretation of the results, the samples were coded into 6 different classes representing the sampling campaigns. Because the data pre-treatment for Xaug-auto would emphasize temporal variations, samples from the same year were given the same symbol, with summer samples in filled symbols and samples in October using unfilled symbols. The codes of the sampling sites are given as a number for each of the samples.

In Fig. 5a, the PC1 vs. PC2 scores plot is shown. In this plot three main groups were distinguished. The first cluster had samples only from the 4th sampling campaign, which took place in October 2005. This cluster of samples presented high negative score values for PC2 combined with also score negative values for PC1, indicating that these samples had the lowest temperatures compared with the rest of sampling campaigns. This group of samples also showed a moderate contamination by the compounds that have negative loadings in PC2 (atrazine, desethyl-atrazine, alachlor, metolachlor and tributylphosphate). The formation of this cluster of samples is related to the episode of very low temperatures that occurred in Spain in autumn and winter of 2005.

A second cluster of samples in Figure 5a had different levels of positive scores on PC1 and with low positive to large negative scores for PC2. These samples mostly belong to sampling campaigns in June and with code numbers of 10 or higher, indicating that they belong to the middle and to lower courses of the river basin. Moreover, all these samples had measurable concentrations of pesticides that increased with the magnitude of score value for PC1.

A third sample cluster was situated around the origin of the PC1-PC2 axes, with a small tendency of positive PC2 score values, indicating that these samples less contaminated by pesticides compared to those belonging to the other clusters. Also, it could be distinguished that samples from October sampling campaigns presented a small tendency to have larger negative values both for PC1 and PC2, towards lower temperatures but not as extreme as was observed for the first samples cluster related to the 2005 sampling campaign.

The PC1 vs. PC3 scores (Fig 5b) plot do not have the cluster formed by the samples of October 2005 and autumn samples did not differ from the other samples collected during the June campaigns, as temperature variations were mostly explained by PC2. In addition, the previously identified cluster (2nd) of summer samples increased now in number, including also some samples from the upper part of the basin, and they were separated into two new subgroups, depending on their score values for PC3. The samples with positive score values for PC3 were mostly contaminated with terbutylazine, terbutryn, tributylphosphate and nonylphenol, which is a contamination of mostly industrial origin.

On the contrary, the samples with negative score values for PC3 were the ones more contaminated with pesticides simazine, desethyl-atrazine and dimethoate. The first group includes samples from June 2005, indicating that during this year there was a more specific contamination due to industrial compounds.

The other two summer campaigns gave negative loading for PC3. The fact that only summer samples appear separated in the plot indicates that these samples were more contaminated with herbicides and pesticides than the autumn ones. Special attention has to be given to sample T11 for October 2004, which presented a high positive score for PC3 and a moderate positive score for PC1. This sample had an extremely high value for nonylphenol and also a very high value for tributylphosphate.

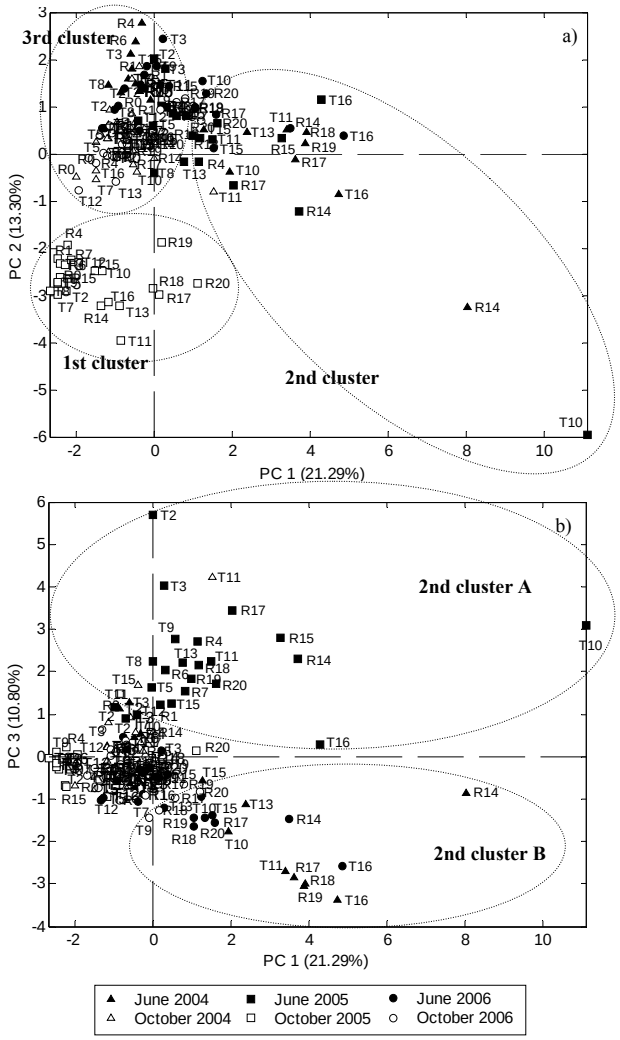

Fig. 5 Scores plots from PCA performed on $X_{aug\text{-}auto}$ data matrix (matrix T in Equation 1 and Fig. 2). (a) for the first two principal components; (b) for the first and the third principal components.

No temporal systematic tendency of steadily increasing or decreasing concentrations over the years 2004-2006 was observed. Concentration of target compounds in the area of Tortosa (R18) was compared with river flow taken as a reference. Although mean river flow had been decreasing during the three year period (from 221 to 96 m3 s^{-1}), but this decrease in the amount of water did not correspond to any increase of concentration of the studied compounds. The absence of a decreasing tendency indicates that there is an input of contaminants that may undergo seasonal variations, but they are detected continuously and thus, actions are needed to reduce the input of contaminants throughout the basin.

Scores plots of the $X_{auto\text{-}aug}$ data matrix are given in Fig. 6. For a better interpretation of the results, the samples have been distributed into 23 classes considering their sampling site. As this kind of pre-treatment is assumed to explain better the geographical than the temporal variations, the samples from the upper course are represented with filled symbols and the ones from the middle and lower course are represented with unfilled symbols. The codes of the sampling campaign are indicated for each of the samples.

In Fig. 6, the PC1 vs. PC2 scores plot is shown. The samples with negative score values for PC1 were mostly coming from the upper part of the river basin (R0-R7, T12). These samples showed a different degree of industrial contamination. On the other hand, samples coming from the middle and lower course are mostly on the positive side of PC1 showing basically contamination from pesticides use, as well as some contribution of industrial contaminants depending on the value for PC2. samples were clearly distributed within this 2 types of contamination sources throughout the 6 sampling campaigns.

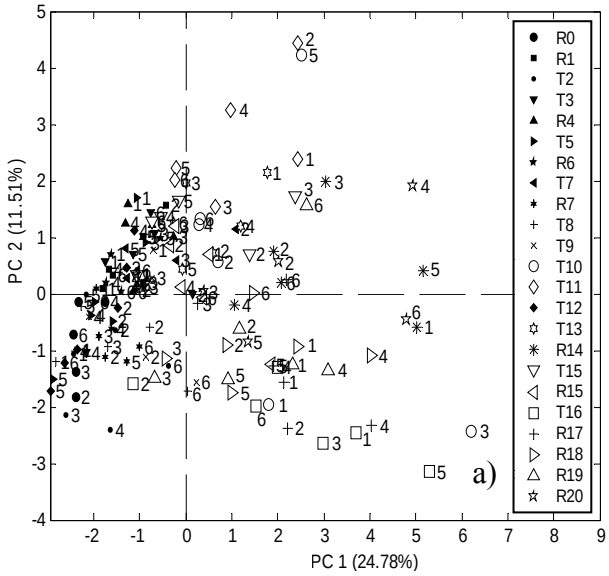

Fig. 6 Scores plots from PCA performed on $X_{auto\text{-}aug}$ data matrix (matrix T in Equation 1 and Fig. 2) for PC1 and PC2

4. Conclusions

Overall, once a large data set is obtained by successive

monitoring campaigns, PCA is a useful tool to assess the temporal and geographical contamination patterns. Two types of data pretreatment (first augmentation and afterwards autoscaling and autoscaling before augmentation) were tested. The second type of data pretreatment, for $X_{auto\text{-}aug}$, better showed the geographical variations among samples. On the other hand, the temporal variations were better displayed for Xaug-auto and they were mostly dominated by the temperature of the sampled waters.

The herbicides atrazine, desethyl-atrazine, alachlor, metolachlor and the organophosphorus insecticide dimethoate showed higher positive correlations among each other suggesting that they are applied together in the fields. On the contrary there are no correlations between the physicochemical parameters and the organic compounds analyzed.

Our analyses revealed two main sources of contamination in water from the Ebro River Basin. The first was mainly associated to pesticides commonly used for agricultural practices, while the second is influenced by industrial compounds. Pesticides were detected at low concentrations in the middle and lower course of the basin at low concentrations, with the exception of 1.2% of the samples containing atrazine, which were above the legislation limits.

In addition, a seasonal trend was observed in the Ebro River basin that reflect agricultural activities, with higher levels of pesticide concentration in early summer and their attenuation in autumn. This seasonality is directly related to the use of pesticides and does not produce an increase of these compounds over the 3 years of investigation.

The second contamination source was dominated by alkylphenols and tributylphosphate which were widely distributed all over the river basin. Specifically, nonylphenol was present above the legislation limits in 75 % of the samples, indicating therefore a potential risk for ecosystems.

This study provides a synoptic tool for monitoring river waters to evaluate the potential sources of toxic organic compounds, vulnerable periods and fate in an area of high ecological interest. The monitoring presented here and the subsequent data treatment provides results on the need to continue monitoring the quality of waters at a basin scale, including wide range of contaminants and main physicochemical parameters.

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4.3.2.- Compostos prioritaris en sediments de la conca de l'Ebre

Degut a l'equilibri que s'estableix entre la fase aquosa i la fase sòlida, els compostos més hidrofòbics tenen més tendència a unir-se a la matèria particulada de l'aigua i sedimenten. Així mateix, aquells contaminants que s'aboquen al medi a concentracions molt elevades, encara que tinguin propietats més hidrofíliques, poden arribar a detectar-se en els sediments de forma puntual. Per això és necessari analitzar aquesta matriu a tota la conca. Els compostos estudiats amb una tendència més hidrofòbica i per tant una major probabilitat de quedar retinguts en els sediments pertanyen als grups de PAHs i compostos organoclorats, a més a més dels APs que es detecten a les dues matrius degut al seu ús massiu (Taules 3.1, 3.2, 3.3 i 3.4). L'objectiu d'aquest estudi va ser determinar la presència i distribució de diversos compostos prioritaris presents en sediments de la conca de l'Ebre degut a les pressions agrícoles, urbanes i industrials.

A part dels compostos analitzats en aigua, que es descriuen a l'apartat 4.3.1, en els sediments també es van analitzar 22 compostos organoclorats, dels quals 9 són insecticides organoclorats i la resta són isòmers o productes de degradació d'aquests. Majoritàriament són pesticides utilitzats històricament a *España* i de tots ells només l'endosulfan s'ha utilitzat més recentment, gràcies a una moratòria fins el desembre de 2007, ja que la UE el va prohibir el desembre de 2005 mitjançant la seva exclusió de l'Annex I de la Directiva 91/414/CEE. Igual que succeeix amb els pesticides hidrofílics descrits anteriorment, la majoria dels compostos organoclorats utilitzats històricament com a insecticides variaven d'uns a altres per simples canvis estructurals en les seves ramificacions amb l'objectiu de defugir la legislació sense necessitat d'una nova inversió en investigació. Un dels casos més clars és la família de les endrines, on s'inclouen les famoses endrina, dieldrina, aldrina i isodrina.

Per altra banda es van analitzar els 16 PAHs per als que l'EPA recomana el seu control per tal d'avaluar la contaminació difusa generada per multitud de fonts com ara el transport, les calefaccions, la indústria i altres processos de combustió.

Els resultats i conclusions de l'anàlisi de tots aquests compostos en sediments de la conca de l'Ebre es recullen a l'article científic 6, reproduït a continuació.

Article científic 6

Occurrence and transport of PAHs, pesticides and alkylphenols in sediment samples along the Ebro River Basin

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Enviat a Journal of Environmental Monitoring

Occurrence and transport of PAHs, pesticides and alkylphenols in sediment samples along the Ebro River Basin

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Abstract

An environmental monitoring program was carried out to determine the impact of 69 pollutants on sediments from the Ebro River basin. PAHs, polar and chlorinated pesticides and alkylphenols were monitored during the period 2004-2006 in 22 sampling sites. Target compounds were determined by pressurized liquid extraction followed by gas chromatography-mass spectrometry. Environmental data were assessed using descriptive statistical analysis and multivariate data analysis with Principal Component Analysis (PCA) to elucidate the relevant contamination patterns and provide a description of their seasonal trends. Comparison with legislated and toxicological values was also carried out. Using descriptive statistics we found that PAHs and APs appeared over 55 % of the samples analyzed and at concentrations up to 4931 and 5999 μg kg^{-1}, respectively, together with some DDTs, although they are forbidden in Spain since 1994. The concentrations found for DDT are over the toxicological values in more than 20% of the samples analyzed. PCA analysis showed two main contamination sources, one due to PAHs and a second one due to chlorine compounds (DDTs and hexachlorobenzene).

Introduction

The Ebro River basin, located in the NE of Spain, covers an area of approximately 85,000 km^2 and has a population of 2,700,000 inhabitants distributed in a very heterogeneous way. Being traditionally agricultural, the region has lately incorporated industrial activities concentrated close to the cities of Zaragoza, Vitoria, Pamplona, Logroño, Lleida, Monzón and Flix. The first five are also the main urban centres, concentrating about 45% of the population.[1] Hence, a wide range of pollutants is generated by urban, industrial and agricultural activities. These toxic compounds, such as pesticides,[2] surfactants[3] and hydrocarbons[4] tend to accumulate on water and river sediments, being then a potential risk for entire ecosystems.[5]

Historically many studies have been performed within the Ebro River Basin, but most of them have been site specific or have been focused on a single chemical family.[2,4,6-10] Little is known about the concentration and patterns of a wide spectrum of priority contaminants in the whole Ebro aquatic ecosystem with temporal continuation. In 2003, a more complete study concerning the complete river basin, various environmental matrices and five different chemical families was carried out.[11] The further inclusion of the Ebro river basin into the European Union project AquaTerra (contract no. 505428), lead to a complete monitoring study of the basin throughout a 3 year period. This survey includes priority compounds according to the Directive 2006/11/EC: (i) polycyclic aromatic hydrocarbons (PAHs), (ii) polar and chlorinated pesticides, (iii) alkylphenols (APs), bisphenol A and tributylphosphate (TBP).

The results presented here are part of this complete monitoring, which included both water and sediments matrices. Organic compounds partitioned in water or sediment providing a different profile of detected contaminants, therefore each matrix has been considered separately. Thus, the main goal of the present study is to evaluate the human impact and the vulnerability of the Ebro River Basin sediments. Different techniques were applied to obtain significant environmental information about temporal and geographical tendencies and to distinguish between the different sources. To complement the application of classical univariate statistic techniques, a deeper study of the presence and correlations of different pollutants is proposed using multivariate exploratory data analysis techniques like Principal Component Analysis (PCA),[12] which is a frequently used multivariate technique that provides a powerful tool for data compression, exploration and interpretation. Sediment data was compared with some legislated and toxicological values and the results of the few compounds that appeared both in water and sediments were also compared.

Materials and methods

Chemicals

Native compounds were purchased as different mixtures from Dr. Ehrenstorfer (Augsburg, Germany) at 100 μg mL^{-1} in ethyl acetate, except PAHs that were purchased at 2000 μg mL^{-1} (see Table 1). Single isotopically labelled surrogates (hexachlorobenzene-13C6, 4,4'-DDE-D8 and 4,4'-DDT-13C12, desethyl-atrazine-D6, atrazine-D5, alachlor-D13, parathion-ethyl-D10 and nonylphenol-D8), a mixture of PAHs labelled surrogates (naphthalene-D8, acenaphtene-D10, phenanthrene-D10, chrysene-D12 and perylene-D12) and two internal

standards (terbuthylazine-D5 and anthracene-D10) were purchased from Dr. Ehrenstorfer at 100 μg mL^{-1} in acetone. Standard working solutions were diluted from the commercial ones in hexane. GC quality solvents were from Merck (Darmstadt, Germany). Florisil powder was of 0.150-0.250 mm for residue analysis quality from Merck, baked at 150 ºC for 4 h to ensure dryness. Hydromatrix was from Varian (Palo Alto, CA USA). 99.995% purity nitrogen from Air Liquide (Paris, France) was used as drying gas.

Table 1 Compounds analyzed and their physico-chemical properties.[13-16] The detected compounds are marked in bold

Compound	Vapour pressure 25 ºC (mPa)	Solubility (mg L^{-1})	log K_{ow}	Canadian limit (μg kg^{-1})	TEC (μg kg^{-1})	PEC (μg kg^{-1})
Polycyclic Aromatic Hydrocarbons (PAHs)[rings]						
Naphthalene (Naph)[2]	10900	31	3.37	34.6	176	561
Acenaphthylene (Acy) [3]	893	16.1	3.98	5.87		
Acenaphthene (Ace) [3]	596	3.9	4.07	6.71		
Fluorene (Flu) [3]	88.1	1.69	4.18	21.2	77.4	536
Phenantrene (Phe) [3]	18	1.15	4.45	41.9	204	1,170
Anthracene (Ant) [3]	0.75	4.34×10^{-2}	4.45	46.9	57.2	845
Fluoranthene (Flut) [4]	1.3	0.26	4.90	111	423	2,230
Pyrene (Pyr) [4]	0.89	0.135	4.88	53	195	1,520
Benzo(a)anthracene (BaA) [4]	8.6×10^{-3}	9.4×10^{-3}	5.61	31.7	108	1,050
Crysene (Cry) [4]	1.3×10^{-2}	0.2×10^{-2}	5.16	57.1	166	1,290
Benzo(b)fluoranthene (BbF) [5]	1.2×10^{-4}	1.5×10^{-3}	6.04			
Benzo(k)fluoranthene (BkF) [5]	5.5×10^{-5}	0.8×10^{-3}	6.06			
Benzo(a)pyrene (BaP) [5]	1.5×10^{-2}	1.62×10^{-3}	6.06	31.9	150	1,450
Indeno(1,2,3-cd)pyrene (InP) [6]	na	0.19×10^{-3}	6.58			
Dibenzo(a,h)anthracene (DahA) [5]	0.8×10^{-3}	2.49×10^{-3}	6.50	6.22	33.0	
Benzo(ghi)perylene (BghiP) [6]	0.02	0.26×10^{-3}	6.84			
Alkylphenols (APs)						
Octylphenol (OP)	63.72	12.6	4.12			
Nonylphenol (NP)	12.56	5.43	4.48	1.4		
Organochlorine compounds (OCs)						
alpha-HCH (a-HCH)	6.00	2	3.80			
beta-HCH	4.80×10^{-2}	0.24	3.78			
gamma-HCH (g-HCH)	5.60	7.3	3.72	35.0	2.37	4.99
delta-HCH	4.69	10	4.14			
Hexachlorobenzene (HCB)	2.40	6.2×10^{-3}	5.73			
Heptachlor	53.32	0.18	6.10			
Heptachlor-exo-epoxide	2.60	0.2	4.98	0.60[a]	2.47[a]	16.0[a]
Heptachlor-endo-epoxide	na	na	na			
Aldrin	16.00	1.7×10^{-2}	6.50			
Endrin	0.40	0.25	5.20	2.67	2.22	207
Endrin aldehyde	0.03	2.4×10^{-2}	4.80			
Isodrin	5.87	1.42×10^{-2}	6.75			
Dieldrin	0.79	0.195	5.40	2.85	1.90	61.8
alpha-Endosulfan	0.40	0.51	3.83			
beta-Endosulfan	0.08	0.45	3.83			
Endosulfan-sulfate	0.04	0.48	3.66			
2,4'-DDE	0.83	0.14	6.00	1.42[a]	4.88[a]	31.3[a]
4,4'-DDE	0.80	4.00×10^{-2}	6.51			
2,4'-DDD	0.26 (30 ºC)	0.10	5.87	3.54[a]	1.9[a]	28.0[a]

4,4'-DDD	0.18	$9.00x10^{-2}$	6.02			
2,4'-DDT	0.18	$8.5x10^{-2}$	6.79	1.19 [a]	3.16 [a]	62.9 [a]
4,4'-DDT	0.02 (20 ºC)	$5.5x10^{-3}$	6.91			
Polar pesticides (PPs)						
Omethoate	3.30	$1x10^{6}$	-0.74			
Dimethoate	0.25	$23.3x10^{3}$	0.70			
Diazinon	12.00	60	3.30			
Dichlofenthion	74.60	0.245	5.14			
Parathion-methyl	0.41	55	3.00			
Fenitrothion	18.00	14	3.43			
Malathion	5.30	145	2.75			
Chlorpyrifos	2.70	1.4	4.70			
Parathion-ethyl	0.89	11	3.83			
Bromophos-methyl	17.06	0.3	5.21			
Chlorfenvinfos	1.00	7.3	3.85			
Bromophos-ethyl	6.13	0.44	6.15			
Ethion	0.20	2	4.28			
Fenchlorfos	10.00	1	4.88			
Azinphos-ethyl	0.32	10.5	3.18			
Propanil	0.05	130	3.30			
Alachlor	2.00	170.31	3.09			
Metolachlor	4.20	488	2.90			
Trifluralin	6.10	0.221	4.83			
Molinate	746.00	990	2.88			
Simazine	$2.95x10^{-3}$	6.2	2.10			
Atrazine	$3.85x10^{-2}$	33	2.50			
Propazine	$3.9x10^{-3}$	5	2.93			
Terbuthylazine	0.15	8.5	3.21			
Terbutryn	0.23	22	3.65			
Desethyl-atrazine	12.43	3200	1.51			
Plasticides						
Bisphenol A	$5.21x10^{-2}$	120	3.32			
Tributylphosphate (TBP)	150.65	280	4.00			

[a] These values are settle up for the sum of the two isomers, na: not analysed

Site selection and sampling

The monitoring comprises three sampling campaigns during three years, between 2004 and 2006, which were carried out in October. The monitoring includes 22 sampling sites covering the whole Ebro river basin (10 at the Ebro River and 12 at the main tributaries) from the most vulnerable sites, according to proximity to big cities, agricultural areas or industrial activities and knowledge on historical contamination episodes. Their specific locations are shown in Table 2, and are numbered from 1 to 20 following the Ebro river flow, from north-west to south-east. "R" indicates a site on the Ebro River whereas "T" indicates a tributary site. Among all the sampling sites, one 6 km downstream the Ebro source (R0) and another one just before the sea (R20) are included. Table 2 lists the locations of each sampling site, the coordinates, the corresponding river, the section of the river basin and the main economical activities in the area.

Sample extraction

The extraction and clean up protocol was briefly optimized according to two previous studies that analyzed multiple compounds in soils.[17,18] Briefly, sediment samples were frozen at -20 ºC and freeze-dried during 48 hours at -40 ºC and under a 10^{-2} mbar vacuum. Samples were then sieved through 500 and 120 µm mesh to obtain a homogeneous sediment material. One gram of this last fraction was spiked by means of a 10 µL syringe with the surrogate solution at 50 µg kg^{-1}-dw and extracted using the pressurized liquid extraction (PLE) system with an accelerated solvent extraction (ASE) 200 from Dionex (Sunnyvale, CA USA). This system was optimized to perform the

extraction and clean-up within the ASE cell in a single step, using Florisil inside the ASE cell.

For the extraction step, 22 mL ASE stainless steel cells were packed as follows: 2 g of Florisil powder were placed at the outflow side of the cell and other 5 g were mixed with the sample. The remaining space was filled with pressed hydromatrix. The mixture hexane:dichloromethane (1:1) was used as extraction solvent. A heat-up time of 5 min was applied to the extraction cell. Temperature was adjusted to 100 ºC and pressure was fixed to 1500 psi (1 psi = 6894.76 Pa). Solvent flow was of 60%. Two cycles of extraction were performed with 10 min in static mode. The purge time was of 90 s with nitrogen gas. Extracts were evaporated at room temperature to nearly dryness using a TurboVap LV from Caliper LifeSciences (Hopkinton, MA USA) and reconstituted into glass amber vials for gas chromatography. The final samples were spiked with the mixture of internal standards at a concentration of 160 µg L^{-1} and the final volume was adjusted to 250 µL with hexane.

Table 2 Description and location of network sampling sites

Code	River	GPS Coordinates		Location	Province	Sector [a]
		Lat.	Long.			
R0	Ebro	42.999N	4.153W	Nestares	Cantabria	Source
R1	Ebro	42.684N	2.951W	Miranda de Ebro	Burgos (Castilla Leon)	I
T2	Zadorra	42.884N	2.486W	Audikana	Álaba (Euskadi)	I
T3	Zadorra	42.833N	2.783W	Villodas	Álaba (Euskadi)	U, I, A
R4	Ebro	42.589N	2.842W	Haro	La Rioja	U, A
T5	Najerilla	42.418N	2.733W	Nájera	La Rioja	A
R6	Ebro	42.470N	2.444W	Logroño	La Rioja	I, U, A
T7	Ega	42.669N	2.031W	Estella	Navarra	I
R7	Ebro	42.067N	1.601W	Tudela	Navarra	U, A
T8	Araquil	42.895N	2.135W	Alsasua	Navarra	I
T9	Arga	42.671N	1.819W	Puente la Reina	Navarra	I
T10	Jalón	41.734N	1.175W	Grisén	Zaragoza (Aragón)	I, A
T11	Huerva	41.614N	0.915W	Zaragoza	Zaragoza (Aragón)	I, U
T12	Gállego	42.402N	0.499W	Jabarrella	Huesca (Aragón)	A
T13	Gállego	41.823N	0.785W	San Mateo de Gállego	Zaragoza (Aragón)	A, I
R14	Ebro	41.567N	0.691W	Presa de Pina	Zaragoza (Aragón)	U, A, I
T15	Cinca	41.725N	0.136E	Monzón	Huesca (Aragón)	I, A
R15	Ebro	41.320N	0.340W	Sástago	Zaragoza (Aragón)	A
T16	Segre	41.536N	0.512E	Torres de Segre	Lleida (Catalunya)	A
R17	Ebro	41.229N	0.552E	Flix	Tarragona (Catalunya)	I, A
R19	Ebro	40.715N	0.581E	Amposta	Tarragona (Catalunya)	A
R20	Ebro	40.714N	0.714E	Deltebre	Tarragona (Catalunya)	A, mouth

[a] A: agricultural, I: industrial, U: urban

Instrumental analysis

GC/MS analysis was performed with a gas chromatograph Thermo Electron (San Jose, CA USA) model Trace 2000 coupled to a mass spectrometer from Thermo Electron. The mass spectrometer was operated in the electron impact ionization mode with an ionizing energy of 70 eV. Compound separation was achieved using a capillary column HP-5MS of 30 m x 0.25 mm i.d. with 0.25 µm film thickness from J&W Scientific (Folsom, CA USA). Each extract was injected three times in a specific GC/MS program to determine: (i) PAHs with the following temperature program: from 60 ºC (holding time 1 min) to 175 ºC at 6 ºC/min (holding time 4 min) to 235 ºC at 3 ºC/min and finally to 300 ºC at 8 ºC/min, keeping the final temperature for 5 min. (ii) Organochlorine compounds (OCs) and (iii) polar pesticides (PPs), together with tributylphosphate, bisphenol A and APs were injected separately but both with the same oven temperature program: from 60 ºC (holding time 1 min) to 130 ºC at 10 ºC min^{-1} to 220 ºC at 3 ºC min^{-1} and finally to 300 ºC at 10 ºC min^{-1} (holding time 5 min). Injection was achieved in the splitless mode keeping the split valve closed for 0.8 min. Helium was used as carrier gas at a flow of 1.2 mL min^{-1}. The injector, transfer and ion source temperatures were set at 280 ºC, 250 ºC and 200 ºC respectively. Acquisition was achieved in time scheduled Selected Ion Monitoring (SIM) mode to increase sensitivity

and selectivity. Identification and quantification were carried out automatically by the Xcalibur software.

Univariate descriptive statistics

Out of the 69 compounds analyzed, 29 were never detected in the samples analyzed (see Table 1) and were not considered in the subsequent analysis. Most of the non detected compounds were part of the group of PPs with also some non detected OCs, 23 and 6 compounds respectively.

Prior to multivariate data analysis, univariate descriptive statistics (minimum, maximum, mean, median, standard deviation and frequency of detection) of each of the measured variables were calculated considering all the samples as a whole. The concentrations obtained were also compared with some legislated and toxicological levels. Pairwise correlations between two variables were preliminary investigated to see the relationships between the studied compounds.[19] This was accomplished by calculating the correlation coefficients between them.

Chemometric methods

Matrix dimension and data pretreatment.

Only those compounds detected in more than 10% of the samples above the limit of detection were finally considered for the chemometrical study. Finally the dimensions of each of the three data matrices used for the multivariate analysis were 22 row samples (sampling sites) and 29 column variables (TOC and 28 compounds).

Two problems were considered before multivariate data analysis was started: a) values below the detection limit and b) presence of missing values. Values below the detection limit were assumed to be equal to half the limit of detection.[20] Approximately 30 % of the entries in the original data were below the limit of detection and they were not distributed uniformly among variables. Detection limits for each variable are given in Table 3. Out of the 66 samples, R15 from 2004 was not analyzed because of the impossibility to find sediment in the river during that specific sampling campaign. To have a consistent set of data matrices (same number of rows and columns), for this sample the values considered for the chemometric analysis were the mean of 2005 and 2006 results, avoiding any distortion of the data. The rest of missing values were handled using the PLS Toolbox (Eigenvector Research, Manson WA, USA) appropriate functions under MATLAB (The Mathworks, Natick, MA, USA). Using this method, missing values were automatically obtained as a result of mathematically undefined operations.[21] Before the application of multivariate methods, the sediment total organic carbon analysis (TOC) was added to each of the data matrices.

The whole data set with the data from the three sampling campaigns can be analyzed by PCA using column-wise matrix augmentation.[1] Matrix augmentation consists of setting the individual data matrices one on top of the other forming a column-wise augmented data matrix. In our case, the whole data set was arranged in an augmented data matrix of dimensions 66 x 29, i.e., with 66 row samples (22 samples x 3 sampling campaigns) and 29 column variables (see Fig. 1).

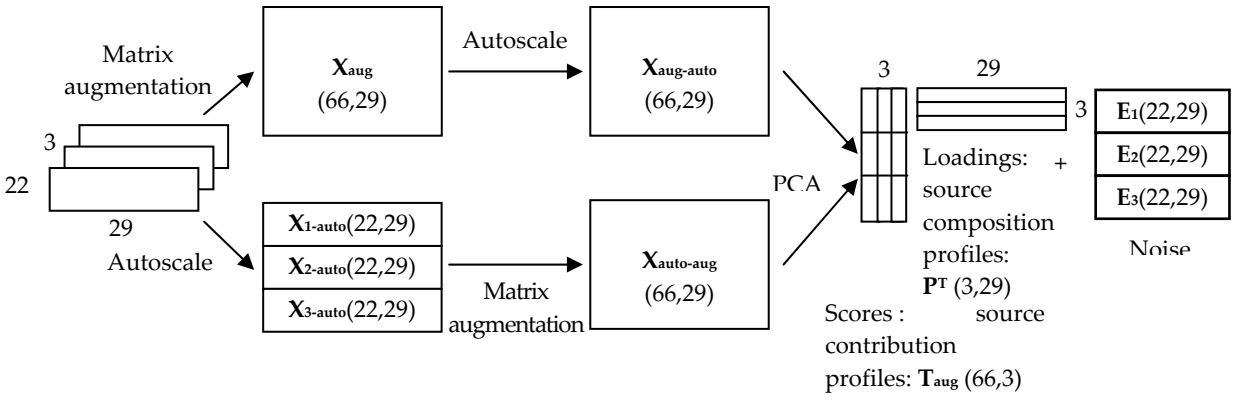

Fig. 1 Data matrix augmentation and pre-treatment scheme and bilinear principal component analysis (PCA) model decomposition (Equation 1). aug: augmented; auto: autoscaled; 1-6: number of sampling campaign

Autoscaling was the pretreatment chosen since it gave a better understanding of the composition and distribution of the different contamination sources.[22] In a previous work using historical data on Ebro river basin five different pretreatments were tested and autoscaling appeared to be the one giving best results for this type of data.[1] With this procedure the mean of the column elements was subtracted from individual elements and divided by their column standard deviation. Consequently, each column has zero mean and unit variance.[23]

Autoscaling was applied to the individual matrices corresponding to each sampling campaign previous and after matrix augmentation. Theoretically the results obtained with these two procedures are different. When the augmented matrix is built up and then autoscaled,

the results of PCA will show the temporal variations (differences between the 3 sampling campaigns). On the contrary, when the matrices are firstly autoscaled separately and then the augmented matrix is built up, the results of the PCA will focus more in geographical variations and less in temporal variations. In Fig. 1 these two different combined procedures of pretreatment and matrix augmentation are illustrated.

In our study, before autoscaling, NP dominated completely the data matrix, as the concentrations found in sediments were much higher than for the other compounds. This fact confirmed the necessity of applying this pre-treatment before using PCA. In our case, the results on the data matrix when applying the two kinds of autoscaling were very similar. In both cases extreme values corresponded to high concentration samples and the rest of the values were skewed towards lower values. When autoscaling was applied before the augmentation the distribution of the values was more homogeneous than when autoscaling was applied after matrix augmentation.

Principal component analysis.

PCA is a data reduction technique that aims to explain most of the variance in the data, while transforming a set of correlated measured variables into a set of a few uncorrelated components (PCs, Principal Components),[20] while attempting to preserve at the same time the relationships present in the original data.[24] The main goal of this multivariate statistical technique is to extract useful information and provide an easier visualization of the existent relationships among objects and variables determined in large or complex data sets.[25] This allows a better understanding of environmental processes[26] where diffuse instead of specific contamination prevails. In this study PCA was applied to the augmented matrices obtained from the two different ways previously explained. PCA bilinear model may be written using the following matrix decomposition equation:

$$\mathbf{X}=\mathbf{T}\mathbf{P}^{\mathbf{T}}+\mathbf{E} \qquad (1)$$

where the matrix **X** is the data matrix obtained when the 18 variables were measured in the water samples from the 23 sampling sites during 6 sampling campaigns (138 samples). It is decomposed to a scores matrix, **T**, and loadings matrix, $\mathbf{P}^{\mathbf{T}}$.[27] The loadings matrix gives information about the composition profiles of the N sources detected during data analysis, which are called Principal Components (PC) and are weighted linear combinations of the original measured variables.[28] PCs are extracted so that the maximum amount of variance is explained in the first PC and progressively less variance is explained for each subsequent component.[20] The scores matrix gives information about the distribution profiles for the samples considering the detected sources. In the case under study, **T** has dimensions of 138 x N and $\mathbf{P}^{\mathbf{T}}$ the dimensions of N x 18. Finally, matrix **E** refers to the residual data variations not modelled by the N detected sources and it has the same dimensions as **X**. In Fig. 2, a detailed description of the data structure for the two augmented matrices and their modelling by PCA are given for the case that the number of resolved components is equal to three, N=3.

A new row space is constructed with the PCs as new axes, in which to plot the scores, which are the redefined original samples into the new axes. The plot of the scores (matrix T) into the space defined by the PCs illustrate the dominant patterns present within the samples.[20]

PCA modelling was conducted using the PLS Toolbox (Eigenvector Research, Manson WA, USA) appropriate functions under the MATLAB computer and visualization environment (The Mathworks, Natick, MA, USA).

Results and discussion

Univariate descriptive statistics

Descriptive statistics data is summarized in Table 3 and compared to legislated levels and toxicological data. The frequency of detection of each compound varied from 2 % to 100 %, with bisphenol A, propanil, alachlor, molinate, heptachlor, dieldrin and endrin aldehyde being the less frequently detected. Another group composed by gamma-HCH, parathion-methyl, endosulfan sulfate and isodrin were found with less than 10 % of frequency of detection. Both groups were not included in the chemometrical analysis with PCA due to its low occurrence in the Ebro sediments. Pyr, Chr and BaP appeared in all the analysed samples while the rest of PAHs had more than 55 % of appearance, confirming that this family is very widespread in the sediments along the Ebro. The four lighter PAHs, with two and three aromatic rings (Ace, Flu, Naph and Ace) were the ones less frequently detected due to their higher volatility, solubility and biodegradability[17] and therefore less bioaccumulated in the sediments. Considering the group of PPs, only 5 out of the 25 analyzed appeared in the sediment matrix and with less than 13 % of appearance. These compounds have higher affinity for the water,[29] and were not supposed to appear in the sediment matrix due to their more polar characteristics. Only diazinon had a 12 % of appearance due to its more widespread use as pesticide.[30] As expected, OCs appear more often than PPs, up to 97 %, with an important presence of DDTs, especially 4,4-DDT, although this compound is forbidden in Spain for agricultural purposes since 1994.[31]

The compounds that presented higher maximum concentration were the most ubiquitous PAHs, all with more than 1,000 μg kg^{-1}, together with NP with a maximum concentration of almost 6,000 μg kg^{-1}. NP presented also a median and a mean concentration of 377 and 876 μg kg^{-1} respectively, more than 5 times higher than the rest of compounds, and also a presence in more than 87 % of the samples. Comparison of mean and median values of all the variables showed that mean values were always higher than the median values, indicating that data were skewed towards low values

Table 3 Descriptive statistics of data

Compounds	Parameters [a]											
	LD (µg kg^{-1})	Min. (µg kg^{-1})	Max. (µg kg^{-1})	Median (µg kg^{-1})	Mean (µg kg^{-1})	SD (µg kg^{-1})	FD (%)	Number of positive samples	% > Canadian limit	% < TEC	TEC-PEC	% > PEC
Pyr	0.05	1.02	4139.14	15.93	117.87	524.50	**100**	65	23	94	5	2
Chr	0.08	0.31	452.71	20.26	54.15	83.98	**100**	65	31	91	9	0
BaP	0.07	1.84	1825.73	55.49	144.15	252.66	**100**	65	65	69	29	2
Flut	0.04	0.90	4931.01	16.93	147.46	642.52	**97**	63	11	95	3	2
BaA	0.09	0.77	259.30	10.61	35.26	58.18	**97**	63	32	94	6	0
4,4-DDT	0.39	0.45	500.57	8.28	48.38	92.95	**97**	63	-	-	-	-
DDT	-	0.45	503.75	8.28	51.26	96.70	**97**	63	95	29	51	21
InP	0.07	0.80	1739.26	54.49	116.93	242.67	**95**	62	-	-	-	-
BghiP	0.06	0.11	1313.81	56.10	110.23	189.41	**94**	61	-	-	-	-
Phe	0.03	0.77	255.75	29.43	45.48	53.61	**89**	58	33	97	3	0
BbF	0.05	0.06	144.47	16.23	23.25	28.57	**89**	58	-	-	-	-
DahA	0.23	2.26	967.92	36.44	77.73	158.20	**89**	58	90	41	59	0
NP	19.57	69.00	5998.92	377.15	876.30	1208.23	**88**	57	21	-	-	-
OP	1.11	1.22	143.13	12.13	27.80	33.31	**86**	56	-	-	-	-
DDE	-	0.50	160.15	2.51	12.56	28.79	**83**	54	69	56	33	11
Ant	0.04	0.17	175.85	5.34	14.71	28.53	**82**	53	4	96	4	0
BkF	0.06	0.17	119.31	13.46	22.29	25.96	**79**	51	-	-	-	-
Acy	0.03	0.03	291.23	1.88	17.77	50.19	**69**	45	24	-	-	-
Flu	0.06	0.54	194.16	4.98	28.01	49.26	**66**	43	30	88	12	0
4,4-DDE	0.24	0.34	141.28	3.58	14.09	28.75	**63**	41	-	-	-	-
Naph	0.03	0.05	52.75	1.65	4.37	9.59	**59**	38	3	100	0	0
Ace	0.05	0.42	695.71	4.56	33.84	117.31	**55**	36	47	-	-	-
2,4-DDE	0.19	0.50	18.87	1.41	2.78	3.87	**55**	36	-	-	-	-
DDD	-	0.30	125.37	4.30	18.65	31.10	**49**	32	56	47	38	16
2,4-DDD	0.20	0.30	77.86	1.75	11.92	19.47	**43**	28	-	-	-	-
4,4-DDD	0.25	0.35	82.96	5.14	10.52	17.93	**39**	25	-	-	-	-
TBP	0.74	1.02	10.66	6.96	6.21	2.96	**20**	13	-	-	-	-
2,4-DDT	0.78	2.21	50.86	9.83	15.10	13.87	**19**	12	-	-	-	-
Alpha-HCH	0.55	1.59	54.41	3.46	11.28	17.12	**14**	9	-	-	-	-
Diazinon	0.60	7.61	72.08	10.45	21.01	21.91	**12**	8	-	-	-	-
HCB	0.97	20.66	263.87	73.36	88.12	88.48	**12**	8	-	-	-	-
Gamma-HCH	0.63	11.67	52.28	19.62	25.80	18.40	**6**	4	100	0	0	100

Parathion-methyl	1.62	10.49	90.32	50.41	50.41	56.45	3	' 2	-	-	-
Pentachlorobenzene	0.41	0.56	0.90	0.73	0.73	0.24	3	2	-	-	-
Endosulfan sulfate	1.92	2.53	9.86	6.20	6.20	5.18	3	2	-	-	-
Isodrin	0.89	17.52	32.68	25.10	25.10	10.72	3	2	-	-	-
Bisphenol A	56.33	57.95	57.95	57.95	57.95	-	2	1	-	-	-
Propanil	1.14	6.89	6.89	6.89	6.89	-	2	1	-	-	-
Alachlor	0.39	7.17	7.17	7.17	7.17	-	2	1	-	-	-
Molinate	0.60	3.45	3.45	3.45	3.45	-	2	1	-	-	-
Heptaclor	0.80	5.75	5.75	5.75	5.75	-	2	1	-	-	-
Dieldrin	0.48	3.24	3.24	3.24	3.24	-	2	1	100	0	100	0
Endrin aldehyde	5.34	112.18	112.18	112.18	112.18	-	2	1	-	-	-	-

[a] LD: limit of detection; Min: minimum; Max: maximum; Median; Mean; SD: standard deviation; FD: frequency of detection; % > Canadian limit: percentage of samples above the limit considered in the Canadian sediment quality guidelines for the protection of aquatic life; % < TEC: percentage of samples below the TEC; TEC-PEC: percentage of samples between TEC and PEC; % > PEC: percentage of samples above PEC

with some peaks of contamination. This behaviour produces also large data dispersion for the different sampling campaigns as well as sampling sites, which can be observed in a standard deviation higher than the mean.

Comparison with legislated and toxicological data

To evaluate the potential risk of Ebro river sediments, the concentrations detected were compared to actual legislation and toxicological data. From a legislative point of view, the Water Framework Directive (WFD) is the most important piece of European water legislation in Europe[32] and has been used as a guideline for the water results evaluation of the Ebro River basin.[29] However the WFD, in force since the year 2000, does not specifically address sediment management.[33] Contrarily, the Canadian quality guidelines for the protection of aquatic life[14] provides data for different types of sediments and include 33 compounds, PAHs and DDTs among them. In addition, for a better environmental interpretation of the sediment data obtained, samples have been classified in the three ranges of concentrations defined by the threshold effect concentration (TEC; below which adverse effects are not expected to occur) and the probable effect concentration (PEC; above which adverse effects are expected to occur more often than not).[13] The legislated and toxicological lists include 20 and 17 of the compounds considered in this study respectively and represent half of the ones detected in the samples. All the PAHs included in the Canadian guideline are present above the limit, ranging from 3 % of the samples for Naph to 90 % for DahA. The toxicological interpretation of PAHs using TEC and PEC indicated that around 90 % of the samples are below the proposed TEC and none or very small percentage will cause an effect in the living organisms. Especial attention has to be paid to BaP and DahA, considered as the most carcinogenic ones among PAHs.[34] These two compounds have 65 and 90 % of samples above the Canadian limit respectively and 29 and 59 % of the samples with concentrations between TEC and PEC, resulting in a high potential risk for the living organisms in the Ebro river ecosystem.

In the case of DDTs, the three compounds (DDT, DDE and DDD) are considered as the sum of both 2,4 and 4,4 isomers. Half of the samples were above the limits proposed by Canadian guideline and DDT had more than 95 % of the samples above this limit. The highest concentration levels, ranging from 15.3 to 351 μg kg^{-1}, also imply that between 10 and 20 % of the samples will probably cause a toxic effect to the environment. Two compounds with very small percentage of appearance in the samples, gamma-HCH and dieldrin, have high concentrations considering the Canadian limits and the four samples with gamma-HCH (2005: R0, R7, T11 and T15) will probable cause toxic effects as all of them are above the PEC.

In contrast with the water results,[29] in which only three compounds were above the Canadian legislated

limits, the sediments present more elevated concentrations that cause that out of the 20 compounds legislated, 18 have some samples above the limits. This different behaviour shows that the water is a more changeable matrix that reflects the current contamination while the sediments behave as a reservoir for the contaminants due to their accumulation potential.

Comparison with water data

In Fig. 2 bar plots of the compounds that appeared simultaneously in both water[25] and sediment matrix were drawn. Among all the compounds analysed, only eight (OP, NP, TBP, bisphenol A, diazinon, propanil, alachlor and molinate) appeared in both matrices. The sampling sites were ordered following the Ebro direction, from north-west to south-east. Fig. 2a shows the bar plot of the three coincident water sampling campaigns and Fig. 2b shows the same graphic for the three sampling campaigns performed in the sediment. The most noticeable fact is that NP is the compound that contributes more in both water and sediments, as it has medium value for log K_{OW} (see Table 1) and the concentrations found are high enough to enable its distribution between the two matrices. The general view of the graphics shows a similar peak of contamination around T11, which is situated in the industrial area of Zaragoza. The rest of the profile shows some differences between water and sediments. Water moves constantly while the sediments are more stable, in consequence the correspondence is not complete as the water indicates the punctual state of the basin and sediments the historical accumulation.

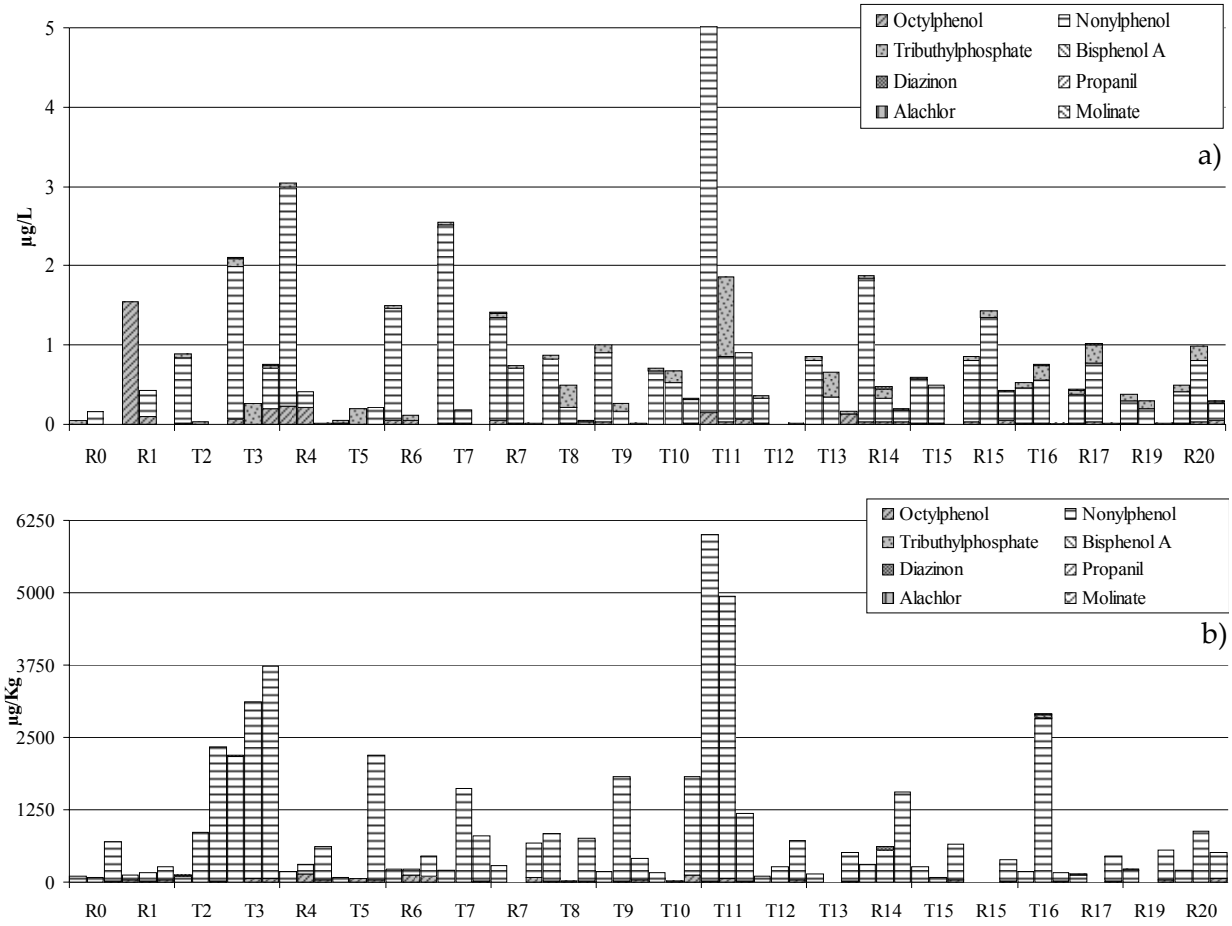

Fig. 2 Concentration of the common compounds between water and sediments observed in the 22 sampling sites along the Ebro. (a) water matrix during the six sampling campaigns (June 2004, October 2004, June 2005, October 2005, June 2006 and October 2006) and (b) sediment matrix during the three sampling campaigns (October 2004, October 2005, October 2006)

Pairwise correlations

In Table 4, pairwise correlations between all the studied variables are given. TOC had very low correlations with the compounds under study, and only shows moderate correlation with NP. Among the compounds, PAHs on one hand and DDTs on the other present moderate to strong correlations. The family of PAHs had correlations among the eight heavier ones, as well as with Phe and Ant. On the other side Naph and Acy had strong correlations with the smaller ones but no correlation is found between the light and heavy PAHs. This first analysis can suggest that the behaviour of the

Table 4 Pairwise correlation coefficients between the variables [a]

	TOC	Naph	Acy	Ace	Flu	Phe	Ant	Flut	Pyr	BaA	Chr	BbF	BkF	BaP	InP	DahA	BghiP	OP	NP	TBP	Diazinon	a-HCH	HCB	2,4-DDE	4,4-DDE	2,4-DDD	4,4-DDD	2,4-DDT	4,4-DDT
TOC	1.00																												
Naph	0.10	1.00																											
Acy	0.01	**0.63**	1.00																										
Ace	-0.10	0.18	**0.25**	1.00																									
Flu	0.04	0.17	**0.50**	**0.50**	1.00																								
Phe	0.24	**0.46**	**0.29**	0.00	0.06	1.00																							
Ant	0.03	**0.71**	**0.47**	-0.04	-0.03	**0.73**	1.00																						
Flut	-0.10	**0.80**	**0.43**	-0.02	-0.06	**0.60**	**0.91**	1.00																					
Pyr	-0.08	**0.82**	**0.43**	-0.02	-0.05	**0.59**	**0.90**	**0.99**	1.00																				
BaA	0.03	**0.39**	**0.26**	-0.06	-0.09	**0.89**	**0.76**	**0.64**	**0.62**	1.00																			
Chr	0.16	**0.27**	0.15	-0.07	-0.09	**0.88**	**0.61**	**0.47**	**0.46**	**0.95**	1.00																		
BbF	0.09	0.03	0.13	-0.06	-0.07	**0.66**	**0.39**	0.20	0.19	**0.79**	**0.83**	1.00																	
BkF	0.10	0.09	0.22	-0.05	-0.03	**0.64**	**0.45**	0.23	0.22	**0.76**	**0.75**	**0.94**	1.00																
BaP	-0.10	0.04	0.04	0.07	0.07	**0.54**	**0.29**	0.17	0.15	**0.65**	**0.60**	**0.48**	**0.43**	1.00															
InP	-0.15	-0.06	-0.03	0.00	-0.01	**0.50**	0.20	0.10	0.07	**0.60**	**0.57**	**0.45**	**0.36**	**0.94**	1.00														
DahA	-0.08	-0.06	-0.02	0.00	-0.01	**0.54**	0.19	0.10	0.07	**0.62**	**0.64**	**0.50**	**0.35**	**0.86**	**0.96**	1.00													
BghiP	-0.14	-0.06	-0.04	0.02	0.01	**0.51**	0.18	0.08	0.06	**0.60**	**0.59**	**0.48**	**0.38**	**0.95**	**0.99**	**0.96**	1.00												
OP	0.17	0.07	0.03	0.05	0.19	0.10	0.08	0.03	0.03	-0.02	0.00	-0.06	-0.04	0.02	0.05	0.06	0.03	1.00											
NP	**0.35**	0.01	-0.02	-0.01	0.03	0.17	0.02	-0.01	0.00	0.02	0.08	-0.01	0.07	-0.09	-0.12	-0.13	-0.11	0.16	1.00										
TBP	-0.27	-0.14	-0.12	-0.09	-0.19	-0.21	-0.15	-0.08	-0.07	-0.09	-0.07	-0.06	-0.06	-0.03	0.00	-0.02	0.01	-0.25	-0.18	1.00									
Diazinon	0.17	-0.02	-0.07	-0.05	-0.10	-0.02	-0.03	-0.05	-0.04	-0.03	-0.01	-0.03	-0.05	-0.04	-0.08	-0.09	-0.10	-0.15	0.01	-0.09	1.00								
a-HCH	0.08	-0.05	-0.03	-0.04	-0.03	0.03	0.01	-0.02	-0.02	-0.01	0.00	-0.03	-0.03	-0.04	-0.03	-0.03	-0.05	-0.07	0.02	-0.09	-0.05	1.00							
HCB	-0.02	-0.07	-0.06	-0.04	-0.03	-0.01	-0.04	-0.04	-0.03	0.11	0.17	**0.35**	**0.25**	0.16	0.16	0.19	0.19	-0.12	-0.13	0.22	-0.07	-0.06	1.00						
2,4-DDE	-0.15	-0.07	-0.07	-0.08	-0.08	-0.02	-0.07	-0.02	-0.02	0.08	0.09	**0.25**	0.22	0.10	0.10	0.09	0.11	-0.10	-0.18	**0.26**	-0.07	-0.10	**0.71**	1.00					
4,4-DDE	-0.01	-0.06	-0.05	-0.05	-0.02	-0.01	-0.07	-0.04	-0.04	0.08	0.13	**0.27**	0.22	0.08	0.10	0.13	0.10	-0.10	-0.09	0.19	-0.07	-0.07	**0.81**	**0.90**	1.00				
2,4-DDD	0.01	-0.10	-0.08	-0.07	-0.09	-0.11	-0.10	-0.06	-0.06	0.01	0.06	0.16	0.07	0.03	0.06	0.13	0.07	-0.09	-0.16	0.20	-0.03	-0.08	**0.70**	**0.78**	**0.83**	1.00			
4,4-DDD	-0.10	-0.07	-0.05	-0.04	0.00	-0.01	-0.06	-0.03	-0.03	0.01	0.02	0.11	0.12	-0.01	0.03	0.02	0.01	-0.02	-0.12	0.05	-0.07	-0.07	**0.45**	**0.86**	**0.77**	**0.63**	1.00		
2,4-DDT	-0.09	-0.10	-0.06	-0.04	0.01	-0.04	-0.05	-0.06	-0.04	0.02	0.04	0.15	0.12	0.07	0.08	0.05	0.09	-0.03	-0.12	**0.30**	-0.09	-0.04	**0.71**	**0.52**	**0.49**	**0.52**	**0.32**	1.00	
4,4-DDT	-0.02	-0.05	-0.10	-0.07	-0.03	-0.05	-0.08	-0.07	-0.06	0.01	0.04	0.15	0.11	0.05	0.06	0.06	0.06	-0.17	-0.11	0.16	-0.03	-0.07	**0.59**	**0.71**	**0.86**	**0.69**	**0.65**	**0.41**	1.00

[a] Higher pairwise correlations (>0.25) are marked in bold (significance 95%)

PAHs can be different depending on the amount of aromatic rings. The group of DDTs, together with HCB, presents also strong correlations since they all are chlorinated compounds, suggesting their simultaneous presence in sediments due to a common use in the past. Better understanding about multivariate correlations among different variables is obtained by the application of PCA.[26]

PAHs sources

The PAHs may originate from pyrolytic sources, including the natural and anthropogenic combustion of organic matter (e.g., forest fires, surface runoff, domestic coal or wood combustion and car exhausts) and from petrogenic sources (e.g., present in subsoil, oil spills).[35,36] According to many references, sources of PAHs may be assessed by some ratios of specific molecular PAHs compounds.[37,38] In this study, Phe/Ant and Flut/Pyr were used to survey the possible sources of PAHs in Ebro river Basin. The ratio of Flut/Pyr<1.0 was usually attributed to petrogenic source, while Flut/Pyr>1.0 was suggested to indicate pyrolytic sources. The ratio of Phe/Ant<10 was usually regarded as an indication of pyrolytic sources, while Phe/Ant>10 was mainly from petrogenic source.[39]

Based on the ratios of Phe/Ant versus Flut/Pyr (Fig. 3), it could be seen that sediments from Ebro river basin were mainly contaminated by pyrolytic PAHs. No sample has a complete petrogenic origin. In 2004 and 2006 sampling campaigns the occurrence of PAHs may be originated from both pyrolytic and petrogenic sources in 30 % of the sites and the rest from only pyrolytic sources while in October 2005 sampling campaign all the samples have a complete pyrolytic origin. For a better interpretation the names of the sites with both petrogenic and pyrolytic origin have been added to the graphic. Most of the sampling sites appear punctually in the group of mixed source but the samples coming from T11 appeared during the 3 year monitoring in the petrogenic/pyrolytic source group. This sampling site is located around the industrial area of Zaragoza, consequently the petrogenic origin can be attributed to chemical industry.

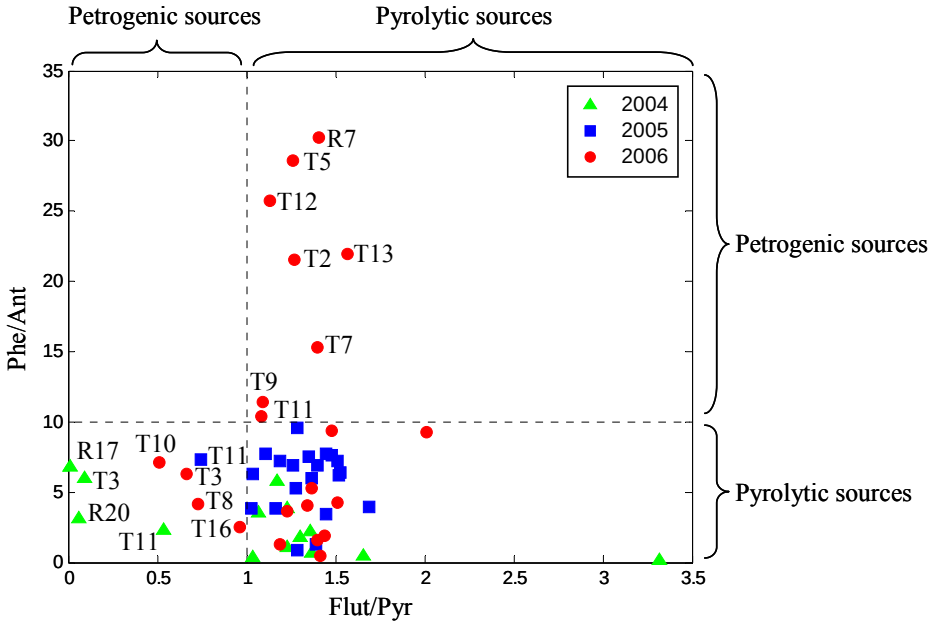

Fig. 3 The ratios of Phe/Ant versus Flut/Pyr in sediments from the Ebro River Basin. The names of the singular samples have been added for better understanding

Principal Component Analysis

Table 5 shows the results of PCA when it was applied to the augmented and autoscaled data matrix ($X_{aug\text{-}auto}$) and also to the autoscaled and augmented data matrix ($X_{auto\text{-}aug}$).

In both cases the explained variances were very similar, slightly higher for the first PC of $X_{auto\text{-}aug}$ PCA. The results obtained with the two different PCAs were also very similar, showing the same contamination sources and an analogous distribution of the samples into the new axis. In addition, no temporal trend was appreciated by PCA of $X_{aug\text{-}auto}$ whereas a better characterization of samples could be observed by PCA of $X_{auto\text{-}aug}$. Consequently, for brevity, and because of their more environmental relevance only the results using the first three PCs of the $X_{auto\text{-}aug}$ PCA are presented here. Using these three PCs the data dimensionality was reduced from the 28 original variables to the new 3 PCs, explaining more than 60 % of the original data variance (for autoscaled data).

In Fig. 4, the loadings plot for the first three PCs is given. The first PC explained 32.28% of the total data variance and it had high positive loadings for the twelve heavier PAHs from Phe to BghiP, approximately at the same level. In the previous analysis of pairwise correlation coefficients, this aspect was also detected.

The group of DDTs together with hexachlorobenzene had a very small contribution to this first PC. Therefore, this PC describes mostly a source of PAHs, i.e. most of the heavier PAHs have a common origin due to incomplete combustions of organic matter except the lighter ones, with only two and three aromatic rings, which behave differently due to their easier biodegradability,[40] as well as their higher volatility and solubility, as can be deduced from values on Table 1. Second PC explained 19.86 % of the total data variance and had high loadings for all DDTs group, together with HCB. All these compounds are chlorinated, and were commonly used as pesticides until they were banned in Spain in 1994.[31] Nowadays DDTs are still present in the environment mostly as a consequence of an historical contamination but also because of their presence as impurity in the manufacture of dicofol.[41] On the other hand, hexachlorobenzene had several uses in agriculture and industry. It continues to be produced as a byproduct during manufacture of some solvents and pesticides, and incineration of municipal waste.[42] The simultaneous presence of these compounds in this second PC at approximately similar levels indicates their common origin. In this PC most of the 2, 3 and 4-ring PAHs present small negative loadings (inversely correlated to OC compounds), together with the TOC. NP and OP have also a small negative loading but TBP have a moderate positive one. The third PC explained a 10.52 % of the total variance and had high positive loadings for TOC and the four lighter PAHs that were not included in the first PC (Naph, Acy, Ace and Flu). These PAHs are moderately negatively correlated with the heavier ones, showing the different behaviour depending on the higher aromaticity of the compound. NP has also a moderate positive loading and DDTs and HCB present a small positive loading. These compounds can be related due to the use of NP as adjuvant in pesticide formulations.[18]

Table 5 Percentages of explained variances obtained by PCA [a]

Matrix	$X_{aug\text{-}auto}$	$X_{auto\text{-}aug}$
PC1	25.72	32.28
PC2	18.75 (44.77)	19.86 (52.13)
PC3	11.71 (56.17)	10.52 (62.65)
PC4	6.85 (63.03)	5.61 (68.26)
PC5	6.42 (69.45)	4.70 (72.96)

[a] The percentage of accumulated explained variance for that particular component is given in parentheses

The absence of a large contribution of NP in the loadings, although its concentration in the environment is the highest among the compounds analyzed, is a consequence of its rather high constant amount in all samples. Since data were autoscaled and a large number of variables were included in the analysis, the contribution of this compound to the variance is not reflected until PC5, which explains a 4.7 % of the total variance. If data were not mean-centred, the influence and presence of NP in the first component is immediately found. NP would be the only contributor to PC1, with a 67.51% of the total variance. This is due to the much higher concentrations of NP in relation with the levels in the analyzed samples of the other compounds considered. Consequently, autoscaling the matrix is a necessary pretreatment for the interpretation of the results but their individually consideration contributes to the complete vision of the Ebro River basin contamination.

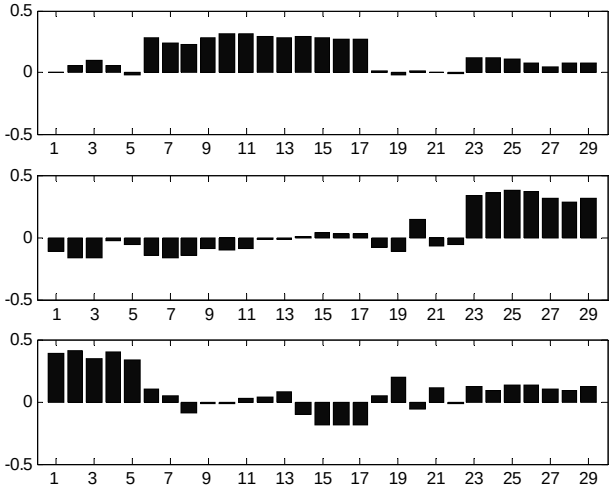

Fig. 4 Loadings for the three principal components (matrix $\mathbf{P}^T$ in Equation 1 and Fig. 2) for $X_{auto\text{-}aug}$ Identification of variables: 1: TOC; 2: Naph; 3: Acy; 4: Ace; 5: Flu; 6: Phe; 7: Ant; 8: Flut; 9: Pyr; 10: BaA; 11: Chr; 12: BbF; 13: BkF; 14: BaP; 15: InP; 16: DahA; 17: BghiP; 18: OP; 19: NP; 20: TBP; 21: Diaz; 22: a-HCH; 23: HCB; 24: 2,4-DDE; 25: 4,4-DDE; 26: 2,4-DDD; 27: 4,4-DDD; 28: 2,4-DDT; 29: 4,4-DDT

Scores plots of $X_{ato\text{-}aug}$ data matrix are given in Fig. 5. The samples have been coded into 3 different classes considering the three sampling campaign (different symbols) and the codes of the sampling sites are indicated for each of the samples (using a letter and a number for their identification according to Table 2).

In the PC1 vs. PC2 scores plot (Fig. 5a), four main groups are distinguished. The most noticeable one includes the three campaign samples in R17 site (collected in 2004-2006). These samples presented high positive values both for PC2 and PC1 indicating that they were very contaminated with PAHs and with OCs. But while the concentration of PAHs decreases within years, the concentration of OCs increases. This sampling site corresponds to a very important industrial area near the city of Flix (close to the Ebro delta), where a large production of chlorinated pesticides has been carried out in the past. A second cluster included samples with different values for PC2 indicating a

different degree of contamination attributed to the presence of OCs. The samples included in this cluster are R20, R19 and R15 from the three sampling campaigns, together with T5, only from 2005 sampling campaign. Most of them are located downstream the chlorinated industry of Flix and also can receive the impact of the agricultural practices in the Ebro Delta, since some of the compounds included of PC2 are present as impurities in the pesticides. R15 and T15 have a small contribution of this PC although they are located in the surroundings of Monzón chlorine industry. The presence of T5 (2005) in this cluster could indicate a punctual contamination by this source due to the application of pesticides, as this sampling site is situated in an agricultural area. The third cluster includes samples from low to high positive values of PC1 scores, describing samples with different contamination levels of PAHs. Included in this cluster are also several samples with no relation between them, indicating a widespread contamination distribution of this source. The two samples that presented larger score values for PC1 belong to R0 sampling site, which is a sampling site only 6 km downstream of the Ebro spring (North of Spain). This highly PAHs contamination area detected in this a priori unpolluted area can be attributed to important active mining activities during the last two

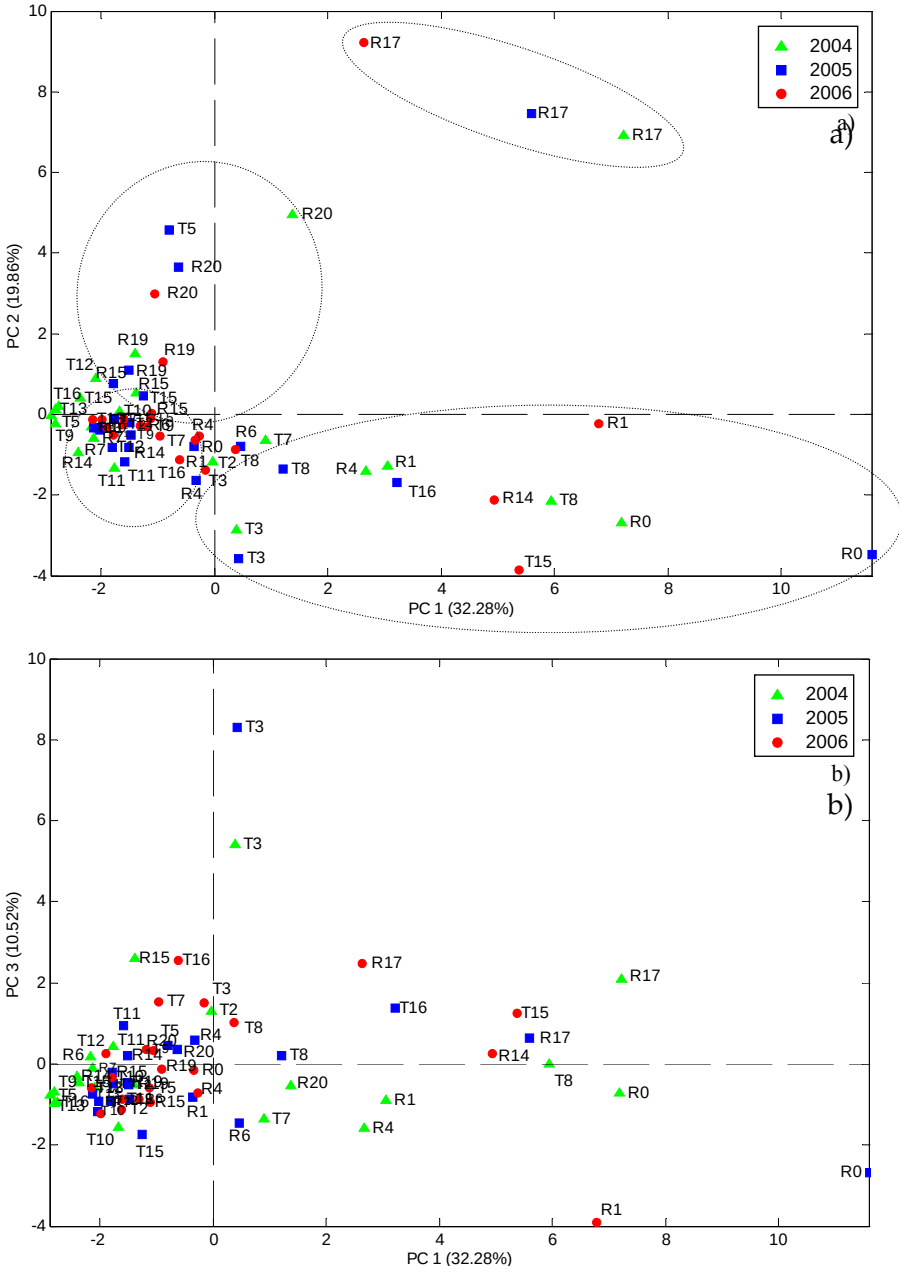

Fig. 5 Scores plots from PCA (matrix $\mathbf{T}_{aug}$ in Equation 3 and Fig. 2). All sediment samples (all sampling sites identified in Table 2 and sampling campaigns given on the upper right of this Figure) were included in the analysis. (a) for the first two principal components; (b) for the first and the third principal components

centuries. The last cluster contains the rest of samples all with small score values for both PCs which indicates a low level contamination.

In Fig. 5b, the PC1 vs. PC3 scores plot is shown. In this case the samples from the two clusters that showed positive score values for PC2 are now separated depending on their score value for PC3. The samples with positive PC3 scores are lighter and generally have two or three aromatic rings together with NP, whereas the samples with negative scores generally have higher presence of five or six aromatic rings. Another cluster is detected from PC3 scores, including the three samples from T3, as well as R15 (2004) and T16 (2006). These samples are contaminated mainly by compounds with high loadings in PC3 and very low loadings in PC1.

Overall, once a large data set is obtained by successive monitoring campaigns, PCA is a useful tool to assess the temporal and geographical contamination patterns. No temporal tendencies were observed for the samples in the scores plot, except for the already explained Flix sample. Differently to what was observed in the previous study of water samples,[29] there was no distinct geographical distribution of the contamination sources over the river basin with the exception of the OC pesticides present close to the chlorine industry of Flix.

Conclusions

A 3 year monitoring of river sediments along the Ebro river basin permit to evaluate the presence and fate of priority compounds. The contamination status can thus be assessed according to toxicological values. Overall, the PCA analysis reveals two main sources of contamination in sediments from the Ebro River Basin. The first one is mostly loaded by PAHs while the second one is loaded by all the DDTs compounds together with hexachlorobenzene. PAHs were widespread all over the sampling area and with concentrations above some legislated levels, although only a small percentage of samples will cause an effect in the living organisms. Among all the pesticides analyzed only DDTs and hexachlorobenzene appeared at high concentrations, although both compounds are forbidden in Spain. Between 11 and 20 % of the samples containing DDTs will have toxic effects on the living organisms. The comparison of the simultaneously detected compounds in water and sediments reveals a predominance of nonylphenol, found at concentrations between 69 and 5999 $\mu g\ kg^{-1}$ in the solid matrix. This compound shows a lower importance in the PCA analysis due to its appearance as punctual pollutant, mainly around the industrial area of Zaragoza. Contrarily to the results obtained for the water matrix, the sediment contamination does not show any temporal or geographical contamination but a widespread presence with some punctual high concentrations.

Acknowledgements

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4.3.3.- Compartimentació de PAHs entre matrius ambientals

Quan els contaminants són emesos al medi, aquests es distribueixen entre la multitud de matrius existents en base a les seves propietats fisicoquímiques, que provoquen una major afinitat per una matriu o una altra. Dins d'una mateixa família podem trobar gradació en les propietats fisicoquímiques dels seus integrants. En el cas del PAHs, per exemple, existeixen congèneres amb pocs anells aromàtics que presenten una major volatilitat que aquells hidrocarburs més grans i pesats. A més a més els PAHs s'emeten en la major part de les ocasions com una barreja en la què estan presents gran quantitat de compostos.

Per a poder estudiar la relació entre la contaminació produïda per PAHs en tres matrius ambientals, *a priori* amb característiques similars, es van analitzar els perfils dels diferents congèneres en sòls, sediments i pins de la conca de l'Ebre. Totes les mostres es van recollir durant una mateixa campanya, considerant a més que per a cada mostra de sòl o sediment es va recollir una mostra del pi més proper. Els resultats obtinguts d'aquest estudi comparatiu per a la família dels PAHs es recullen a l'article científic 7, reproduït a continuació.

Article científic 7

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Abstract
The content of 16 polycyclic aromatic hydrocarbons (PAHs) in 60 samples of three major environmental matrices (soils, sediments and pine needles) was determined in an effort to assess their distribution at a river basin scale. A sampling campaign was carried out in autumn 2006, selecting the sampling locations by their urban, industrial and agricultural activities along the Ebro River Basin (NE Spain). Techniques used involved pressurized liquid extraction (PLE) and solid-liquid ultrasonic extraction followed by gas chromatography-electron impact ionisation mass spectrometry. Established PAH ratios and Principal Component Analysis were used to identify the origins and profiles of PAHs. While sediments showed contamination patterns with the full range of compounds attributed to historical inputs, soils and pine needles presented a compartmentalization of the PAHs, with lighter air-borne PAHs accumulated in pine needles and more heavy ones in soils. It can be concluded that the monitoring of several matrices is necessary to elucidate the contamination sources and accumulation patterns of PAHs.

Keywords: sediment; soil; pine needles; PAHs; ratios; PCA

1. Introduction

The Ebro River basin, located in the NE of Spain, covers an approximate area of 85,000 km^2 and has a population density of 38 inhabitants per km^2 with a highly uneven distribution [1]. Being mainly agricultural, the region also incorporates industrial activities concentrated close to the cities of Zaragoza, Vitoria, Pamplona, Logroño, Lleida, Monzón and Flix. The first five are also the main urban centres, harbouring up to 45% of the population [2]. Previous studies in the Ebro basin area report a wide range of pollutants generated by urban, industrial and agricultural wastes which tend to accumulate on water, sediments, soils, plants or animals [3]. Heavy metals [4], organophosphorous and organochlorinated compounds [5, 6], pesticides [7-10], pharmaceuticals [11], dioxins and furans [12, 13], polychlorinated biphenyls (PCBs) [12, 14], polybrominated diphenyl ethers (PBDEs) [15, 16] and polycyclic aromatic hydrocarbons (PAHs) [15, 17, 18] have been identified in environmental samples from this basin.

Due to the industrial and agricultural activities of the basin and to the high population density in some cities, PAHs in particular have an important impact on the Ebro ecosystem. Their widespread presence is of concern given their proven carcinogenic and mutagenic properties [19] and also because they are considered priority pollutants [20]. With this panorama, it is needed to implement monitoring programs that include different environmental compartments to better evaluate the presence, compartmentalization and fate of PAHs over a large geographical area which in turn will permit a deeper risk assessment and decision making actions.

Traditionally, PAHs have been monitored in water, sediments, soils and air and their contamination patterns are well depicted [21]. However, vegetation has been only recently been incorporated as an important matrix to be used for the biomonitoring of PAHs [22]. Among others, pine needles have been used because of their perennial character and the fact that the lipidic-waxy cover has a big tendency to accumulate air-borne contaminants [23]. Therefore, pine needles may provide complementary information on the presence of PAHs in monitoring programs. This matrix has already been used to determine the concentration and sources of PAHs [24], to evaluate their spatial distribution [25] and as passive samplers [26].

When several matrices are analysed in basin-scale monitoring programs, multivariate factor analysis and related tools are frequently used to help the understanding of experimental results and elucidate the trends of organic pollutants. The use of chemometric methods may identify main point and diffuse sources of contamination and establish the respective distribution profiles among samples [1]. Furthermore, and in the case of PAHs, the critical step of source assessment for risk evaluation and management of contaminants [27] was determined in many environmental studies by PAH molecular diagnostic ratios [28].

The purpose of this work was to evaluate the contamination patterns and compartmentalization of

sixteen PAH in three environmental matrices (soils, sediments and pine needles) collected in 30 vulnerable sites along the Ebro River basin. Soils were collected in 9 sites and sediments in the remaining 21 sites, whereas pine needles were sampled in all sites. Comparison between the retention patterns according to each individual PAH was performed using principal component analysis (PCA) and the appraisal of the possible sources of pollution for each matrix was done employing combinations of PAH diagnostic ratios.

2. Experimental data

2.1. Chemicals

Sixteen PAHs considered of primary environmental concern according to the Environmental Protection Agency (EPA) were analysed: naphthalene (Naph), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flut), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene (IcdP), dibenzo(a,h)anthracene (DahA) and benzo(g,h,i)perylene (BghiP). The mixture of these 16 PAHs was purchased from Supelco (Bellefonte, PA, USA) at 2000 μg mL^{-1} in ethyl acetate. A mixture of isotopically labeled PAHs surrogates was also from Supelco (naphthalene-D8, acenaphthene-D10, phenanthrene-D10, chrysene-D12 and perylene-D12) while the internal standard (anthracene-D10) was purchased from Dr. Ehrenstorfer at 100 μg mL^{-1} in acetone. Standard working solutions were diluted from the commercial ones in hexane. Gas chromatography (GC) quality solvents were from Merck (Darmstadt, Germany). SPE Alumina cartridges (5 g, 25 mL) were purchased from International Sorbent Technology (Mid Glamorgan, UK). Florisil powder was of 0.150-0.250 mm for residue analysis quality from Merck, baked at 150 ºC for 4 h to ensure dryness. Hydromatrix was from Varian (Palo Alto, CA USA). Nitrogen (99.995% purity) from Air Liquide (Paris, France) was used as drying gas.

2.2. Sampling

Sampling points were chosen to reflect a wide coverage of the Ebro River Basin (NE Spain) and included sites from the source of the river in Cantabria to its delta, in Catalonia. The study covered vulnerable sites, according to their proximity to urban conglomerates, agricultural areas or industrial activities. Thirty sites were sampled in October 2006 and included: (i) 21 sites in the Ebro River and in its main tributaries (R) and (ii) 9 sites in open agricultural areas (A). In every sampling site two samples were taken: (i) sediment samples in Ebro River and its tributaries and soil in the open agricultural areas and (ii) pine needles from the closest pine tree to soil or sediment collected samples. Four species were sampled according to its availability in the designated sites: *Pinus halepensis, Pinus pinea, Pinus nigra* and *Pinus pinaster*. Table 1 lists the locations of each sampling site, the corresponding river, the main economical activities in the area and the pine tree species sampled in any case.

The upper 10 cm sediment layer was taken with a Van Veen grab from a bridge at the middle of the water bed whenever possible or from the shore. Soil composite samples were gathered by collecting randomly four quantitatively similar sub-samples (about 300 g each) with at least 3 m between each other and 3 m away from the extreme of the field. Sub-samples were taken with a Dutch auger at 0-10 cm top soil. Both sediment and soil samples were then stored in glass jars covered with aluminium foil. For pine needles, to assure high homogeneity between them, only second-year needles from the bottom branches of the trees were chosen. This guaranteed at least one year of exposure to contamination. The needles were removed from the twigs in one piece and kept in sealed polyethylene bags. All samples were stored at + 4 ºC in the dark until reaching the laboratory, where they remained frozen at -20 ºC for a period no longer than 20 days.

2.3. Soil and sediment sample extraction

The extraction and clean up protocol was briefly optimized from a previous study [10]. Soil and sediment samples were freeze-dried during 48 hours at -40 ºC and under a 10-2 mbar vacuum. Samples were then sieved through 500 and 120 μm mesh to obtain a homogeneous material. One gram of this last fraction was spiked with 50 ng of surrogate standard and extracted using the pressurized liquid extraction (PLE) system ASE 200 from Dionex (Sunnyvale, CA USA). This system was optimized to perform the extraction and clean-up within the ASE cell in a single step, using Florisil as described below.

For the extraction step, 22 mL ASE stainless steel cells were packed as follows: 2 g of clean-up Florisil were placed at the outflow side of the cell and other 5 g were mixed with the sample. The remaining space was filled with pressed hydromatrix. A mixture of hexane:dichloromethane (1:1) was used as extraction solvent. A heat-up time of 5 min was applied to the extraction cell. Temperature was adjusted to 100 ºC and pressure to 1500 psi (1 psi = 6894.76 Pa), with a 60% solvent flow. Two cycles of extraction were performed with 10 min in static mode. The purge time was of 90 s with nitrogen gas. Extracts were evaporated at room temperature to nearly dryness using a TurboVap LV from Caliper LifeSciences (Hopkinton, MA, USA) and reconstituted into glass amber vials for gas chromatography. The final sample extracts were spiked with the internal standard at a concentration of 200 μg L^{-1} and their volume was adjusted to 250 μL with hexane.

Table 1
Location and description of sampling sites. Ordered according to the matrix studied and within each matrix, from source to mouth.

Code	GPS Coordinates		Location	River	Sector[a]	Pine species
	Lat.	Long.				
A1	42.326N	1.931W	San Adrián (Navarra)	-	A	*P. halepensis*
A2	42.371N	1.937W	Andosilla (Navarra)	-	A	*P. halepensis*
A3	41.981N	1.681W	Cascante (Navarra)	-	A	*P. halepensis*
A4	41.964N	1.685W	Monteagudo (Navarra)	-	A	*P. pinea*
A5	41.824N	1.549W	Maleján (Zaragoza)	-	A	*P. pinea*
A6	41.772N	0.821W	Villanueva de G. (Zaragoza)	-	A	*P. halepensis*
A7	41.639N	0.796W	Movera (Zaragoza)	-	A	*P. pinea*
A8	41.618N	0.907E	Mollerussa (Lleida)	-	I	*P. halepensis*
A9	41.694N	1.051E	Tornabous (Lleida)	-	I	*P. halepensis*
R1	42.999N	4.153W	Nestares (Cantabria)	Ebro	A, Spring	*P. nigra*
R2	42.684N	2.951W	Miranda de Ebro (Burgos)	Ebro	I	*P. pinaster*
R3	42.833N	2.783W	Villodas (Álaba)	Zadorra	U, I, A	*P. pinea*
R4	42.589N	2.842W	Haro (La Rioja)	Ebro	U, A	*P. nigra*
R5	42.418N	2.733W	Nájera (La Rioja)	Najerilla	A	*P. halepensis*
R6	42.470N	2.444W	Logroño (La Rioja)	Ebro	U, I, A	*P. halepensis*
R7	42.669N	2.031W	Estella (Navarra)	Ega	I	*P. halepensis*
R8	42.067N	1.601W	Tudela (Navarra)	Ebro	U, A	*P. halepensis*
R9	42.895N	2.135W	Alsasua (Navarra)	Araquil	I	*P. nigra*
R10	42.671N	1.819W	Puente la Reina (Navarra)	Arga	I	*P. halepensis*
R11	41.734N	1.175W	Grisén (Zaragoza)	Jalón	I, A	*P. halepensis*
R12	41.614N	0.915W	Zaragoza (Zaragoza)	Huerva	U, I	*P. halepensis*
R13	42.402N	0.499W	Jabarrella (Huesca)	Gállego	A	*P. halepensis*
R14	41.823N	0.785W	San Mateo de G. (Zaragoza)	Gállego	I, A	*P. halepensis*
R15	41.567N	0.691W	Presa de Pina (Zaragoza)	Ebro	U, I, A	*P. pinea*
R16	41.725N	0.136E	Monzón (Huesca)	Cinca	I, A	*P. pinea*
R17	41.320N	0.340W	Sástago (Zaragoza)	Ebro	A	*P. halepensis*
R18	41.536N	0.512E	Torres de Segre (Lleida)	Segre	A	*P. pinea*
R19	41.229N	0.552E	Flix (Tarragona)	Ebro	I, A	*P. halepensis*
R20	40.715N	0.581E	Amposta (Tarragona)	Ebro	A	*P. halepensis*
R21	40.714N	0.714E	Deltebre (Tarragona)	Ebro	A, Mouth	*P. halepensis*

[a] A: agricultural, I: industrial, U: urban

2.4. Pine needle sample extraction

Each sample consisted in 5 g of needles cut into 1 cm segments. 10 ng of the surrogate mixture was added to all samples previous to extraction. The extraction and clean up was described previously by Ratola et al. [22]. In brief, the needles were placed in glass tubes with 30 mL of hexane:dichloromethane (1:1) and sonicated for 10 minutes in a 360 W ultrasonic bath from J.P. Selecta (Barcelona, Spain). Samples were centrifuged at 2000 rpm and the solvent was recovered. This procedure was repeated three times always using fresh solvent. Samples were collected in pear-shaped flasks and reduced to less than 1 mL in a Buchi R-200 rotary evaporator (Flawil, Switzerland) prior to a clean-up procedure. First, SPE alumina cartridges were conditioned with 50 mL of hexane:dichloromethane (1:1) and elution was done with 50 mL of hexane:dichloromethane (1:1), followed by 50 mL of dichloromethane. The eluted fractions were collected together in pear-shaped flasks, concentrated in the rotary evaporator to circa 0.5 mL, transferred to 2 mL amber glass vials for GC and evaporated to nearly dryness under a gentle stream of nitrogen. The final sample extracts were spiked with the internal standard at a concentration of 160 μg L^{-1} and finally reconstituted in 1 mL of hexane.

2.5. Instrumental analysis

GC-MS analysis was performed with a Thermo Electron gas chromatograph (San Jose, CA, USA) model Trace 2000 coupled to a mass spectrometer from Thermo Electron. The mass spectrometer was operated in the electron impact ionization mode with an ionizing energy of 70 eV. Compound separation was achieved using a capillary column HP-5MS of 30 m x 0.25 mm i.d. with 0.25 μm film thickness from J&W Scientific (Folsom, CA USA). The temperature program was as follows: from 60 ºC (holding time 1 min) to 175 ºC at 6 ºC/min (holding time 4 min) to 235 ºC at 3 ºC/min and finally to 300 ºC at 8 ºC/min, keeping the final temperature for 5 min. Injection was achieved in the splitless mode keeping the split valve closed for 0.8 min.

Helium was used as carrier gas at a flow of 1.2 mL min^{-1}. The injector, transfer and ion source temperatures were set at 280 ºC, 250 ºC and 200 ºC respectively. Acquisition was achieved in time scheduled Selected Ion Monitoring (SIM) mode to increase sensitivity and selectivity. Identification and quantification were carried out automatically with the Xcalibur software from Thermo Electron (San Jose, CA, USA) using internal standard quantification.

2.6. Matrix dimension and data pretreatment

For the univariate statistics, data were arranged in three tables, one for each of the matrices considered (sediment, soil and pine needle). Univariate descriptive statistics (minimum, maximum, mean, standard deviation and frequency of detection) for each of the measured compounds were calculated considering each matrix separately.

For the multivariate analysis the three initial matrices were used to obtain the augmented matrix [2, 29]. The dimensions of the new matrix were 60 row samples (9 soils, 21 sediments and 30 pine needles) and 16 column variables (16 analyzed PAHs). Values below the detection limit were assumed to be equal to half the limit of detection [30]. Less than 10 % of the entries in the original data were less than the limit of detection.

In a previous work using historical data on the Ebro river basin, five different data pretreatments were tested. Autoscaling appeared to be the one giving best results for this type of data [2] since it gave a better understanding of the composition and distribution of the different contamination sources. With this procedure the mean of the column elements was subtracted from individual elements and divided by their column standard deviation. Consequently, each column has zero mean and unit variance [31, 32].

2.7. Principal component analysis (PCA)

PCA is a data reduction technique that aims to explain most of the variance in the data, while transforming a set of correlated measured variables into a set of a few uncorrelated components (PCs, Principal Components) [30], attempting to preserve at the same time the relationships present in the original data [33]. The main goal of this multivariate statistical technique is to extract useful information and provide an easier visualization of the existent relationships among objects and variables determined in large or complex data sets [34]. This allows a better understanding of environmental processes [35] where diffuse instead of specific contamination prevails. PCA bilinear model may be written using the following matrix decomposition equation:

$$X=TPT + E \quad (1)$$

where the matrix X is the data matrix obtained when the 16 variables were measured in the sediment, soil and pine needle samples from the 30 sampling sites (60 samples). It is decomposed to a scores matrix, T, and a loadings matrix, PT [36]. The latter gives information about the composition profiles of the N sources detected during data analysis, which are called Principal Components (PC) and are weighted linear combinations of the original measured variables [37, 38]. PCs are extracted so that the maximum amount of variance is explained in the first PC and progressively less variance is explained for each subsequent component [30]. The scores matrix gives information about the distribution profiles for the samples considering the detected sources. In the case under study, T has dimensions of 60 x N and PT the dimensions of N x 16. Finally, matrix E refers to the residual data variations not modeled by the N detected sources and has the same dimensions as X.

A new space is constructed with the PCs as new axes, in which to plot the scores, which are the redefined original samples into the new axes. The plot of the scores into the space defined by the PCs illustrate the dominant patterns present within the samples [30].

PCA modeling was conducted using the PLS Toolbox (Eigenvector Research, Manson, WA, USA) appropriate functions under the MATLAB computering and visualization environment (The Mathworks, Natick, MA, USA).

3. Results and discussion

3.1. Univariate descriptive statistics

Table 2 summarizes the statistical values for all the compounds. The mean concentrations for the sum of PAHs were of 290 < 598 < 1628 ng g^{-1} in pine, soil and sediment samples respectively. All compounds are present in at least 95% of the sediment samples. The same happens with the pine needles matrix, with the exception of IcdP, DahA and BghiP that were present in 63%, 83% and 80% respectively. In the soil matrix Naph, Acy, Ace, Flu and Phe were never detected and Ant, Flut and Pyr were detected in 33 %, 11% and 22% of the soils respectively, while the other heavier PAHs were detected in all the samples.

The minimum values obtained range from 0.2 to 11.2 ng g^{-1} in the pine needle matrix and from 0.1 to 12.7 ng g^{-1} in sediments. The minimum concentrations found in soil samples are higher than in the other matrices, especially for Pyr, IcdP and DahA. Maximum values for pine needles and soils have a similar range, from 1.0 to 487.9 ng g^{-1}, although the compounds that present higher maximum values change between the two matrices. In both cases the standard deviation is within the same order of magnitude than the corresponding mean. Contrarily, the sediment matrix presents much higher maximum levels for all the PAHs analyzed. Standard deviations for almost all the compounds are higher than the mean, indicating high dispersion of the results. In the case of soil and pine needle matrices, median values are very similar to the mean values but for the sediment matrix, all the median

values are smaller than their mean. This phenomenon is especially noticeable for those PAHs with higher concentrations (Flut, Pyr, BaP, IcdP and BghiP) and indicates that, in sediments, the data is skewed to low values with the presence of extreme high levels in some areas. Considering the total PAHs concentration, pine needle samples present the lowest concentrations and sediment samples the highest ones.

Table 2
Descriptive statistics of data by compound and matrix collected along the Ebro river basin [a]

PAH (rings)	Pine needles (n=30)				Soils (n=9)				Sediments (n=21)			
	Min (ng g $^{-1}$)	Max (ng g $^{-1}$)	Median (ng g $^{-1}$)	Mean ± SD (ng g $^{-1}$)	Min (ng g $^{-1}$)	Max (ng g $^{-1}$)	Median (ng g $^{-1}$)	Mean ± SD (ng g $^{-1}$)	Min (ng g $^{-1}$)	Max (ng g $^{-1}$)	Median (ng g $^{-1}$)	Mean ± SD (ng g $^{-1}$)
Naph (2)	3.9	47.2	10.5	12.3 ± 8.6	-	-	-	-	0.1	52.7	2.4	7.1 ± 12.6
Acy (3)	1.5	14.7	4.2	4.9 ± 3.2	-	-	-	-	0.7	291	6.0	36.8 ± 69.5
Ace (3)	2.0	51.5	5.1	6.8 ± 8.6	-	-	-	-	1.3	696	10.2	56.8 ± 151
Flu (3)	2.8	158	43.0	52.4 ± 47.7	-	-	-	-	2.2	194	27.8	56.2 ± 61.3
Phe (3)	11.2	488	94.1	124 ± 111	-	-	-	-	12.7	224	34.5	62.9 ± 62.0
Ant (3)	0.4	16.7	3.9	4.1 ± 3.5	0.1	1.4	0.8	0.8 ± 0.7	0.6	176	7.0	24.4 ± 42.5
Flut (4)	1.1	69.7	12.3	20.7 ± 19.8	6.2	6.2	6.2	6.2 ± 0.0	2.9	4931	33.8	360 ± 1091
Pyr (4)	2.2	106	20.5	30.8 ± 27.5	23.2	37.4	30.3	30.3 ± 10.1	2.1	4139	28.4	294 ± 907
BaA (4)	0.4	32.8	5.3	7.5 ± 7.0	1.4	14.1	4.9	5.9 ± 3.5	1.5	238	12.8	47.2 ± 74.4
Chr (4)	1.8	40.9	17.4	16.9 ± 9.0	7.5	24.0	12.0	12.8 ± 5.5	1.6	259	20.2	62.5 ± 84.1
BbF (5)	0.4	12.7	2.9	3.8 ± 3.1	2.7	15.0	6.7	7.6 ± 3.5	2.3	105	21.8	24.4 ± 23.2
BkF (5)	0.9	8.1	3.0	3.7 ± 1.8	1.9	13.4	5.2	6.3 ± 3.3	2.2	119	21.4	25.0 ± 25.6
BaP (5)	0.4	3.9	1.2	1.4 ± 0.9	10.0	99.2	47.7	46.5 ± 25.1	8.6	1826	62.1	206 ± 390
IcdP (6)	0.2	2.0	0.6	0.4 ± 0.5	38.8	465	256	237 ± 142	5.5	1739	71.7	151 ± 367
DahA (5)	0.2	1.3	0.7	0.6 ± 0.4	51.1	269	179	160 ± 67.4	4.6	968	41.9	87.3 ± 204
BghiP (6)	0.2	1.0	0.5	0.4 ± 0.3	9.2	254	145	132 ± 83.8	7.4	1314	55.8	130 ± 276
∑PAHs	**44.4**	**837**	**241**	**290 ± 207**	**107**	**1125**	**688**	**598 ± 326**	**90.4**	**10577**	**829**	**1628 ± 2632**

[a] Min: minimum; Max: maximum; SD: standard deviation; - : below LOD

For the sake of a better comparison and interpretation of the achieved results in the three analyzed matrices, bar plots of the sum of the 16 PAHs were drawn in Fig 1. The sampling sites are divided depending on whether they correspond to river sediment (R) or to an agricultural soil (A) and next to each terrestrial sample a bar plot of the corresponding pine needle is also shown. Furthermore the pine needle samples are also classified into the corresponding species in order to appreciate their different behavior. The levels of PAHs than the ones found in sediments or soils. In the case of soils, there is a good correspondence between their total concentration and the one found in the pine needle (R2=0.5, n=8). On the contrary, for the sediment matrix there is no correspondence (R2=0.05, n=21). The concentrations found in *Pinus halepensis* are higher than the ones found in other pine species, also in the sites where the concentrations found in sediments and soils are higher. This phenomenon indicates a higher accumulation potential of this particular species in comparison to the others studied.

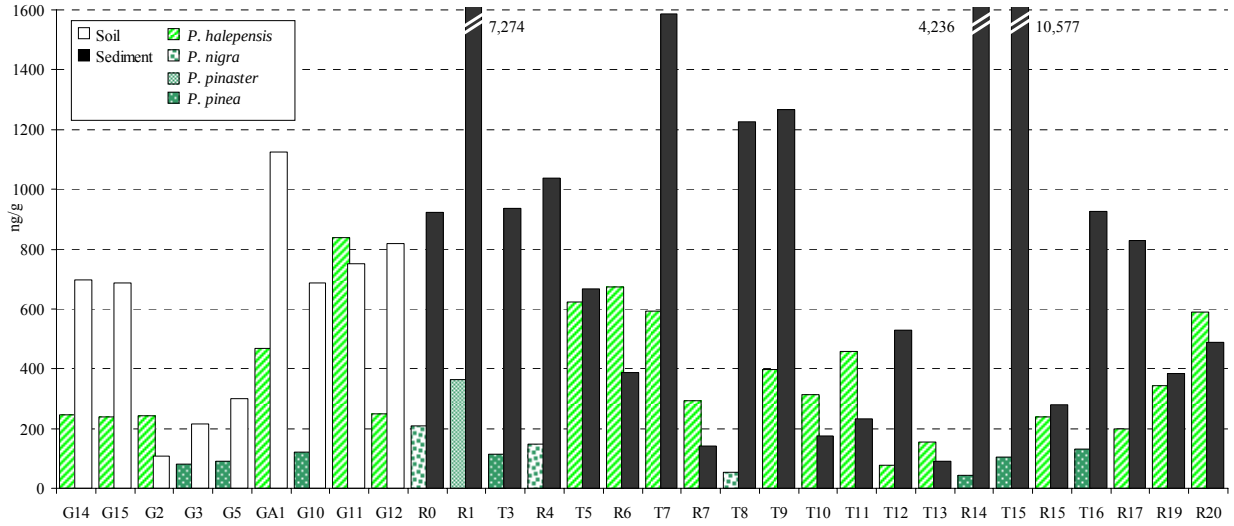

Fig. 1. Total concentration of PAHs in sediments, soils and pine needles in the 30 sampling sites along the Ebro River basin

3.2 Compartmentalization of PAHs

The PAH distribution patterns for the pine needle samples are plotted in Fig 2(a) and in Fig. 2(b) the ones for soil and sediment. The three matrices show a very different pattern, especially between pine needles and terrestrial samples. Pine needles show a predominance of 3 and 4-ring PAHs, ranging from 65 to 95 % of the total concentration of PAHs. On the contrary, 5 and 6-ring PAHs are found as the most abundant compounds in the soil samples. The pattern for sediment is more heterogeneous, with contributions of all the PAHs but different distribution depending on the sample considered. The origin of the contamination found in the sediments depends on what is transported or spilled into the river as well as the accumulated PAHs during the years, while the other two matrices are more influenced by human activities and atmospheric inputs and show a recent contamination profile. Consequently, the contamination in the sediments does not follow a clear pattern but depends on the sampling site. On the other hand, pine needles and the corresponding soil are exposed to the same contamination source and the PAHs pattern observed is complementary and it can be attributed to compartmentalization of the PAHs among the two matrices. While pine needles accumulate the lighter PAHs, soils tend to accumulate the heavier ones.

To the best of our knowledge there are no works that consider deeply the PAHs into pine needles, soil and sediment simultaneously. Among the ones that study the matrices separately most of them show a similar profile for sediments [39, 40], soils [39, 40] and pine needles [24, 41], indicating the same compartmentalization phenomena as in the Ebro River basin.

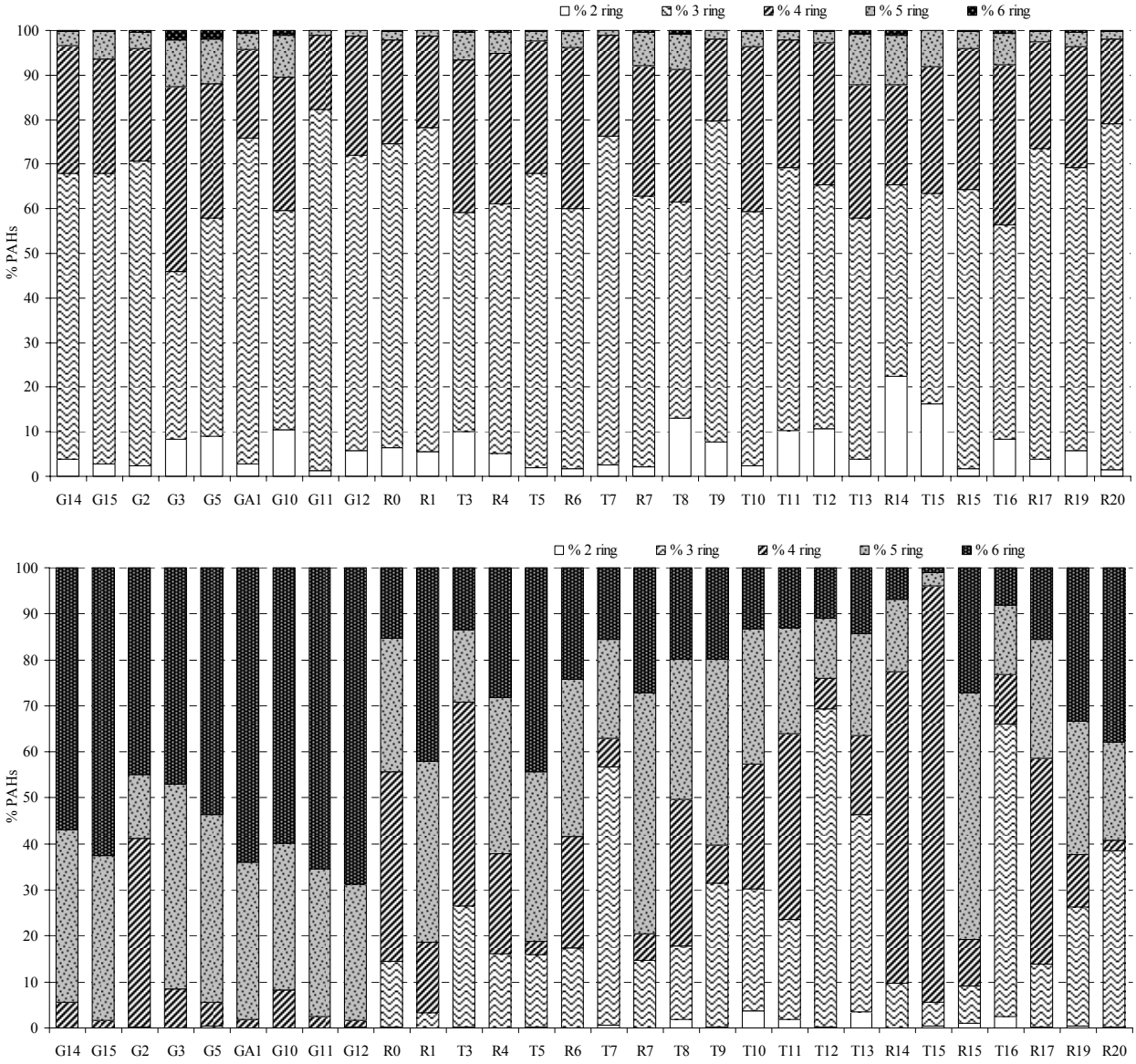

Fig. 2. Ring patterns in percentage of the total concentration of PAHs (a) for pine needle matrix and (b) for soil samples (G) and sediment (R and T) samples

3.3 PAHs sources

PAHs may originate from pyrolytic sources, including the natural and anthropogenic combustion of organic matter (e.g., forest fires, domestic coal or wood combustion and car exhausts) and from petrogenic sources (e.g., present in subsoil, oil spills) [42]. According to many references, sources of PAHs may be assessed by some ratios of specific molecular PAHs compounds [43], [44]. When PAH ratios are used to determine the source of emission, it is assumed that the paired compounds are diluted to a similar extent during transport, and consequently, the ratios remain constant en route from sources to receptors [27]. In this sense, Hwang et al. [24] concluded that the comparison of PAH ratios in different sample matrix like vapour phase in the air and pine needles may not cause significant differences. Contrarily, Zhang et al. [27] reported that this assumption is not always valid and consequently, changes in diagnostic ratios from sources to receptors are almost unavoidable. In this study, two combinations of ratios were used to survey the possible sources of PAHs in the Ebro River basin and to check if the three matrices considered indicate the same or different origin: Phe/Ant vs. Flut/Pyr and BaA/(BaA+Chr) vs. IcdP/(IcdP+BghiP). The first one has been chosen due

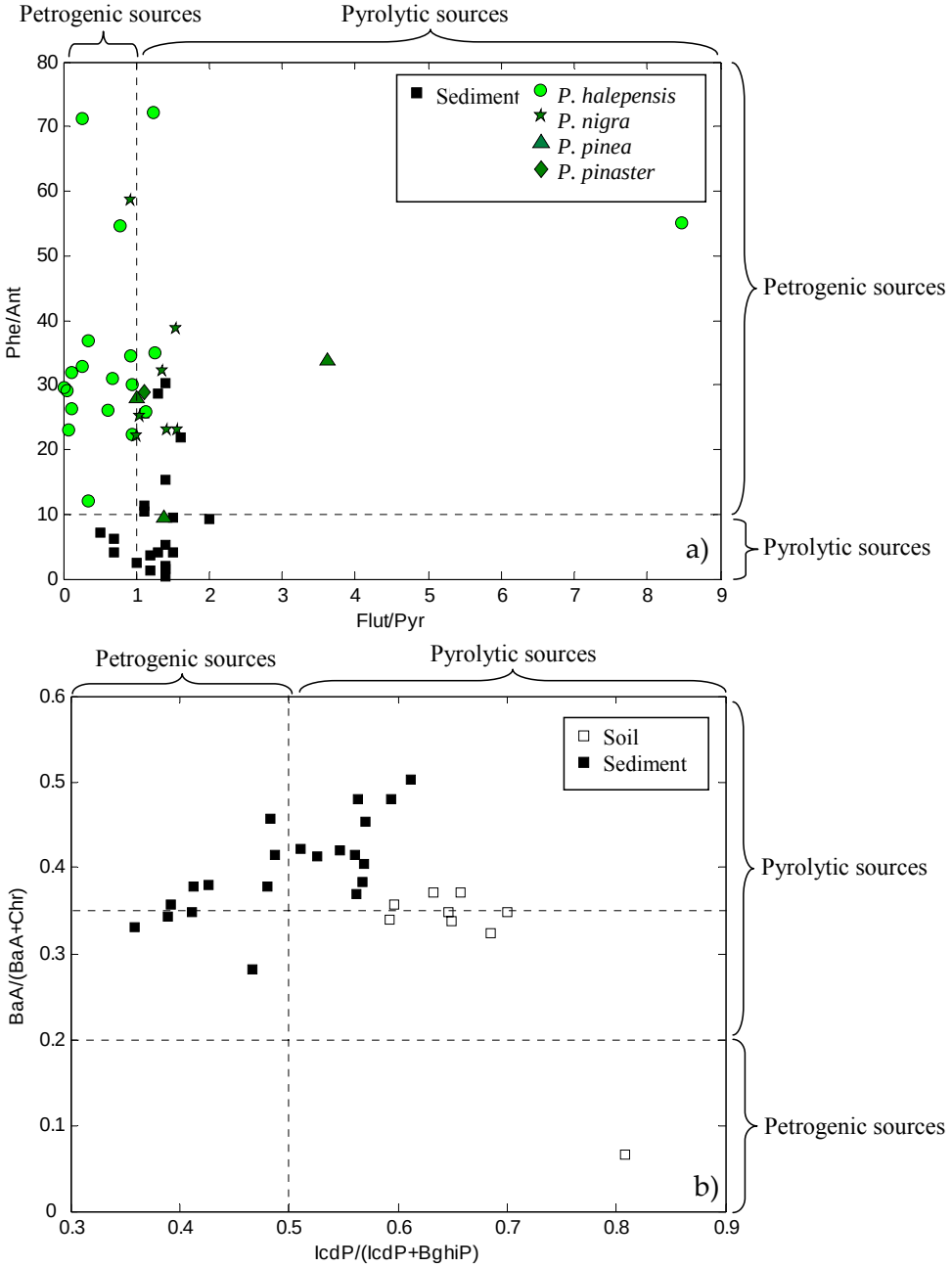

Fig. 3. PAHs ratios in sediments, soils and pine needles from the Ebro River Basin. (a) Phe/Ant *vs.* Flut/Pyr and (b) BaA/(BaA+Chr) *vs.* IcdP/(IcdP+BghiP)

to its widespread use while the latter permits the inclusion of the soil samples that can not be seen in the ones performed with Flut and Phe. In the latter only sediments and soils are included

Based on the first ratio (Fig. 3a), it can be seen that although some overlap was expected, pine needle samples are completely separated from sediment samples. The comparison between pine needles and soils is not possible as none of the established ratios can be calculated for these two matrices simultaneously. In Fig. 3a sediment samples show a predominance of pyrolytic sources while pine needles show a predominance of petrogenic sources, although both matrices have samples with mixed origin. It is also noticeable that only sediment samples indicate a pure pyrolytic origin while among the pine needle samples, the *P. halepensis* samples tend to indicate pure petrogenic origin of the contamination while *P. nigra, P. pinaster and P. pinea* pointed to mixed contamination. In Fig. 3b a similar behavior for sediments is observed, with the addition of soil samples that show pyrolytic contamination due to the burning of weeds and vegetal wastes after harvesting. From the observation of these plots it can be concluded that the matrix affects the elucidation of the contamination source from the established PAHs ratios, in agreement with the work by Zhang et al. [27]. The predominance of mixed origin among *P. nigra* samples is also observed in the work form Lehndorff and Schwark [41] as well as the mainly pyrolytic origin of the soil samples agrees with Ma et al. [45].

3.4. Principal Component Analysis results

The PCA performed with all the samples was completely dominated by the sediment samples R2, R15 and R16, although the data was previously autoscaled, due to their concentration one order of magnitude higher than the rest of the sediments. R2, R15 and R16 were not considered in the analysis to better analyze the main group of samples.

In Fig. 4, the loadings plot for the first three PCs is given. The first PC explained 32.3% of the total data variance and it had high positive loadings for Ant, Flut, Pyr, BaA, Chr, BbF, BkF and BaP, mainly 4-5 ring PAHs. The second PC explained 24.6% of the total data variance and had high loadings for the three heaviest PAHs, 5-6 rings, and negative moderate loadings for Naph, Flu, Phe and Pyr. The third PC explained 12.0% of the total variance and had high positive loadings for the lighter PAHs, 2-3 rings, together with BaP. Usually the PCs obtained when applying PCA to the data are associated with the PAHs sources found in the samples. In this case, the composition of the first two PCs agree with the compartmentalization found between the three matrices, so to say, the first PC includes the PAHs found in sediments while the second PC reflects the contamination present in pine needles with the negative loadings and the one found in soils with positive loadings.

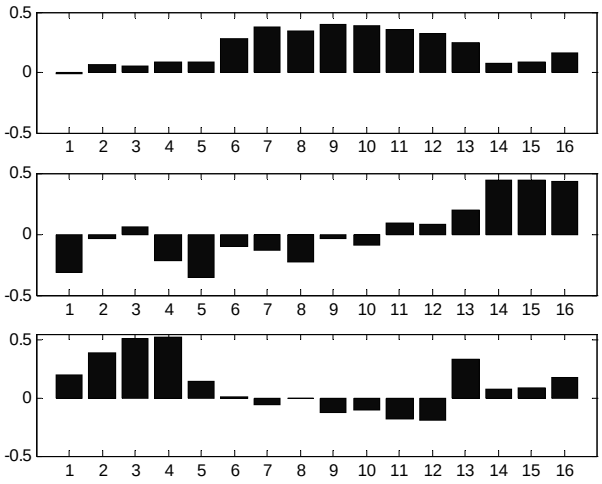

Fig. 4. Loadings for the first three principal components. Identification of variables: 1: Naph; 2: Acy; 3: Ace; 4: Flu; 5: Phe; 6: Ant; 7: Flut; 8: Pyr; 9: BaA; 10: Chr; 11: BbF; 12: BkF; 13: BaP; 14: IcdP; 15: DahA; 16: BghiP.

The scores plot of data matrix is given in Fig. 5. Samples have been coded into 3 different classes considering the matrix: white squares correspond to soil samples, black squares to sediment samples and the other symbols to pine needles. In addition, the pine needle class has been divided into four subclasses corresponding to the four pine species considered in this study. The samples from each matrix are perfectly separated from the other two, generating three groups with different degree of contamination. On one hand, soil samples are distributed along the positive side of PC2 while pine needles are along the negative side. Therefore, PC2 divides these two groups and indicate that soil samples are contaminated by the compounds with positive loadings on PC2 (IcdP, DahA and BghiP) and pine needle samples are contaminated by the ones with negative loading in this PC (Naph, Flu, Phe and Pyr), confirming the compartmentalization found between these two matrices. On the other hand, sediment samples are situated along PC1 with a small variance in PC2, showing contamination with Ant, Flut, Pyr, BaA, Chr, BbF, BkF and BaP, mainly 4-5 ring PAHs. PC1 has almost no influence in the samples from soil and pine needle samples. Therefore, PC1 describes only the sediment matrix and indicates the separate behavior of this matrix in comparison with the other. In addition, pine needle samples from *P. nigra, P. pinaster* and *P. pinea* are grouped into the area closest to zero for PC2 showing less contamination than the *P. halepensis* samples.

4. Conclusions

A global monitoring covering the analysis of several environmental matrices is necessary to evaluate the presence and fate of PAHs within a river basin that receives different pollution inputs. The comparison among the three studied environment al matrices using

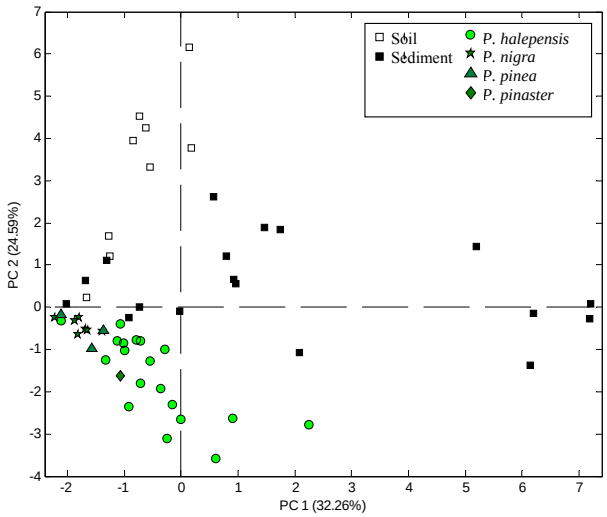

Fig. 5. Scores plot from PCA for the first two principal components.

univariate descriptive statistics and PCA reveals a compartmentalization of PAHs between pine needle and soil samples, with a high contribution of 5-6 ring PAHs (IcdP, DahA and BghiP) in the soil matrix, while pine needle samples show a higher presence of lighter PAHs (Naph, Flu, Phe and Pyr). This contamination pattern can be attributed to human activities and atmospheric deposition. On the contrary, sediment matrix shows a more independent PAHs contamination profile, resulting of the historical pressures and the accumulation of compounds released to the river. Total concentrations between the three matrices rises from pine needles to soil and to sediment indicating the increasing accumulation capacity of these three matrices. The contamination source was evaluated with some established PAHs ratios that indicated different origin depending on the matrix. Consequently, it can be concluded that the matrix affects the elucidation of the contamination source using the PAHs ratios.

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4.4.- Discussió addicional als articles

4.4.1.- Procediment per a la interpretació dels resultats

Un cop obtinguts els resultats, ja sigui a través d'una base de dades com en l'article científic 4 o per l'anàlisi de gran quantitat de mostres com en els articles científics 5, 6 i 7 es van seguir dos camins per a la seva interpretació. Aquesta va resultar complexa i va constar de moltes etapes diferents que no es veuen totalment reflectides en els articles científics. Bàsicament es van seguir les dues línies que es descriuen a continuació i s'esquematitzen a la Figura 4.3:

~ **Estadística descriptiva univariable:** es basa en el càlcul de descriptors, com ara el mínim, màxim, freqüència de detecció, mitjana, mediana i desviació estàndard, i en representacions gràfiques senzilles com poden ser gràfics de barres o histogrames. Va ser sempre el primer tipus d'anàlisi utilitzat, per ser simple i de fàcil interpretació, a més de servir com a base per a una posterior aplicació de mètodes quimiomètrics.

~ **Anàlisi quimiomètric multivariable:** en aquesta tesi com a mètode quimiomètric es va utilitzar el PCA. Per a l'aplicació d'aquest mètode quimiomètric és necessària la preparació de taules de dades adequades en les que no hi hagi cap buit, per a fer això es van estimar els valors absents i es van substituir per la meitat del LD aquells valors que es trobaven per sota del LD. Abans de l'aplicació del PCA es va seguir un esquema similar de processament de dades en tots els casos per a tenir una primera visió d'aquestes i poder escollir el pretractament més adequat. Però la millor manera de triar un pretractament adequat al tipus de dades consisteix en provar-ne diversos i realitzar un PCA amb cadascun d'ells per a poder escollir el que dóna lloc a una millor interpretació dels resultats. A més a més el PCA es va aplicar tant a les matrius individuals com a les matrius augmentades tant abans com després de realitzar el pretractament escollit.

Tot i que sembla un procés d'interpretació ben estructurat cal dir que no és una seqüència lineal sinó un estudi en el que es van portar a terme moltes anàlisis de forma simultània, els resultats de les quals influïen tant en les fases posteriors com en les anteriors. Per aquesta raó és molt comú haver de repetir certs passos durant tot el procés. D'altra banda, tenint en compte la gran quantitat de dades que es van tractar, tot el procés va donar lloc a una gran quantitat

d'informació, la major part de la qual va permetre avaluar els avantatges i limitacions dels mètodes emprats.

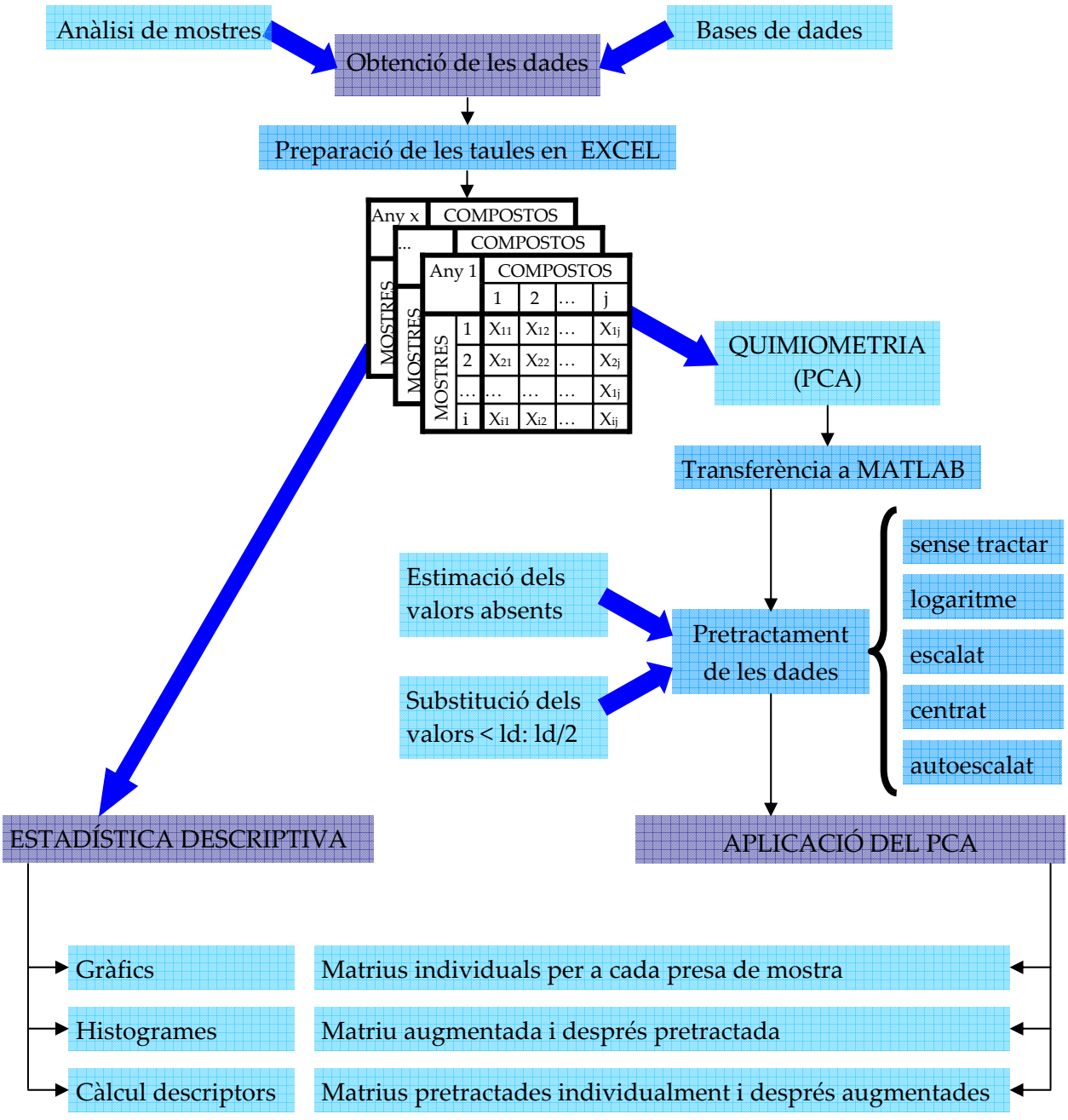

Figura 4.3: Esquema del processament de dades realitzat en aquest estudi

A continuació es mostren els resultats més rellevants obtinguts durant el processament previ a l'aplicació del PCA per a l'estudi de les dades històriques de la CHE, recollit a l'article científic 3, ja que aquest va ser el primer que es va realitzar. La naturalesa d'aquests valors és

molt similar a la resta de dades de la conca de l'Ebre, de forma que els resultats obtinguts en aquest primer estudi van servir de base per a la posterior aplicació del PCA a la resta de dades.

Per a una primera caracterització, les dades es van representar gràficament dividint els valors segons el punt de presa de mostra o segons el compost analitzat. A la Figura 4.4 es presenta un d'aquests gràfics, corresponent a l'any 2003. En el gràfic s'observa clarament que el punt de presa de mostra amb uns nivells més alts de contaminants analitzats va ser el T9 (vermell), seguit del R18 (verd) i el R1 (blau), que també van presentar unes concentracions més elevades que la resta de punts. Per altra banda es dedueix que els compostos amb unes concentracions més elevades van ser els PAHs, a excepció del naftalè, i els DDTs, mentre que altres compostos van presentar unes concentracions molt baixes.

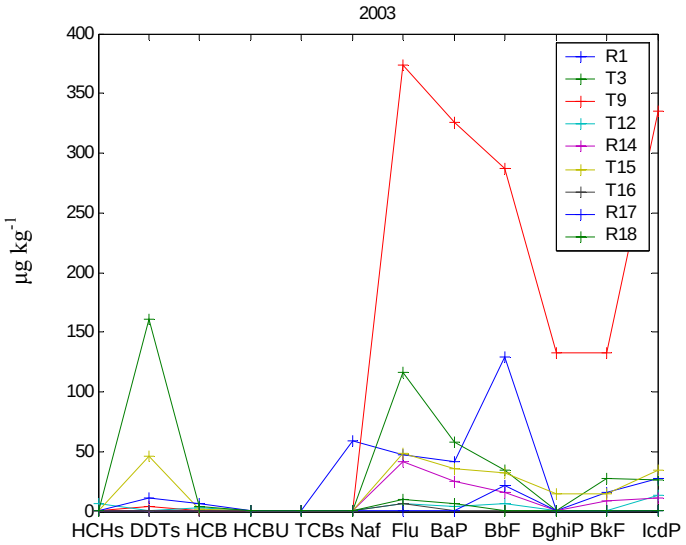

Figura 4.4: Concentracions de compostos orgànics en sediments corresponents a la campanya del 2003 de la RCSP (article científic 3)

També es van realitzar histogrames per a cadascun dels compostos (Figura 4.5). Amb aquest tipus de gràfics es va veure la distribució de les concentracions obtingudes, ja que indica amb una barra la quantitat de mostres amb una determinada concentració. Les dades estaven molt esbiaixades cap a valors baixos i el percentatge de valors per sota del LD era molt elevat. Aquesta va ser una tònica constant en tots els resultats de la CHE. Hi ha, però, algunes diferències entre els compostos, els PAHs van presentar una distribució lleugerament més homogènia, especialment el naftalè, tot i que les concentracions van ser molt més baixes. En el

cas dels DDTs, tot i que d'entrada la distribució semblava igualment esbiaixada cap a valors molt baixos cal considerar que les concentracions que apareixen als histogrames són dos ordres de magnitud més altes que per als altres compostos.

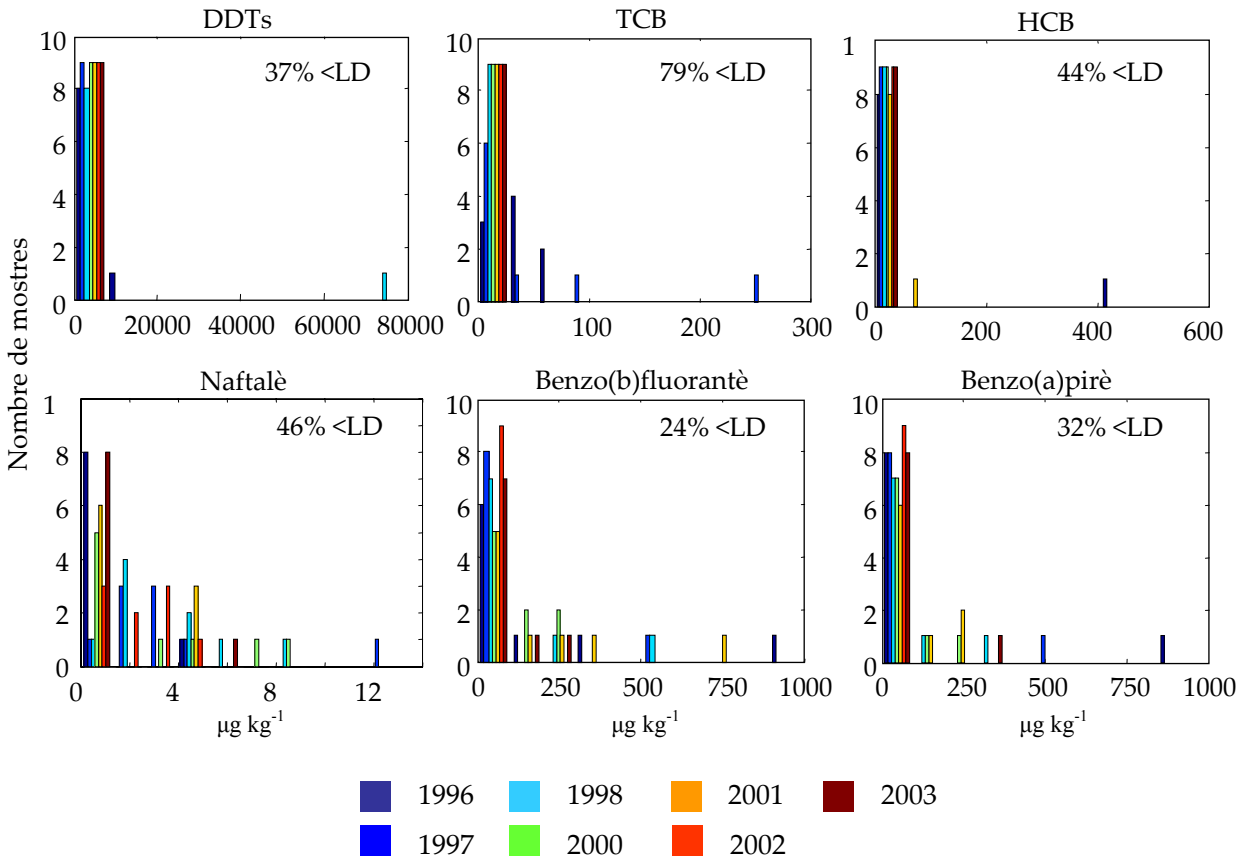

Figura 4.5: Histogrames dels compostos orgànics en sediments corresponents l'estudi històric de les dades de la RCSP (article científic 3)

En base a aquests tipus de dades, pretractaments com la transformació logarítmica, el centrat o l'autoescalat semblaven ser els més idonis per a intentar donar més importància als valors baixos i evitar d'aquesta manera que els valors atípics dominessin els resultats. D'entre els diferents pretractaments que es van provar, el que va donar lloc a una millor interpretació dels resultats en tots els casos va ser l'autoescalat. En la Figura 4.6 es mostra el diagrama de caixes per a les dades sense tractar i un cop s'havia realitzat l'autoescalat. Aquest tipus de diagrama és útil per veure la dispersió de dades i decidir si el tractament aplicat és adequat. En el primer diagrama podem veure que els valors atípics dominaven tant la distribució dels resultats, que la caixa, on hi ha el 50% dels valors, estava completament comprimida a valors

baixos i es feia difícil la comparació entre les diferents variables o compostos analitzats. En aplicar l'autoescalat, com mostra el segon diagrama, les caixes es van eixamplar i els valors atípics es van concentrar. La distribució obtinguda en aquest cas va ser molt similar a la de la resta de dades, amb medianes en general per sota de la meitat de la caixa, indicant una prevalença de valors baixos i una gran quantitat de valors atípics (+) però només per al valors alts.

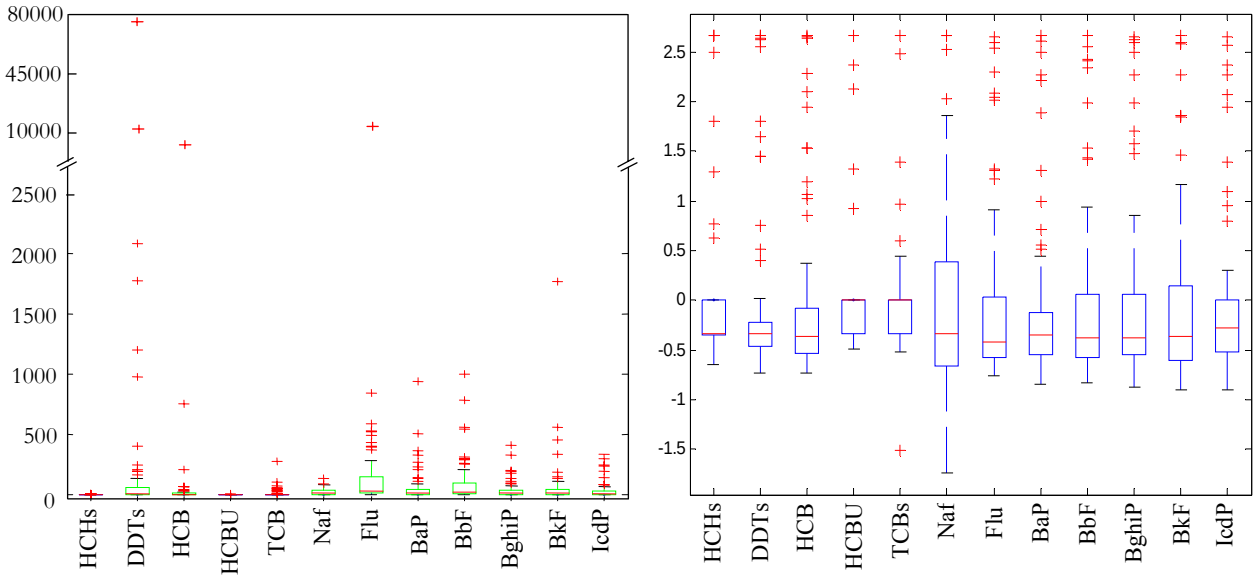

Figura 4.6: Diagrama de caixes abans i després de l'aplicació de l'autoescalat per als compostos orgànics en sediments corresponents a la campanya del 2003 de la RCSP (article científic 3)

Un cop triat el pretractament es van realitzar els PCAs comprovant en tots els casos les diferències entre autoescalar abans o després d'augmentar la matriu així com diverses agrupacions de matrius. Els resultats més rellevants i la seva interpretació són els que apareixen als articles científics 4, 5, 6 i 7.

4.4.2.- Anàlisi del carboni orgànic total

A part dels compostos orgànics, a totes les mostres es va analitzar també el COT com un altre paràmetre de caracterització. Els resultats obtinguts per a les dues matrius es recullen a l'annex B. El COT en aigües es va mantenir entre 0,4 i 15,4 mg L^{-1} i en sediments el rang es va situar entre 0,2 i 10,7%, que a priori són valors normals per a les mostres estudiades. A la Figura 3.4 es representen els valors de COT de les matrius aigua i sediment per a tots els punts de

presa de mostra. S'hi representen els valors de COT obtinguts a les 3 campanyes d'octubre, hi ha per tant un total de 6 barres per a cada punt de mostreig, 3 per a aigües (blau) i 3 per a sediments (groc-marró). Els punts de presa de mostra estan ordenats seguint el curs del riu Ebre, des del naixement al nord-oest fins a la desembocadura al sud-est. Per als sediments es considera que un valor mitjà del COT es troba entre el 2 i el 2,5% *(Serrassolses, 1999)*, per sobre d'aquest valor es considera que el sediment és ric en matèria orgànica i per sota és pobre. De forma que al gràfic es mostra amb una banda horitzontal el rang mitjà i les barres tenen un color més fosc si estan per sobre del 2,5% i un color més clar si estan per sota del 2%. A primer cop d'ull es van poden distingir 3 grans grups de mostres segons el seu contingut en carboni orgànic.

- **R0 – R4:** és el primer tram del riu, entre el naixement i *Haro* (*La Rioja*). Va mostrar en general uns valors molt elevats per al COT tant a les aigües com als sediments, on destacava el punt T3 corresponent a *Villodas* (*Araba*) on els sediments dels tres anys tenien un contingut de carboni orgànic superior al 6%. Cal dir també que les mostres del punt R0, al naixement van presentar un COT menor que la resta d'aquest grup.
- **T5 – T13:** és el tram central de la conca i va presentar uns valors de COT més baixos, tant en aigües com en sediments. Tot i que en alguns casos puntuals els valors estaven per sobre del 2,5%, la tendència general va ser que el COT estigués per sota del 2% en els sediments i de 2 mg L^{-1} en l'aigua, com va ser el cas del punt T5, on totes les mesures de COT van estar per sota d'aquests límits.
- **R14 – R20:** és la part baixa del riu i va presentar uns valor mitjans en quant a COT. Tot i que la singularitat no va ser tant notable com en els altres dos grups, hi va haver menys abundància de valors baixos.

A més a més de les relacions entre el COT de diverses campanyes i matrius, també es van buscar relacions entre el COT, tant d'aigües com de sediments, i les corresponents concentracions totals de compostos orgànics així com els sumatoris parcials per a cadascuna de les famílies i compostos individuals. No es va trobar cap correlació entre les dues variables, ni quan es van relacionar el total de les mostres ni quan es van separar segons si presentaven un COT baix (menor de 2,5% en sediments i 2,5 mg L^{-1}) o un COT alt.

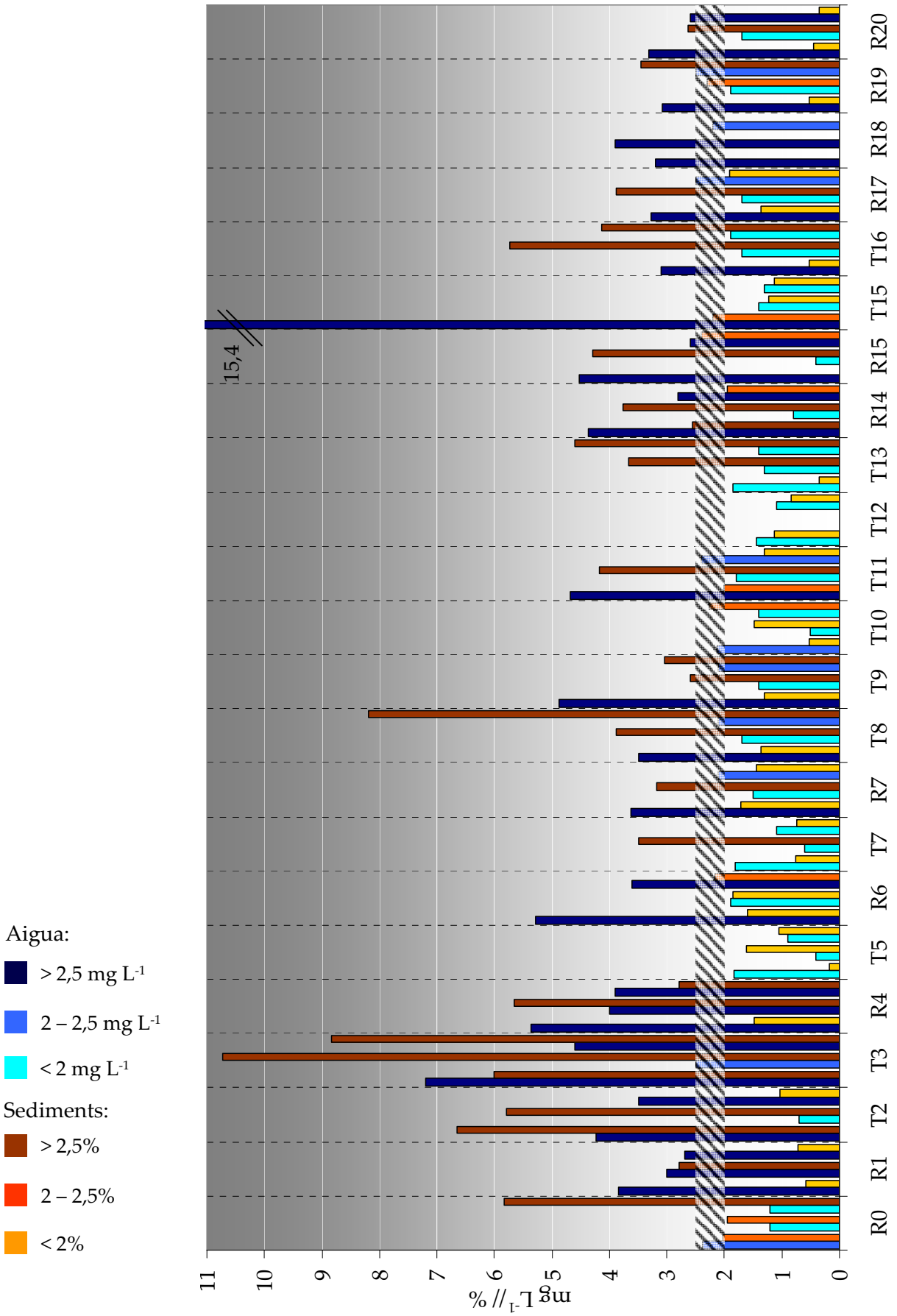

Figura 4.7: Relació del COT per aigües i sediments a tots els punts i campanyes

4.4.3.- Nivells obtinguts en aigües i sediments

Durant els 3 anys en els que van tenir lloc les campanyes de presa de mostra del projecte AquaTerra es van recollir i analitzar 132 mostres d'aigua i 65 mostres de sediments que van donar lloc a un total de 9.938 resultats. Per a resumir les tendències temporals tant en aigües com en sediments es van agrupat els diversos compostos en els 5 grups següents: PAHs, APs, plastificants, pesticides organoclorats i pesticides polars. A les Figures 4.8 i 4.9 es mostren els màxims trobats per a cadascun dels grups així com la freqüència d'aparició a les mostres analitzades, per a aigües i sediments respectivament.

En el cas de les aigües, els pesticides polars es van detectar en un percentatge molt elevat de mostres (86%), seguits dels APs i els plastificants (BPA i TBP), amb 75 i 73% respectivament. Cal remarcar també que els APs es van detectar a concentracions un ordre de magnitud superior a pesticides polars i a plastificants. Els PAHs a part d'aparèixer en un percentatge molt menor de les mostres (29%, el 2004) es van detectar a concentracions molt baixes i els pesticides organoclorats no es van detectar en aquesta matriu. L'absència de PAHs i pesticides organoclorats en les aigües de 2004 va comportar que a partir del 2005 aquests compostos no s'analitzessin més en aquesta matriu.

La situació en canvi va ser oposada per a la matriu de sediments on els PAHs i els pesticides organoclorats es van detectar a un 100% de les mostres mentre que els pesticides polars només es van detectar a un 29% i el plastificants a un 54% i ambdós es van trobar a concentracions molt més baixes que els altres grups. La distribució d'aquests cinc grups entre les dues matrius es deu a les propietats físiques dels diferents compostos, si tenen més afinitat pels sediments (compostos més lipofílics com els PAHs o els pesticides organoclorats) o per la fase aquosa (compostos més hidrofílics com els plastificants o els pesticides polars). Ens vam trobar però amb el cas dels APs, que es van detectar a un percentatge elevat de mostres en les dues matrius (75% en aigües i 100% en sediments) i a més a més a concentracions altes, reflectint el seu abocament al medi aquàtic degut a l'ampli ús dels surfactants no iònics en moltes activitats domèstiques i industrials, així com l'ús directe de NP en formulacions de pesticides. En el cas d'aquesta família de compostos les concentracions trobades es van deure quasi únicament al NP. En general les concentracions d'aquests cinc grups en les mostres de

sediments va ser molt més elevades que per a les aigües, arribant a nivells superiors a 6000 ng g^{-1} en el cas dels APs i dels PAHs.

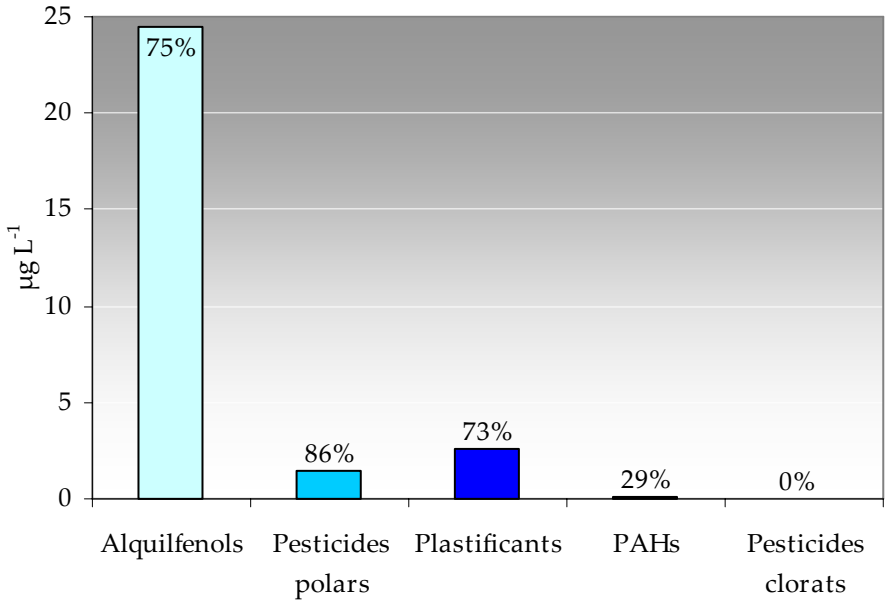

Figura 4.8: Concentració i percentatge d'aparició dels 5 grups de compostos en aigües

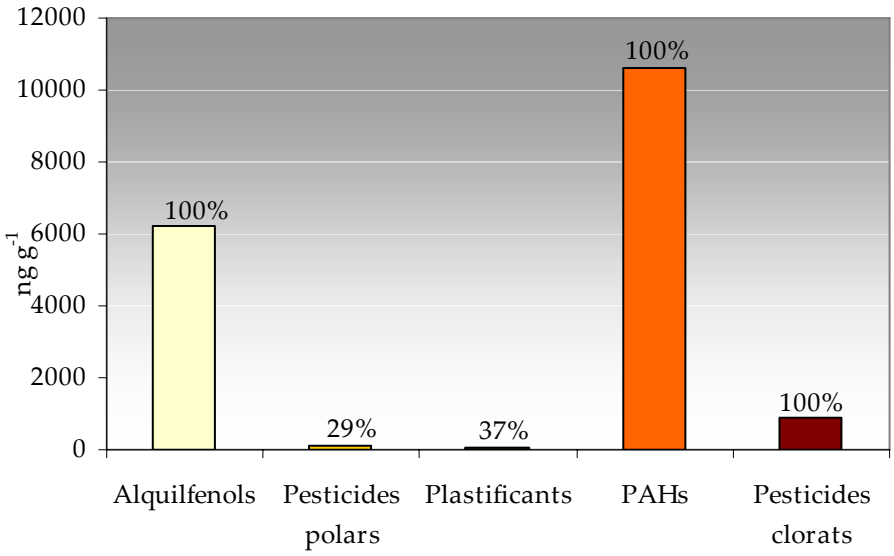

Figura 4.9: Concentració i percentatge d'aparició dels 5 grups de compostos en sediments

Per a una visió més concreta de la distribució temporal i geogràfica dels contaminants les Figures 4.10, 4.11 i 4.12 mostren les concentracions totals per grups considerant cadascun dels punts de presa de mostra i totes les campanyes per separat. En aquests gràfics els punts de presa de mostra estan ordenats seguint el curs del riu Ebre, és a dir, de nord-oest a sud-est,

intercalant els punts situats als afluents allà on aquests desemboquen a l'Ebre, de forma que es poden apreciar les possibles distribucions geogràfiques.

A la Figura 4.10 es mostren els gràfics de barres per als plastificants en aigües i en sediments, ja que aquests compostos es van detectar a les dues matrius, tot i que la concentració en sediments va ser un ordre de magnitud superior a la de les aigües. Cal considerar que en tots els casos el compost majoritari va ser el TBP. No es va veure una tendència temporal ni geogràfica sinó més aviat contaminacions puntuals a certes zones de la conca, especialment aigües avall de *Gasteiz* (T3 als sediments i R4 a les aigües), entre *Gasteiz* i *Pamplona* (T8 a les dues matrius) al voltant de *Zaragoza* (T10 i T11 només a les aigües) i entre Lleida i Flix (T16 als sediments i R17 a les aigües).

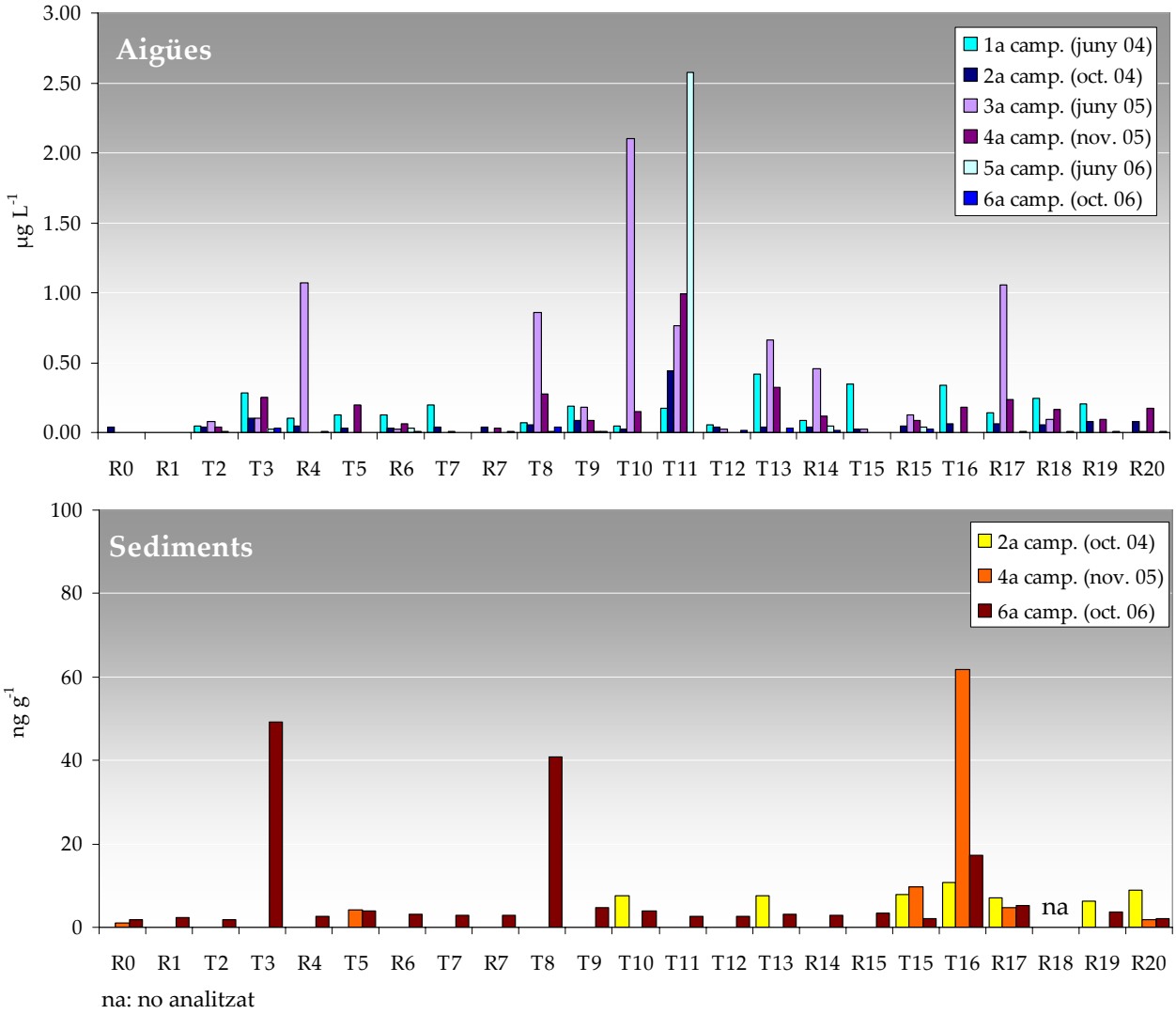

Figura 4.10: Concentració de plastificants en aigües i sediments

A la Figura 4.11 es mostra la distribució geogràfica dels pesticides organoclorats en sediments. Tot i que aquests es van detectar de forma constant al llarg tota la conca amb concentracions de fins a 200 ng g^{-1}, no va ser fins a la part baixa de la conca, a partir de la localitat de Flix (R17) on les concentracions van augmentar considerablement. Els pesticides organoclorats estan prohibits en la seva aplicació agrícola *(European Council, 1991b)*, de forma que les concentracions trobades al llarg de la conca poden reflectir contaminació agrícola històrica ja que es van utilitzar àmpliament en el passat. Tanmateix observant la contribució de cada compost a la concentració total es pot veure com el 4,4'-DDT va ser el compost majoritari a gran part dels punts de presa de mostra. Aquest fet indica que la contaminació per aquest tipus de compost és relativament recent, ja que amb el temps el DDT es degrada a DDE *(Wang et al., 2008)*. A partir del punt R17 (Flix) es va observar la presència d'HCB amb una contribució important, compost que s'ha generat durant molt anys al complex electroquímic situat en aquesta zona *(Sunyer et al., 2008)*. Aquest mateix esquema es va observar per als pesticides organoclorats analitzats a les mostres de la RCSP corresponent a l'any 2003, tal i com reflecteix l'article científic 3.

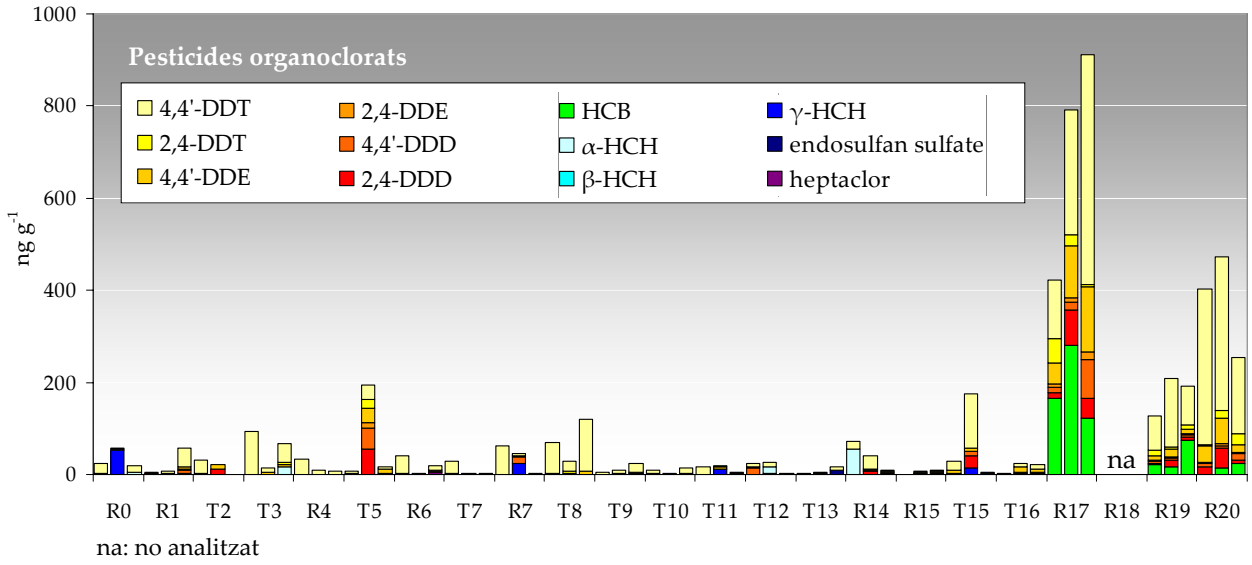

Figura 4.11: Concentració i perfil de pesticides organoclorats en sediments

A la Figura 4.12 es mostra el gràfic de barres per als PAHs en sediments. Els perfils de les tres campanyes van ser molt similars. En tots els punts de presa de mostra es van trobar

presents tots els compostos d'aquesta família, però amb una lleugera predominança dels PAHs més pesats, especialment del benzo(a)pirè, que és el més cancerigen *(IARC), 2008)*. Pel que fa a la seva distribució, no es va observar cap tendència geogràfica al llarg de la conca, però si que es van apreciar concentracions més elevades d'aquesta família de compostos a R0 (*Reinosa*), R1 (*Miranda de Ebro*), R14 (aigües avall de *Zaragoza*), T15 (aigües avall de *Monzón*) i amb una concentració una mica inferior a R17 (Flix). Tots aquests punts, a excepció del R0, es corresponen amb zones amb una certa activitat industrial. En canvi el R0, que es va triar com a blanc, es troba només 6 km aigües avall del naixement de l'Ebre i per tant *a priori* s'esperava trobar concentracions més baixes que a la resta de punts. Malgrat tot, es va descobrir que la zona va tenir activitat minera d'extracció de carbó durant molts anys *(López, 2008)*, fet que podria ser l'origen de la contaminació per PAHs.

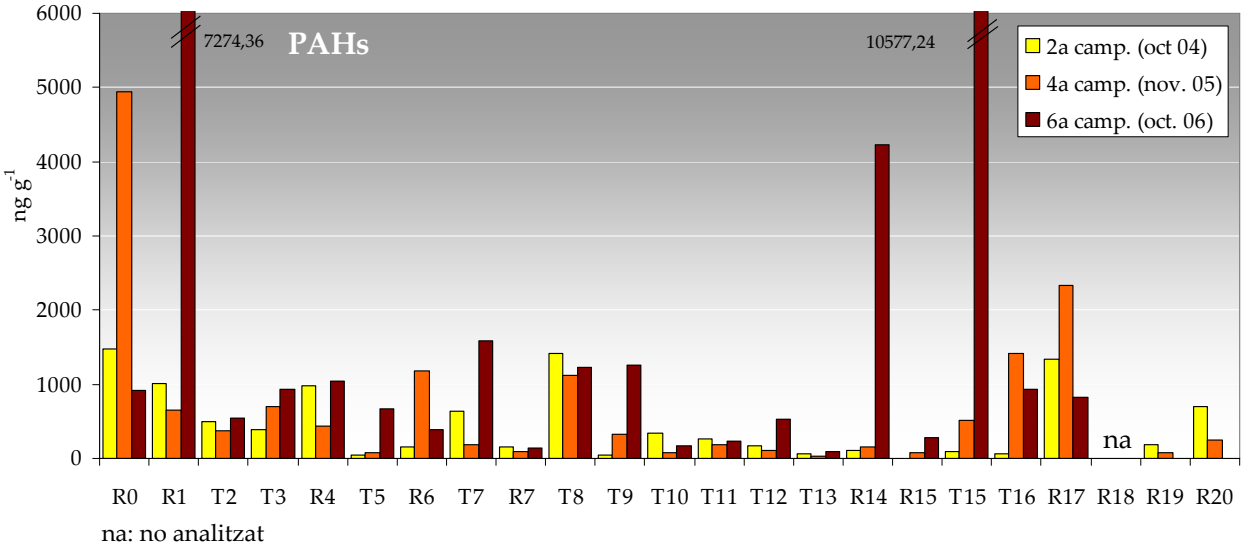

Figura 4.12: Concentració de PAHs en sediments

4.4.4.- Comparació de les concentracions de contaminants en sediments per la CHE i el projecte AquaTerra

Es va portar a terme una comparació utilitzant eines quimiomètriques entre les concentracions de contaminants en sediments obtingudes en el projecte AquaTerra durant els tres anys de presa de mostra (2004-2006) i aquelles disponibles a la base de dades de la CHE per

al mateix període de temps. Aquest procés només va ser possible per a la matriu de sediments, ja que en la RCSP de la CHE només existeixen dades per als paràmetres físicoquímics de l'aigua, però no per a les concentracions de les substàncies prioritàries. Els resultats de sediments de la RCSP no són tant exhaustius com els de l'estudi realitzat en aquesta tesi i per tant l'aplicació del PCA es va restringir a aquelles dades comunes entre la CHE i l'AquaTerra. Pel que fa als punts de presa de mostra, hi havia 18 punts en comú, si bé és cert que per a la CHE dos d'ells estaven doblats (R1 i T15) i ambdós es van considerar per a la comparació. La quantitat de compostos estudiats en canvi és molt menor en el cas de la RCSP, que només analitza 20 dels 68 compostos orgànics inclosos en aquesta tesi. D'aquests 20, però, n'hi ha 5 (naftalè, aldrina, endrina, dieldrina i isodrina) que no es van detectar a cap mostra durant els 3 anys considerats i 2 (NP i OP) que només es van analitzar en algunes mostres de forma puntual. A més, igual que en els altres estudis quimomètrics, es van descartar tots aquells compostos amb menys del 10% de presència. Segons totes aquestes consideracions finalment el nombre de variables va ser d'onze (7 PAHs, HCB i 3 congèneres del DDT). També cal tenir en compte que la RCSP està dissenyada de forma que tots aquests compostos només s'analitzen a 9 dels punts de presa de mostra, mentre que als altres 9 punts únicament s'analitzen els PAHs. Tot i això es va optar per agafar els 11 compostos, ja que sinó l'anàlisi s'hagués reduït només a una família, i es va considerar la meitat del LD en aquells punts on no s'havien analitzat els DDTs i l'HCB. L'establiment del LD va resultar més complicat que en altres casos, ja que els límits de detecció instrumentals variaven per a un mateix compost i entre les diferents campanyes donant lloc en alguns casos a límits de detecció superiors a concentracions trobades en les altres campanyes. Per tal d'homogeneïtzar i evitar sobrevaloracions es va utilitzar sempre la meitat del LD més baix per a cada compost. Finalment es van obtenir un total de 6 matrius de dades, 3 per a la CHE i 3 per a l'AquaTerra, amb 18 punts i 11 variables. En l'estudi quimiomètric de les dades històriques de la RCSP reflectit a l'article científic 4 es van estudiar els resultats des del 1996 fins el 2003. Els compostos inclosos en l'estudi de les dades històriques són relativament similars als de la comparació que ens ocupa, si més no pel que fa a les famílies (PAHs i pesticides organoclorats) però en el cas de les dades històriques només es van considerar els 9 punts en els que s'analitzen tots els compostos. Tot i això la comparació dels resultats dels PCAs és també possible de forma general si es tenen en compte aquestes diferències.

Després de realitzar l'anàlisi de les 6 matrius agrupades de diverses formes i amb les dues tècniques de pretractament considerades en els altres estudis (articles 5, 6 i 7), es van obtenir uns resultats similars, de forma que només es presenten a tall d'exemple els resultats de les matrius autoescalades per separat i posteriorment augmentades segons la seva procedència, RCSP o AquaTerra. A la Taula 4.1 es mostren els percentatges de variància explicada tant dels dos PCAs per a les dades actuals com del PCA de les dades històriques inclòs a l'article científic 4. En els tres casos s'explicava un percentatge molt alt amb només 3 PCs i només amb el primer PC ja s'explicava entre el 48,7 i el 63,1% de la variància de les mostres originals. Tot i que el PCA per a les dades històriques de la RCSP no incloia exactament les mateixes variables es va observar també un comportament molt similar en quant a variància explicada que per als altres dos.

Taula 4.1: Percentatges de variància explicada per als 3 PCAs realitzats

	RCSP històriques	RCSP actuals	AquaTerra actuals
PC1	52,3	63,1	48,7
PC2	16,8 (69,1)*	19,4 (82,5)	21,9 (70,6)
PC3	9,4 (78,5)	10,3 (92,8)	11,8 (82,4)

* (entre parèntesis): variància acumulada

A la Figura 4.13 es mostren els *loadings* obtinguts per als dos grups de dades. *A priori* sembla que els *loadings* són diferents, però en els dos casos aquests van assenyalar l'existència de dues fonts de contaminació, una de PAHs i una altra de pesticides organoclorats, però en cada cas expressat de forma diferent. Mentre que per a la CHE les dues fonts van ser completament independents, ja que la de PAHs va aparèixer únicament al PC1 i la de pesticides organoclorats només al PC2, per a les dades de l'AquaTerra hi va haver una font de contaminació general pels dos grups de compostos reflectida al PC1 i la contraposició entre els dos grups de compostos es va donar únicament al PC2 amb *loadings* positius per a pesticides organoclorats i negatius per a PAHs, indicant una correlació inversa. Va resultar curiós que per a les dades de la CHE en el PCs 3 només van contribuir els pesticides organoclorats i en canvi en el cas de l'AquaTerra en aquest PC van contribuir només els PAHs.

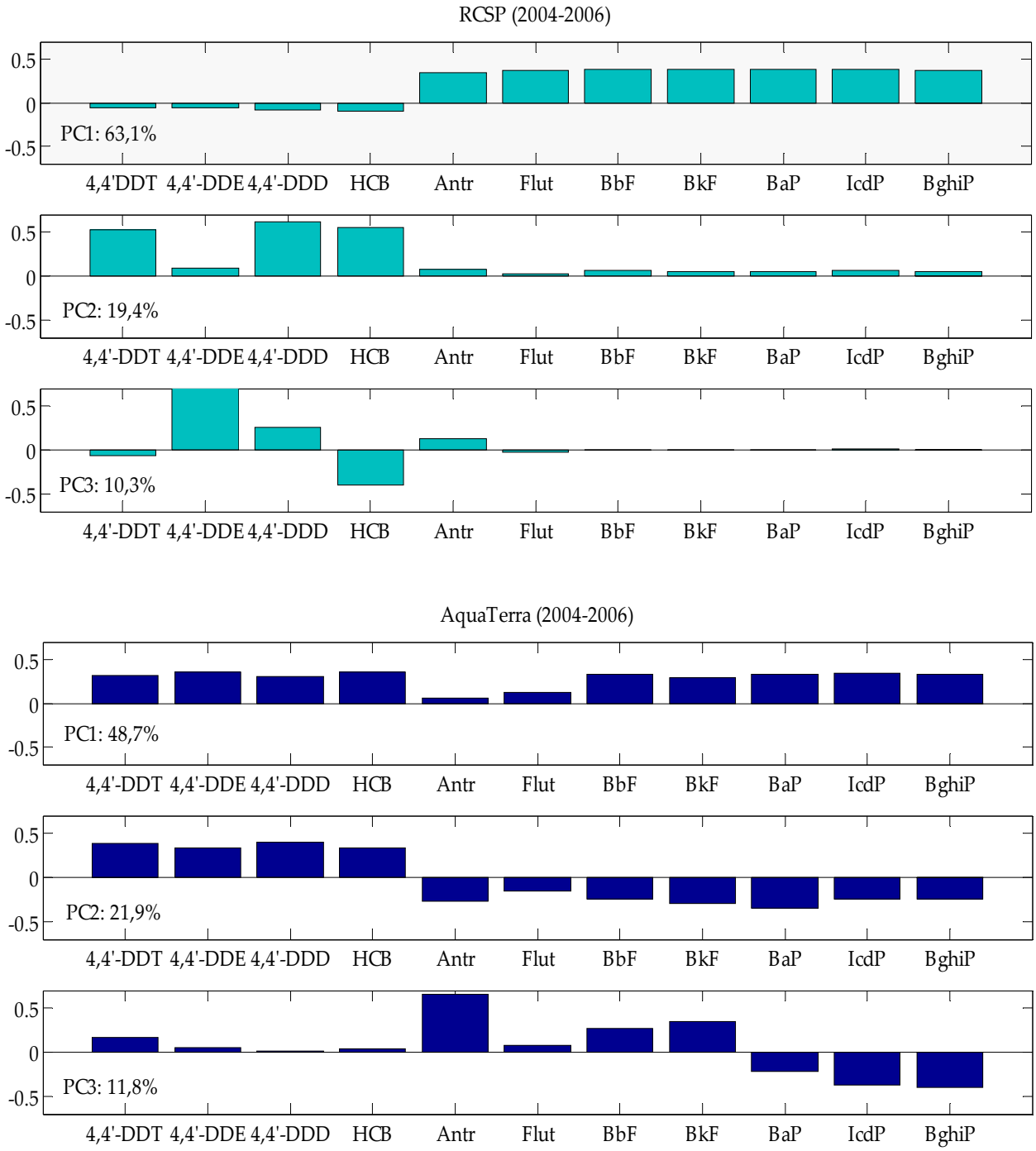

Figura 4.13: *Loadings* dels 4 primers PCs per a les dades de la RCSP i del projecte AquaTerra

A la Figura 4.14 es mostren els gràfics d'*scores* de PC1 *versus* PC2 tant per al PCA aplicat a les dades de la RCSP com per a les dades de l'AquaTerra. Així com amb l'observació dels *loadings* es va veure una certa similitud de les fonts de contaminació, aquestes no van veure reflectides de la mateixa manera en les 18 mostres considerades. Per a la RCSP les mostres es

van trobar distribuïdes només al llarg dels eixos, indicant una contaminació diferenciada segons la font de contaminació. Els punts de presa de mostra al llarg del PC1 tan sols contenien PAHs (T3 i T9) i els que es troben al llarg del PC2 només estaven contaminats amb pesticides organoclorats (R17, R18, T15 i T12). La resta de punts de presa de mostra van aparèixer molt concentrats al voltant del zero, indicant que aquests no presentaven cap contribució per a aquestes dues fonts de contaminació majoritàries. La situació va ser una repetició del que es va trobar a l'estudi de les dades històriques de la RCSP ja que els 9 punts de presa de mostra que no es van considerar en l'estudi històric apareixen ara al grup situat al voltant de l'origen, indicant la poca influència d'aquests punts en el PCA. En resum, en els dos casos que es van considerat dades de la RCSP els valors van ser molt extrems, amb la major part dels punts que no presentaven contaminació i només uns quants van estar molt contaminats per la barreja de compostos estudiada.

En el cas dels *scores* obtinguts dels resultats de l'AquaTerra la situació va ser molt diferent ja que les mostres no estaven tan concentrades al voltant del zero sinó que van presentar diferents graus de contaminació, que a més a més era combinació dels PAHs i pesticides organoclorats. Totes les mostres amb valors positius per a PC1 presentaven una contribució dels dos grups de compostos però es van diferenciar segons el valor de PC2, aquelles situades a la part positiva de PC2 tenien una major contribució de pesticides organoclorats i aquells situades a la part negativa tenien més contribució de PAHs. D'entre tots els punts de presa de mostra l'únic amb una contaminació semblant per a la RCSP i l'AquaTerra va ser el R17.

Tot i la poca quantitat i varietat de compostos que es van poder incloure, la comparació va donar uns resultats força similars en quant a fonts de contaminació. Pel que fa a la contaminació de les mostres només el punt R17 va seguir un patró similar en els dos casos amb una forta contribució de pesticides organoclorats. La falta de correspondència entre la contaminació present a les mostres de les dues bases de dades és atribuïble a la manca de constància de les dades de la CHE, i a certes diferències durant la presa de mostres, com per exemple que les mostres de la RCSP es van recollir a finals d'agost i les de l'AquaTerra a finals d'octubre.

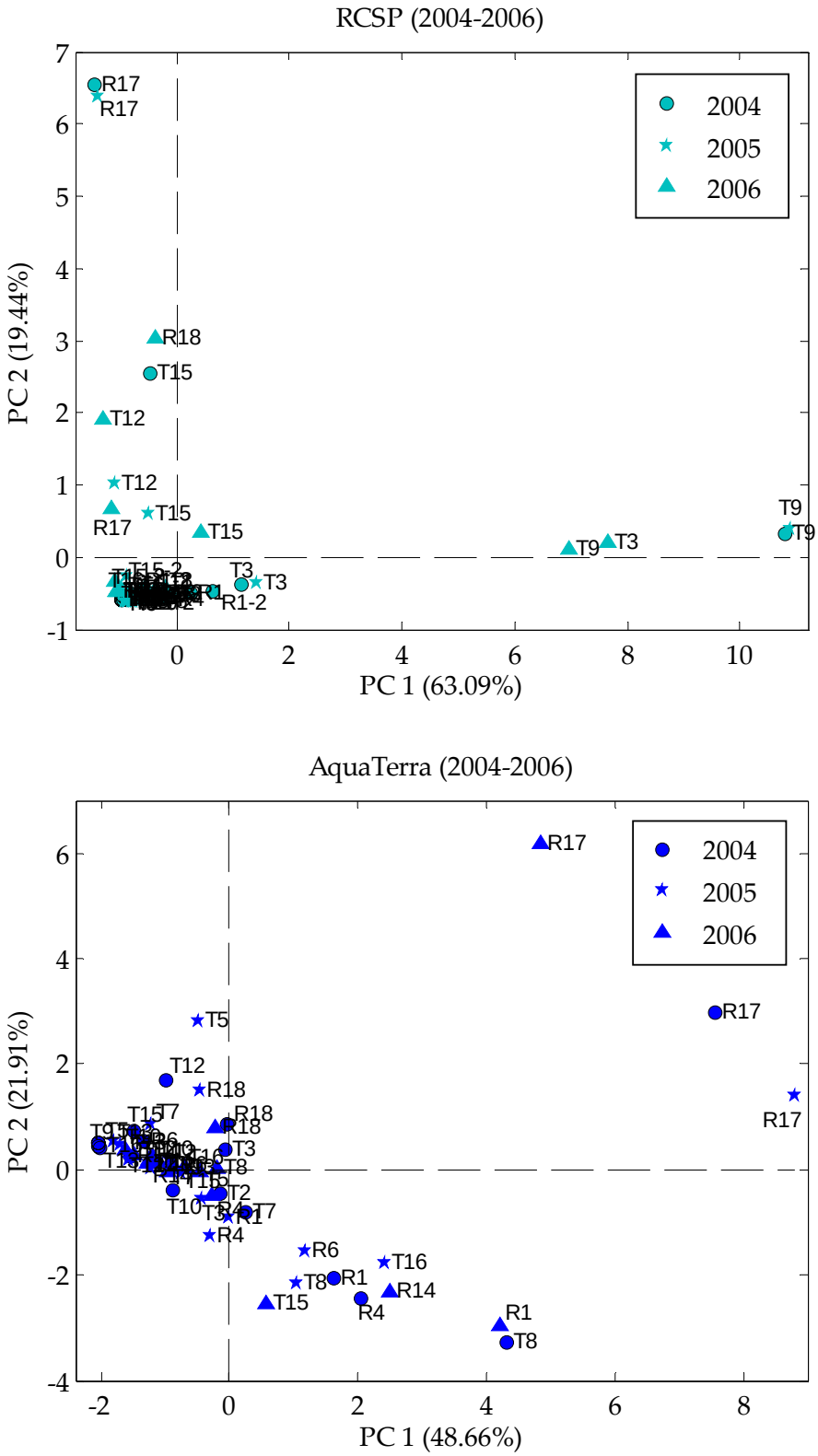

Figura 4.14: ***Scores*** **dels PCs 1 i 2 per a les dades de la RCSP i del projecte AquaTerra**

En base a aquest estudi de la contaminació de l'Ebre, especialment a la part comparativa entre les diferents fonts d'informació, es pot concloure que per a obtenir una bona descripció de

la contaminació i captar les possibles variacions temporals que tenen lloc en una conca hidrogràfica és necessari portar a terme programes de vigilància ambiental molt exhaustius. Per a caracteritzar una conca de les dimensions de les de l'Ebre cal un nombre elevat de punts de presa de mostra així com la utilització d'un mètode multiresidual en el que s'incloguin gran quantitat i varietat de compostos. També és imprescindible que la informació sigui constant en el temps, és a dir, que en cadascuna de les campanyes de presa de mostra es recullin les mostres dels mateixos punts i s'analitzin els mateixos compostos, en cas contrari les dades són molt difícils de comparar i per tant resulta complicat establir cap tipus de tendència. Per altra banda la freqüència dels mostrejos cal que sigui més elevada que la que s'ha donat en qualsevol dels dos programes de vigilància considerats en la present Tesi, amb preses de mostra mensuals que assegurin la continuïtat de la informació i permetin d'aquesta forma captar les variacions temporals, enlloc de només la contaminació puntual en el moment de presa de mostra. Aquest fet queda palès en la comparació entre els resultats del projecte AquaTerra i la RCSP on una única presa de mostra amb una diferència de dos mesos va donar lloc a resultats diferents.