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Analysis and occurrence of pharmaceuticals, estrogens, progestogens and polar pesticides in sewage treatment plant effluents, river water and drinking water in the Llobregat river basin (Barcelona, Spain)

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Monitoring

Summary This work investigated the presence of 21 emerging contaminants of various chemical groups (7 estrogens, 3 progestogens, 6 pharmaceuticals and personal care products (PPCPs), and 5 acidic pesticides) in the Llobregat river basin (NE Spain). Waters from the outlet of various sewage treatment plants (STP) and waterworks located along the river basin, as well as water samples from the river or its tributaries upstream and downstream of these plants were analysed in two pilot monitoring studies. Chemical analyses were performed by means of on-line or off-line solid-phase extraction followed by liquid chromatography-electrospray-tandem mass spectrometry. Methods detection limits (in ng/L) were $\leqslant$0.85 for estrogens, $\leqslant$3.94 for progestogens, $\leqslant$30 for PPCPs, and $\leqslant$0.99 for pesticides. Of the estrogens and progestogens analysed, only estrone-3-sulfate, estrone, estriol and progesterone were found to be present in the low nanogram per liter range in some of the samples investigated. Except for atenolol, all PPCPs studied (ibuprofen, diclofenac, clofibric acid, salicylic acid, and triclosan) could be identified at levels usually

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lower than 250 ng/L and up to 1200 ng/l (diclofenac). Of the various pesticides investigated (2,4-D, bentazone; MCPA, mecoprop and propanil) MCPA and 2,4-D were the most ubiquitous and abundant and bentazone the only one not detected. Individual concentrations were most often below 100 ng/L and never surpassed the EU limits.

Introduction

In addition to the classical priority pollutants (e.g. pesticides) the non regulated so-called emerging contaminants are causing high environmental concern. Emerging contaminants can be broadly defined as any synthetic or naturally occurring chemical or any micro-organism that is not commonly monitored in the environment but has the potential to enter the environment and cause known or suspected adverse ecological and (or) human health effects (http://toxics.usgs.gov/regional/emc). These chemicals are produced on a large scale worldwide, are used in a wide range of applications, and have become indispensable to our modern society.

Progress in instrumental analytical chemistry and robust extraction techniques have enabled the detection of more compounds and at lower concentrations contributing to the awareness of this environmental problem.

The Llobregat river is one of the major, and at the same time limited, water resources available in Catalonia (NE of Spain). In the Baix Llobregat area (lower reach of the Llobregat river) there is an important concentration of industries, agricultural activities and populated areas, with important demands of water, which also act as important sources of both point and diffuse pollution.

A variety of organic micro pollutants such as pesticides, surfactants and plasticizers has been detected in this stretch of the Llobregat river basin (Castillo et al., 2000; Cespedes, 2005; Petrovic, 2002a) and several studies have also investigated the estrogenic activity in this area (Castillo et al., 2000; García-Reyero, 2001; Petrovic, 2002a,b; Solé, 2000). However, to the authors knowledge, estrogens, progestogens, PPCPs and the acidic pesticides included as target analytes in the present study, have never or only occasionally, been investigated in this area. PPCPs, for instance, have only been studied in the upper part of the river (Farré, 2001); acidic pesticides have only been determined at the inlet of one of the waterworks included in this study (Kampioti et al., 2005); and estrogens and progestogens have only been determined in the Anoia and Cardener tributaries of the Llobregat river and in the inlet and outlet of some related STPs (Solé, 2000; Petrovic et al., 2002b), but not in the Llobregat river itself (with exception of the intake of one of the waterworks included in this study (Rodriguez-Mozaz et al., 2004a,b)) neither in the STPs connected to it. In this context, the aim of this study was to investigate the levels of 21 selected organic contaminants, belonging to the classes of estrogens, progestogens, PPCPs, and pesticides in the lower reach of the Llobregat river basin (NE of Spain). Analysis was performed by means of off-line and on-line solid-phase extraction (SPE) followed by liquid chromatography–tandem mass spectrometry (LC–MS/MS). The list of target compounds included eight estrogens and progestogens, as well as two conjugated estrogen metabolites. Within the class of PPCPs, five active pharmaceutical ingredients and a personal care product have been studied. Furthermore, a total of five polar pesticides (acidic and neutral) were tested. Table 1 lists the chemical class and uses of all target analytes included in this study.

Adverse estrogenic effects have been assigned to the class of steroidal estrogens at very low concentrations of $\sim$1 ng L^{-1} (Hansen, 1998). Acidic anti-inflammatory drugs and acidic pesticides are receiving increasing attention because of their high polarity and therefore high mobility in the water media (Petrovic et al., 2005) with potential to contaminate groundwater sources (Heberer, 2006). The pharmaceuticals clofibric acid, triclosan and atenolol are investigated for the very first time in this area in this work, and most of the river sampling sites, two of the three waterworks, and all three STPs included in this study had never been investigated before for the presence of the target contaminants considered. Thus, virtually all the environmental data generated in the present study is new. This work provides a first, general picture about the occurrence of these compounds in this area, which is essential for future work regarding potential environmental and human health risks.

Experimental

Chemicals and standards

Pure standards of the target estrogens, progestogens, PPCPs and acidic pesticides were purchased from Sigma–Aldrich (St Louis, MO, USA). Individual stock solutions of the analytes were initially prepared at 1 μg/mL in methanol (100 μg/mL for PPCPs). Standard mixtures of the various compound classes were then prepared in methanol at different concentrations by appropriate dilution of the individual stock solutions. The standard mixtures were used as calibration solutions or, in on-line methods (for estrogens, progestogens and acidic pesticides), as spiking solutions for preparation of the aqueous calibration standards (content of methanol < 0.1%).

HPLC-grade water, acetonitrile and methanol, and hydrochloric acid 37% and ammonium acetate were purchased from Merck (Darmstadt, Germany). Nitrogen for drying 99.995% of purity was from Air Liquide (Spain).

Site description and sampling procedure

The Llobregat river is located in the northeast of Catalonia (Spain) and flows into the Mediterranean Sea south of the city of Barcelona. This is a densely inhabited area, thus urban wastewaters represent a significant input in the river. The river also receives industrial wastewaters and surface runoff from agricultural areas. Water from the Llobregat

Table 1 Chemical class and use of the studied target analytes

Group	Analyte	Chemical class	Use
PPCPs			
	Acetylsalicylic acid	Benzoic acid	Anti-inflammatory
	Diclofenac	Phenylacetic acid	Anti-inflammatory
	Ibuprofen	Phenylpropanoic acid	Anti-inflammatory
	Atenolol	Acetamide	Beta-blocker
	Clofibric acid	Methylpropanoic acid	Blood lipid regulator
	Triclosan	Phenoxyfenol	Antiseptic
Estrogens			
	Estradiol	Sex steroid	Contraceptive and HRT
	Estrone	Sex steroid	Contraceptive and HRT
	Estriol	Sex steroid	Contraceptive and HRT
	Estradiol-17-glucuronide	Sex steroid metabolite	–
	Estrone-3-sulfate	Sex steroid metabolite	–
	Ethynyl estradiol	Synthetic sex steroid	Contraceptive
	Diethylstilbestrol	Bisphenol	Contraceptive
Progestogens			
	Progesterone	Sex steroid	Contraceptive
	Levonorgestrel	Synthetic sex steroid	Contraceptive
	Norethindrone	Synthetic sex steroid	Contraceptive
Pesticides			
	Bentazone	Benzothiadiazine	Herbicide
	MCPA	Alkanoic acid	Herbicide
	2,4 D	Alkanoic acid	Herbicide
	Mecoprop	Alkanoic acid	Herbicide
	Propanil	Acetanilide	Herbicide

HRT, hormone replacement therapy.

river basin is used for production of drinking water at various locations.

In the present study a total of nineteen water samples (including the effluents from 3 STPs, river water, and drinking water from 3 waterworks) were collected at eleven selected sites of the lower reach of the Llobregat river basin (see Fig. 1) in two different sampling campaigns performed in October 2003 and April 2004.

River water was collected from three locations along the Llobregat river (sites 7, 9, and 10 in Fig. 1), and one location from each of its two tributaries Rubí creek (2) and Anoia river (4) at sites close to the investigated STPs and waterworks. The three STPs – Rubí (3), Abrera (5), and Martorell (8) – included in this study receive high amounts of urban and industrial wastewaters, with capacity to serve 75,000, 76,000 and 100,000 inhabitants, respectively. Their treatment is based on a classical biological treatment: primary sedimentation, activated sludge oxidation and secondary settling. The STPs effluents are discharged into the Llobregat river, except for the Rubí STP that releases its effluent into the Rubí creek (used for irrigation). Drinking water was collected from the drinking water treatment plants (DWTPs) of Cardedeu (1), Abrera (6), and San Joan Despí (11) (SJD). The two latter DWTPs use water from the Llobregat river and the Cardedeu DWTP from the Rubí Creek. The complexity of the operation of these plants varies considerably from each other since the surface raw water at the intake of the Sant Joan Despí DWTP is by far the most polluted one and experiences frequent peak episodes that require continuous adjustments of the different treatment units (prechlorination or predioxichlorination,

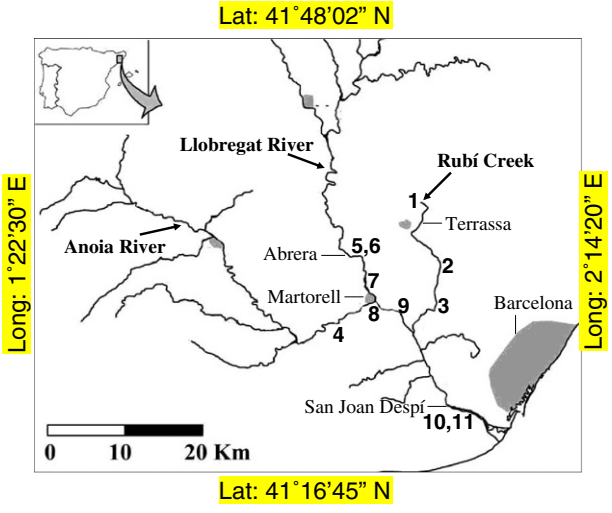

Figure 1 Sampling locations; 1. DWTP Cardedeu; 2. Rubí Creek; 3. STP Rubí; 4. Anoia River; 5. STP Abrera; 6. DWTP Abrera; 7. Llobregat River before Anoia River confluence; 8. STP Martorell; 9. Llobregat River after Anoia River confluence; 10. Llobregat River at entrance of DWTP SJD; 11. DWTP SJD.

coagulation, sand filtration, ozonation, activated carbon filtration, blending with groundwater, postchlorination, etc.), not necessary in the other DWTPs.

Water samples (1 L) were collected in amber glass bottles (previously washed with water, methanol and acetone and heated at 400 °C for 12 h), and transported to the laboratory under cooled conditions (4 °C). Upon reception, samples were filtered through 0.45 μm Nylon filters (Whatman, Maidstone, UK) to eliminate particulate matter and other suspended solid matter and then stored at 4 °C in the dark until analysis which was always carried out within 48 h of collection to keep microbial degradation to a minimum. Samples suspected to contain residual chlorine (drinking waters) were amended with sodium thiosulfate anhydrous (80-100 ng/L) immediately after collection.

Analytical methods

An overview of the analytical methods employed in this work is presented in Table 2. Estrogens, progestogens and pesticides were analysed by on-line SPE-LC-MS/MS (Kampioti et al., 2005; Rodriguez-Mozaz et al., 2004b) and PPCPs by off-line SPE LC-MS/MS (Hernando et al., 2006; Hernando et al., 2004).

Sample preparation

Off-line solid-phase extraction of PPCPs was performed using an automated solid-phase extraction (SPE) sample processor ASPEC XL (automated sample preparation with extraction columns), fitted with a 817 switching valve and an external 306 LC pump for selection and dispensing of samples, respectively, all from Gilson (Villiers-le-Bel, France).

The water samples (250 mL of river and drinking water, 50 mL of effluent wastewater) were loaded onto Isolute SPE C18 cartridges (500 mg) (4 mL/min) from Biotage (Hengoed, UK) previously conditioned with 5 mL methanol and 3 mL HPLC water (4 mL/min). A washing step with HPLC water (3 mL) was applied after sample loading. The cartridges were then dried by vacuum for 30 min and PPCPs were eluted with 2 × 4 mL of methanol. Prior to extraction, the pH of the collected samples was adjusted to 2.8 with 1 M HCl for the determination of the acidic PPCPs (acetylsalicylic acid, diclofenac, ibuprofen and clofibric acid) and to pH 10 for determination of the basic PPCPs (triclosan and atenolol), thus performing two separate extractions.

Fully automated on-line trace enrichment and analysis of estrogens, progestogens and acidic pesticides in samples, aqueous standards and blanks was performed with an automated on-line SPE sample processor Prospekt-2 (Spark Holland, Emmen, The Netherlands) configured for high sample volumes and connected in series with the LC—MS/MS instrument. This system allows performing the extraction of one sample in a sequence while the elution and analysis of the previously extracted one is simultaneously carried out.

Extraction was performed with disposable trace enrichment polymeric cartridges: PLRP-s (cross linked styrene-divinylbenzene, 10 × 2 mm i.d., 15—25 μm particle size) for estrogens and progestogens and Hysphere Resin GP (polydivinyl-benzene, 10 × 2 mm i.d., 10—12 μm particle size) for pesticides (both from Spark Holland, Emmen, The Netherlands). The samples (20 mL) were loaded at a flow-rate of 8 mL/min onto the cartridges previously conditioned with 1 mL acetonitrile and 1 mL water (flow-rate 5 mL/min). Subsequent washing of the cartridges was carried out with 1 mL of water (flow-rate 5 mL/min). Elution of the target analytes directly onto the chromatographic column was performed with the chromatographic mobile phase. All steps of the sample preparation procedure were programmed on and automatically controlled by means of SparkLink version T2.20-01 software (Spark Holland).

Liquid chromatography

LC—MS/MS analyses were carried out in a system consisting of a Waters Alliance 2690 LC pump equipped with an

Table 2 Analytical procedures for analysis of PPCPs, estrogens, progestogens and pesticides in environmental waters

	PPCPs	Estrogens	Progestogens	Pesticides
SPE				
Mode	off-line	on-line	on-line	on-line
Sample volume	250; 50 mL[a]	20 mL	20 mL	20 mL
Cartridge	Isolute C18	PLRPs	PLRPs	Hysphere Resin GP
Elution	2 × 4 mL MeOH	LC mobile phase	LC mobile phase	LC mobile phase
Drying	N_2 stream	—	—	—
Reconstitution	500 μL MeOH	—	—	—
LC				
Column	Purospher STAR RP-18e (125 × 2 mm I.D., 5 μm)			
Mobile phase	MeOH/Am. acet[b]	ACN/water	ACN/water	ACN/water
ESI				
Ion mode	NI/PI[c]	NI	PI	NI
MS/MS				
Analyser	Triple quadrupole operating in SRM mode			

[a] 250 mL, drinking and river water; 50 mL, wastewater effluent.
[b] ACN/water for atenolol.
[c] PI for atenolol.

auto-sampler with the volume injection set to 20 μL (Waters, Milford, MA, USA) and connected in series with the MS/MS detector. The auto-sampler was only used for the off-line analysis of PPCPs.

Chromatographic separation was performed in all cases using a reversed-phase Purospher STAR-RP-18e analytical column (125 × 2 mm, 5 μm particle diameter) preceded by a guard column (4 × 4 mm, 5 μm) of the same packing material from Merck (Darmstadt, Germany).

The LC-gradient program used for the off-line analysis of PPCPs at 0.2 mL/min began with a hold for 1 min at 30% methanol in water increasing to 95% in 25 min, which was held constant for 5 min. Finally after readjusted to initial conditions equilibration time was 10 min.

On-line elution of the trapped estrogens, progestogens and pesticides onto the chromatographic column was performed at 0.2 mL/min with a 45 min gradient starting from 10% acetonitrile in water, increasing to 50% acetonitrile in 5 min and continuing to 80% in 20 min. During the following 5 min the column was cleaned with 100% acetonitrile, readjusted to the initial conditions in 2 min, and equilibrated for further 13 min.

Tandem mass spectrometry

Detection was performed using a Quattro triple—quadrupole mass spectrometer from Micromass (Manchester, UK), equipped with an orthogonal electrospray (ESI) ionization source. Two different selected reaction monitoring (SRM) transitions were monitored per compound (except for acetyl salicylic acid, ibuprofen and triclosan): the first and more abundant transition was used for quantitation and the second transition for confirmation of the results (see Table 3). To maximize sensitivity, data acquisition was performed under time-scheduled conditions (time windows are shown in Table 3). Due to the different preferential ionization of the target compounds, the electrospray interface was operated in negative ion mode for analysis of estrogens, acidic pesticides and all PPCPs except atenolol and in the positive ion mode for progestogens and atenolol.

Other MS/MS experimental conditions were as follows (on-line/off-line analysis): capillary voltage, 3.5 kV; source temperature, 150/120 °C; desolvation temperature, 450/370 °C; extractor voltage, 2 V; and RF lens, 0.4 V. Nitrogen was used as both the nebulizing and the desolvation gas. The flow-rate of the nebulizing gas was set at 60/109 L h^{-1}, and that of the desolvation gas at 550/510 L h^{-1}. Argon was used as collision gas with a pressure of 2.58×10^{-3} mbar.

Instrument control, data acquisition and evaluation were performed by Masslynx 4.0 software (Micromass, Manchester, UK).

Table 3 Time scheduled SRM conditions used for the analysis of PPCPs, estrogens, progestogens and pesticides

Analyte	Time window	Retention time (min)	SRM transitions (m/z) [a] Prec. ion → Prod. ion	Cone (V)	Collision (eV)
PPCP (NI)					
Acetylsalicylic acid	00.00—05.00	2.25	137 → 93	20	20
Clofibric acid	10.00—15.00	12.27	213 → 127; 213 → 85	20	10; 15
Diclofenac	10.00—18.00	14.10	294 → 250; 294 → 214	20	20; 15
Ibuprofen		14.35	205 → 161	20	10
Triclosan	12.00—18.00	16.73	289 → 253	20	15
PPCP (PI)					
Atenolol	00.00—10.00	4.13	267 → 145; 267 → 190	25	25; 25
Estrogens (NI)					
Estradiol—17—glucuronide	00.00—13.25	10.04	447 → 113; 447 → 271	40	30; 50
Estrone—3—sulfate		11.17	349 → 269; 349 → 145	40	40; 40
Estriol		12.09	287 → 171; 287 → 145	50	40; 40
Estradiol	13.25—25.00	15.51	271 → 145; 271 → 183	50	45; 45
Ethynyl estradiol		16.44	295 → 145; 295 → 159	50	40; 40
Estrone		17.14	269 → 145; 269 → 143	50	40; 45
Diethylstilbestrol		17.75; 20.42	267 → 222 (2 peaks)	30	35; 45
Progestogens (PI)					
Norethindrone	00.00—17.00	16.20	299 → 171; 299 → 145	30	20; 20
Levonorgestrel	17.00—20.50	18.33	313 → 245; 313 → 185	30	20; 20
Progesterone	20.50—25.00	22.48	315 → 109; 315 → 97	30	25; 25
Pesticides (NI)					
Bentazone	00.00—14.00	4.86	239 → 132; 239 → 197	35	25; 20
MCPA		9.52	199 → 141; 201 → 143	20	15; 15
2,4-D		9.61	219 → 161; 219 → 125	15	10; 30
Mecoprop		9.98	213 → 141; 215 → 143	15	15; 15
Propanil	14.00—30.00	17.07	216 → 160; 218 → 162	30	15; 15

[a] Quantitation; confirmation transition.

Results and discussion

Table 4 summarizes the main analytical quality control parameters of the methods used for monitoring of estrogens, progestogens, PPCPs, and pesticides. All methods showed satisfactory performance in terms of linearity (correlation coefficients (r^2) equal to or higher than 0.99 for all compounds), sensitivity (limits of detection (LODs) in the pg/L or low ng/L range for all compounds except atenolol (17 ng/L), ibuprofen (12 ng/L) and triclosan (30 ng/L)), and extraction efficiency (recovery percentages (%R) higher than 69% for all compounds except acetyl salicylic acid (30%)).

In all cases quantitation was performed by the external standard method and for a positive confirmation the retention time of the compound in the sample must be within 2% of that in the standard calibration solution and the relation of abundances between the first and second transition must be within the signal ion ratio of the standard ± 20%.

The results of the sampling program (number of positive samples, and average and maximum concentrations per each detected compound) are summarized in Table 5. In total, twenty one compounds and nineteen samples were analysed. Of the twenty one compounds monitored, thirteen were found to be present in one or more samples.

The levels of the compounds detected varied considerably between classes (see Fig. 2). PPCPs and pesticides were found at higher concentrations, up to 1.20 μg/L (diclofenac) and 1.28 μg/L (MCPA), respectively, in STP effluent. Estrogens and progestogens were detected at much lower levels, in the pg/L or low ng/L range (except for one sample where estriol was found at 11.6 ng/L). The following subsections discuss in more detail the results obtained for each compound class.

Levels of PPCPs

As shown in Table 5 all PPCPs investigated except atenolol were detected at least once, although the levels found and their temporal and spatial distribution was quite erratic (see Fig. 3). Diclofenac was found in both sampling campaigns, clofibric acid and acetylsalicylic acid only in the first campaign, and ibuprofen and triclosan only in the second one. The highest observed concentration corresponded to diclofenac, which was found in the effluent of the STP of Rubí at a concentration of 1200 ng/L. All other results were distinctly lower (below 150 ng/L in most instances). With regards to the different types of matrix, effluent waters contained higher levels of PPCPs than river waters. However since both matrices show a very random distribution of both compounds and levels (Fig. 3) no further conclusions can be drawn. The drinking water samples were all free of the PPCPs investigated.

No trend or further observation can be made either with respect to the concentration profiles found in the autumn and spring campaigns because they were completely different (see Fig. 3).

Table 4 Methods performance parameters

Analyte	r^2	% Rec	RSD	LOD (ng/L)	LOQ (ng/L)
PPCPs					
Acetylsalicylic acid	0.996	30	2.5	9.00	24.0
Atenolol	0.997	70	10	17.0	45.3
Clofibric acid	0.996	92	3.3	7.50	20.0
Diclofenac	0.999	92	1.7	7.50	20.0
Ibuprofen	0.998	87	2.8	12.0	32.0
Triclosan	0.997	85	3.9	30.0	80.0
Estrogens					
Estradiol-17-glucuronide	1.000	92	4.5	0.50	1.33
Estrone-3-sulfate	0.999	104	7.0	0.03	0.08
Estriol	1.000	70	4.0	0.62	1.65
Estradiol	0.993	85	3.6	0.85	2.27
Ethynyl estradiol	1.000	89	1.7	0.62	1.65
Estrone	1.000	99	2.0	0.24	0.64
Diethylstilbestrol	0.997	77	3.6	0.67	1.79
Progestogens					
Norethindrone	0.993	72	5.1	3.94	10.5
Levonorgestrel	0.994	100	8.0	1.84	4.90
Progesterone	0.997	95	7.5	0.24	0.64
Pesticides					
Bentazone	0.993	69	7.0	0.99	2.64
MCPA	0.990	88	5.6	0.29	0.77
2,4-D	0.991	80	3.7	0.48	1.28
Mecoprop	0.998	108	4.8	0.22	0.59
Propanil	0.993	96	4.1	0.03	0.08

Table 5 Summary of the results (ng/L) obtained in both sampling campaigns

Compound class	Detected compounds	October 2003 (n = 9)			April 2004 (n = 10)		
No. of analytes		Positive samples	Average concentration	Maximum concentration	Positive samples	Average concentration	Maximum concentration
PPCPs (6)	Diclofenac	1	1200	1200	5	86	241
	Clofibric acid	5	44	107	—	—	—
	Acetylsalicylic acid	2	41	50	—	—	—
	Ibuprofen	—	—	—	6	65	134
	Triclosan	—	—	—	2	45	45
Estrogens (7)	Estrone	1	0.75	0.75	6	0.95	1.68
	Estrone sulfate	6	0.81	1.32	7	0.44	0.79
	Estriol	—			1	11.60	11.60
Progestogens (3)	Progesterone	—			3	0.88	1.39
Pesticides (5)	MCPA	9	30.05	67.20	8	234	1286
	Mecoprop	9	3.97	6.95	8	6.49	25.10
	2,4-D	8	21.40	76.10	9	33.39	109
	Propanil	1	0.09	0.09	4	0.09	0.10

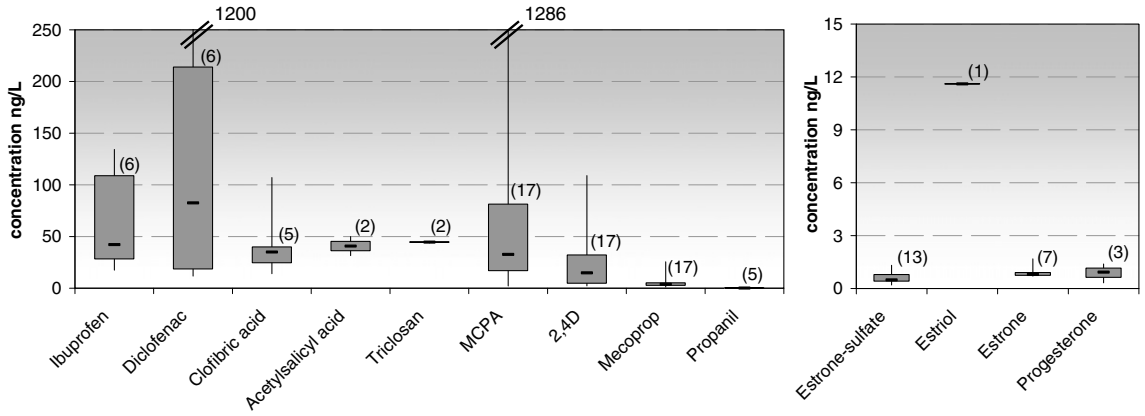

Figure 2 Box—plot summarizing the median and the values between the 25th and 75th percentile of the compounds detected during this study. Values given in parentheses represent the number of positive samples out of 19 analysed.

The alarmingly high concentrations of pharmaceuticals found previously (Farré, 2001) were not observed in the present study, suggesting an improvement of the environmental status. In order to confirm this observation additional work is currently being performed in this area.

Levels of estrogens

The list of target estrogens included seven substances comprising both natural (free and conjugated) and synthetic compounds. However only three of them were detected in the studied samples (see Table 5). Estrone and estrone sulfate were found in both sampling campaigns and estriol in only one sample of the campaign performed in April (see Fig. 4). The compounds with higher environmental concern due to its high estrogenic potency estradiol and ethynyl estradiol were not detected in any sample.

Estrone sulfate was detected in six and nine samples of the first and second campaigns, respectively, being the most ubiquitous sex steroid found in this study, followed by estrone. The levels of these two compounds did not surpass 1.7 ng/L, which in terms of estrogenic activity, should not pose a high risk to aquatic organisms (Petrovic et al., 2004; Routledge, 1998). Significantly higher levels of estrone and estrone-sulfate (4—22 ng/L and 4—7 ng/L respectively) were found a year earlier by Rodriguez-Mozaz et al. (2004a). In our study the highest load of estrogens was found to happen in river waters (see Fig. 4) more than in STP effluents, leading us to the conclusion that there must be other sources (uncontrolled, non-treated discharges) for their introduction in the environment.

The pattern found for estrone and estrone sulphate seems to be a result of natural variability or unknown factors. The average temperatures in the studied area were higher in autumn 2003 (18.3 °C in September and 13.2 °C in October) than in the following spring (8.0 °C in March and 10.7 °C in April) and therefore do not explain the higher levels of estrone sulphate detected in October, since microbial deconjugation is favoured in warm periods. The dilution factor cannot explain either those values as the total pre-

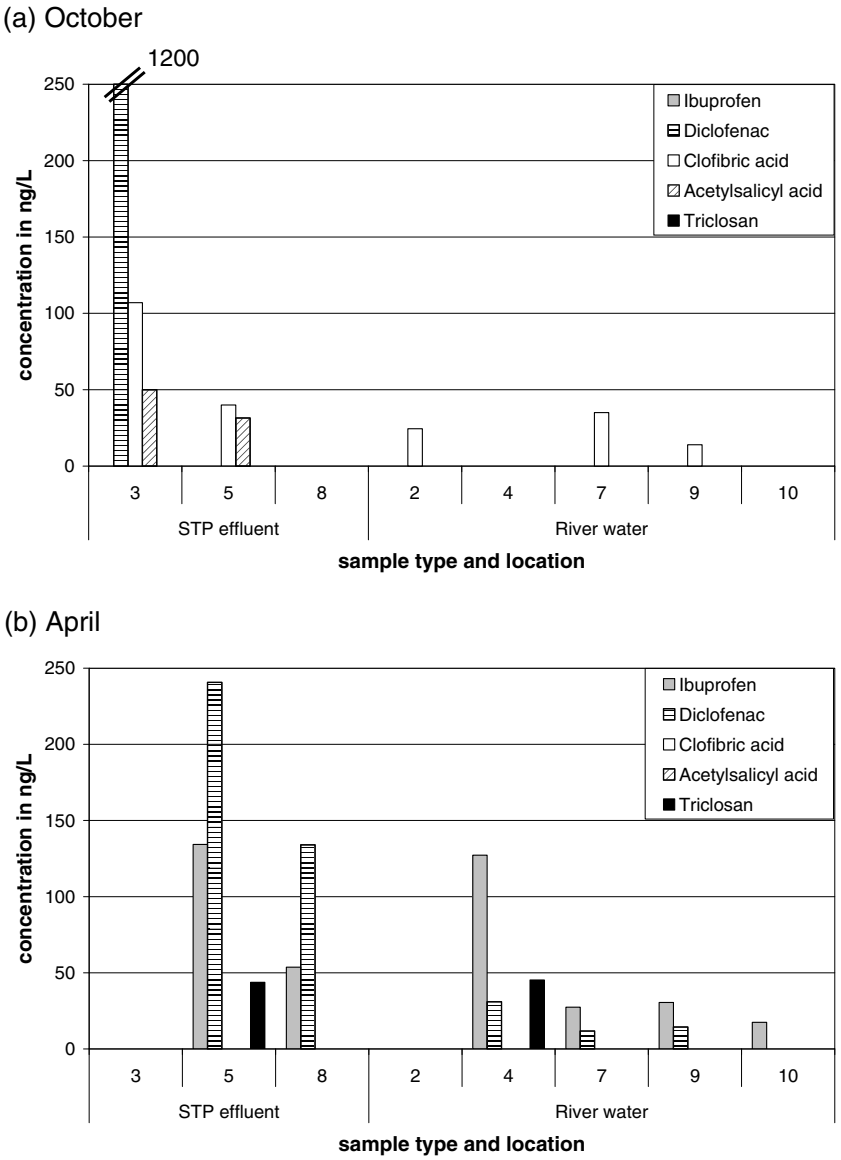

Figure 3 Levels of PPCPs found in STP effluents and river waters in both sampling campaigns. Atenolol was not detected.

cipitations in the months of October 2003 and April 2004 were 125 and 119 mm, respectively (http://www.darrera.com/darrera.htm).

Drinking water samples were all free of estrogens with the only exception of the sample collected in the DWTP of Abrera in the second campaign. This sample contained estriol at a concentration of 11.6 ngL. This concentration, if in surface water, could represent a risk for aquatic organisms since estriol is about one order of magnitude less active than estradiol (Petrovic et al., 2004) and estradiol has been shown to cause deleterious effects in aquatic organisms at concentrations of 0.1—1 ng/L (Hansen, 1998; Pelissero, 1993). For humans this finding is not worrisome; the estriol intake derived from the consumption of such drinking water would be negligible as compared to the human daily production of endogenous estrogens (0.05—0.60×10^3 μg/day estrogens) (Kuster et al., 2007). Fig. 5 illustrates the chromatograms corresponding to the analysis of estriol in this sample and in a standard solution at the two selected SRM transitions. As can be seen, the criteria that have to be fulfilled for a positive identification/confirmation, relative to the retention time and the SRM1/SRM2 ratio, are complied with.

Levels of progestogens

The list of target progestogens included two synthetic compounds norethindrone and levonorgestrel of wide use in contraceptive formulations and the natural hormone progesterone. Of all three compounds only progesterone was detected and in just three samples of the second campaign. Progesterone levels were very low (see Fig. 2), between 0.32 and 1.39 ng/L. The drinking water sample from Abrera that was positive in the analysis of estriol also contained progesterone (0.93 ng/L), but neither in this case nor in the others is the presence of progesterone relevant with regard to human toxicity.

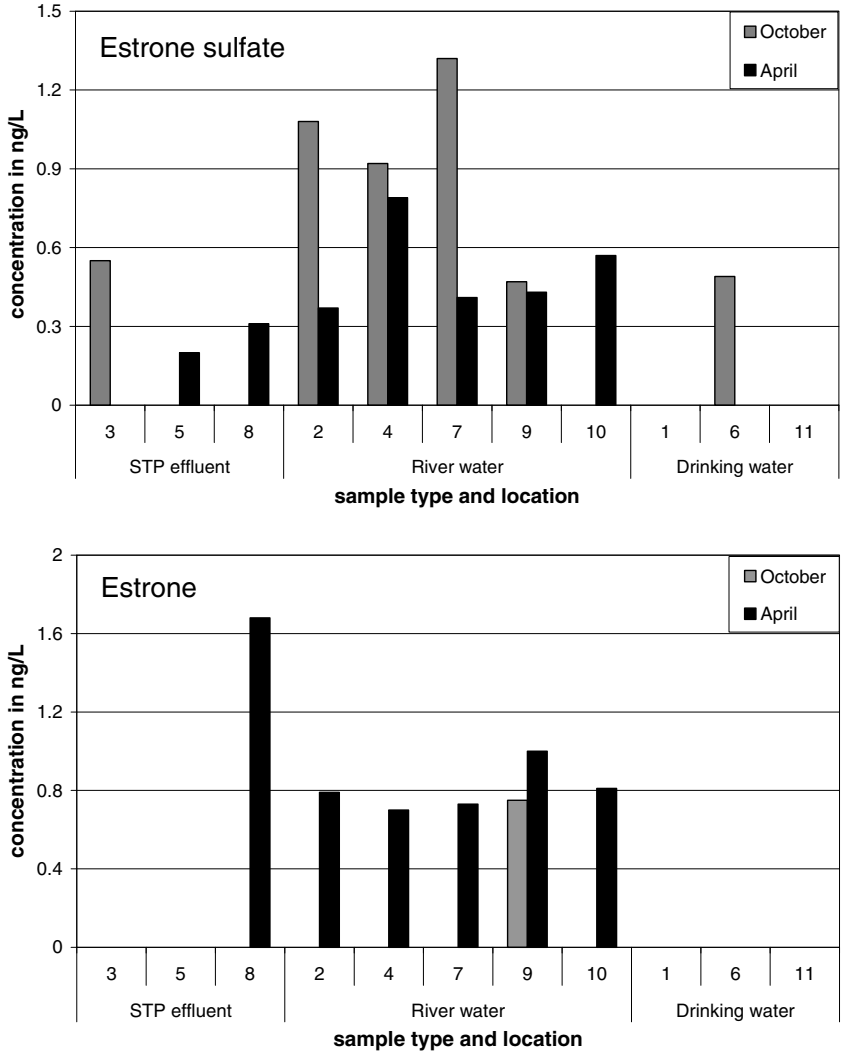

Figure 4 Estrone-3-sulfate and estrone concentrations detected in both sampling campaigns.

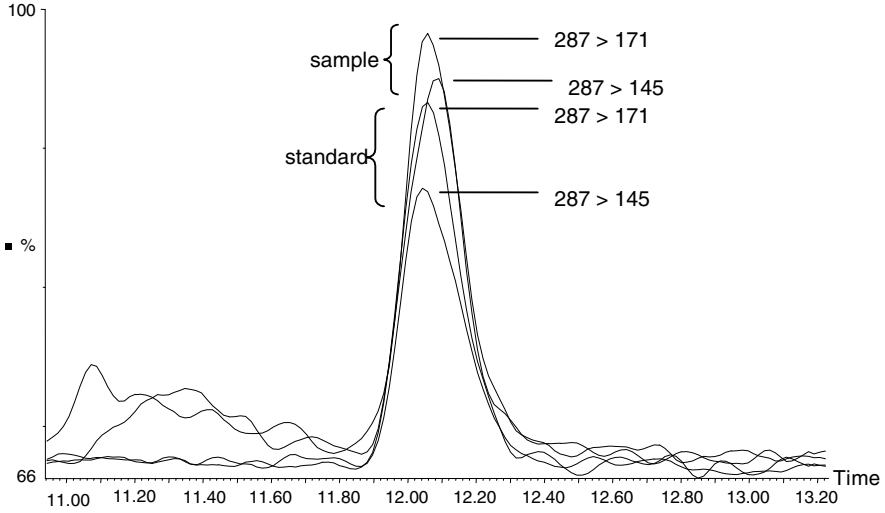

Figure 5 Analysis of estriol in the drinking water sample from *Abrera* (11.60 ng/L) and in a standard solution (10 ng/L) at the SRMs used for quantitation (287 → 171) and confirmation (287 → 145).

Levels of pesticides

Of the five pesticides analysed only bentazone was not detected. This pesticide is widely used in rice crops further south of the studied area, in the Delta of the Ebro river, where it has been detected at very high concentrations (up to 127 μg/L) (Kuster et al., 2008). Hence, based on the present results this pesticide seems to receive very little use in the lowest reach of the Llobregat river.

The cumulative levels of the detected pesticides are shown in Fig. 6. As can be seen in this figure and in Table 5, the most ubiquitous and abundant compounds were MCPA and 2,4-D, with more than 90% samples positive and concentrations up to 1286 and 109 ng/L, respectively.

In Europe, pesticide residue tolerances in drinking water are set by the European Union Commission to 100 ng/L for an individual compound, and 500 ng/L for the sum of pesticide residues (Council of the European Communities, 1998) whereas the maximum concentration for total pesticides in surface water intended for abstraction of drinking water varies between 1 and 5 μg/L depending on the method of water treatment applied (Directive 75/440/EEC). As shown in Table 5 and Fig. 6, these levels have not been surpassed in any of the samples investigated. The individual pesticides levels in the first sampling campaign (autumn) were always, regardless of the water matrix, below 100 ng/L. In the second sampling campaign this limit was surpassed in a few occasions (by 2,4-D in the Anoia river (108.98 ng/L) and by MCPA in the effluent of the STP of Abrera (1286 ng/L) and in the Llobregat river at two different sites (121.8—187.2 ng/L)) but not in drinking water samples.

The total pesticide concentration was in all but one sample below the limit set for drinking waters (500 ng/L). The exception corresponded to the effluent of the STP of Abrera (1310 ng/L).

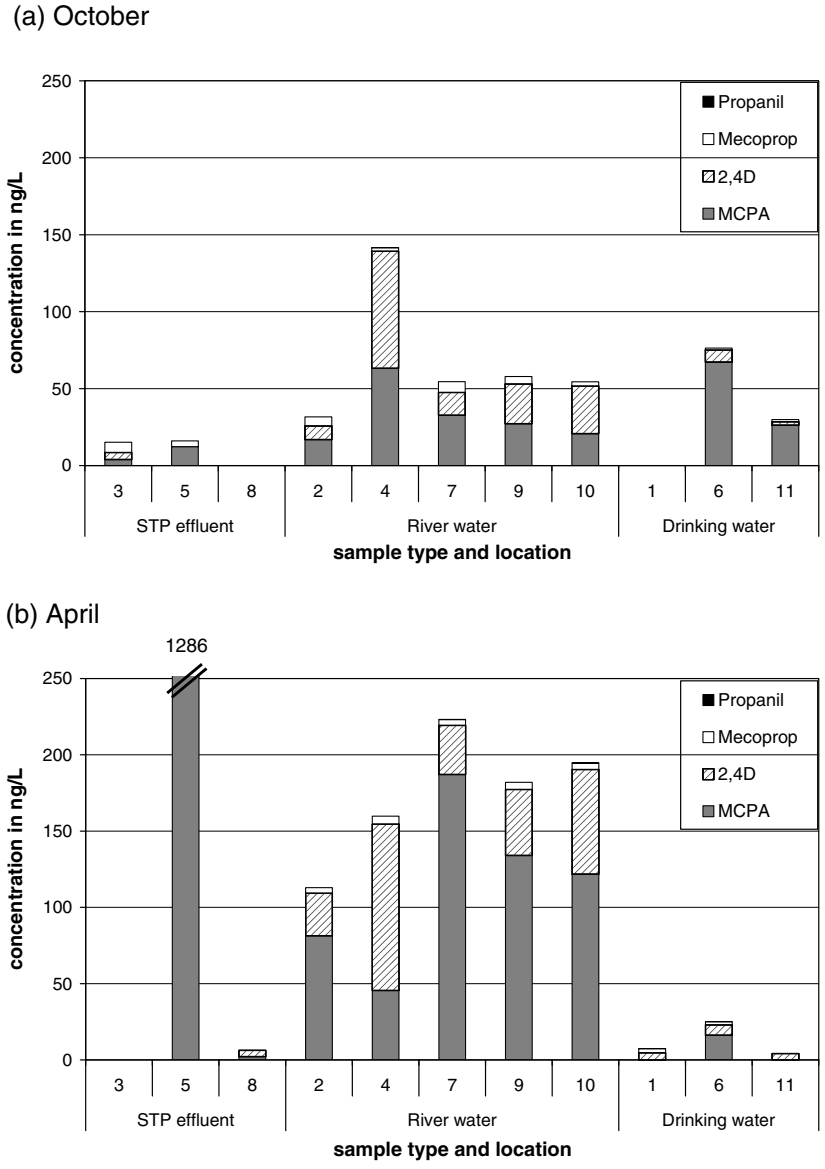

Figure 6 Cumulative levels of the pesticides found in autumn (a) and spring (b). Bentazone was not detected.

Conclusions

The Llobregat river is characterized by a typical Mediterranean regime and, as such, it suffers from extreme and frequent flow (10—300 m^3/s) fluctuations. In addition, in the lower reach there is an important concentration of urban and industrial activities with the corresponding pollution pressures. However, the results of this study indicate that the contamination load by estrogens, progestogens, PPCPs and the acidic pesticides investigated is not very important in the studied area, except in the case of a few occasionally high concentrations found for some species.

Both pharmaceuticals and pesticides were detected at levels below 100 ng/L in most instances. The compounds showing the highest concentrations within each class were the analgesic/anti-inflammatory diclofenac (1200 ng/L in a STP effluent) and the acidic pesticide MCPA (1286 ng/L in another STP effluent). Individual and total pesticides concentrations never surpassed the limits set by the EU. Estrogens and progestogens were found also at very low levels, in the pg/L or low ng/L range, except for estriol that was detected at a concentration of 11.60 ng/L in a drinking water.

Of all samples investigated the effluents of the STPs of Abrera and Rubí and the Anoia river water were the most contaminated.

Studies performed in 2000 (Farré, 2001) and 2002 (Rodriguez-Mozaz et al., 2004a) reported significantly higher concentrations that were of environmental concern. This reduction of contamination is most likely the result of the implementation in the last years of a considerable number of new STPs and the incorporation of tertiary treatments in already existing STPs along the river basin (López de Alda et al., 2003). To gain a more complete knowledge of the environmental status in this region more detailed investigations are currently being carried out.

In terms of toxicity, the levels found for estrogens, progestogens and pesticides are considered not to pose a risk. In the case of PPCPs the lack of environmental toxicity information does not allow to discard potential long term effects on the aquatic environment. In addition, synergistic effects of different compounds classes, especially at low levels, remain unknown and therefore adverse effects cannot be precluded.

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