Consell Superior d'Investigacions
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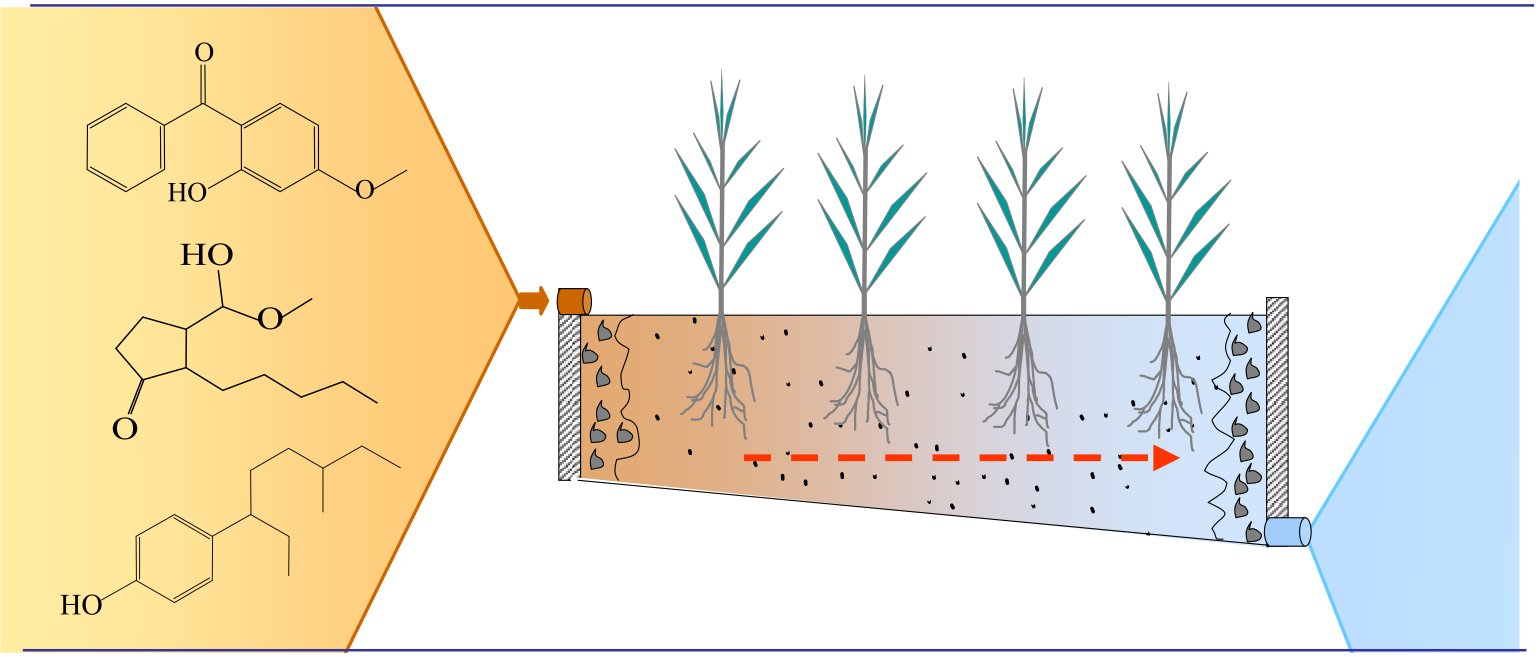
# COMPORTAMENT DE CONTAMINANTS ORGÀNICS EN AIGUAMOLLS CONSTRUÏTS
# I
# FORMACIÓ DE SUBPRODUCTES DE DESINFECCIÓ DURANT EL PROCÉS DE REGENERACIÓ D'AIGÜES RESIDUALS



Víctor Matamoros i Mercadal

*Octubre del 2007*

# **CAPÍTOL 2:** Sistemes Tous per al Tractament d'Aigües Residuals. El Cas dels Aiguamolls Construïts

……

*Jala bahule srustinasa, jaba bihurne srustinasa.*
Tant l'excés com la manca d'aigua destrueixen la creació.

Dita del poble Oriya

……

# 2.1. Tractament d'aigües residuals mitjançant sistemas tous

Els sistemes tous de tractament d'aigües residuals, principalment d'origen domèstic, fan referència a la creació d'uns equipaments de baix cost i impacte paisatgístic. La diferència principal envers les EDARs convencionals rau en que en els sistemes tous els requeriments energètics són baixos o nuls, mentre en les EDARs els fenòmens de tractament d'aigües residuals transcorren de forma seqüencial en tancs i reactors, i amb un cost energètic elevat. Tot i que dintre d'aquesta definició hi entrarien tant les foses sèptiques, els llacunatges, els biofiltres, els filtres verds i els aiguamolls construïts, en la present Tesi només s'han considerat els darrers sistemes. A més, encara que aquests sistemes són capaços de tractar aigües procedents d'escorrenties agrícoles, aigües residuals urbanes, aigües urbanes de pluja, llots d'EDARs, efluents industrials i agües procedents de sobreeixidors d'EDARs, la present Tesi només es centrarà en el tractament d'aigües residuals d'origen domèstic.

Així doncs, l'aplicació d'aiguamolls construïts per al tractament d'aigües residuals es basa en l'aprofitament de l'elevada productivitat dels aiguamolls naturals pel reciclatge de carboni, tot dissenyant-los per fer-los fins i tot més eficients que els sistemes naturals.

De entre totes les definicions que hom pot trobar a la literatura sobre els aiguamolls construïts, caldria esmentar la feta per Cole basant-se amb la seva funcionalitat: "*Els aiguamolls construïts són sistemes de tractament d'aigües residuals que consisteixen en llacunes poc profundes, llits o basses que contenen vegetació emergent, arrelada o flotant*" (Cole, 1998).

L'ús d'aiguamolls construïts per al tractament d'aigües residuals té els seus inicis a l'Institut Max Planck d'Alemanya prop de la dècada dels 60 amb els estudis pioners duts a terme per Seidel i Kickuth. A partir d'aquesta primera aproximació, John i Bob Kadlec van estendre la seva investigació durant la dècada dels 70' als Estats Units (Kadlec i Knight, 1996). A partir d'ençà, aquesta tecnologia s'ha anat estenent arreu del planeta. En l'actualitat existeixen de l'ordre de milers d'instal·lacions d'aquest tipus en funcionament en tot el món.

Pel que fa a la Unió Europea, els aiguamolls construïts són considerats com la millor tecnologia de cara al compliment de la Directiva Europea 91/271, la qual va establir que tots els estats membres haurien d'haver garantit la depuració de les aigües residuals urbanes de totes les poblacions de menys de 2000 habitants per abans del desembre del 2005. Així doncs, aquests sistemes han sorgit com una alternativa a les EDARs convencionals en poblacions petites.

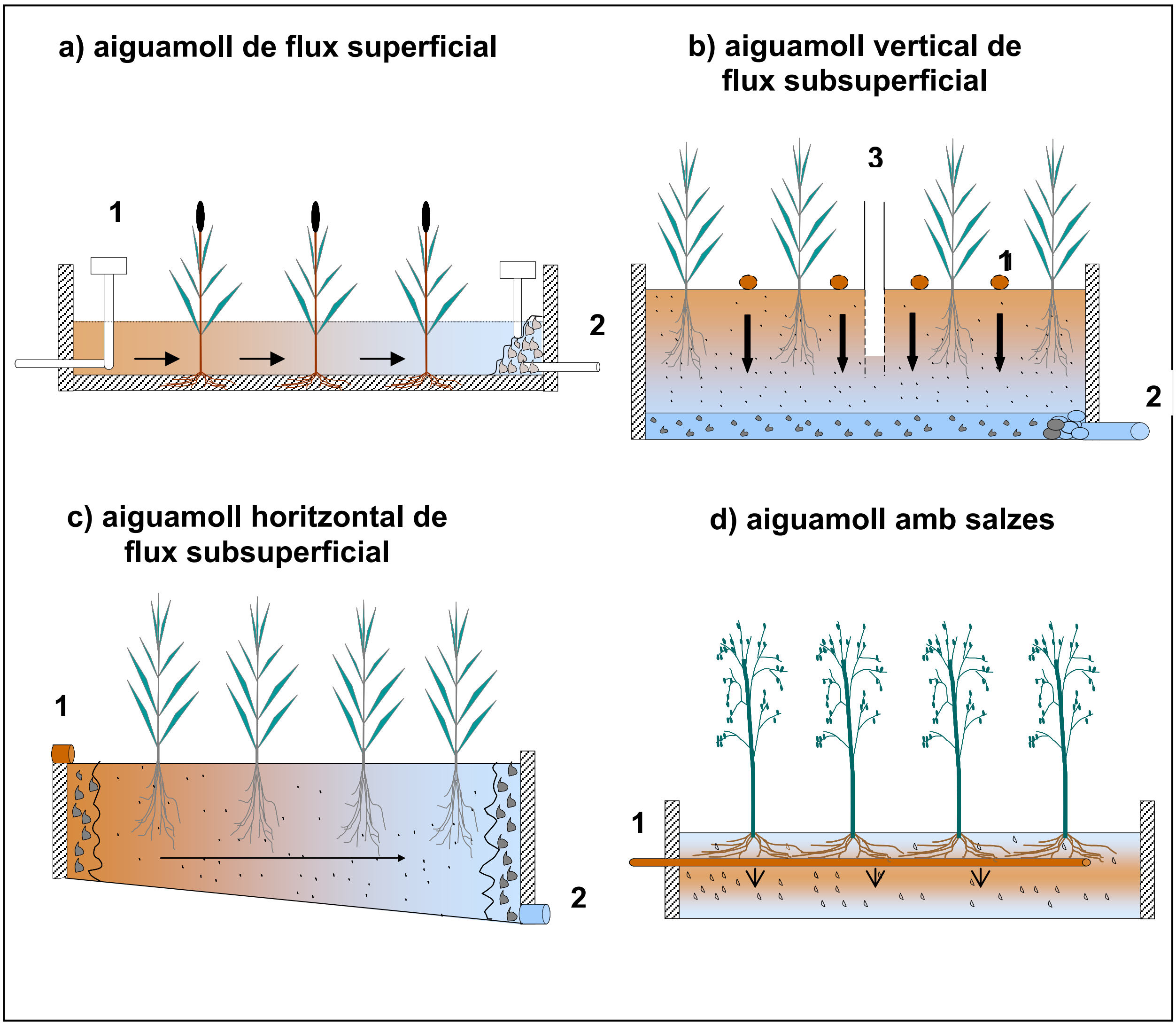
## 2.1.1. Tipus d'aiguamolls construïts

Els aiguamolls construïts es poden classificar en dos grans grups, aiguamolls construïts de flux subsuperficial (*subsurface flow constructed wetlands* SSFCWs) o aiguamolls construïts de flux superficial (*surface flow constructed wetlands* , SFCWs), atenent a si l'aigua residual flueix per la superfície del sistema o no (Kadlec i Knight, 1996; Wallace i Knight, 2006).

Els SFCWs (Fig 2.1a) consisteixen en canals o basses vegetades on l'aigua flueix per la superfície. En aquest sentit, es subdivideixen atenent a si la vegetació és emergent, flotant o submergida (Wallace i Knight, 2006). Aquests sistemes s'empren generalment per depurar efluents secundaris d'EDARs o escorrenties agrícoles (*agricultural runoff*). Es caracteritzen per requerir grans àrees superficials amb llargs temps de residència hidràulica (*hydraulic residence time*, HRT). Els SSFCWs en canvi, encara que també consisteixen en bases o canals amb vegetació, aquestes estan emplenades amb graves o arenes de diferent diàmetre (entre 0.2 i 0.6 mm). L'aigua residual és tractada quan percola a través de l'entramat de grava, rizomes i arrels del sistema. Se'ls divideix entre, aiguamolls construïts de flux vertical (*vertical flow constructed wetlands*, VFCW) (Fig. 2.1b) o aiguamolls construïts de flux horitzontal (*horitzontal flow constructed wetlands*, HFCWs) (Fig. 2.1c) atenent a la direcció en que flueix l'aigua residual. Aquests sistemes són emprats típicament per depurar aigües residuals procedents d'efluents primaris, amb un pas de sedimentació anterior, tot i que n'hi ha que funcionen directament tractant les aigües residuals crues.

La vegetació que hi predomina són *Phragmites australis*, *Scirpus sp.* o *Typha sp.*, tot i que en aiguamolls de flux superficial també s'hi pot plantar el papir (*Cyperus papyrus*) o les espigues d'aigua (*Pontederia sp.*), entre d'altres (Wallace i Knight, 2006).

Finalment, i com a un últim grup aparegut recentment, apareixen els sistemes plantats amb salzes (*willow systems*, WS) desenvolupats els últims anys al nord d'Europa (Arias i Brix, 2005). Cal destacar la manca d'efluent d'aquests últims sistemes (Fig. 2.1d). L'eliminació de l'aigua residual es du a terme mitjançant l'elevada evapotranspiració dels salzes, mentre l'eliminació de la matèria orgànica es realitza via degradació, incorporació per les plantes o bactèries i/o mineralització.



a) aiguamoll de flux superficial

b) aiguamoll vertical de flux subsuperficial

c) aiguamoll horitzontal de flux subsuperficial

d) aiguamoll amb salzes

**Figura 2.1.** Classificació típica dels aiguamolls construïts. Les fletxes indiquen la direcció del flux. 1. entrada, 2. sortida, 3. tub d'aeració.

L'eliminació de contaminants en aiguamolls construïts es pot dur a terme mitjançant diferents processos, tant físics, químics com biològics, atenent al tipus d'aiguamoll que es tracti en cada cas. Les principals vies d'eliminació de contaminants orgànics són les interaccions fisicoquímiques amb la matèria orgànica, mineralització, biodegradació, incorporació via plantes i/o bactèries, volatilització i fotodegradació. Tot i que aquest últim procés només té lloc en els SFCWs i que les rutes metabòliques aeròbiques són les predominants en els VFCWs i SFCWs, mentre les anaeròbiques ho són en els HFCWs i WS, la major part de processos poden coexistir en un mateix aiguamoll (Cooper et al., 1996).

## 2.1.2. Limitacions i avantatges dels aiguamolls construïts

Tal i com queda palès a la Taula 2.1, hi ha una sèrie d'avantatges i inconvenients que sorgeixen alhora de comparar aquests sistemes amb les EDARs convencionals.

**Taula 2.1.** Principals inconvenients i avantatges dels aiguamolls construïts en front les EDARs convencionals.

| | **Aiguamolls construïts** | **EDARs** |
|---|---|---|
| Cost d'explotació i consum energètic | Baix | Elevat |
| Producció de fangs | Baixa | Elevada |
| Impacte ambiental | Baix, integrat en el medi | Elevat |
| Disseny | difícil[a] | Estandarditzat |
| Vida útil | 10-20 anys[b] | depèn del equipament |
| Adaptació a la variabilitat de càrrega estacional | Elevada | Baixa |
| Males olors | Baixes | Elevades |
| Superfície requerida | Elevada[c] | Baixa |

[a] Tot i que hi ha força guies de construcció a l'abast, el disseny és funció del tipus d'aigua residual i la climatologia de la zona.
[b] hi ha l'opció de fer buidat de fangs en SFCWs i neteja de graves en SSFCWs.
[c] 3-5 $m^2$ per habitant equivalent (Brix i Arias, 2005; Vymazal, 2005)

A més, cal tenir en compte el possible ús de la biomassa com a font de combustible, així com la potenciació de la biodiversitat, l'educació ambiental i les zones d'esbarjo. Així doncs, donada l'elevada superfície requerida per habitant equivalent (*population equivalent*, PE), els aiguamolls construïts s'empren generalment per poblacions inferiors a 2000 habitants, principalment nuclis aïllats de difícil accés a la xarxa general de clavegueram.

## 2.1.2. El cas de Catalunya.

Pel que fa a l'ús d'aquests sistemes a les nostres contrades, actualment hi ha 13 aiguamolls horitzontals de flux subsuperficial (Velayos et al., 2006) emprats per al tractament d'aigües d'origen domèstic i 60 més que tracten aigües procedents del riu Besòs (Huertas et al., 2006). Per altra banda, els aiguamolls de flux superficial, tot i que poden ser emprats per tal d'atenuar l'impacte dut a terme per les EDARs, actualment només existeixen tres plantes en ús a Catalunya (exemple: estany Europa als aiguamolls de l'Empordà). Així doncs, la manca d'altres tipus d'aiguamolls, com ara els sistemes amb salzes o verticals de flux subsuperficial, fa que al nostre país resti encara molt camí per recórrer en aquest àmbit.

## 2.2. Estructura i contingut general del capítol

L'objectiu fonamental d'aquest capítol és el de conèixer el comportament dels contaminants orgànics en aiguamolls construïts tant en HFCWs (*secció 2.3*) com en VFCWs (*secció 2.4*) i en SFCWs (*secció 2.5*). A més, en el cas del HFCW, s'estableixen dos subapartartats més, en relació als compostos d'estudi en cada cas, PPCPs (*secció 2.3.1*) o contaminats de la Directiva Marc de l'Aigua (*secció 2.3.2*).

Hi ha dues vies plausibles a l'hora de modelar el comportament d'aquests compostos en un aiguamoll construït. Aquestes són, l'estudi de l'eliminació dels compostos d'interès mitjançant l'increment del cabal d'aigua residual a tractar per unitat de superfície o la injecció directa dels anàlits a estudiar simultàniament amb un traçador. Tot i que aquesta segona opció és la més emprada en sistemes de microcosmos, en aquesta Tesi es va emprar tant a nivell de microcosmos (*article 2*) com de planta a escala pilot (*articles 1 i 4*). Per altra banda, l'estudi de les concentracions de contaminants preexistents en les pròpies aigües residuals, s'empra normalment en aiguamolls a escala real. En aquest sentit, en els HFCW i SFCW es va estudiar l'eliminació d'aquests compostos en relació a l'àrea superficial del sistema (afluent, punts intermedis i efluent) (*articles 3 i 6*) , mentre en els VFCWs aquest estudi es va fer a través de l'increment de la càrrega hidràulica del sistema (*article 5).* A partir d'aquests treballs, es poden calcular les cinètiques d'eliminació per a cada grup determinat de compostos, permetent conèixer les necessitats de disseny d'un sistema per tal d'eliminar-los correctament. Aquest desenvolupament s'ha dut a terme per a un ampli ventall de PPCPs, tant en VFCWs com en HFCWs.

Per tal d'analitzar aquests compostos en matrius ambientals en el marc de la present Tesi es van desenvolupar mètodes analítics i se'n van adaptar d'altres ja existents. Primerament, es van tractar mètodes que feien referència a l'estudi d'aquests anàlits tant en la fase aquosa dissolta com en els sòlids suspesos, i seguidament mètodes d'aplicació a mostres de graves i sediments.

Els compostos analitzats en aquesta Tesi són el resultat d'una criba prèvia de les entrades de cada sistema de forma individual mitjançant la identificació de compostos específics amb ajuda de la llibreria NIST 98.

## 2.3. Aiguamolls construïts de flux subsuperficial horitzontal

Aquests sistemes es caracteritzen per tenir condicions anaeròbies molt marcades, amb concentracions d'oxigen normalment inferiors a 1 mg/L i potencials rèdox fortament reductors (entre -250 mV i -400 mV). Tot i que les rutes metabòliques que hi predominen són la sulfatoreducció, la desnitrificació i la metanogènesi, hi poden coexistir microambients on hi hagi oxigen i per tant es doni lloc alguna de les rutes metabòliques aeròbies, com per exemple la nitrificació.

Per tal de conèixer el funcionament d'un aiguamoll construït s'estudien els seus paràmetres hidràulics, normalment a través de compostos inerts anomenats traçadors. A continuació es fa una breu definició dels paràmetres hidràulics emprats en aquesta Tesi (Levenspiel, 1999).

**Temps de residència mig ($\tau$)**, és el temps mig que triguen les molècules d'un traçador en travessar l'aiguamoll. En aquest cas dóna idea del temps mig que roman un contaminant en l'aiguamoll.

**Variança normalitzada ($\sigma^2/\tau^2$),** s'utilitza per a mesurar el procés de dispersió que els contaminants tenen en el si dels aiguamolls. Es calcula dividint la variança de la funció de distribució del temps de residència al quadrat ($\sigma^2$) pel temps mig de residència al quadrat ($\tau^2$). Serveix per veure l'existència de camins prioritaris i zones mortes en la zona de flux d'aigua. Dóna idea de les interaccions dels contaminants en l'aiguamoll.

**Temps de retard normalitzat ($\Phi^0$),** permet comparar el temps de retard d'aiguamolls amb diferent temps mig de residència. El temps de retard té unitats de temps i fa referència al moment en que surt la primera molècula de traçador, per tal de normalitzar-lo aquest es divideix pel temps de residència mig.

Hi ha dues aproximacions a l'hora d'estudiar el comportament dels contaminants mitjançant traçadors, la injecció d'aquests de forma continuada o amb una sola dosificació puntual (Figura 2.2). Sigui de la manera que sigui, les interpretacions hidràuliques a les que es pot arribar són semblants. En aquest sentit, la injecció de fàrmacs es va realitzar de forma continuada, mentre la injecció de substàncies prioritàries de la Directiva Marc de l'Aigua va ser per injecció puntual. Aquesta diferència rau en el diferents tipus d'abocaments que es donen en el medi aquàtic per a aquest compostos, crònic o puntual, respectivament per fàrmacs i gran part de les substàncies prioritàries estudiades (plaguicides).

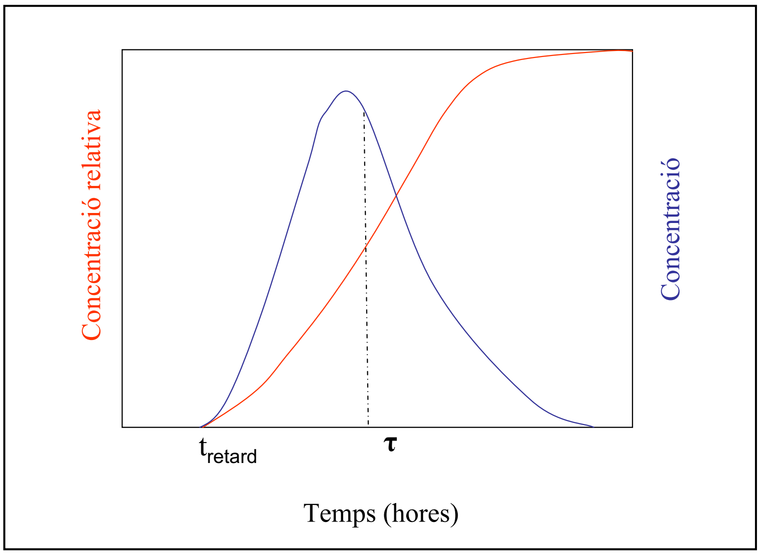


**Figura 2.2.** Representació gràfica de l'injecció continuada (en vermell) o puntual (en blau) d'un traçador en un aiguamoll, tot assenyalant els diferents paràmetres hidràulics.

## 2.3.1. Eliminació de fàrmacs i productes d'higiene personal

### 2.3.1.1. Introducció

Tot i que l'estudi del comportament de PPCPs en EDARs de fangs activats s'ha documentat extensament en els últims anys, aquest és el primer cop que s'estudia el seu comportament en HFCWs. Així doncs, els articles que es presenten a continuació, pretenen donar a conèixer aquest comportament, tant des del punt de vista dels processos d'interacció com d'eliminació dels PPCPs en el sí d'aquests tipus de sistemes de tractament d'aigües residuals.

En el primer article, "*Behavior of Selected Pharmaceuticals in Subsurface Flow Constructed Wetlands: A Pilot-Scale Study* ", es realitza la injecció de tres fàrmacs, ibuprofèn, àcid clofíbric i carbamazepina, conjuntament amb el bromur com a traçador, en un aiguamoll a escala real. En el segon article, "*Pharmaceutical products behaviour in horizontal subsurface flow constructed wetland. A microcosms experiment*" s'empren les propietats de l'àcid clofibric com a traçador, conclusió de l'article anterior, per tal d'aprofundir en el comportament de la carbamazepina, l'ibuprofen així com dels seus productes de biodegradació a escala microcosmos. Finalment en un tercer article, "*Elimination of Pharmaceuticals and Personal Care Products in Subsurface Flow Constructed Wetlands* ", s'estudia el comportament de 13 PPCPs presents en condicions normals d'explotació en l'aiguamoll a escala real descrit en el primer article.

### 2.3.1.2. Article 1: Behavior of Selected Pharmaceuticals in Subsurface Flow Constructed Wetlands: A Pilot-Scale Study

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*Environ. Sci. Technol.* **2005**, *39,* 5449–5454

# Behavior of Selected Pharmaceuticals in Subsurface Flow Constructed Wetlands: A Pilot-Scale Study

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Subsurface flow constructed wetlands (SSFs) constitute a wastewater treatment alternative to small communities due to the low operational cost, reduced energy consumption, and no sewage sludge production. Although much information is available about conventional water quality parameters in SSF constructed wetlands, few data are available regarding specific contaminants. In this paper, we focus on the behavior of three widely used pharmaceuticals (clofibric acid, ibuprofen, and carbamazepine) in two pilot SSF constructed wetlands planted with *Phragmites australis* and characterized by different water depths (i.e., 0.3 and 0.5 m). These SSFs partially treat the urban wastewater from a housing development (ca. 200 inhabitants). The three pharmaceuticals and bromide were continuously injected into the two SSFs during a period of 150–200 h, and the effluent concentration was simultaneously measured as 6 h composite samples. Their removal efficiency was calculated from the injected concentration, and the hydraulic parameters were evaluated and compared to bromide as tracer. In this regard, the behavior of clofibric acid was similar to that of bromide, and no sorption into the gravel bed occurred. On the other hand, carbamazepine showed a higher sorption than bromide and clofibric acid, which is attributable to its interaction on the gravel bed. Accordingly, the use of clofibric acid as a hydraulic tracer is proposed, taking into account its low residence time. Ibuprofen removal was 81% in the shallow SSF and 48% in the deep one. Differences in removal efficiency could be explained by the less anaerobic environment of the shallow wetland.

## Introduction

Constructed wetlands are land-based wastewater treatment systems that consist of shallow ponds, beds, or trenches that contain floating or emergent-rooted wetland vegetation (*1*). In subsurface flow constructed wetlands (SSFs), wastewater is infiltrated through vegetated gravel beds confined by a liner. The wastewater treatment relies on biological, chemical, and physical processes in a natural environment. The potential of SSFs for the removal of contaminants occurring in urban wastewater has attracted increasing interest over the past decade with a view of treating wastewaters from small populations to comply with environmental regulations such as the European Union Directive 91/271 and the U.S. EPA Clean Water Act and to attenuate diffuse agricultural contamination runoff to surface waters (*2*, *3*). Nevertheless, the available information on the performance of these systems is limited to common contamination parameters, such as suspended solids, COD, $BOD_5$, nutrients, and bacteria (*4*). At present, very little information is available on specific organic contaminants, and it mainly deals with herbicides, pesticides (*5*–*7*), and nonionic (*8*) and anionic surfactants (*9*).

Pharmaceutical products are becoming a new environmental problem due to the widespread use of antiinflammatory, lipid regulator, and analgesic drugs. In recent years, several authors have reported the occurrence of pharmaceuticals in the aquatic environment (*10*). Pharmaceutical substances have been detected in wastewater treatment plant (WWTP) effluents, even at low parts-per-billion concentrations (*11*–*19*). Moreover, it is known that if pharmaceutical compounds are not totally removed, then variable concentrations of pharmaceutical drugs can reach surface, ground, and coastal waters (*20*–*22*).

In this paper, we focus on the fate of clofibric acid (the active metabolite of the blood lipid regulators clofibrate, ethofyllin clofibrate, and ethofibrate), ibuprofen (an analgesic), and carbamazepine (an antiepileptic, antineuralgic, and antipsychotic drug) in SSFs. These drugs were chosen according to their high production volume and widespread use (ca. 100 t $year^{-1}$ for ibuprofen and carbamazepine and 16 t $year^{-1}$ for clofibric acid in Germany (*23*)) and their widespread occurrence in WWTP effluents.

Furthermore, their different physicochemical structure and characteristics shown in Table 1 can presumably lead to different behavior in SSFs. In fact, whereas clofibric acid and carbamazepine show a remarkably high persistence in the aquatic environment, ibuprofen is readily removed in conventional WWTPs (*20*, *24*).

The objectives of this work were to evaluate the response curves and removal behavior of clofibric acid, ibuprofen, and carbamazepine in two pilot SSFs with different water depths serving a housing development. In a former paper, we showed that water depth is a key design parameter affecting the redox status of the SSFs and therefore the relative importance of the biochemical reactions responsible for the degradation of organic matter (*25*). Moreover, because of the sorption of contaminants in the organic matter retained in the gravel bed and in the biofilm, a continuous injection experiment of the pharmaceuticals was designed to equilibrate the sorption sites of the bed and thus obtain a more reliable estimate of their biodegradation potential. Furthermore, the use of some of these pharmaceuticals as a hydraulic tracer is evaluated and compared to widely used bromide.

## Experimental Procedures

**Chemicals.** HPLC grade methanol, acetonitrile, and water were obtained from Merck (Darmstadt, Germany). Analytical grade formic acid and trifluoracetic acid were obtained from Panreac (Barcelona, Spain). Suprasolv grade isooctane, hexane, and ethyl acetate were obtained from Merck. Pyromellitic acid, triethanolamine, hexamethonium hydroxide, clofibric acid, ibuprofen, carbamazepine, and dihydrocarbamazepine were purchased from Sigma-Aldrich (Steinheim, Germany). Mecoprop and 2,4,5-trichlorophenoxy-

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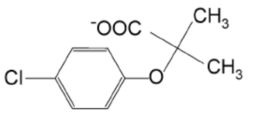
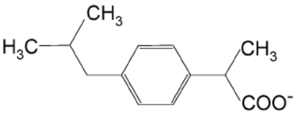
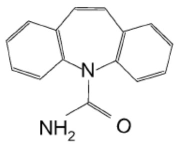
**TABLE 1. Chemical Structures and Physicochemical Properties of the Pharmaceutical Products**

| Compound | Clofibric acid | Ibuprofen | Carbamazepine |
|---|---|---|---|
| function | lipic regulator | anti-inflammatory | antiepileptic |
| pka | 2.84 | 4.31 | 7.00 |
| log Kow[a] | 2.84 | 3.97 | 2.45 |
| log Kow (pH 8)[b] | -0.97 | -0.11 | 2.25 |
| Structure[c] |  |  |  |
| CAS RN. | 882-09-7 | 15687-27-1 | 298-46-4 |

[a] Experimental values of neutral compounds (*40*). [b] Estimated value calculated for the compounds in the injection experiment conditions (*40*). [c] Obtained from ChemSketch 5.0.

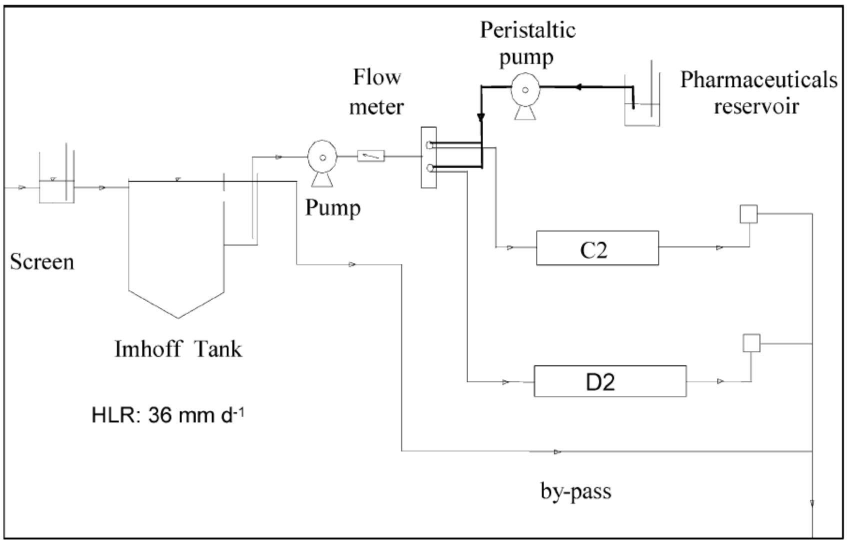


**FIGURE 1. Schematic diagram of the pharmaceutical injection experiment in two wetlands of the SFCW pilot plant at Les Franqueses del Vallès, Barcelona. The hydraulic loading rate was 36 mm day$^{-1}$.**

propionic acid (2,4,5-TPA) were obtained from Reidel-de-Haen (Seelze, Germany) and sodium bromide from Fluka (Buchs, Switzerland). Bis(trimethylsilyl)-trifluoro-acetamide (BSTFA) was purchased from Merck.

**SSF Pilot Plant.** The pilot plant consists of eight parallel horizontal SSFs and is located in the municipality of Les Franqueses del Vallès (Barcelona, Spain). The plant partially treats the urban wastewater generated by a housing development (ca. 200 inhabitants). The water is screened and then flows to an Imhoff tank (Figure 1). This tank is connected to another one from which the water is pumped to a distribution chamber where the flow is split by means of a weir into eight lines flowing to each wetland. The flow rate was adjusted through the operation time of the pump and a valve that allowed the flow rate of the influent to be controlled. An electromagnetic flow meter was installed to measure the instantaneous flow rate. These mechanisms allow the flow rate in the SSF plant to be controlled. Each bed has a surface area of 54−56 m$^2$ and was planted with *Phragmites australis*. The plant began operation in March 2001. In this study, only two SSFs were used, one with an aspect ratio (length × width) of 2:1 (named C2) and one with an aspect ration of 2.5:1 (named D2). The size of the granular granitic medium measured as $D_{60}$ ($D_x$ is the size of the material that corresponds to %$x$ of the total material weight) was 3.5 mm, and the uniformity coefficient was 1.7. The average water depth is approximately 0.3 m in bed D2 and 0.5 m in bed C2. The plant was operated at a hydraulic loading rate of 36 mm day$^{-1}$ during the injection experiment.

**Experimental Design.** About 20 L of distilled water contained in a glass bottle was spiked with 250 mg of each pharmaceutical compound and 200 g of sodium bromide. This mixture was homogenized and injected into the weir lines corresponding to both SSFs using a Minipuls 2 peristaltic pump (Gilson, Villiers le Bel, France) synchronized to the wastewater pumping system. A previous conditioning of the silicon tubing and connectors for 72 h with the experimental pharmaceutical concentration was carried out to minimize the analyte sorption losses during the experiment. Flow rates were adjusted to obtain a final influent concentration of 25 $\mu$g L$^{-1}$ for each pharmaceutical and 15 mg L$^{-1}$ for bromide. The injection experiment was run for 9 days in the D2 wetland and 7 days in the C2 wetland from April to May 2004.

**Sampling.** Effluent composite samples were collected every 6 h with an American Sigma 900 autosampler (Sigma, Loveland, CO) for a time period of 21 days. To ensure the absence of the injected pharmaceuticals in the influent, samples of the inflow were also collected and analyzed. All the samples were collected in 500 mL amber glass bottles and kept refrigerated during transportation to the laboratory, where they remained refrigerated at 4 °C until analysis. The sample holding time was less than 2 days. Furthermore, a gravel sample with biofilm was sampled at 2 m from each wetland inlet and kept under −20 °C prior to analysis.

**Wastewater Analysis.** All sewage samples, influents, and effluents were filtered through 0.45 $\mu$m glass filter of 47 mm from Millipore (Bedford, MA) and then acidified to pH 2 with concentrated hydrochloric acid. A sample volume of 200 mL was mixed with a solution of surrogate standards, 2,4,5-TPA (25 mg L$^{-1}$) and dihydrocarbamazepine (25 mg L$^{-1}$), giving a final concentration of 25 $\mu$g L$^{-1}$. The spiked sample was percolated through a polymeric SPE cartridge packed with 100 mg of Strata X from Phenomenex (Torrance, CA) conditioned previously with 5 mL of *n*-hexane, 5 mL of ethyl acetate, 10 mL of MeOH, and 10 mL of MilliQ water (pH = 2). The flow rate was approximately adjusted to 10 mL min$^{-1}$. Thereafter, the cartridges were allowed to dry for 30 min; finally, the analytes were eluted with 5 mL of ethyl acetate. The extract was evaporated to dryness under a gentle nitrogen stream, reconstituted in 175 $\mu$L of methanol, and mixed with 5 $\mu$g of mecoprop as an internal standard since it was not detected in the influent wastewater.

The resulting extracts were analyzed using a dual pump HPLC equipped with a UV diode array detector from Shimadzu (Kyoto, Japan) at 230 nm. Injection was carried out using a Rheodyne valve (Rohnert Park, CA) with a sample loop volume of 20 $\mu$L fitted to a Shimadzu autosampler. Chromatographic separation was performed on a LichroCART column 125 × 4 mm packed with Lichrospher 100 RP-18 (5 $\mu$m) from Merck. The mobile phase used in the chromatographic separation consisted of a binary mixture of solvents A (acetonitrile) and B (water adjusted to pH 3 with formic acid) at a flow rate of 0.8 mL min$^{-1}$. Initial conditions were 40% A for 20 min, then linearly programmed up to 80% A for 10 min followed by a linear program to 100% A for 5 min, and then held for 5 min. The LODs, LOQs ($\mu$g L$^{-1}$), and RSDs ($n = 3$) were analyte-dependent, ranging from 0.15 to 0.24, 0.48 to 1.05, and 1.4 to 4.2%, respectively, and recoveries were from 80 to 98%. LOD and LOQ of the analytical procedure were carried out by using a water effluent from a microcosm pilot plant effluent without pharmaceuticals. LOD was calculated 3 times for the area of the procedural blank and 10 times for the LOQ. Linear regression coefficients from a 10-point linear calibration curve (concentration rage 0.6−50 $\mu$g mL$^{-1}$) were always higher than 0.998 for all compounds.

The bromide determination was carried out with a Hewlett-Packard (Palo Alto, CA) 3DCE capillary electrophoresis (CE) system equipped with a UV diode array detector. A 60 cm capillary length of 50 $\mu$m i.d. from Agilent Technologies was used. The electrolyte solution was pyromellitic acid (2.25 mM), triethanolamine (1.6 mM), and hexamethonium hydroxide (0.75 mM) adjusted to pH 8.5. The injection was performed at 50 mbar for 4 s, at a constant voltage of −30 kV, and a column temperature of 30 °C was used with a detection signal of 350 nm and a reference of 245 nm. Quantitation was performed as described elsewhere (*26*).

**Gravel Analysis.** Following a freeze-drying step for 24 h, 40 g of dry wt gravel samples were extracted by a pressurized solvent extraction apparatus (PSE, Applied Separations, Allentown, PA) using a 33 mL extraction cell. The extraction conditions were 80 °C, 100 bar, and 3 cycles of 5 min with ethyl acetate containing 0.2% TFA acid (v/v) added into the extraction cell. The obtained extract was concentrated to 0.2 mL with a rotary evaporator (Heidolph Instruments, Schwabach, Germany) and reconstituted with 200 mL of distilled water (*27*), adjusted to pH 2 with concentrated hydrochloric acid, and cleaned up by percolation through a 100 mg Strata X cartridge (Phenomenex, Torrance, CA) eluting with 5 mL of ethyl acetate.

The cleaned extracts were evaporated to dryness and derivatized with 200 $\mu$L of BSTFA for 1 h at 70 °C. Then, the BSTFA excess was evaporated to dryness and reconstituted with isooctane. The sample was injected onto a TRACE-GC-MS (Thermo-Finnigan, Dreieich, Germany) fitted with a 30 m × 0.25 mm i.d. × 0.25 $\mu$m DB-5 (J&W Scientific, Folsom, CA). The carrier gas was helium (purity: 99.9995%) at a constant linear average velocity of 40 cm s$^{-1}$. The oven temperature was held at 90 °C for 1 min, then temperature programmed at 20 °C min$^{-1}$ to 120 °C, then to 3 °C min$^{-1}$ to 160 °C followed by 12 °C min$^{-1}$ to 260 °C, and finally held for 12 min. A volume of 2 $\mu$L of sample was injected in the splitless mode at an injector temperature of 260 °C with an injector purge activation time of 1 min. The transfer line was set at 280 °C and the ion source at 200 °C. The electron impact energy was 70 eV. Acquisition was performed in the scan mode ranging from $m/z$ 50 to 350 at two scans s$^{-1}$. The following diagnostic ions were monitored for target analyte quantification (in bold) and identification: ibuprofen **161**/2347263; clofibric acid, **128**/169/143; carbamazepine, **193**/236/192; mecoprop, **169**/286/142; 2,4,5-TPA, **196**/282/198; and dihydrocarbamazepine,**195**/238/160. The LODs and LOQs (ng g$^{-1}$) were compound-dependent, ranging from 1.1 to 1.3 and 4.8 to 8.4, respectively. The recoveries for the acid pharmaceuticals were ca. 75%, and the correlation coefficients ($r^2$) of the calibration curve were always higher than 0.998.

**TABLE 2. Average Concentration and SD of Several Contaminants in Influent and Effluents ± SD of Pilot SSFs**

| parameter | *N* | influent | effluent C2 | effluent D2 |
|---|---|---|---|---|
| TOC, mg/L[a] | 20 | 50 ± 20 | 21 ± 7.1 | 17 ± 7.4 |
| BOD$_5$, mg/L[a] | 103 | 136 ± 54 | 55 ± 28 | 20 ± 19 |
| NH$_4^+$, mg N/L[a] | 103 | 45 ± 16 | 31 ± 12 | 18 ± 12 |
| LAS, mg/L[b] | 20 | 3.65 ± 0.97 | 2.6 ± 0.89 | 1.07 ± 0.84 |

[a] Garcia et al. 2005. [b] Huang et al. 2004.

## Results

**Pharmaceutical Behavior in Injection Experiment.** The injection experiment was carried out in shallow SSF (bed D2) because after 3 years of operation, it was found to have a better performance in terms of the removal efficiency of a broad spectrum of contaminants (i.e., BOD$_5$, TOC, NH$_4^+$, and linear alkylbenzenes sulfonates) (Table 2). A deeper SSF (bed C2) was also considered in the injection experiment to compare its performance with bed D2. The aspect ratio of the two wetlands is also different, but it has been demonstrated that in the range used in the present study, it is not a relevant design parameter in terms of contaminant removal efficiency (*9*, *25*).

Effluent concentrations were corrected by the actual influent flow and the background target pharmaceutical concentration, ibuprofen being the only target pharmaceutical that occurred in the SSF influent at low concentrations (i.e., 4.7−5.8 $\mu$g L$^{-1}$). Effluent concentrations of target compounds were normalized by their influent concentration, and the results are shown in Figure 2. There is a different behavior of the pharmaceutical compounds response curves, which is attributable to their different physicochemical characteristics (Table 1). The anionic form of clofibric acid showed a similar curve to bromide because it has the lowest log $K_{\mathrm{ow}}$ (−0.97), and no degradation was observed in two wetlands. On the other hand, the behavior of carbamazepine is different to that of bromide and attributable to its sorption behavior, which is higher than that of clofibric acid with a log $K_{\mathrm{ow}}$ of 2.25. The behavior of ibuprofen was affected by its degradation, and its trend is not similar to that of bromide.

**Hydraulic Behavior.** The effluent concentrations were used to obtain the hydraulic parameters, namely, the mean hydraulic retention time ($\tau$), the normalized variance ($\sigma^2/\tau^2$), and the normalized delay time ($\phi_\theta$) (*28*). Moreover, these parameters were correlated with the different behavior of

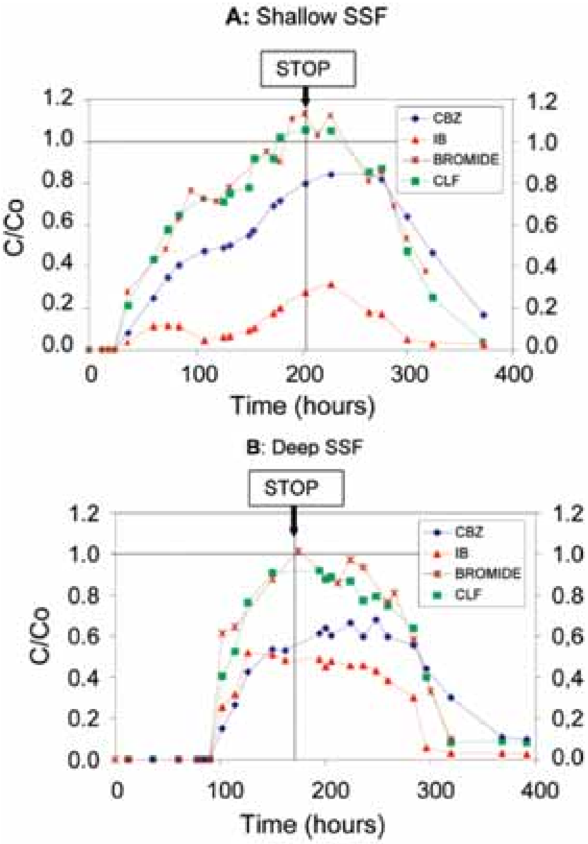


**FIGURE 2. Behavior of the pharmaceuticals (carbamazepine: CBZ; ibuprofen: IB; clofibric acid: CLF) and bromide in wetlands type D2 and C2 (STOP: end of pharmaceutical injection). Horizontal line at $C/C_o = 1$ denotes the equilibration for conservative tracers.**

**TABLE 3. Values of the Hydraulic Parameters Estimated from Different Substances Used as Tracers**

| | deep bed (C2) | | | shallow bed (D2) | | |
|---|---|---|---|---|---|---|
| | $\tau$ (h) | $\sigma^2/\tau^2$ | $\phi_\theta$ | $\tau$ (h) | $\sigma^2/\tau^2$ | $\phi_\theta$ |
| bromide | 154.9 | 0.80 | 0.58 | 114.4 | 1.10 | 0.21 |
| clofibric acid | 151.8 | 0.80 | 0.59 | 111.5 | 1.16 | 0.22 |
| carbamazepine[a] | 226.7 | 0.92 | 0.40 | 167.7 | 1.10 | 0.14 |

[a] Sorption observed.

these pharmaceuticals as compared to bromide. The first step was to transform the response curves to residence time distribution curves (RTD or E curves), from which the hydraulic parameters were obtained (Table 3). The similarity in the shape of the response curve, $\tau$, and $\sigma^2/\tau^2$ for both clofibric acid and bromide in the two SSFs indicates that clofibric acid can be used as a nonreactive tracer. The normalized variance can be used to measure the internal dispersive process in SSFs (*29*). In SSFs with a similar depth, the normalized variance will decrease with the increase in aspect ratio, while the normalized delay time will increase (*28*). In the present experiment, the opposite trend was observed because the shallow SSF had a higher aspect ratio than the deep SSF, and the shallow one had a higher normalized variance and a lower normalized delay time. Moreover, carbamazepine and ibuprofen acid will be described in this work as reactive compounds by sorption and degradation, respectively, and cannot be used as conservative tracers.

**Gravel Bed Accumulation.** Since suspended particles from the wastewater are retained in a wetland bed (*30*), sorption of dissolved organic contaminants in that organic matter and on the biofilm coating the gravel bed can be a significant mechanism for their removal (*4*). Therefore, the concentration of pharmaceutical products was measured near the inlet because, in accordance with previous results, this is the place where most of the organic matter is retained (*32*). The highest retention in the gravel bed was obtained for carbamazepine (97 ng $g^{-1}$; 77%) in relation to clofibric acid (14 ng $g^{-1}$; 11%) and ibuprofen (15 ng $g^{-1}$; 12%). These results are consistent with its response curves and could be explained by its higher hydrophobicity (Table 1) and the electrostatic interactions between the acidic compounds charged negatively and the negative charged biofilm covering the gravel bed (*31*).

**Degradation.** The removal rate (Table 4) was evaluated using the cumulative mass recovery curves in the effluents (Figure 3). Recoveries of clofibric acid and bromide in the deep bed were nearly 100%, but in the shallow bed it was 108%. The approximately 8% in excess may be attributable to evapotranspiration in this bed. The different behavior of the two SSF wetlands could be attributable to a higher rhizome contact with the water in the shallower bed. Accordingly, the removal rate was corrected. After more than 400 h from the pharmaceutical injection, a different removal efficiency for ibuprofen was observed in two wetlands: 81 and 48% for the shallow and deep beds, respectively. Carbamazepine was not completely recovered, in agreement with its higher sorption in the bed (*33*). Clofibric acid has been identified as a refractory contaminant in several WWTPs (*14*) and in river biofilm systems (*34*). No removal was observed in the two wetlands studied.

## Discussion

The removal of contaminants in SSFs involves a variety of processes including biodegradation, sorption onto the bed, sedimentation, and plant uptake (*4*). The complexity of such processes makes it difficult to understand the primary removal mechanism for each class of contaminants, and design parameters are usually based on empirical approaches. Further understanding of the removal and degradation pathways and mechanisms of organic matter is needed to improve the efficiency of such systems.

In this regard, we have demonstrated that the redox potential ($E_H$) was higher in the water inside the shallow beds (ranging from −144 to −131 mV) than in the deep ones (from −183 to −151 mV) (*31*). More oxidized conditions in the shallow SSFs can promote more energetically favorable biochemical reactions, leading to a higher removal efficiency of the organic matter. Therefore, in the present study, we considered two pilot SSFs with a different depth, and the shallow bed showed a better ibuprofen removal than the deep bed. Similar results were reported in an experimental biofilm microcosm reactor (*35*). The lower concentration of the pharmaceutical in the bed under the equilibrium conditions indicates that ibuprofen is biodegraded in the wetland environment. Although much information is available on the removal of this pharmaceutical in conventional WWTPs, to the best of our knowledge, this is the first time that its removal has been reported in a SSF system. The removal efficiency of the SSF system used in this study is not directly comparable with that of a conventional WWTP because the inlet concentration is usually lower than in our case; however, the results can be compared in Table 4, and it can be seen that the efficiencies are quite similar in the case of the shallow bed.

A completely different behavior was obtained in the case of carbamazepine. First, it exhibited a different response curve to the other pharmaceutical compounds (Figure 2), consistent with its higher hydrophobicity (Table 1). Second, its removal is lower than that of ibuprofen, being more efficient in the deep bed wetland than in the shallow one (Table 4). The

**TABLE 4. Removal Efficiency (%) of Pharmaceuticals during Injection Experiment Obtained from Total Mass Recovery and Comparison with Conventional WWTPs**

| | SSFs | | WWTPs | | | | |
|---|---|---|---|---|---|---|---|
| | deep | shallow | ref *18* | ref *14* | ref *20* | ref *17* | |
| country | this paper | this paper | Spain | Germany | Germany | Brazil[a] | Brazil[b] |
| ibuprofen | 48 | 81 | 60–70 | n.d.[c] | 90 | 75 | 22 |
| clofibric acid | n.r. | n.r. | n.d. | n.r | 51 | 34 | 15 |
| carbamazepine | 26 | 16 | n.d. | 8 | 7 | n.d. | n.d. |
| samples (*n*) | *d* | *d* | 3[e] | 20–27[f] | 6[e] | 6[e] | 6[e] |

[a] Actived sludge. [b] Biological filter (trickling filter). [c] n.r.: no removal observed; n.d.: no data available. [d] Continuous injection. [e] Integrated samples of 24 h. [f] Integrated samples of 24 h from three different WWTPs in Berlin.

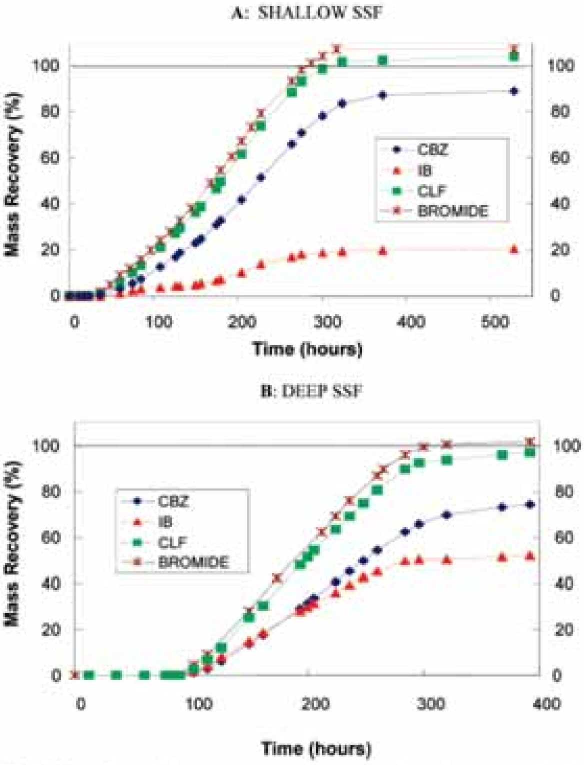


**FIGURE 3. Cumulative percent mass recovery for pharmaceuticals and bromide for two wetlands. See Figure 2 caption for abbreviations.**

different behavior of this pharmaceutical is attributable to its higher hydrophobicity and its refractory behavior to biodegradation. In fact, carbamazepine is one of the most recalcitrant pharmaceutical compounds occurring in the aquatic environment (*36*). It has even been suggested as a conservative tracer of urban pollution in ground and surface waters (*37*). According to its refractory character to biodegradation and its high concentration in the gravel, the removal of this compound in the wetlands could be explained by the sorption onto the available organic surfaces. The difference between the two wetlands could be explained because of the greater volume of the deep wetland than the shallow one, which implies a higher specific surface for sorption processes than in the shallow bed. The comparison of its removal in the SSF and in conventional WWTPs is favorable to the former, probably due to the presence of media for sorption.

Finally, clofibric acid behaves in a different way than the other two pharmaceuticals. Its low hydrophobicity leads to a low sorption onto the wetland bed, and its refractory behavior leads to negligible removal. Clofibric acid was described as a refractory compound in WWTPs (*13*). Nevertheless, in WWTPs in Berlin and Brazil (*20, 17*), moderate removal is reported (Table 4). However, no removal was also reported in an exhaustive study in Berlin WWTP (*14*). Additional microcosm studies support the recalcitrant behavior of clofibric acid (*34, 35*), as found in our study. A possible explanation of the different results could be explained by the required microbiological adaptation to achieve a significant degradation rate or/and the high fluctuation of the pharmaceutical concentrations in the WWTP influents; this fluctuation is avoided when the pharmaceuticals are injected as in our study. Moreover, the presence of clofibric acid in the North Sea (*22*) supports the recalcitrant behavior of this compound.

In summary, SSF depth is a key design factor for the removal of pharmaceuticals. Less refractory compounds such as ibuprofen are removed more efficiently in the shallow SSF, presumably linked to more oxidized conditions. On the other hand, the more refractory pharmaceuticals such as clofibric acid show no removal, in good agreement to the limited removal observed in WWTPs.

The retention of pharmaceuticals in wetlands is dependent on their physicochemical properties and is close to the behavior of bromide for acidic pharmaceuticals. In this regard, clofibric acid has a similar retention behavior to bromide in the two wetlands studied. Therefore, it can be used as a tracer to describe the hydraulic parameters. In addition, using clofibric acid in a lower concentration (ng mL$^{-1}$) than bromide (mg mL$^{-1}$) avoids the effect of density stratification in the characterization of the hydraulic parameters (*38*) and bromide uptake by plants (*39*), which has not been demonstrated in the case of pharmaceuticals.

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## 2.3.1.3. Article 2: Pharmaceutical products behaviour in horizontal subsurface flow constructed wetland. A microcosms experiment

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# Pharmaceutical products behaviour in horizontal subsurface flow constructed wetland. A microcosms experiment

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## 0- Abstract

Horizontal subsurface flow constructed wetlands (SSF CWs) constitute a cost-effective and sustainable alternative to conventional wastewater treatment plants (WWTP) for sanitation of small communities. SSF CWs are designed to remove suspended solids and organic matter from wastewater but the characteristics of organic mater on the removal efficiency of specific contaminants are not well established. In this paper, selected pharmaceuticals namely carbamazepine, ibuprofen and clofibric acid were continuously injected in two microcosms SSF CWs fed with synthetic wastewaters with different organic matter sources, dissolved (glucose) and particulate (starch). Response curves of carbamazepine and ibuprofen were compared with the clofibric acid, which was used as a conservative tracer. Ibuprofen was partially removed and the identification of the two main biodegradation intermediates namely carboxy and hydroxy derivatives support that the main ibuprofen elimination pathway occurs through aerobic conditions. Moreover, the removal efficiency of that pharmaceutical was shown to be independent of the organic matter characteristics (i.e. dissolved or particulate).

**Keywords:** constructed wetland, ibuprofen, ibuprofen metabolites, clofibric acid, carbamazepine, removal.

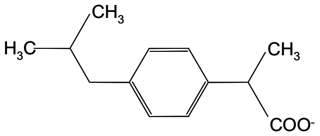
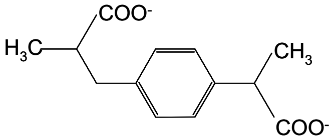
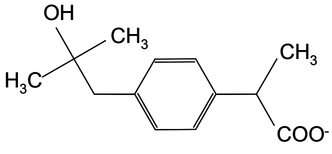
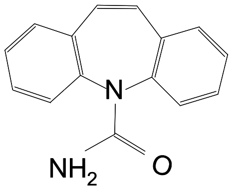
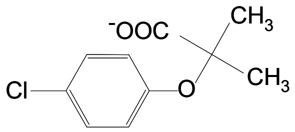
## 1. Introduction

Horizontal subsurface flow (SSF) constructed wetlands (CWs) are low-cost treatment technology where wastewater flows slowly across gravel, roots and rhizomes of the emergent vegetation. The removal of contaminants occurs as a result of complex physical, chemical and microbial interactions (Kadlec and Knight, 1996). The rates of these processes may vary in time and space, and depend on many factors such as the surface loading rate, the water depth and the availability of electron acceptors (García et al, 2004; Aguirre et al, 2005).

Pharmaceuticals and personal care products (PPCPs) have been continuously discharged into the aquatic environment for more than one century without any restriction (Daugthon and Ternes, 1999; Sedlak et al., 2000). They have been detected in a variety of wastewater treatment plant (WWTP) effluents ranging their concentrations from parts per trillion to low parts per billion (Heberer, 2002; Ternes et al., 2004). Although the behaviour of pharmaceutical products in WWTP is reported in several publications (Daugthon and Ternes, 1999), the amount removed is compound dependent. Indeed, in real-scale WWTPs (Heberer, 2002), microcosmos experiments (Winkler et al., 2001) and pilot SSF CWs (Matamoros et al., 2005) carbamazepine and clofibric acid are almost not eliminated. Accordingly, clofibric acid can be classified as a refractory compound in gravel systems (Drillia et al., 2005) and SSF CWS, and it has been proposed as a potential tracer (Matamoros et al., 2005). Other compounds like ibuprofen are readily removed in a variety of treatment processes including SSF CWs (Buser et al., 1999; Matamoros and Bayona, 2006).

In this study, the behaviour of ibuprofen and carbamazepine and clofibric acid was evaluated in two different mesocosm SSFs. Clofibric acid was used as a tracer because its previously demonstrated refractory properties (Matamoros et al., 2005). During the experiments both wetlands were fed with synthetic wastewater with the same organic load but with different organic matter sources, one with glucose (as a readily biodegradable dissolved organic matter) and the other one with starch (as a slowly biodegradable particulate organic matter). The effect of the type of organic matter source on the removal efficiency of the wetlands was the purpose of a previous study (Caselles-Osorio and García, 2006) but the effect on the removal of specific contaminants never accomplished. The fact the wetlands were fed with different organic sources gave us the opportunity to study the interaction between the type of organic matter and the behaviour of the pharmaceutical products. In a previous study, it was not found significant differences on the removal efficiency of organic matter between two systems were found. In the present work, ibuprofen degradation products were also considered. In fact, it is reported both the biodegradation of ibuprofen in oxic and anoxic biofilm reactors (Zweiner et al., 2002). Nevertheless, no information about their generation in SSF CWs is available. Furthermore, the structure and physico-chemical characteristics of pharmaceutical compounds were also considered to get further insight into the different behaviour among the target compounds (Table 1).

**Table 1**. Chemical structures and physicochemical properties of the pharmaceutical products and their main degradation products.

| Trade Name/CAS | pka | log Kow[a] | function | Structure |
|---|---|---|---|---|
| Ibuprofen<br>15687-27-1 | 4.31 | 0.45 | Analgesic / anti-inflammatory |  |
| Carboxy-ibuprofen | -- | -/- | Ibuprofen metabolite |  |
| Hidroxy-ibuprofen<br>51146-55-5 | -- | -/- | Ibuprofen metabolite |  |
| Carbamazepine<br>298-46-4 | 7 | 2.25 | Antiepileptic |  |
| Clofibric acid<br>882-09-7 | 2.84 | -0.97 | Lipid regulator |  |

[a] estimated value at pH 8 (EPI suite, 2007)

## 2. Experimental section

**SSF CWs.** The two wetlands were built using PVC containers (0.93 m long, 0.59 m wide and 0.52 m high) filled with wetted gravel extracted from a pilot SSF CWs system in operation since 2001 (García et al., 2004). The containers had a drainage pipe located on the bottom of one of their sides with a flat bottom. Gravel depth was 0.35 m with a $D_{60}$= 3.5 mm (the diameter at which 60% of the material passed through the sieve), the uniformity coefficient was ($C_u = D_{60}/D_{10}$) was 1.7 and the initial porosity 40%. The water level was maintained at 0.05 m under the gravel surface to give a water depth of 0.30 m. In April 2004, reed rhizomes (*Phragmites australis*) were planted in the wetlands and stored in the Environmental Engineering Laboratory (Technical University of Catalonia, Barcelona) in order to avoid great temperature variations. In addition to environmental and laboratory light, each experimental SSF CW was illuminated using racks with 15 Grolux lamps that provided an additional total power of 540 W.

Both wetlands were fed with synthetic wastewater. As organic carbon source one SSF CW was fed with starch $(C_6H_{11}O_5)_n$ as slowly biodegradable organic matter, and the other with an influent containing glucose ($C_6H_{12}O_6$ ) as readily biodegradable organic matter (Mino et al., 1995; Gujer et al., 1999). During the experiments, the surface organic loading rate was maintained approximately constant and identical in both SSF CWs. For preparing the influent wastewater 3.8 g of starch or 4.0 g of glucose were added on a daily basis to 10 L of tap water. The starch and glucose loads were calculated in order to have the same theoretical influent chemical oxygen demand (COD) concentration in both SSF CWs as reported in Caselles-Osorio and Garcia (2006). Other major nutrients such are N and P were supplied to the tap water in order to have a C:N:P ratio of approximately 100:16:1. Both SSF CWs were fed in batch; thus the daily volume was added for a 20 min period.

**Pharmaceutical products injection experiment.** It was carried out in July 2004, when the reeds were quite well developed. Note that during this study the plants grew with thinner and shorter steams than it is observed in the field. The 10 L of synthetic wastewater were daily spiked with 250 μg of each pharmaceutical compound in order to reach a final concentration of 25 μg $L^{-1}$. The injection experiment lasted 30 days. No adaptation of the biofilm to the pharmaceutical products was done prior to the injection.

**Sampling.** Effluent samples were daily collected when the microcosms were fed with the synthetic wastewater. All the samples were collected in 500 mL amber glass bottles, and kept refrigerated during transportation to the CSIC laboratory where they were stored before analysis. The sample holding time was less than 2 days.

**Wastewater Analysis.** General wastewater parameters such are ammonium, sulfate and total COD were analyzed following conventional methods (APHA-AWWA-WPCF., 2001).

Temperature and dissolved oxygen (DO) were measured *in situ* using a Chektemp-1 Hanna thermometer and an oxymeter respectively.

Samples for the pharmaceutical product analyses were filtered through 0.45 μm glass filter of 47 mm diameter from Millipore (Bedford, MA) and then acidified to pH 2 with concentrated hydrochloric acid. A sample volume of 200 mL was mixed with a solution of surrogate standards, fenoprop and dihydrocarbamazepine, giving a final concentration of 25 μg $L^{-1}$. The spiked sample was percolated through a polymeric SPE cartridge packed with 100 mg of Strata X from Phenomenex (Torrance, CA) conditioned previously with 5 mL of *n*-hexane, 5 mL of ethyl acetate, 10 mL of MeOH, and 10 mL of MilliQ water (pH = 2). The flow rate was approximately adjusted to 10 mL $min^{-1}$. Thereafter, the cartridges were allowed to dry for 30 min. Finally, the analytes were eluted with 5 mL of ethyl acetate. The extract was evaporated to dryness under a gentle nitrogen stream, reconstituted in 175 μL of methanol, and mixed with 5 μg of mecoprop as internal standard since it was not detected in the influent wastewater. The resulting extracts were analyzed using a dual pump HPLC equipped with a UV diode array detector from Shimadzu (Kyoto, Japan) at 230 nm. Injection was carried out using a Rheodyne valve (Rohnert Park, CA) with a sample loop volume of 20 μL fitted to a Shimadzu autosampler. Chromatographic separation was performed on a Lichro-CART column 125 x 4 mm packed with Lichrospher 100 RP-18 (5 *μ*m) from Merck. Mobile phase program and quantification details are described elsewhere (Matamoros, 2005).

**Biodegradation metabolites analysis.** About 50 μL extracts were derivatized with 5 μl of trimethylsulfonium hydroxide and then the sample was injected on to a TRACE-GC-MS (Thermo-Finnigan, Dreieich, Germany) fitted with a 30 m x 0.25 mm i.d. x 0.25 μm DB-5 (J&W Scientific, Folsom, CA). The carrier gas was helium (purity: 99.9995%) at a constant linear average velocity of 40 cm $s^{-1}$. The oven temperature was held at 90 °C for 1 min, then temperature programmed at 20 °C $min^{-1}$ to 120 °C, then to 3 °C $min^{-1}$ to 160 °C followed by 12 °C $min^{-1}$ to 260 °C, and finally held for 12 min. A 2 *μ*L of sample volume was injected in the splitless mode at 270 °C with an injector purge activation time of 1 min. The transfer line was set at 280 °C and the ion source at 200 °C. The electron impact energy was 70 eV. Acquisition was performed in the scan mode ranging from *m*/*z* 50 to 450 at 2 scans $s^{-1}$. The following diagnostic ions were monitored for target analyte quantification (in bold) and identification: ibuprofen **161**/177/220; carboxy-ibuprofen **205**/145/177 hydroxy-ibuprofen **119**/118/178 clofibric acid, **128**/169/228; carbamazepine, **193**/ 165/194; mecoprop,

**169**/142/228; fenoprop, **196**/223/282; and dihydrocarbamazepine, **195**/180/196. Quantification details are reported elsewhere (Matamoros and Bayona, 2006).

## 3. Results and discussion

**General parameters.** During the experiments the surface loading rate was 6 g COD $m^{-2}$ $d^{-1}$ in both wetlands. The high sulfate removal and low oxygen concentration in effluents suggest that anaerobic pathways like sulfate reduction are of major importance for the removal of organic matter (Table 2). Furthermore, removal efficiencies for the COD, ammonium and sulfates were not significantly different between the two systems evaluated. These results suggest that SSF CWs are not sensitive to the type of organic matter in the influent whether it is readily (glucose) or slowly (starch) biodegradable, at least during the initial period of operation.

**Table 2.** Mean concentration values, standard deviation (in brackets) and removal efficiencies of the $SO_4^{-2}$, COD, $NH_4^+$-N and DO.

| | **glucose** | | | **starch** | | |
|---|---|---|---|---|---|---|
| | Inf. | Eff. | Rem (%) | Inf. | Eff. | Rem (%) |
| **DO (mg $L^{-1}$)** | 6.4 (0.7) | <0.05 | - | 7.9(1.0) | <0.05 | - |
| **$SO_4^{-2}$ (mg $L^{-1}$)** | 160(36) | 40(25) | 75 | 160(32) | 50(22) | 69 |
| **COD (mg $L^{-1}$)** | 330(28) | 40(17) | 88 | 300(42) | 25(139 | 91 |
| **$NH_4^+$-N (mg $L^{-1}$)** | 23.1(2.7) | 10.0(1.9) | 57 | 23.4(2.9) | 10.9(2.5) | 48 |

**Response curves of the pharmaceutical products.** The pharmaceutical products behaviour in the two wetlands can be observed in the Figure 2, where the clofibric acid behaves like a tracer (Matamoros et al. 2005). The response curves correlate with the log Kow of the pharmaceutical products shown in Table 1. In fact, carbamazepine, that has a higher log Kow than clofibric acid, seems to be the compound that takes longer to reach the equilibrium. Ibuprofen behaves different to the other two pharmaceutical products because it was removed to some extend, whereas carbamazepine and clofibric were far to show any removal and ended reaching the equilibrium concentration (25 μg $L^{-1}$). From the response curve of the clofibric acid it is demonstrated that both systems had a very similar hydraulic retention time (206 hours for the wetland fed with glucose and 183 hours for the wetland fed with starch).

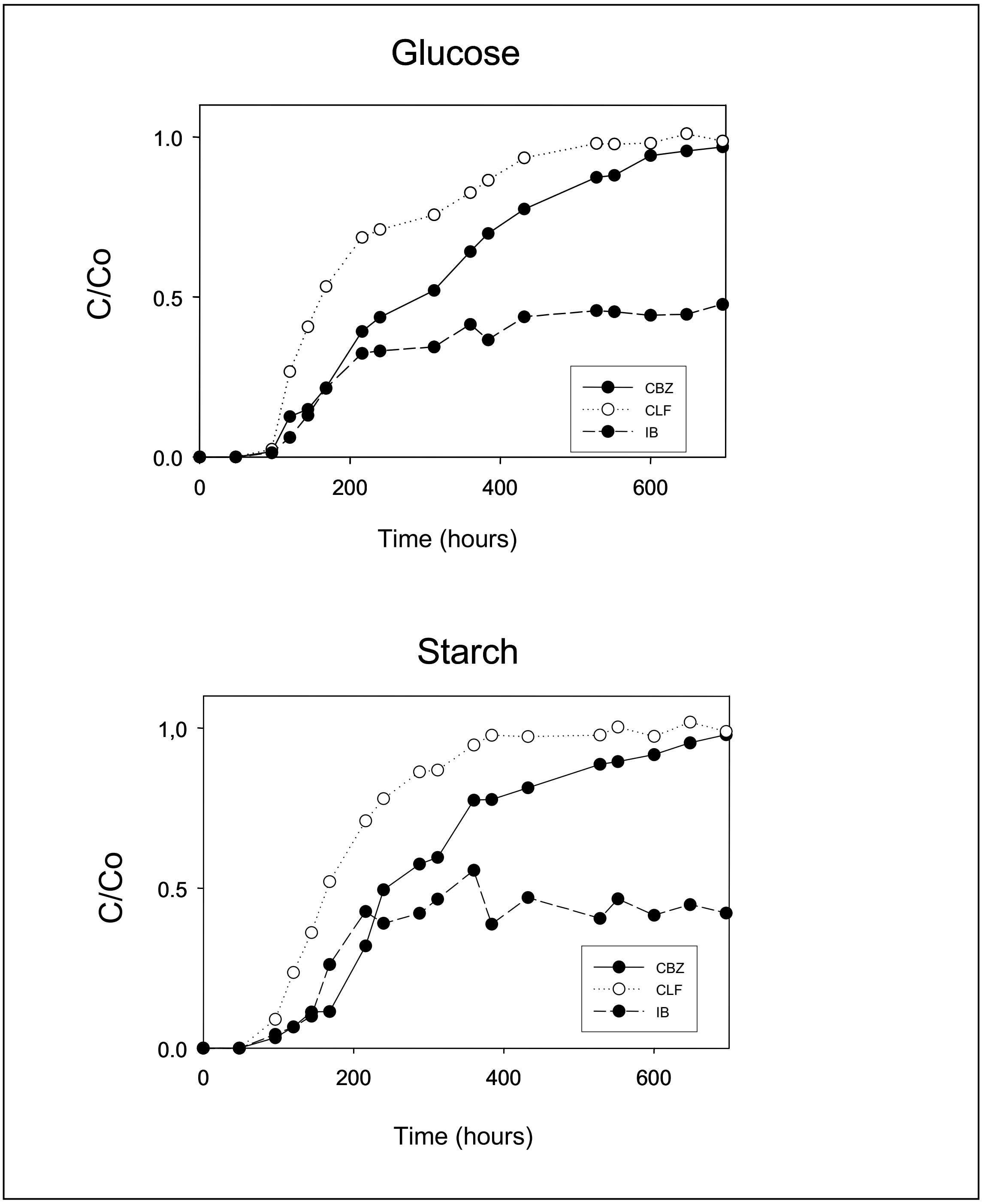


**Figure 1**. Response curves of the pharmaceuticals products in the two microcosms SSF CWs studied. (CBZ: carbamazepine, CLF: clofibric acid and IB: ibuprofen).

**Removal efficiency of the pharmaceutical products.** Removal efficiency was calculated from the response curves at the latest sampling point (see Figure 1) when the compounds reached the equilibrium. As reported previously, carbamazepine and clofibric acid were not removed in the SSF CWs due their recalcitrant properties (Matamoros *et al.* 2005). Only ibuprofen was partially removed, and with very similar rates in both systems, i.e. 53% and

57% for SSF CW fed with glucose and starch respectively. Thus, at a first insight it seems that there is not interaction between the type of organic source and the removal of the organic compounds. Nevertheless, when the response curve of the ibuprofen is carefully observed, it is possible to see that at the middle of the experiment the wetland fed with starch had lower removal rates than the wetland fed with glucose (Figure 1). In fact, when the ibuprofen removal difference between both systems is plotted *vs* time (Figure 2), lower removals are clearly observed over the middle of the injection period for the SSF CW fed with starch. Nevertheless, at the equilibrium very similar removal rates were attained in both systems. The different removal behaviour observed for ibuprofen over the middle of the experiment could be attributable to the higher biofilm development in the SSF CW fed with a readily biodegradable source of organic matter (Caselles-Osorio and García 2006) than in the other one.

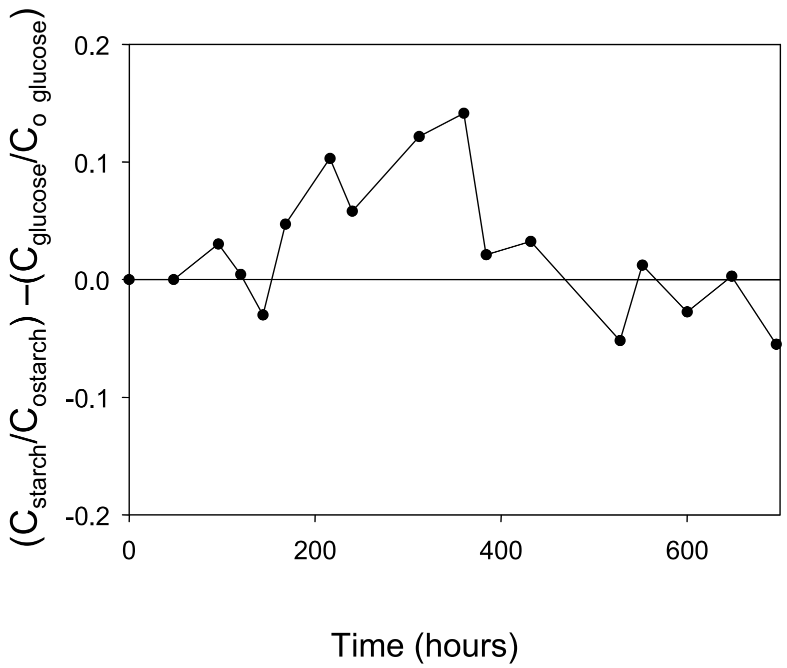


**Figure 2.** Ibuprofen removal difference between the SSF CW fed with starch and that fed with glucose.

Indeed, the removal rates observed for ibuprofen in this study were lower than those previously reported for conventional WWTPs, in which the removal rates are approximately 90% (Carballa et al. 2004; Ternes et al. 2004). Nevertheless, the removal rates observed in this study were in accordance with the rates observed for pilot SSF CWs (i.e. 48%) with prevailing anaerobic conditions (Matamoros et al. 2005), but higher than ones in anoxic biofilm reactors (i.e. 22%) (Zwiener et al. 2002). From the results of the present study, and those of the previous work conducted in a pilot SSF CW (Matamoros et al. 2005), we can conclude that to reach a good ibuprofen removal in the wetlands it is necessary to promote as much as possible aerobic conditions.

**Biodegradation intermediates.** Ibuprofen degradation products (i.e. CA-IB and OH-IB) were analysed in selected effluent samples from the two systems. Concentrations found in the two wetlands were very similar for both products (n=4). Therefore, in this paper it is only reported the results concerning the system fed with glucose.

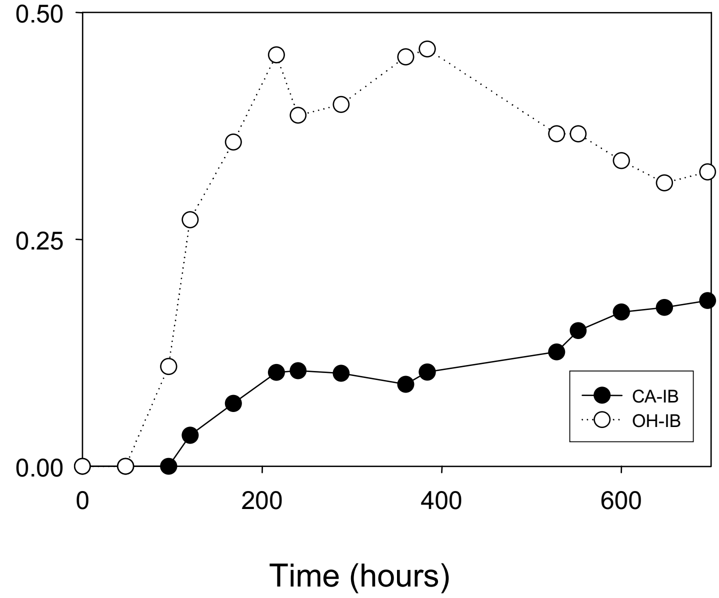


**Figure 3**. Ibuprofen degradation products (CA-IB: Carboxy-ibuprofen and OH-IB: hydroxy-Ibuprofen) observed in the SSF CW fed with glucose.

Figure 3 shows the behaviour of the two biodegradation products identified. No more than 5% of the degraded ibuprofen could be accounted for the sum of the two major metabolites analysed. Similar results (ca. 10%) were obtained in a biofilm reactor experiments, which was obtained from activated sludge of WWTP and fed with synthetic wastewater (Zwiener and Frimmel, 2003). In fact, CA-IB and OH-IB could be intermediate products because consumption kinetics is similar to their formation leading to almost no net accumulation. Nevertheless, the presence of CA-IB and OH-IB in the influent and effluent of pilot SSF CWs treating urban watewater at a similar concentration as ibuprofen (Matamoros and Bayona,

2006) suggest that the biodegradation intermediates do not accumulate in the system. In Figure 3, it can be seen that CA-IB reached its equilibrium at 400 h, whereas OH-IB decreased from the 400 to 650 h since the experiment started. This could be linked to the fact that biofilm was not adapted to the OH-IB biodegradation. In fact, OH-IB was shown to be biodegradable especially after certain adaptation of the biofilm microorganisms to higher amounts of this metabolite (Winkler et al. 2001).

On the other hand, the predominance of OH-ibuprofen over CA-ibuprofen could lead to the assumption that the biodegradation occurs under oxic prevailing conditions (Zwiener and Frimmel 2003), which contrasts with the oxygen depleted effluent. Although it appears as a contradiction, in SSF CWs different aerobic or anaerobic reactions can occur simultaneously but at different wetland microsites subjected to different redox conditions (Cooper et al. 1996). In this sense, the ibuprofen removal percentage accounted to aerobic and anaerobic pathways was estimated through the equations 1 and 2. Whereas ibuprofen anaerobic biodegradation gives rise to both CA-IB and OH-IB, under aerobic only OH-IB was identified (Zwiener et al. 2002).

*Equation 1*

$$Aerobic\_ratio = \frac{C_{OH-IB} + x * C_{CA-IB}}{C_{OH-IB} + C_{CA-IB}}$$

*Equation 2*

$$Anaerobic\_ratio = \frac{y * C_{CA-IB}}{C_{OH-IB} + C_{CA-IB}}$$

where *Ca* represents the concentration of metabolite *a* and *x* and *y* the percentages of CA-IB obtained in aerobic and anaerobic pathways respectively. The values of these percentages were obtained from Zwinner that reported a maximum of 0.4% and 1.8% of degraded ibuprofen under oxic and anoxic batch experiments respectively (Zwiener et al. 2002). According to our calculations whereas at the start of the experiment the anaerobic pathways explained almost the 10% of ibuprofen degradation, it rised up to approximately 30 % at the end of the experiment. This increase over time could be explained by a faster aerobic, biofilm adaptation to ibuprofen removal than the anaerobic ones.

## 4. Conclusions

The data of 700 h injection experiment in a microcosmos reveals that the evaluated pharmaceutical products have similar removal rates in SSF CWs fed with two different types of organic sources, glucose and starch. In both wetlands the removal of ibuprofen ranged from 53 to 57 %, while carbamazepine and clofibric acid passed through the wetlands without a detectable decrease.

Carboxy-ibuprofen and hydroxy-ibuprofen were identified as metabolite products of ibuprofen degradation. Nevertheless, they accounted for approximately the 5% of ibuprofen degradation, suggesting that no accumulation occurred in the wetlands. Indeed, when the contribution of aerobic and anaerobic pathways were studied, aerobic ones were postulated as the major importance in the ibuprofen removal.

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Removal efficiency and elimination rates of 11 pharmaceuticals and personal care products (PPCPs) were measured in two subsurface horizontal flow constructed wetlands (SSFs) characterized by different water depths (i.e. 0.3 and 0.5 m) in a 2-year study. Dissolved and particulate phases of wastewater and gravel samples were collected and analyzed. The PPCP influent concentration ranged from 1 to 25 $\mu$g L$^{-1}$. The best removal efficiency was found in the shallower bed SSF due to its less negative redox potential. PPCPs were classified in four groups according to their removal behavior: (i) the efficiently removed (>80%) namely caffeine, salicylic acid, methyl dihydrojasmonate, and carboxy-ibuprofen, (ii) the moderately removed (50−80%) namely ibuprofren, hydroxy-ibuprofen, and naproxen, (iii) the recalcitrant to the elimination namely ketoprofen and diclofenac, and, finally, (iv) compounds that were eliminated by hydrophobic interactions namely polycyclic musks (i.e. galaxolide and tonalide). These compounds were removed more than 80% from the effluent but occurred at high concentrations (up to 824 $\mu$g kg$^{-1}$) in the gravel bed. Accordingly, their elimination by sorption onto the organic matter retained is the predominant removal mechanism. Furthermore, the constructed wetland clogging appears to induce a negative effect in the PPCP degradation in the SSF evaluated. The PPCP elimination classified as efficiently and moderately removed through the shallow bed fitted to either zero- or a first-order areal kinetics. Finally, the apparent distribution coefficients between suspended solids ($Kd'_{ss}$) or gravel bed ($Kd'_{gb}$) and water were determined in the different sampling points of the wetland obtaining values comparable to the described previously for sewage sludge.

## Introduction

Some pharmaceuticals and personal care products (PPCPs) are being considered as emerging contaminants of environmental concern because of their continuous release into the aquatic environment, their persistence, and increasing evidences for ecotoxicological effects (*1*). In fact, the occurrence of PPCPs in the aquatic environment, mainly by domestic usage, has been extensively documented (*2, 3*). PPCPs have been detected in a variety of sewage treatment plants (STPs) effluents from parts per trillion to low parts per billion concentrations (*4−8*). Consequently, variable concentrations of these compounds have been detected in surface, ground, and coastal waters receiving treated sewage effluents (*9*).

Despite PPCPs including a large class of chemicals that can be originated from different human and veterinary usage (i.e. prescription drugs, fungicides, shampoos, and deodorants) (*9*), analgesics and anti-inflammatory compounds (i.e. salicylic acid, ibuprofen, and diclofenac) are the most abundant in raw sewage (*10*).

Regarding the possible toxicity of the studied compounds, PPCPs and especially polycyclic musks have been reported to act as inhibitors of multixenobiotic resistance (MRX) in aquatic organisms (*11, 12*). Moreover, recent studies on environmental risk assessment of polycyclic musks showed that their concentrations in the aquatic environment could exceed the predicted no-effect concentration (PNEC) (*13*).

Constructed wetlands are land-based wastewater treatment systems that consist of shallow ponds, beds, or trenches that contain floating or emergent-rooted wetland vegetation (*14*). More specifically, in the subsurface horizontal flow constructed wetlands (SSFs), wastewater is infiltrated through vegetated gravel beds confined by a liner. The wastewater treatment relies on biological, chemical, and physical processes in a natural environment. The potential of SSFs for the removal of contaminants occurring in urban wastewater has attracted increasing interest over the past decade with a view of treating wastewaters from small populations. The objective is to comply with environmental regulations and to attenuate diffuse agricultural contamination runoff to surface waters (*15, 16*).

According to the PPCP removal in STPs, they could be classified as efficiently eliminated (i.e. ibuprofen, caffeine) or as recalcitrant (i.e. clofibric and carbamazepine) (*17*). However, limited information exists in relation to their elimination in engineered natural systems such as constructed wetlands. Until now, existing studies are basically related to herbicides, pesticides (*18−20*), and surfactants (*21, 22*), and only two studies are focused on pharmaceutical elimination in these systems (*23, 24*). In one of these studies, a surface flow constructed wetland was used for the PPCP attenuation in an effluent dominated river showing a low removal (*23*). In the second paper a continuous injection experiment in a SSF used for the treatment of wastewater showed that the ibuprofen removal was comparable to the STP efficiency (*24*).

To the best of our knowledge, this is the first time that the occurrence and the removal of PPCPs are studied in real wastewater treated by a pilot SSF constructed wetland. In this regard, the removal of 11 PPCPs was studied in order to get preliminary information about the behavior of these emergent pollutants in SSFs, where the areal kinetics were also calculated. Furthermore, the structure and physicochemical characteristics of PCPPs were also considered to get further insight into the different behavior among the studied contaminants (Table 1, Supporting Information).

## Experimental Section

**Chemicals.** GC grade (Suprasolv) hexane, methanol, ethyl acetate, acetone, and dichloromethane were obtained from Merck (Darmstadt, Germany). Analytical grade acetic acid and hydrogen chloride were obtained from Panreac (Barcelona, Spain). Ibuprofen, caffeine, methyl dihydrojasmonate, galaxolide, tonalide, ketoprofen, salicylic acid, diclofenac, 2,2′-dinitrophenyl, and dihydrocarbamazepine were purchased from Sigma-Aldrich (Steinheim, Germany). The 2,4,5-

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**TABLE 1. Mean Value of the Pharmaceutical Removal (%) Obtained in the Three Campaigns and Its Comparison with Conventional STPs**

| | May-2004 | | May-2005 | | July-2005 | |
|---|---|---|---|---|---|---|
| | C2 0.5 m | D2 0.27 m | C2 0.5 m | D2 0.27 m | D2 0.27 m | STP activated sludge |
| | | | **Pharmaceuticals** | | | |
| salicylic acid | 97 ± 1 | 98 ± 1 | 77 ± 5 | 92 ± 2 | 97 ± 1 | 99 (*8*) |
| ibuprofen | 52 ± 1 | 80 ± 2 | 17 ± 11 | 71 ± 8 | 62 ± 2 | 60–70 (*7*)/90 (*2*) |
| OH-ibuprofen | 33 ± 7 | 69 ± 9 | 20 ± 10 | 67 ± 11 | 50 ± 4 | 95 (*40*) |
| CA-ibuprofen | 73 ± 8 | 92 ± 2 | 25 ± 9 | 94 ± 3 | 75 ± 6 | 95 (*40*) |
| naproxen | 0 ± 10 | 90 ± 3 | 47 ± 22 | 85 ± 4 | 80 ± 9 | 40–55 (*7*)/66 (*2*) |
| diclofenac | 11 ± 42 | 45 ± 17 | 0 ± 12 | 0 ± 5 | 0 ± 10 | 9–75 (*2*)/17 (*5*) |
| ketoprofen | 0 ± 14 | 69 ± 5 | 0 ± 29 | 0 ± 8 | 45 ± 5 | 48–69 (*2*) |
| caffeine | 94 ± 1 | 98 ± 1 | 85 ± 2 | 94 ± 1 | 99 ± 1 | 99 (*5*) |
| | | | **Fragances** | | | |
| methyl dihydrojasmonate | 94 ± 2 | 99 ± 1 | 61 ± 8 | 99 ± 1 | 99 ± 1 | 98 (*33*) |
| galaxolide | 31 ± 11 | 45 ± 1 | 44 ± 6/80[a] | 50 ± 7/85[a] | 61 ± 10/88[a] | 70–85 (*7*)[a]/89 (*33*)[a] |
| tonalide | 32 ± 10 | 44 ± 9 | 53 ± 6/84[a] | 65 ± 3/87[a] | 64 ± 4/90[a] | 75–90 (*7*)[a]/88 (*33*)[a] |

[a] Removal when the particulate and dissolved phase were balanced (%Removal=[(($IC_{wastewater}$+$IC_{SPM}$)−($EC_{wastewater}$+$EC_{SPM}$))/($IC_{wastewater}$+$IC_{SPM}$)]*100), IC and EC correspond to the influent and effluent concentration.

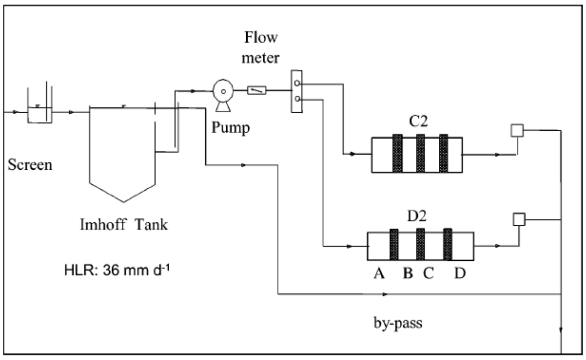

**FIGURE 1. Schematic diagram of the two wetlands of the SFF pilot plant at Les Franqueses del Vallès, Barcelona. The bed is divided in four sections attending to the three piezometric sampling points.**

trichlorophenoxypropionic acid (2,4,5-TPA) was obtained from Reidel-de-Haen (Seelze, Germany). Trimethylsulfonium hydroxide (TMSH) was purchased from Fluka (Buchs, Switzerland), and the 0.45 μm glass fiber filters of 47 mm were purchased from Millipore (Bedford, MA).

**Subsurface Flow (SSF) Pilot Plant Description.** The pilot plant consists of eight parallel horizontal SSFs and is located in the municipality of Les Franqueses del Vallès (Barcelona, Spain). The plant partially treats the urban wastewater generated by a housing development (ca. 200 inhabitants). The water is screened and then flows through an Imhoff tank (Figure 1). Each bed has a surface area of 54–56 $m^2$ and is planted with *Phragmites australis*. Three different perforated tubes (0.1 m in diameter) are inserted in the middle section of the gravel bed and uniformly distributed throughout the bed length, which allow the intermediate sampling. The plant was operated at a hydraulic loading rate of 36 mm $d^{-1}$. In this study, only beds C2 and D2 with 0.27 and 0.5 m water depth were sampled. Further information about the pilot plant description and operation is reported elsewhere (*25, 26*).

**Sampling Regime.** Influent and effluent samples were daily collected during 1 week in three different periods (i.e. May 2004 and 2005 and July 2005). Moreover, in the second campaign, samples from intermediate piezometers were also collected. Grab samples were taken due to the buffer effect of the Imhoff tank that provides an efficient wastewater homogenization.

All the samples were collected in 1 L amber glass bottles and kept refrigerated during transportation to the laboratory, where they were stored at 4 °C until analysis. The sample holding time was less than 2 days. Furthermore, bed D2 was subdivided into a grid of 12 sections in reference to the three piezometers as shown in Figure 1, and gravel samples with biofilm were sampled from each section and kept at −20 °C prior to analysis. The gravel samples were grouped by sections and processed as composite samples (A–D).

**Total Organic Carbon Determination.** The gravel composite samples were analyzed in order to measure the extractable (EOM) and the nonextractable organic matter (NEOM). About 100 g of gravel was dried at 70 °C during 24 h and extracted twice with 100 mL of dichloromethane/hexane (1:1) for 15 min in a sonication bath. Finally the extract was filtered through a 1.2 μm GF/F (Whatman, Maidstone, U.K.) and evaporated to dryness, and the EOM was determined by gravimetry. The NOEM was analyzed in the silt fraction (previously removed the EOM) by combustion using an elemental carbon analyzer (Carlo Erba, Milano, Italy).

**Wastewater Analysis.** Wastewater samples were filtered and processed as reported previously (24). Briefly, a sample volume of 500 mL was spiked with 1 μg of a surrogate standard mix (i.e. fenoprop for the acidic compounds, 2,2′-dinitrophenyl for musk, and dihydrocarbamazepine for neutral compounds). The spiked sample was percolated through a polymeric solid-phase extraction cartridge (100 mg Strata X) from Phenomenex (Torrance, CA). Activated cartridges were eluted with 10 mL of hexane/ethyl acetate (1:1). The extract was evaporated until ca. 20 μL under a gentle nitrogen stream, and a 186 ng of triphenylamine as internal was added. Then the vial was reconstituted to 300 μL with ethyl acetate.

Methylation of the acidic carboxyl group was performed online in a hot GC injector by adding 10 μL of TMSH solution (0.25 mol $L^{-1}$ in methanol) to a 50 μL sample before injection as described previously (*27*). Derivatized samples were injected onto a TRACE GC-MS (Thermo-Finnigan, Dreieich, Germany) in the electron impact mode (70 eV ionization energy) fitted with a 30 m × 0.25 mm i.d. × 0.25 μm DB-5 (J&W Scientific, Folsom, CA). Helium was used as carrier gas (99.9995% purity) at a constant linear average velocity of 40 cm $s^{-1}$. The oven temperature was held at 65 °C for 2 min and then the temperature was programmed at 15 °C $min^{-1}$ to 120 °C, at 4 °C $min^{-1}$ to 160 °C, at 7 °C $min^{-1}$ to 220 °C, at 5 °C $min^{-1}$ to 290 °C, and finally at 15 °C $min^{-1}$ to 320 °C holding the final temperature for 5 min. A volume of 2 μL of sample was injected in the splitless mode at an injector temperature of 270 °C with an injector purge activation time of 0.8 min. The transfer line and ion source were set at 280 °C and at 200 °C, respectively. Acquisition was performed in the scan mode ranging from $m/z$ 50 to 500 at two scans $s^{-1}$. The following diagnostic ions were monitored by time-scheduled acquisition for target analyte quantification (in bold) and identification: ibuprofen **161**/177/220; hydroxy-ibuprofen, **119**/118/178; carboxy-ibuprofen, **205**/177/145; naproxen, **185**/244/170; diclofenac, **214**/242/309; ketoprofen, **209**/268/191; galaxolide, **243**/258/213; tonalide, **243**/258/201; caffeine, **194**/193/109; dihydrojasmonate, **153**/156/83; salicylic acid, **120**/152/92; fenoprop, **196**/223/282; 2,2′-dinitrophenyl, **198**/168/139; and dihydrocarbamazepine, **195**/238/160. The linearity range was from 0.01 to 5 ($\mu$g $mL^{-1}$) for acidic compounds and from 0.08 to 5 ($\mu$g $mL^{-1}$) for the neutral compounds. The LODs and LOQs in ng $L^{-1}$ were compound dependent, ranging from 0.5 to 2.2 and from 1.7 to 7.5, respectively, for the acidic compounds and from 22 to 150 and 67 to 500 for the neutral compounds. The correlation coefficient ($r^2$) of the calibration curve was always higher than 0.998. The surrogate recoveries were always higher than 90%. Because no authentic ibuprofen metabolites standards were commercially available, their quantification was based on the ibuprofen ion calibration.

**Suspended Particulate Matter (SPM) Analysis.** Filters were lyophilized for 24 h and then spiked with surrogate standards of 1 $\mu$g of fenoprop and dihydrocarbamazepine and 2.5 $\mu$g of 2,2′-dinitrophenyl. Filters were kept 12 h at 4 °C to equilibrate the surrogates with the SPM collected. Neutral compounds were extracted by sonication in an ultrasonic bath with 2 × 10 mL of hexane/acetone (1:1) in a 40 mL Pyrex flask. The acidic compounds were recovered from the previously extracted filter in a subsequent step by using acetone/acetic acid (20:1). The obtained extracts were concentrated to 0.2 mL with a rotary evaporator (Heidolph Instruments, Schwabach, Germany), then reconstituted with 500 mL of MilliQ water, and finally analyzed as described previously in the wastewater analysis section. The recoveries for the acid pharmaceuticals were ca. 70% and 90% for the polycyclic musks and 80% for the remaining analytes. The LOD and LOQ (in $\mu$g $L^{-1}$) were from 0.97 to 1.1 and 2.5 to 3.2, respectively, for neutral compounds and 0.02 and 0.04 for ibuprofen, respectively.

**Gravel Analysis.** About 40 g of freeze-dried samples were spiked with the same surrogate standard solution used for the SPM, keeping an equilibration time of 12 h and extracted by a pressurized solvent extraction apparatus (Applied Separations, Allentown, PA) using a 33 mL extraction cell. Extraction conditions for the nonpolar analytes were 80 °C, 100 bar, and 3 cycles of 5 min with hexane/acetone (3:1) followed by a subsequent step with acetone/acetic acid (20:1) in order to recover the acidic compounds. The recovered extracts were concentrated to 0.2 mL with a rotary vacuum evaporator, reconstituted with 1 L of MilliQ water (*28*), and adjusted to pH 2 with concentrated hydrochloric acid. Then, the reconstituted samples were analyzed following the wastewater methodology as described previously in this section. The recoveries for the acid pharmaceuticals were ca. 70% and 90% for polycyclic musks and 70% for the remaining analytes. The LODs and LOQs ($\mu$g $Kg^{-1}$) were between 2.9 to 4 and 4.3 to 10, respectively, for neutral. For ibuprofen the LOD and LOQ ($\mu$g $Kg^{-1}$) were 0.16 and 0.35.

**Statistical Analysis.** SPSS v13 package (Chicago, IL, U.S.) was used for statistical data analysis. Normal distribution of all data populations was ensured for the applicability of parametric statistics (*Kolmogorov-Smirnov test*). The removal comparison was analyzed by independence-samples *t*-test.

**TABLE 2. Zero-Order and First-Order Areal Rate Constants from the Shallow Wetland on PPCP Removal and Their Correlation Coefficients**[a]

| | zero-order kinetic | | | first-order kinetic | |
|---|---|---|---|---|---|
| | $K_{0\,A}$ (mg $m^2$ $day^{-1}$) | $K_{0A}/C_0$[b] ($m^{-1}$) | $r^2$ | $K_A$ (m $day^{-1}$) | $r^2$ |
| ibuprofen | 0.523 | 0.028 | 0.888 | 0.051 | **0.951** |
| OH-ibuprofen | 0.514 | 0.026 | 0.930 | 0.044 | **0.973** |
| CA-ibuprofen | 0.973 | 0.035 | **0.671** | 0.130 | 0.505 |
| naproxen | 0.057 | 0.036 | **0.984** | 0.076 | 0.919 |
| galaxolide | 0.204 | 0.015 | 0.504 | 0.050 | 0.497 |
| tonalide | 0.133 | 0.022 | 0.540 | 0.060 | 0.589 |
| caffeine | 0.270 | 0.038 | 0.796 | 0.137 | **0.863** |
| methyl dihydrojasmonate | 0.632 | 0.034 | 0.603 | 0.208 | **0.907** |
| TOC (*26*) | 1.587[c] | 0.029 | 0.658 | 0.065 | **0.753** |

[a] In bold is indicated the best fitting kinetic rate. [b] Denotes the normalized values from the zero-order rate constant. [c] TOC units are in g $m^2$ $day^{-1}$and in the same bed.

## Results and Discussion

**PPCP Occurrence and Removal in SSF Wetlands.** Concentrations of the selected PPCPs in the influent and the gravel bed are shown in Table 2, Supporting Information. This set of PPCPs compounds was selected on the basis of their concentration and a high detection frequency. These values are comparable to those reported in the literature for raw sewage. The most abundant compounds in the urban wastewater analyzed were ibuprofen, caffeine, and methyl dihydrojasmonate due to their widespread use. On the other hand, galaxolide and tonalide were found to be the most abundant on the gravel bed and in the SPM due to their high hydrophobicity and low biodegradability properties (Table 1SI, Supporting Information).

Contaminant removal was calculated by analyzing both dissolved and particulate phases from the influent and effluent concentrations (Table 1). From these data, it is apparent that the shallow wetland performs a more efficient removal than the deeper one. Similar results have already been reported for organic matter ($BOD_5$) (*26*), linear alkylbenzene sulfonates (*22*), and selected pharmaceuticals (*24*). It has been attributed to a less negative redox potential (shallow bed ranging from −144 to −131 mV and deep ones from −183 to −151 mV) (*25*) leading to a more efficient biodegradation pathway of the organic matter.

According to their removal efficiency, the PPCPs identified in this study can be classified as (i) efficiently removed whenever their removal exceeded the 80% (i.e. caffeine, salycilic acid, methyl dihydrojasmonate, and CA-ibuprofen), (ii) the moderately removed, when it was nearly 50% (i.e. ibuprofren, OH-ibuprofen, and naproxen), and, finally, (iii) the recalcitrants (i.e. ketoprofen and diclofenac) (Table 1). Fragances analyzed are also quantitatively removed (i.e. galaxolide and tonalide), but they are retained in the gravel bed (see the Gravel Bed Accumulation section), and it has been attributed to hydrophobic interactions with the OM and the biofilm. Accordingly, polycyclic musks were classified as eliminated by hydrophobic interactions.

Several mechanisms could explain the low removal of the group defined as recalcitrant. It is known that sorption of these compounds onto organic matter retained in the gravel bed is not an important removal mechanism due to their hydrophilic structure. Its refractory character to biodegradation could be ascribed to specific structural characteristics, two extended aromatic rings (Table 1SI, Supporting Information). In fact, the estimated time frame for the biodegradation of diclofenac according to the Biodegradation

**TABLE 3. K'd Values Calculated in the Influent Samples ($n$=5) and in the Gravel Bed ($n$=1)**

| | SPM | gravel bed | | |
|---|---|---|---|---|
| compounds | log $K'd_{ss}$ | log $K'd_{gb}$[a] | log $K'oc_{gb}$[b] | references log Kd/log Koc |
| galaxolide | 4.81 | 2.92 | 5.7 | 3.3−3.7 (*39*)/4.80 (*41*); 4.14 (*39*) |
| tonalide | 5.00 | 3.05 | 5.83 | 3.4−3.7 (*39*)/4.86 (*41*); 4.17 (*39*) |
| methyl dihydrojasmonate | 3.32 | 0.70 | 3.48 | 2.61 (*33*)/− |
| ibuprofen | 2.37 | −0.44 | 2.23 | 0.9 (*39*); 1.69 (*42*)/1.75 (*39*); 2.21 (*42*) |

[a] Sorbed on the gravel bed, accounted to the gravel and O.M. [b] Accounted to the extractable and bound O.M.

Probability Program (*29*) is weeks, whereas most of the other pharmaceuticals present have a biodegradation of days (Table 1SI, Supporting Information). Moreover, ketoprofen and diclofenac behave as recalcitrant in microcosm experiments and membrane bioreactor systems (*30*), oxic biofilm reactors (*27*), and also activated sludge STP (*17*).

The low concentrations of the PPCPS in the gravel bed are attributable to their hydrophilic character which makes it not interact with the OM retained. However, the hydrophobic compounds such as galaxolide and tonalide (i.e. log $K_{ow} > 5$) are sorbed onto the OM as previously reported in sewage sludge (*31, 32*). The removal efficiency of these hydrophobic contaminants from wastewater (particulate and dissolved phases) is similar to that reported previously in STPs (*7, 33*).

**Water Depth Effect.** A different behavior according to the water depth is found to be compound dependent. It is apparent that the shallow wetland performs a more efficient removal than the deeper one. Similar results have already been reported for organic matter ($BOD_5$) (*26*), linear alkylbenzene sulfonates (*22*), and selected pharmaceuticals (*24*). It has been attributed to a less negative redox potential (*25*) leading to more efficient biodegradation pathways of the organic matter. In this regard, the PPCPs classified as efficiently removed, moderately removed, and recalcitrant were eliminated more efficiently ($p<0.05$) in the shallow bed than in the deep one (Table 3SI, C2 vs D2, Supporting Information). This removal observed in the shallow bed is similar to that reported in STPs (*10*). Nevertheless, the polycyclic musks removal was not water depth dependent ($p>0.05$) due to their elimination which was ascribed mainly to the hydrophobic interaction with the gravel bed.

**Clogging Effect.** A clogging effect occurred in the deeper wetland along this study, and it was investigated in terms of PPCP removal. In fact, it is known that wetland clogging leads to a decline in its hydraulic retention time and as, a consequence, to a decrease in the nutrient removal efficiency from wastewaters (*34, 35*). It is important to notice that in the months prior to the 2004 campaign, it was rainy, meanwhile the sampling campaigns carried out in 2005 were in dry months. In the second campaign (May 2005), a clogging effect was apparent in the first 3 m of the deep wetland with a statistically significant decrease in the removal efficiency for most of the biodegradable compounds (Table 3SI, C2; 2004 vs 2005, Supporting Information). This evidence suggests that the elimination of these PPCPs might occur mainly by biodegradation, because the decrease in the SSF residence time is directly related to a decrease in removal. Furthermore, no evident clogging effect was observed in the shallow wetland showing no statistical differences (Table 3SI, D2; 2004 vs 2005, Supporting Information) on the removal of the compounds, with the exception of salicylic acid and caffeine.

**PPCPs Removal Kinetics.** The PPCP removal kinetics was calculated in the shallower wetland (D2), because it was found to be the most efficient. The zero- and first-order areal kinetic constants were calculated by the equations shown as 1 and 2, respectively (*36, 37*)

$$K_{0A} = \frac{Q(\text{Cin} - \text{Cout})}{A} \tag{1}$$

$$K_A = \frac{Q(\ln \text{Cin} - \ln \text{Cout})}{A} \tag{2}$$

where $A$ is the wetland surface area between the inlet and the sampling point (i.e. piezometer or effluent), $Q$ is the volumetric flow rate (2 $m^3\ d^{-1}$), Cin and Cout are the inlet and outlet PPCP concentrations collected at each sampling point (i.e. piezometer or effluent), and $K_{0A}$ and $K_A$ are the zero- and first-order kinetic constants, respectively.

In this way, the PPCP removal through the bed was adjusted to a first-order kinetics where areal constants are shown in Table 2, with values from 0.04 to 0.21. The fact that these values are in accordance with the $BOD_5$ elimination rates reported by SSF (0.06−1.00 m $d^{-1}$) (*38*) might suggest that the kinetics of PPCP removal is comparable to the biodegradable organic matter. The only exception is the CA-ibuprofen and naproxen that fit better to a zero-order kinetics (Table 2). The naproxen biodegradation rate fit better to zero-order kinetics, showing that it has a linear dependence with the distance to the wetland inlet. This behavior could be explained by the limiting catalyst assumption in the elimination of a reactant (*37*). However, CA-ibuprofen does not adequately fit to any two kinetic equations described due to its fast removal in the first and second piezometers. Galaxolide and tonalide removal did not fit to the zero- or first-order kinetics evaluated. It is attributable to their substantial phase association with the particulate phase (Figure 2), and their elimination is attributable to retention (particulate phase) and sorption into the OM (dissolved phase).

**Gravel Bed Accumulation and Correlation with Organic Matter Distribution.** Although all the studied PPCPs were analyzed in the gravel bed, only the polycyclic musks concentrations exceeded the LOQs of the analytical methodology in all the gravel bed sections. In addition, low concentrations of these compounds were found in the wastewater, inducing to the classification of these compounds as eliminated by interaction with the OM. Figure 1SI (Supporting Information) shows the distribution of galaxolide, tonalide, EOM, and NEOM in the three sections of the shallow bed. As it is shown in this figure, the accumulation of the polycyclic musks and OM is basically localized in the first section of the wetland. Whereas 94% of the total polycyclic musk and EOM was reduced in the first wetland section, only 75% of NEOM was reduced in it. The similarity between the concentration profile of the EOM and the polycyclic musk versus the wetland inlet distance suggests that sorption into the EOM is rather than to the NOEM (Figure 1SI, Supporting Information). In fact the NOEM exhibited a less pronounced concentration decline with the wetland inlet distance than the polycyclic musk analyzed.

**Apparent Distribution Coefficients Measurement.** To estimate the PPCP amount retained in the gravel bed (gb) and in the suspended solids (ss) retained in the wetlands, the apparent distribution coefficients in relation with dis-

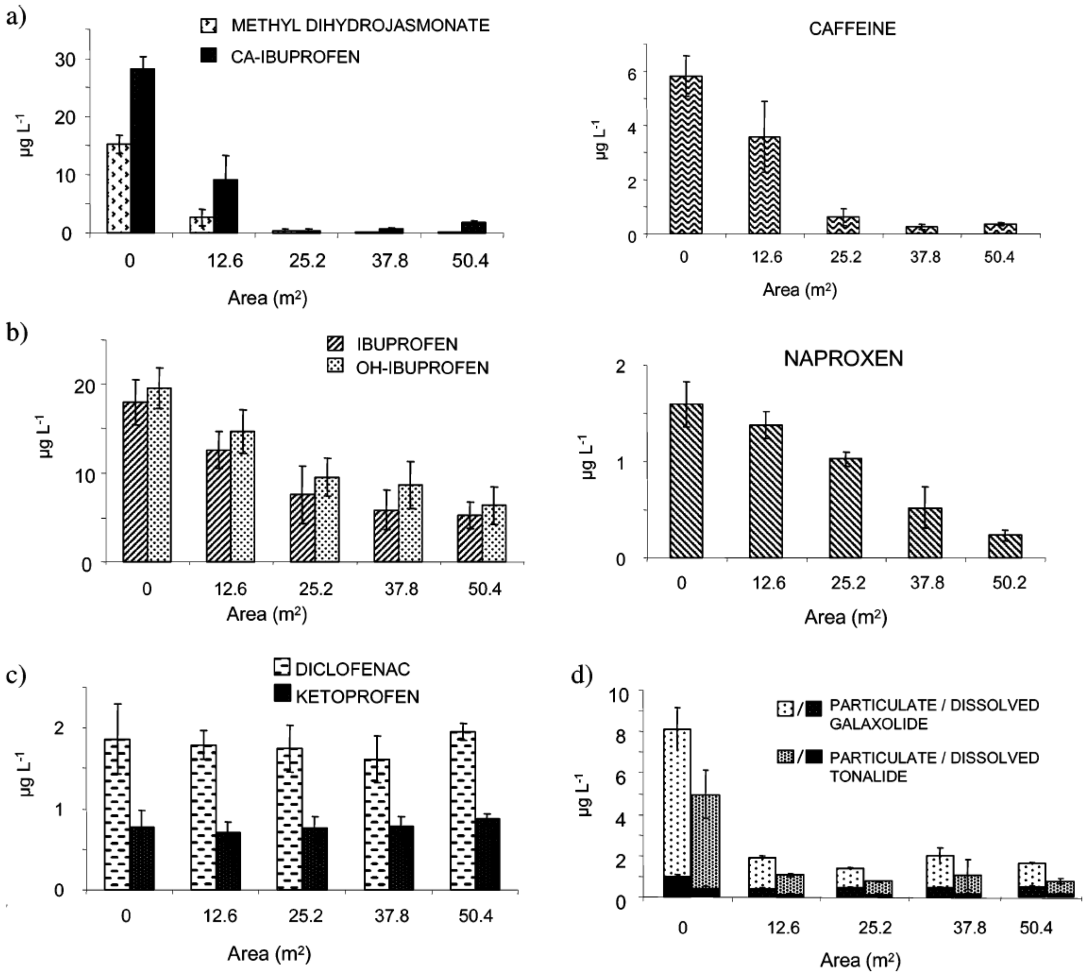

**FIGURE 2. PPCP behavior through the shallow SSF according with their surface area: (a) efficiently removed; (b) moderately removed; (c) recalcitrant; and (d) those removed by hydrophobic interaction. The dual histogram column filling for galaxolide and tonalide denotes the particulate phase (upper part) and the dissolved phase (lower part) concentrations.**

solved concentrations were calculated. Moreover, they were compared with those reported for these compounds in sewage sludges (Table 3). The apparent distribution coefficients (K'$d_{ss}$ and K'$oc_{gb}$) for the musk compounds, ibuprofen, and methyl dihydrojasmonate (compounds that were upper to the LOQ in the influent SS and in the first section of the gravel bed) were closer to the reported Koc than to the Kd. This could be explained by the high level of OM in the suspended solids and by the characteristics of the OM present in water samples. On the other hand, the differences between the K'd and K'$d_{ss}$ values could be ascribed to the differences in the filtration procedures applied (filter pore size). In fact, while we used the 0.45 $\mu$m pore size, values reported in the literature refer to filtration through 1 $\mu$m (*39*).

In the gravel bed, the K'$d_{gb}$ were close to the reported Koc, meanwhile the obtained K'$d_{gb}$ were lower than the reported due to the gravel size (6 mm diameter) and could be used to estimate the PPCP budget in the gravel bed from the wetland volume. In fact, the PPCP accumulation in the shallow bed could be estimated through gravel density (1300 kg m$^{-3}$), volumetric area (3.42 m$^3$), and PPCP concentration for each gravel section (Figure 1). After 5 years of operation the polycyclic musks accounted for nearly 4.2, 2.2, and 0.63 g for galaxolide, tonalide, and methyl dihydrojasmonate, respectively. Conversely, the accumulation of pharmaceuticals in the gravel bed can be considered as negligible except for caffeine and ibuprofen with an accumulation of 0.53 and 0.06 g, respectively. It could be accounted for its high influent concentration. Furthermore, it is possible to calculate the theoretical PPCP eliminations that were detected in the gravel bed through the zero-order kinetic rates (Table 2). Following the 5 years of operation, the highest total removal values correspond to ibuprofen, methyl dihydrojasmonate, and caffeine with 47.8, 57.7, and 24.6 g, respectively. On the other hand, the polycyclic musk showed lower values (18.6 and 12.0 g for galaxolide and tonalide). To sum up, nearly 25% of the total polycyclic musk that were removed from the SSF effluent during the last 5 years still remain accumulated in the gravel bed contrasting with less than 0.1% for ibuprofen.

## Acknowledgments

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## Supporting Information Available

Musk, EOM, and NEOM behavior in the gravel bed (Figure 1SI) and chemical and physicochemical properties of PPCPs studied (Table 1SI), levels of PPCPs in the wastewater influent, SPM, and gravel (Table 2SI); and statistic results (Table 3SI). This material is available free of charge via the Internet at http://pubs.acs.org.

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# Elimination of Pharmaceuticals and Personal Care Products in Subsurface Flow Constructed Wetlands

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Summary of Supplement Information

Number of tables: 3 tables SI

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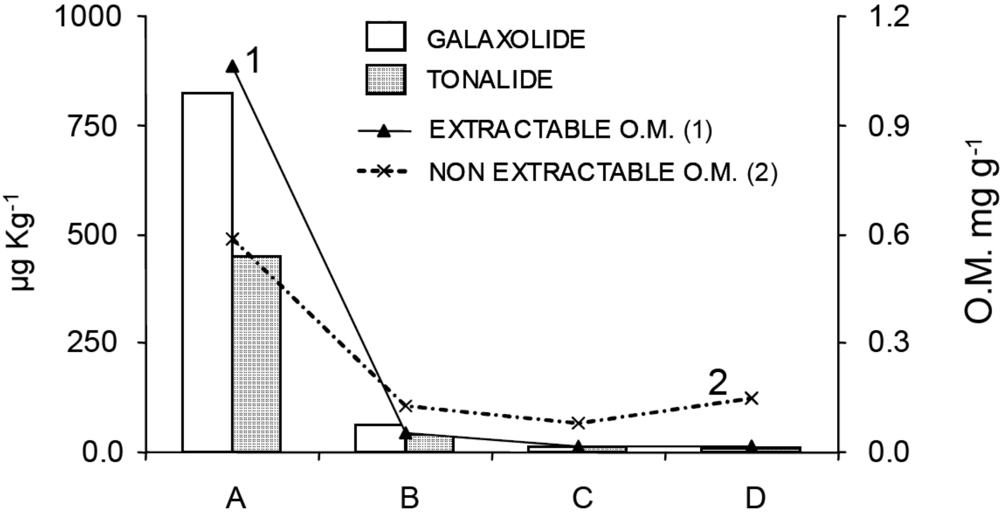
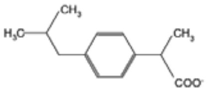
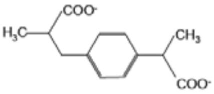
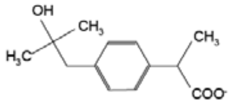
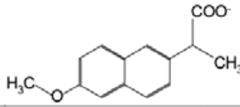
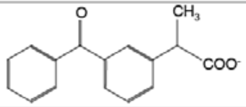
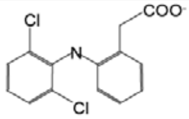
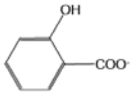
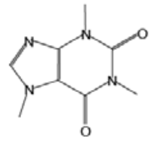
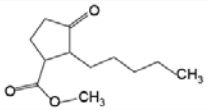
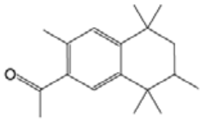
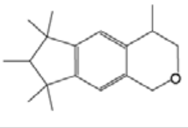


**Figure 1SI.** PPCPs retained on the gravel and their correlation with the extractable organic matter. EOM and NEOM are referred to the total weight in mg per g gravel.

**Table 1SI.** Chemical Structures and Physicochemical Properties of PPCPs studied.

| Trade Name/CAS | pka | log Kow$^{a/b}$ | Biodegradation$^{c}$ | function | Structure |
|---|---|---|---|---|---|
| Ibuprofen<br>15687-27-1 | 4.31 | 3.97/0.45 | 2.9582 | Analgesic/ anti-inflammatory |  |
| Carboxy-ibuprofen | -- | -/- | -- | Ibuprofen metabolite |  |
| Hidroxy-ibuprofen<br>51146-55-5 | -- | -/- | -- | Ibuprofen metabolit |  |
| Naproxen<br>22204-53-1 | 4.2 | 3.18/-0.34 | 2.9219 | Analgesic/ anti-inflammatory |  |
| Ketoprofen<br>22071-15-4 | 4.45 | 3.12/-0.44 | 2.9265 | Analgesic/ anti-inflammatory |  |
| Diclofenac<br>15307-86-5 | 4.2 | 4.51/0.7 | 2.2863 | Analgesic/ anti-inflammatory |  |
| Salycilic acid<br>69-72-7 | 3.5 | 2.26/-2.42 | 3.0382 | Acetyl Salycilic metabolit |  |
| Caffeine<br>58-08-2 | -- | 0.16 | 2.7700 | Stimulant |  |
| Methyl dihydrojasmonate<br>24851-98-7 | -- | 3.0 | 3.1151 | Fragance |  |
| Galaxolide<br>1506-02-1 | -- | 5.7 | 2.1066 | Fragance |  |
| Tonalide<br>1222-05-5 | -- | 5.9 | 2.1204 | Fragance |  |

[a] log Kow for neutral compounds; [b] log Kow obtained at pH 8; [c] ultimate biodegradation timeframe coefficients (≥4 days; ≥3 weeks and ≥2 months )(29

**Table 2SI.** Maximum, minimum and average concentration of the PPCPs in the wastewater influent, SPM, gravel and its comparison with conventional WWTPs.

| | May-2004 | May-2005 | | | Jul-2005 | | WWTP |
|---|---|---|---|---|---|---|---|
| | influent | Influent | | | influent | | influent |
| | ($\mu g\ L^{-1}$) | Wastewater ($\mu g\ L^{-1}$) | SPM ($\mu g\ L^{-1}$) | Gravel* ($\mu g\ Kg^{-1}$) | ($\mu g\ L^{-1}$) | SPM ($\mu g\ L^{-1}$) | ($\mu g\ L^{-1}$) (references) |
| **Pharmaceuticals** | | | | | | | |
| Salicylic acid | (0.67-1.26) 0.87 | (0.41-0.51) 0.45 | <0.02 | <0.18 | (1.65-1.90) 1.77 | <0.02 | 57±1.2(8)/0.34(5) |
| Ibuprofen | (7.77-12.20) 9.76 | (15.60-22.13) 17.95 | 0.48 | 6.47 | (18.71-24.57) 21.64 | 0.44 | (2.64-5.70) 3.70(7)/ (0.99-3.30)2.12(39) |
| OH-ibuprofen | (10.86-19.06)13.38 | (10.00-21.51) 17.61 | <0.02 | <0.26 | (18.48-23.81) 21.14 | <0.02 | -- |
| CA-ibuprofen | (16.74-24.61) 19.57 | (17.11-30.33) 24.15 | <0.02 | <0.33 | (13.20-21.26) 17.23 | <0.02 | -- |
| Naproxen | (7.98-18.78) 10.10 | (0.95-1.89) 1.47 | <0.02 | <0.26 | (2.45-3.02) 2.82 | <0.02 | (1.79-4.60) 3.28(7) |
| Diclofenac | (0.19-0.96) 0.60 | (1.38-3.28) 2.14 | <0.02 | <0.30 | (1.11-1.65) 1.34 | <0.02 | (7.10)3.02(5) |
| Ketoprofen | (0.85-1.46) 1.16 | (0.54-1.00) 0.77 | <0.01 | <1.15 | (0.89-1.38) 1.01 | <0.01 | 0.60(4) |
| Caffeine | (17.32-34.86) 26.65 | (4.19-6.28) 5.50 | 1.37 | 68.7 | (36.20-37.52) 37.61 | 2.35 | (640) 230(5) |
| **Fragances** | | | | | | | |
| Methyl-dihydrojasmonate | (6.88-10.80) 9.06 | (13.28-17.30) 15.27 | 3.79 | 99 | (37.02-38.71) 38.0.1 | 19.2 | 4.48 ± 0.675(33) |
| Galaxolide | (0.23-0.43) 0.36 | (0.85-1.14) 0.98 | 7.12 | 824 | (0.60-1.32) 0.92 | 11.2 | (2.10-3.18) 2.89(7) / 10.7 ± 0.62(33) |
| Tonalide | (0.14-0.21) 0.17 | (0.35-0.45) 0.40 | 4.52 | 449 | (0.20-0.38) 0.28 | 4.33 | (0.9-1.69) 1.37(7) / 13.7 ± 1.5(33) |
| Samples per campaign | 4 | 5 | 2 | 1 | 4 | 3 | |

* related to the first section of the shallow bed.

**Table 3SI.** In parenthesis the probabilities of the t-test on the removal between C2 vs. D2 and 2004 vs. 2005 campaigns.

| | *C2 vs. D2* | C2 0.5m | D2 0.27m |
|---|---|---|---|
| | *(0.5m/0.27m)* | *(2004 vs. 2005)* | *(2004 vs. 2005)* |
| **Pharmaceuticals** | | | |
| Salicylic acid | *(0.27)* | *(0.00[&])* | *(0.03[&])* |
| Ibuprofen | *(0.00[&])* | *(0.09[&])* | *(0.14)* |
| OH-ibuprofen | *(0.00[&])* | *(0.05)* | *(0.47)* |
| CA-ibuprofen | *(0.00[&])* | *(0.00[&])* | *(0.25)* |
| Naproxen | *(0.00[&])* | *(0.01[&])* | *(0.06)* |
| Diclofenac | *(0.00[&])* | *(0.09)* | *(0.01[&])* |
| Ketoprofen | *(0.00[&])* | *(0.39)* | *(0.01[&])* |
| Caffeine | *(0.00[&])* | *(0.00[&])* | *(0.00[&])* |
| **Fragances** | | | |
| Methyl-dihydrojasmonate | *(0.02[&])* | *(0.00[&])* | *(0.76)* |
| Galaxolide | *(0.25)* | *(0.06)* | *(0.42)* |
| Tonalide | *(0.13)* | *(0.00[&])* | *(0.00[&])* |

[&] Statistically significant differences at a significance level of 0.050.

## 2.3.1.5. Discussió de les metodologies analítiques

Tot i que les metodologies analítiques ja han estat explicades en els articles corresponents, en el següent apartat s'assenyalen alguns dels detalls que no han estat comentats en ells. La Taula 2.2 té com a objectiu recollir-les totes elles fent especial referència al tipus de mostra.

**Taula 2.2.** Resum dels diferents mètodes analítics emprats per l'anàlisi dels PPCPs*.

| Nº | Tipus de mostra | Preparació de mostra | | | Sep. | Det. | Rec. (%) | LOD/ LOQ ($\mu$g L$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | | Extracció | | Purificació addicional | | | | |
| 1 | Aquosa (200 mL) | SPE | | No requerida | HPLC (C-18) | UV | 80-98 | 0.15-0.24/ 0.48-1.05 |
| 2 | Aquosa (500 mL) | SPE | | No requerida | GC (DB-5) | MS | >90 | 0.5-150/ 1.7-500$^{a}$ |
| 3 | Sòlids suspesos | Vòrtex i ultrasons | FI | Dilució amb aigua Milli Q + SPE | | | 80-90 | 0.97-1.1/ 2.5-3.2 |
| | | | FII | Dilució amb aigua Milli Q + SPE | | | 70 | 0.02/0.04 |
| 4 | Grava i sediment | PSE | FI | Dilució amb aigua Milli Q + SPE | | | 70-90 | 2.9-4.0/ 4.3-10$^{b}$ |
| | | | FII | Dilució amb aigua Milli Q + SPE | | | 70 | 0.16/0.35$^{b}$ |

*vegi's *articles 1 i 3* per més detall sobre les condicions analítiques, Sep. Separació, Det. Detecció, Rec. Recuperació, FI i FII corresponen a la fracció de compostos neutres i àcids, respectivament, $^{a}$ en ng L$^{-1}$, $^{b}$en $\mu$g kg$^{-1}$.

### a) Preparació de mostra

**Anàlisi de la fase dissolta**

Per tal de dur a terme l'extracció dels anàlits d'interès en mostres d'aigües residuals es van fer proves tant en cartutxos reblerts de fase polimèrica (STRATA X,100 mg, Phenomenex, Torrance, USA) com en cartutxos d'octadecilsilà (C-18, 100 mg Interchrom, França). A la Taula 2.3 es resumeixen les recuperacions obtingudes per als tres fàrmacs inclosos en *l'article 1 i 2*. Els cartutxos polimèrics van mostrar recuperacions molt millors, especialment pel que fa a la carbamazepina i l'àcid clofíbric. Així doncs, es van seleccionar els cartutxos STRATA X per tal de realitzar l'anàlisi de les mostres.

**Taula 2.3.** Percentatges de recuperació d'una mostra de 200 mL d'aigua residual fortificada amb 5 µg de cada fàrmac individual i acidificada a pH 2 (**Mètode 1**).

| | ***STRATA X (%)*** | ***C-18 (%)*** |
|---|---|---|
| Carbamazepina | 98 | 12 |
| Àcid clofíbric | 97 | 48 |
| Ibuprofèn | 99 | 98 |

Per altra banda, es va realitzar l'estudi del volum de ruptura dels cartutxos STRATA X per a tots els PPCPs estudiats en els tres articles. Es van percolar diferents volums (200, 500 i 1000 mL ) d'aigua residual acidificada i fortificada amb 0.5 µg $L^{-1}$ de cada compost individual. Tal i com s'observa en la Figura 2.3, el volum de ruptura per a la major part dels compostos àcids s'assolia quan es percolàven 1000 mL mostra aquosa fortificada. Així doncs, en aquesta Tesi es va optar per treballar amb volums de mostra que no excedeixin els 500 mL.

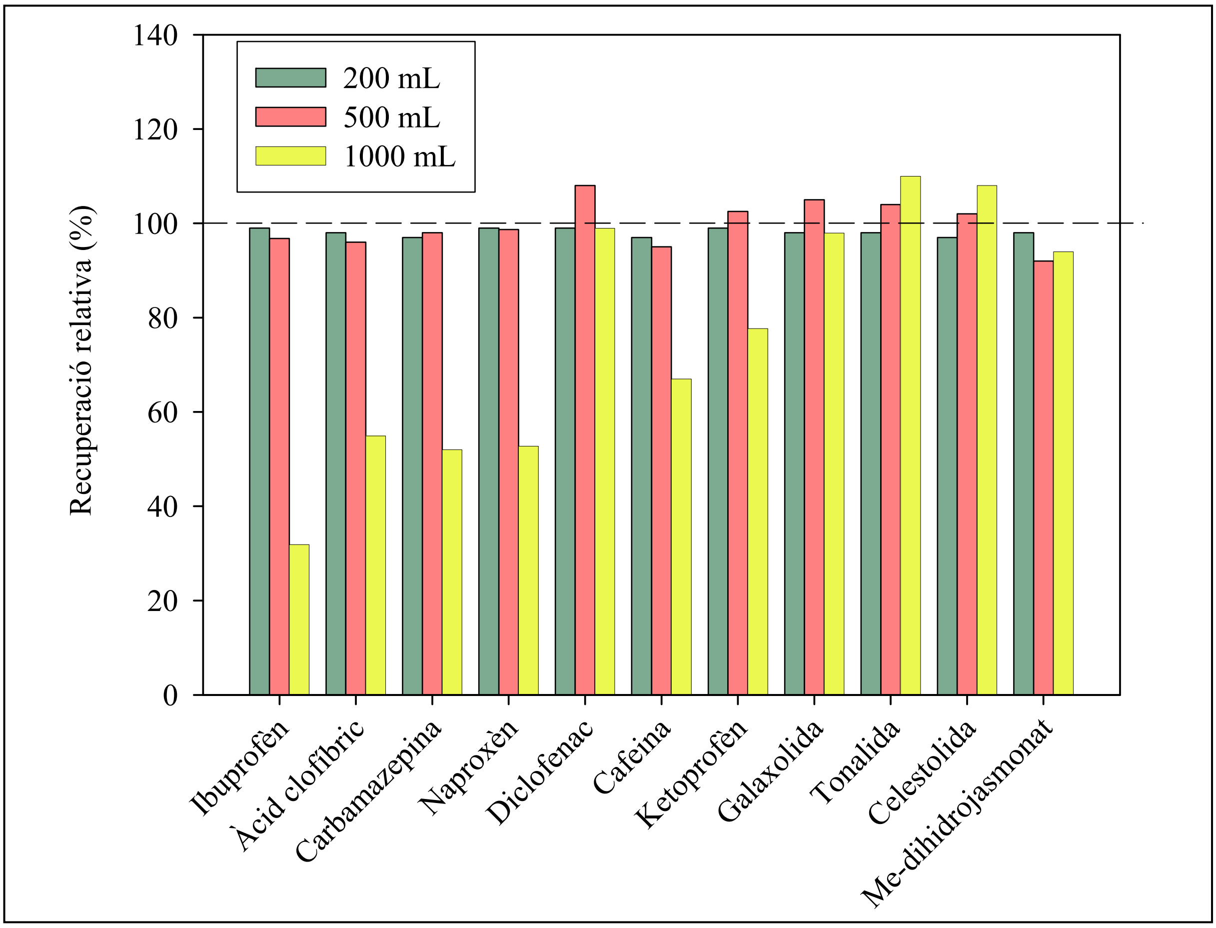


**Figura 2.3.** Avaluació del volum de ruptura dels cartutxos STRATA X per als PPCPs seleccionats (**Mètode 2**).

Seguidament es va dur a terme l'ajust de l'elució dels anàlits. Inicialment es va emprar metanol com a eluent, però posteriorment, per tal d'eliminar al màxim l'efecte de la matriu en la mostra eluïda, aquesta elució es va optimitzar fins a una barreja hexà/acetat d'etil (1/1, v/v).

**Anàlisi de PPCPs en mostres de graves i sòlids suspesos**

La primera aproximació a l'extracció de PPCPs en graves es va dur a terme en *l'article 1*, on es va adaptar una metodologia prèviament descrita (Loffler i Ternes, 2003). Tot i això, posteriroment, i amb la idea de poder tenir els compostos separats en dues fraccions, una que inclogués els compostos neutres i una altra els àcids, es va optar per realitzar una extracció en dos passos (*article 3*). Tot i que el fraccionament dels diferents tipus de PPCPs en mostres de grava va donar resultats molt favorables, separant ambdós grups de compostos (mètode 4), el fraccionament dut a terme per a les mostres de filtres va ser molt dependent de la quantitat de matèria orgànica present (mètode 3). Els paràmetres de qualitat analítica es presenten a l'apartat corresponent dins d'aquesta mateixa secció.

**b) derivatització.**

Per tal de realitzar la derivatització dels compostos es van assajar dos agents derivatitzants diferents; el BSTFA i el TMSH. Tot i que ambdós agents eren capaços derivatitzar tots el PPCPs que presentaven un grup carboxílic, la resposta senyal/soroll per part de l'agent metilant era superior a la de l'agent sililant. A més, donat que el procés de derivatització mitjançant TMSH no requereix passos intermedis d'evaporació i escalfament, es va escollir el TMSH com a agent derivatitzant per a l'estudi de PPCPs, tant en mostres aquoses com en les procedents del lixiviat de graves i sediments.

**c) Separació cromatogràfica i detecció.**

En les Figures 2.4 i 2.5 es mostren els cromatogrames resultants de l'anàlisi dels PPCPs per GC-MS i per HPLC-UV, respectivament. Tal i com es pot observar, ambdós cromatogrames mostren una resolució satisfactòria dels anàlits d'interès.

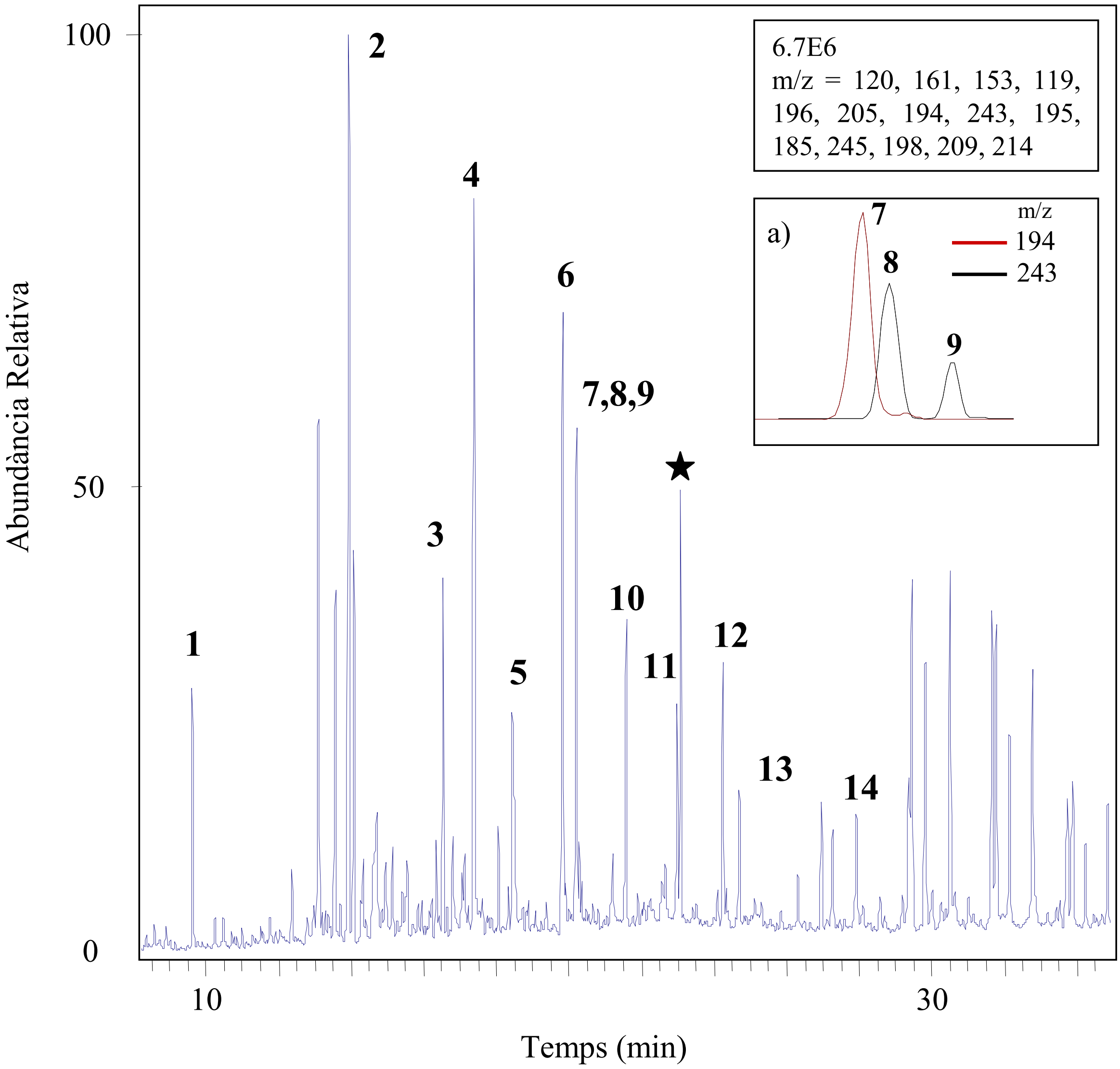


**Figura 2.4.** Registre dels ions d'interès d'una mostra d'aigua residual procedent de l'afluent als aiguamolls de les Franqueses del Vallès. Identificació dels compostos: (1) àcid salicílic, (2) ibuprofèn, (3) Me-dihidrojasmonat, (4) OH-ibuprofèn, (5) fenoprop (*surrogate*), (6) CA-ibuprofèn, (7) cafeïna, (8) galaxolida, (9) tonalida, (10) dihidrocarbamazepina (*surrogate*), (11) naproxèn, (12) 2,2'-dinitrofenil (*surrogate*), (13) ketoprofèn, (14) diclofenac, (*) trifenilamina (patró intern) (**mètode 3**). a) ampliació de la regió corresponent als analits 7, 8 i 9.

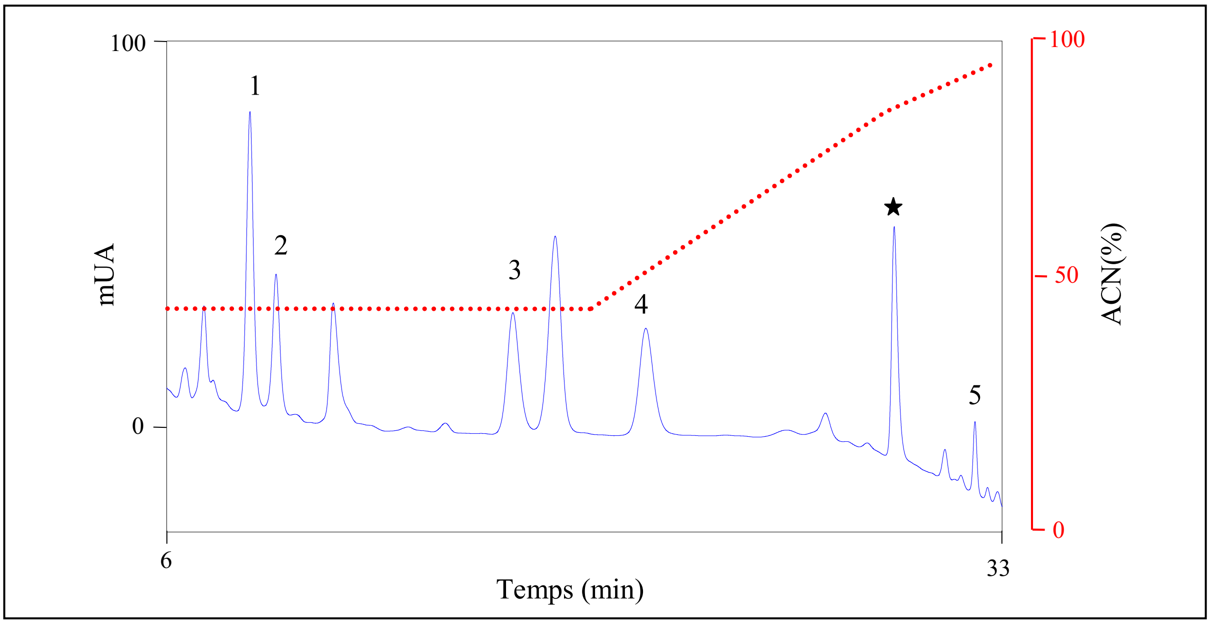


**Figura 2.5.** Cromatograma d'una mostra real de l'efluent de l'aiguamoll somer durant l'experiment d'injecció (*article1*), (1) carbamazepina, (2) dihidrocarbamazepina (*surrogate*); (3) àcid clofíbric, (4) fenoprop (*surrogate*), (*) mecoprop (*patró intern*), (5) ibuprofèn. La ratlla discontínua representa el percentatge de fase B corresponent al gradient binari (fase A, $H_2O$ amb d'àcid fòrmic al 0.2% (v/v) i fase B, acetonitril) (**mètode 1**).

### c) Paràmetres de qualitat analítica

Avaluació de la precisió dels mètodes

L'avaluació de la repetibilitat es va dur a terme amb una mostra d'aigua residual procedent de l'afluent de l'aiguamoll de les Franqueses del Vallès. Es va dividir la mostra en tres submostres de 500 mL cadascuna, i seguidament es va processar tot seguint la metodologia experimental descrita en *l'article 3* (mètode 2). El mateix es va fer per una mostra d'aigua residual sintètica dopada amb 25 μg $L^{-1}$ d'àcid clofibric, ibuprofèn i carbamazepina, dividida en tres submostres de 200 mL (mètode 1).

Per tal d'avaluar la repetibilitat de la metodologia d'anàlisi dels PPCPs en els sòlids suspesos, es van filtrar 500 mL d'aigua residual per triplicat, tractant els tres filtres resultants tal i com es descriu en *l'article 3* (mètode 3). Finalment, en quant a la repetibilitat en graves, aquesta es va dur a terme novament agafant tres mostres de grava de la part anterior de l'aiguamoll D2 descrit en els *articles 1 i 3* (mètode 4).

**Taula 2.5.** Repetibilitat (n=3) expressada com a percentatge de desviació estàndard relativa (RSD) en cadascun dels mètodes emprats en aquesta Tesi.

| **Mètode*** | **1 (%)** | **2 ( %)** | **3 (%)** | **4 (%)** |
|---|---|---|---|---|
| | **Rep.** | **Rep.** | **Rep.** | **Rep.** |
| Àcid salicílic | - | 5.3 | 14.5 | - |
| Ibuprofèn | 1.7 | 2.0 | 9.3 | 15.0 |
| Àcid clofíbric | 1.4 | - | - | - |
| Carbamazepina | 4.2 | - | - | - |
| Naproxèn | - | 5.7 | - | - |
| Diclofenac | - | 3.8 | - | - |
| Cafeïna | - | 7.7 | 7.2 | 5.2 |
| Ketoprofèn | - | 4.1 | - | - |
| Galaxolida | - | 6.9 | 9.6 | 9.8 |
| Tonalida | - | 5.0 | 11.8 | 11.1 |
| Me-dihidrojasmonat | - | 7.0 | 15.6 | 13.1 |

* Numeració descrita seguint la Taula 2.2. Rep. repetibilitat

Tal i com es mostra en la Taula 2.5, la repetibilitat corresponent a la *metodologia 1* va resultar ser la menor de totes, degut a la major concentració dels anàlits. Així mateix, la repetibilitat obtinguda tant en graves com en sòlids suspesos, en general, va resultar ser més elevada que les corresponents a mostres d'aigua, bàsicament degut a la complexitat de la matriu.

Avaluació dels LOD i LOQ dels mètodes

Els límits de detecció i quantificació (LODs i LOQs) de les metodologies es recullen a la Taula 2.6. El càlcul d'aquests per als mètodes analítics 1 i 2 es va realitzar mitjançant aigua procedent d'un sistema d'aiguamolls a nivell microcosmos alimentat amb aigua residual sintètica (*article 2*) i aigua Milli Q (article 3), respectivament. Pel que fa a l'estudi dels blancs dels filtres es van emprar filtres als quals se'ls havia percolat prèviament 500 mL d'aigua Milli Q. Finalment el blanc de graves es va dur a terme mitjançant una mostra de sorra muflada i processada tal i com s'indica en el mètode 4. El càlcul es va realitzar mesurant l'àrea del blanc més tres o deu cops la seva desviació, segons si es tractava del LOD o el LOQ (n=3).

**Taula 2.6**. LOD i LOQ dels diferents mètodes (*articles 1 i 3)*.

| **Mètode*** | **1** (μg $L^{-1}$) | | **2** (ng $L^{-1}$) | | **3** (μg $L^{-1}$) | | **4** (μg $kg^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | LOD | LOQ | LOD | LOQ | LOD | LOQ | LOD | LOQ |
| Àcid salicílic | - | - | 2.0 | 6.5 | 0.02 | 0.06 | 0.18 | 0.48 |
| Ibuprofèn | 0.15 | 0.48 | 2.2 | 7.5 | 0.02 | 0.04 | 0.16 | 0.35 |
| Àcid clofíbric | 0.25 | 1.05 | 0.5 | 1.7 | 0.03 | 0.08 | 0.11 | 0.35 |
| Carbamazepina | 0.19 | 0.67 | 22 | 67 | 0.10 | 0.44 | 0.60 | 1.20 |
| Naproxèn | - | - | 1.2 | 3.8 | 0.02 | 0.07 | 0.26 | 0.75 |
| Diclofenac | - | - | 1.0 | 4.2 | 0.02 | 0.08 | 0.30 | 1.20 |
| Cafeïna | - | - | 150 | 500 | 0.97 | 2.87 | 2.85 | 4.30 |
| Ketoprofèn | - | - | 1.1 | 3.3 | 0.01 | 0.05 | 1.15 | 3.50 |
| Galaxolida | - | - | 40 | 120 | 1.10 | 3.20 | 4.00 | 10.0 |
| Tonalida | - | - | 90 | 320 | 0.90 | 2.50 | 2.50 | 6.00 |
| Me-dihidrojasmonat | - | - | 120 | 220 | 0.98 | 2.60 | 3.00 | 6.10 |

* Numeració descrita seguint la Taula 2.1.

A la Taula 2.6 s'observa com els LODs i LOQs dels compostos analitzats mitjançant GC-MS, prèvia derivatització, van ser molt més baixos que no els corresponents a aquells compostos que no es van derivatitzar, (LOD i LOQ en cursiva a la Taula 2.6).

Avaluació de la recuperació dels mètodes

Donada la impossibilitat d'obtenir mostres reals sense aquests compostos, l'avaluació de la recuperació dels compostos individuals per a les diferents matrius plantejades (aigua, sòlids suspesos i grava) es va realitzar recollint sis submostres de cada tipus de matriu amb la màxima homogenitat possible, fortificant la meitat d'elles a l'inici de la metodologia, mentre l'altra meitat van ser dopades en el pas final abans de la seva injecció en el GC-MS. De la comparació de la senyal estandarditzada dels dos subgrups de mostres es va calcular el percentatge de recuperació individual. En tots els casos la fortificació individual es corresponia entre un 100 i 200% de la concentració dels compostos presents en les mostres. El temps d'equilibri entre la matriu i els anàlits va ser de 12 hores.

Així doncs, per una banda l'avaluació de la recuperació de cada compost individual en aigua es va dur a terme amb una mostra d'aigua residual decantada procedent del clavegueram situat a la part nord del campus de la UPC (Barcelona). Aquesta mostra es va dividir en sis submostres de 500 mL cadascuna, seguidament es va filtrar cada submostra i els filtres resultants es van emmagatzemar a -20 ºC per al seu posterior anàlisi. Totes les mostres d'aigua, fortificades a l'inici o al final de la metodologia (amb concentracions d'entre 1 i 6 µg $L^{-1}$), van seguir el processat experimental descrit en *l'article 3* (mètode 2).

Per altra banda, per tal d'avaluar la recuperació de cada compost individual en els sòlids suspesos, es van recuperar els 6 filtres anteriors. Donada la major presència dels compostos apolars en els sòlids suspesos, els filtres es van fortificar amb concentracions entre 5 i 7.5 µg $L^{-1}$ per a les fragàncies (galaxolida i tonalida) i entre 0.2 i 0.4 µg $L^{-1}$ per a la resta de compostos. Finalment les mostres es van processar tal i com es descriu en *l'article 3* (mètode 3). L'avaluació de la recuperació en mostres de graves es va dur a terme amb sis mostres de grava procedents de la part anterior d'un dels aiguamolls de les Franqueses del Vallès (mètode 4). En funció de les concentracions trobades en aquestes mateixes mostres (*article 3*) es van fortificar les mostres amb concentracions entre 400 i 800 µg $kg^{-1}$ per a les fragàncies (galaxolida i tonalida) i entre 6 i10 µg $kg^{-1}$ per a la resta de compostos.

**Taula 2.7.** Recuperació i percentatge de desviació estàndard relativa (RSD) en cadascun dels mètodes emprats en aquesta Tesi.

| **Mètode*** | **2 (%)** | | **3 (%)** | | **4 (%)** | |
|---|---|---|---|---|---|---|
| | **Rec.** | **RSD** | **Rec.** | **RSD** | **Rec.** | **RSD** |
| Àcid salicílic | 94 | 9 | 80 | 8 | 90 | 5 |
| Ibuprofèn | 93 | 10 | 92 | 2 | 89 | 10 |
| Àcid clofíbric | 95 | 12 | 97 | 4 | 94 | 6 |
| Carbamazepina | 84 | 16 | 87 | 7 | 85 | 10 |
| Naproxèn | 91 | 11 | 91 | 3 | 92 | 9 |
| Diclofenac | 89 | 11 | 91 | 6 | 94 | 8 |
| Cafeïna | 82 | 4 | 85 | 9 | 80 | 11 |
| Ketoprofèn | 86 | 11 | 96 | 3 | 94 | 6 |
| Galaxolida | 88 | 10 | 98 | 6 | 87 | 10 |
| Tonalida | 85 | 10 | 94 | 4 | 90 | 9 |
| Me-dihidrojasmonat | 95 | 8 | 89 | 3 | 87 | 11 |
| *Dihidrocarbamazepina* | *82* | *5* | *95* | *5* | *77* | *14* |
| *Fenoprop* | *97* | *5* | *82* | *5* | *89* | *8* |
| *2,2'-dinitrofenil* | *93* | *10* | *92* | *6* | *95* | *7* |

* Numeració descrita seguint la Taula 2.1. En cursiva s'assenyalen els compostos emprats com a *surrogates.*

Tal i com mostra la Taula 2.7, la recuperació de cada compost per totes les metodologies considerades va ser superior al 80%, i sempre dintre dels valors descrits anteriorment per altres metodologies emprant GC-MS, tant en aigües (Gomez et al., 2007) com en sediments (Loffler i Ternes, 2003). Per altra banda, es va observar una relació estreta entre el comportament dels *surrogates* i els PPCPs, posant de manifest la idoneïtat del seu ús.

### 2.3.1.6. Discussió general dels resultats

La injecció directa de tres fàrmacs en un aiguamoll construït, permet esbrinar el processos fisicoquímics i biològics que hi tenen lloc. Així doncs, atès a que molts dels productes estudiats en aquesta Tesi poden ionitzar-se, el seu *log Dow* és un bon descriptor a l'hora de predir el seu comportament. En aquest sentit, l'àcid clofibric no presenta cap tipus de retenció en el sistema, recuperant-se la totalitat del producte injectat i podent-se assimilar el seu comportament al d'un traçador (*article 1*). Per altra banda, la carbamazepina presenta certa retenció, tal i com ho mostra el seu major HRT i presència més elevada en les graves. Aquest mateix comportament ha estat descrit en estudis duts a terme en sediments (Loffler et al., 2005), sòls de diferent concentració de matèria orgànica (Drillia et al., 2005) i en llots de depuradores (Ternes et al., 2004). De fet, l'àcid clofíbric és el compost més abundant en les aigües subterrànies procedents de la zona de Berlin, on durant anys s'ha realitzat la recàrrega d'aqüífers mitjançant aigües residuals tractades, bé sigui per injecció directa o filtració a través de llits de sorra (*bank filtration*, BF) (Heberer, 2002).

Per altra banda, tot i que l'ibuprofèn presenta un log Dow semblant al de l'àcid clofíbric, el primer es degrada i el segon compost és refractari. L'avaluació de la ruta d'eliminació d'ibuprofèn no es va poder dur a terme a l'experiment d'injecció de fàrmacs de *l'article 1* donat que els metabòlits descrits en la literatura també tenen el seu origen en el metabolisme humà d'aquest fàrmac i per tant eren presents a elevades concentracions en l'afluent de l'aiguamoll. Per tal d'eliminar aquesta interferència, es va treballar amb un aiguamoll a escala microcosmos alimentat amb aigua residual sintètica (*article 2*). Els resultats atribueixen condicions aeròbies i anaeròbies al sistema, degut primerament a les condicions de saturació d'oxigen de l'aigua residual sintètica dosificada. Tot i que aquest oxigen es consumeix ràpidament en la primera part del HFCW, aquest conjuntament amb la transferència d'oxigen a través de la grava i les plantes facilitaria que entre un 10 i 30 % de rutes d'eliminació de l'ibuprofèn fossin en condicions aeròbies.

La major eficiència de l'aiguamoll somer a l'hora d'eliminar gran part de PPCPs estudiats (*articles 1 i 3*) s'explica pel fet que al comparar dos sistemes, que disposen de la mateixa superfície i diferent profunditat, la relació entre la superfície i el volum és un paràmetre clau. Aquesta relació és més elevada en un sistema somer que en un de profund, per tant, la transferència d'oxigen a través de la vegetació o de les graves és major. Aquesta idea ve recolzada per la major presència de nitrats en l'efluent del sistema somer (Garcia et al., 2005), resultat de la nitrificació de l'amoni present en l'aigua residual, procés que té lloc en condicions aeròbies.

A partir dels estudis cinètics duts a terme en *l'article 3,* es pot concloure que l'eliminació de la major part dels PPCPs estudiats en els aiguamolls construïts es dóna en la part anterior del sistema, on es produeix la retenció de la major part dels sòlids suspesos que porta l'aigua residual. Així doncs, la major presència de matèria orgànica i biofilm en aquesta secció, produeix una major eficiència en l'eliminació dels PPCPs que no la que es dóna en les seccions restants (*Figura 2, article 3, pàgina 64*). Això pot ser degut tant a interaccions hidrofòbiques o electrostàtiques, com per biodegradació o incorporació dels contaminants per part del biofilm. En aquest sentit, les cinètiques individuals d'eliminació dels PPCPs es poden emprar per tal de dissenyar aiguamolls que tinguin com a finalitat l'eliminació d'uns contaminants concrets.

## 2.3.2. Eliminació de substàncies prioritàries de la Directiva Marc

### 2.3.2.1. Introducció

Tot i que l'ús dels aiguamolls construïts a l'hora d'eliminar o atenuar l'abocament de PPCPs al medi ja ha estat demostrat en els articles anteriors, la possibilitat d'emprar-los per controlar l'abocament de substàncies establertes com a perilloses en la Directiva Marc de l'Aigua encara no ha estat estudiada. Així doncs, en aquesta secció es pretén avaluar el grau d'idoneïtat d'aquests sistemes per tal d'atenuar la contaminació d'aquestes substàncies al medi aquàtic.

En aquest sentit, en l'article "*Behavior of selected priority organic pollutants in horizontal subsurface flow constructed wetlands: A preliminary screening*" es realitzà la injecció puntual de 8 substàncies perilloses establertes com a tals per la Decisió nº 2455/2001/CE, conjuntament amb un traçador. El traçador seleccionat va ser l'àcid clofíbric ja que en l'article anterior (*article 1*) es va veure que es comportava de la mateixa manera que el bromur. Tot i que la injecció es va realitzar en pocs minuts, per tal de simular els abocaments puntuals que es realitzen d'aquestes substàncies en el medi ambient, la recollida de mostres de l'efluent de l'aiguamoll es va dur a terme durant les tres setmanes posteriors.

Per a aquesta finalitat es van posar a punt metodologies analítiques per analitzar aquests compostos de manera simultània en la fase dissolta i particulada. Per altra banda, també es van dissenyar metodologies per tal d'analitzar aquests compostos en les graves de l'aiguamoll.

### 2.3.2.2 Article 4: Behavior of selected priority organic pollutants in horizontal subsurface flow constructed wetlands: A preliminary screening.

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# Behavior of selected priority organic pollutants in horizontal subsurface flow constructed wetlands: A preliminary screening

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## Abstract

A discrete injection experiment was carried out in a constructed wetland to evaluate the behavior of selected priority pollutants. A horizontal subsurface flow pilot plant located in the NE of Spain was selected for this study. A total of eight European Priority Pollutants listed in the Water Framework Directive were considered, including a commonly used herbicide (mecoprop). The pollutants encompassed a variety of chemical classes and physicochemical properties. They included organochlorine, organophosphorus, phenols, chloroacetanilides, triazine, phenoxycarboxylic acid and phenylurea pesticides. A time series of composite effluent samples and discrete gravel bed samples from the wetland were analyzed. Response curves for all the pollutants injected from effluent concentrations were obtained and compared with the tracer (clofibric acid). On the basis of an analysis of the samples taken 21 days after the injection, priority pollutants were classified into four groups according to their removal efficiency. These groups were (i) the highly efficiently removed (>90%), namely lindane, pentachlorophenol, endosulfan and pentachlorobenzene; (ii) the efficiently removed (80–90%), namely alachlor and chlorpyriphos; (iii) poorly removed (20%), namely mecoprop and simazine; and (iv) recalcitrant to elimination, namely clofibric acid and diuron. Taking into account the poor accumulation of the injected contaminants in the gravel bed (0–20%), biodegradation and plant uptake are postulated as the most likely elimination pathways for the pollutants.

*Keywords:* Constructed wetland; European water framework directive; Priority pollutants; Gravel bed accumulation; Pollutant injection; Removal efficiency

## 1. Introduction

Decision no. 2455/2001/EC of the European Parliament and the Council of 20 November 2001 established a list of 33 priority pollutants in the water policies that had to be implemented by the Member States. This list includes a variety of biocides, additives and by-products, whose emission, discharge and loss from water effluents need to be controlled in order to improve aquatic environmental conditions throughout the EU. In accordance with Article 1(c) of Directive 2000/60/EC, future reviews of the priority substances list will contribute to the cessation of the emission, discharge and loss of all hazardous substances by 2020. Therefore, technologies to remove or attenuate the contamination of surface waters by priority pollutants must be implemented.

Constructed wetlands (CWs) used for municipal wastewater treatment have a certain capacity for removing specific organic pollutants (Haberl et al., 2003; Huang et al., 2004; Matamoros et al., 2005; Matamoros and Bayona, 2006). Nevertheless, to date few studies have assessed the feasibility of using horizontal subsurface flow (SSF) CWs to remove most of the priority pollutants in the European Water Framework Directive.

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In this study, we focused on the behavior of eight EU-regulated pollutants (simazine, alachlor, chlorpyriphos, pentachlorobenzene, pentachlorophenol, endosulfan, diuron and lindane) and one commonly used acid herbicide (mecoprop) in a pilot SSF CW system planted with *Phragmites australis.* The behavior of these pollutants was compared to that of clofibric acid, which was used as a conservative tracer (Matamoros et al., 2005).

## 2. Material and methods

### 2.1. Chemicals

GC grade (Suprasolv) hexane, methanol, ethyl acetate, acetone and dichloromethane were obtained from Merck (Darmstadt, Germany). Analytical grade acetic and hydrogen chloride acids were obtained from Panreac (Barcelona, Spain). Clofibric acid (97%), mecoprop (99%), simazine (99%), alachlor (99%), chlorpyriphos (97%), pentachlorobenzene (99%), pentachlorophenol (98%), endosulfan (99%), diuron (99%), lindane (97%), triphenylphosphate (99%), terbutylazine (98%), fenoprop (99%), endrin (99%), pentabromophenol (96%), monuron (99%), metalachlor (99%), PCB 166 (99%) and trimethylsulfonium hydroxide (TMSH) were purchased from Sigma–Aldrich (Steinheim, Germany).

### 2.2. Pilot plant

Experiments were carried out in a horizontal SSF CW pilot plant located in the municipality of Les Franqueses del Vallès (Barcelona, Spain). The plant partially treats the urban wastewater generated by a housing development (ca. 200 inhabitants). First, the municipal wastewater is screened. It then flows into an Imhoff tank from which it is conveyed to eight wetlands in parallel (further information about the plant configuration and its performance can be found in García et al., 2005). One of these eight wetlands was selected for the purposes of this study because in a long-term evaluation, it was the most efficient at removing BOD, ammonia, and specific organic contaminants (Huang et al., 2004; García et al., 2005; Matamoros et al., 2005; Matamoros and Bayona, 2006). The wetland is characterized by an average water depth of 0.3 m, a surface area of 55 $m^2$, an average granular size of 3.5 mm and a hydraulic loading rate (HLR) at the time of the experiments of 36 mm $d^{-1}$.

### 2.3. Experimental design and sampling

About 40 l of distilled water contained in a glass bottle were spiked with 100 mg of each pollutant. The pollutants had previously been dissolved in 100 ml acetone. This mixture was homogenized and injected by a single-shot into the inlet tubing of the SSF CW in July 2005, when vegetation was well developed. Effluent composite samples were collected every 6 h using an American Sigma 900 autosampler (Sigma, Loveland, CO) for a time period of 450 h (more than four times the nominal hydraulic retention time). All the samples were collected in 1 l amber glass bottles and kept refrigerated during the sampling and transportation to the laboratory, where they were held at 4 °C until analysis. The sample holding time was less than two days. In addition, two gravel samples with accumulated organic matter and biofilms were sampled at 2 m from the wetland inlet and kept at −20 °C until analyzed for priority pollutants. Moreover, during the injection experiment, seven influent and effluent grab samples were taken on different days and immediately analyzed as a composite sample for general wastewater parameters. Samples from gravel, water influent and effluent were taken before injection to ensure that the pollutants were not present.

### 2.4. Wastewater analysis

General wastewater parameters, including $BOD_5$, ammonium, sulfates, total COD and soluble COD (of samples filtered through 0.2 μm), were analyzed following the APHA-AWWA-WPCF methods (APHA-AWWA-WPCF, 2001). Temperature and pH were measured *in situ* using a Chektemp-1 Hanna thermometer and a Crison pH-meter, respectively.

In order to analyze pollutants from wetland effluent, an unfiltered sample volume of 200 ml was acidified to pH 2 with concentrated hydrochloric acid. The samples were then spiked with 1250 ng of a surrogate standard mix (fenoprop for clofibric acid and mecoprop; terbutylazine for simazine; triphenylphosphate for chlorpyriphos; monuron for diuron; metalachlor for alachlor; endrin for endosulfan; and PCB 166 for pentachlorobenzene and pentachlorophenol). The spiked samples were percolated through Strata X solid-phase extraction cartridges (Phenomenex, Torrance, CA). These cartridges consist of a surface modified styrene-divinylbenzene copolymer with a modified pyrrolidone surface. The detailed preconcentration procedure has been published elsewhere (Matamoros et al., 2005). The loaded cartridges were eluted with 10 ml hexane/ethyl acetate (1:1). The extract was evaporated under a gentle nitrogen stream until it reached ca. 20 μl, and 186 ng of triphenylamine was added as an internal standard. The vial was then reconstituted to 300 μl with ethyl acetate.

Two aliquots of 50 μl of each sample were taken. The first aliquot was derivatized using 10 μl of TMSH, whilst the second was not derivatized (apart from alachlor, due its degradation by TMSH in the injector). The aliquots were then injected onto a TRACE-GC-MS (Thermo-Finnigan, Dreieich, Germany) equipped with an electron impact ion source (70 eV ionization energy). A 30 m × 0.25 mm i.d. × 0.25 μm DB-5 (J&W Scientific, Folsom, CA) was used. Instrumental conditions have been reported elsewhere (Matamoros and Bayona, 2006). The following diag-

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nostic ions were monitored by time-scheduled acquisition for target analyte quantification (in bold) and identification: diuron as isocyanate, **187**/189/124; pentachlorobenzene, **250**/252/215; simazine, **201**/186/173; lindane, **181**/219/109; alachlor, 160/188/146; chlorpyriphos, 267/269/323; endosulfan, **195**/237/159; pentachlorophenol, **266**/268/264; clofibric acid, 128/169/228; mecoprop, **169**/228/142; monuron as isocyanate, **153**/155/125; terbutylazine **214**/229/173; triphenylphosphate, **326**, 325, 215; metalachlor, **162**/238/240; PCB 166, **360**/362/358; endrin, **263**/265/261 and fenoprop, **196**/223/282.

The linearity range was from 0.1 to 100 (μg ml$^{-1}$) for all compounds. The LODs and LOQs were compound dependent. They ranged from 0.1 to 0.4 μg l$^{-1}$ and from 0.2 to 0.9 μg l$^{-1}$, respectively. The linear correlation coefficient ($R^2$) of the calibration curve was always higher than 0.998. The surrogate recoveries were always higher than 90%. Recoveries were confirmed by the addition of 1000 ng of pollutants and surrogates to a 200 ml sample of water effluent collected before the single-shot injection. Recoveries were higher than 90% for all compounds and surrogates. Phenylureas (diuron and monuron) were analyzed as their respective isocyanates, due to their thermal degradation in the GC injector port (Peña et al., 2002).

### *2.5. Gravel analysis*

About 40 g of freeze-dried gravel samples were analyzed using a pressurized solvent extraction (PSE) device (Applied Separations, Allentown, PA) with a 33 ml extraction cell. Extraction conditions were as follows: (a) a first fraction was extracted with 100 °C, 100 bar, and three cycles of 5 min with hexane/acetone (1:1); (b) a second fraction was extracted with acetone/acetic acid (20:1) in order to recover the acidic compounds. The recovered extracts were concentrated to 0.2 ml with a rotary vacuum evaporator. The first fraction, corresponding to non-acidic pollutants, was cleaned up using sodium sulfate (0.5 g) and alumina (1 g) deactivated with Milli-Q water (5%). Then, the analytes were eluted with 10 ml hexane/ethyl acetate. The second fraction, corresponding to acidic analytes (clofibric acid and mecoprop), was reconstituted using 500 ml of Milli-Q water and adjusted to pH 2 with concentrated hydrochloric acid. Then, the reconstituted samples were analyzed following the wastewater methodology, as described previously in this section. The recoveries were from 50% to 100% for neutral compounds and 70% for acidic compounds. The LODs and LOQs (μg Kg$^{-1}$) were between 0.1 to 0.2 and 0.2 to 0.5 respectively.

The LOD and LOQ of the analytical procedures were determined using effluent and gravel samples collected before the injection experiment. The LOD and LOQ were determined from the mean background noise plus three or ten times the standard deviation of the background noise respectively.

## 3. Results and discussion

### *3.1. General parameters*

During the experimental period, the average surface organic loading rate was 6.8 g $BOD_5$ m$^{-2}$ d$^{-1}$. The $BOD_5$ removal efficiency obtained during the experiment was consistent with the average efficiencies found in a long-term evaluation carried out in this wetland (Table 1). The long-term data series shows that ammonium removal efficiency was quite high in this wetland, as the removal rates in horizontal SSF CWs are usually under 50% (García et al., 2005). The low removal of sulfates suggests that sulfate reduction and other anaerobic reactions such as methanogenesis were not of major importance to the removal of organic matter at the time of the experiment. The absence of nitrate (in conjunction with the high ammonia removal efficiency of the wetlands) and the low oxygen concentration (<1 mg l$^{-1}$) in the effluent suggest that anoxic reactions such as denitrification were of major significance at the time of the experiments. Note that $BOD_5$ removal in horizontal SSF CWs occurs through a combination of different biochemical reactions (aerobic respiration, denitrification, sulfate reduction and methanogenesis). The predominant reaction is determined by the environmental conditions and particularly by the redox status (Aguirre et al., 2005). These reactions can also occur simultaneously in different wetland microsites subjected to different redox conditions (Cooper et al., 1996). This trend was observed in a long-term evaluation of this pilot plant (García et al., 2005).

### *3.2. Priority pollutant behavior*

The use, physicochemical properties and toxicity of the pollutants selected in this study are summarized in Table 2. As can be seen, the pollutants encompass a large variety of chemical classes and physicochemical properties.

Table 1
Average and ±SD of the physico-chemical properties of the influent and the effluents of the pilot horizontal SSF CW during the experiments ($n = 7$) and over a long-term evaluation ($n = 103$, (García et al., 2005))

| | Influent | Effluent | Efficiency (%) |
|---|---|---|---|
| *Experimental period* | | | |
| Temperature (°C) | 19.9 ± 0.8 | 16.5 ± 1.4 | na |
| pH | 7.5 ± 0.2 | 7.7 ± 0.4 | na |
| COD (total) (mg/l) | 283 ± 37 | 49 ± 16 | 82 |
| COD (soluble) (mg/l) | 72 ± 23 | 36 ± 21 | 49 |
| $BOD_5$ (mg/l) | 190 ± 17 | 19 ± 5 | 89 |
| $SO_4^{2-}$ (mg/l) | 102 ± 27 | 93 ± 22 | 9 |
| *Long-term evaluation* | | | |
| COD (total) (mg/l) | 200 ± 82 | 41 ± 24 | 80 |
| $BOD_5$ (mg/l) | 140 ± 54 | 20 ± 19 | 86 |
| [a]$NH_4^+$ (mg N/l) | 44.5 ± 15.9 | 18.1 ± 11.8 | 59 |

Na – not applicable.

[a] During the experiments nitrate was detected neither in influent nor effluent.

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Table 2
Use, toxicity and physicochemical properties of the studied pollutants

| | Use | Log $K_{ow}$[a] | EC50[b] | Toxicity class[c] |
|---|---|---|---|---|
| Simazine (triazines) | Herbicide | 2.18 | 1.1 ppm | Moderately |
| Alachlor (chloroacetanilides) | Herbicide | 3.52 | 27 ppm | Slightly |
| Chlorpyriphos (organophosphorus) | Insecticide nematicide | 4.96 | 0.1–0.6 ppb | Very high |
| Pentachlorobenzene (organochlorine) | Fungicide and flame retardant | 5.17 | 0.3–5.3 ppm | Highly/Moderately |
| Pentachlorophenol (organochlorine) | Insecticide and fungicide | 5.12 | 0.038–2.79 ppm | Highly/Moderately |
| Endosulfan (organochlorine) | Acaricide | 3.84 | 0.16–0.72 ppm | Highly |
| Lindane (organochlorine) | Insecticide | 3.72 | 0.46 ppm | Highly |
| Diuron (phenylurea) | Herbicide | 2.80 | 1.4–8.4 ppm | Moderately |
| Mecoprop[d] (phenoxycarboxylic acid) | Herbicide | −0.87 | 100 ppm | Negligible |
| Clofibric acid[d] | Lipid regulator | −1.30 | 92 ppm | Negligible |

[a] Obtained from EPI suite 3.12 (EPI, 2006).
[b] *Daphnia magna* test, 48 h obtained from PAN (PAN, 2006) pesticides data base.
[c] Classification according to Zucker (Zucker, 1985).
[d] Log Kow were calculated from the ionized form at pH 8 by using SMILES. Pesticide group (in parenthesis).

The behavior of the nine pollutants injected, including a tracer (clofibric acid), is shown in Fig. 1. Clofibric acid was used as a tracer to follow up the wetland response from the injection time. It was chosen because in a previous study it had been found to behave as a conservative pollutant (Matamoros et al., 2005). Furthermore, clofibric acid behaves more conservatively than bromide in wetlands (García et al., 2004; Xu et al., 2004). The absence (<LOD) of endosulfan, lindane and pentachlorobenzene in the wetland effluent could be attributable to their elimination by degradation, plant and/or microbial uptake and/or sorption within the organic matter and biofilm accumulated onto the gravel (see the next section).

An analysis of the response curves shows that some pollutants exhibited either low (i.e. mecoprop, diuron and simazine) or strong attenuation (i.e. alachlor, chlorpyriphos, pentachlorophenol, lindane, pentachlorobenzene and endosulfan) throughout the wetland. The shape of the curves reveals that mecoprop behaves as a tracer, due its similar log $K_{ow}$ (−0.31 and −1.30, respectively). In contrast, diuron and simazine show more dispersion than the other pollutants, due to their higher log $K_{ow}$ (2.8 and 2.2, respectively). The other pollutants cannot be compared at all.

The mean hydraulic retention time (HRT) of the wetland was estimated for compounds that were totally recovered (clofibric acid and diuron, Fig. 2) at 5.4 and 6.2 days, respectively. The difference in the HRTs of the two compounds could be due to the fact that the log $K_{ow}$ for diuron is higher than that of clofibric acid, which occurs in its ionized form (Matamoros et al., 2005).

### 3.3. *Removal efficiency*

The removal was calculated by using cumulative percent mass recovery curves (Fig. 2), as has been described previously for other pollutants (Matamoros et al., 2005). Priority pollutants were classified in four groups according to their removal efficiency (Table 3). These groups were as follows: (i) highly efficiently removed pollutants (>90%), namely lindane, pentachlorophenol, endosulfan and pentachlorobenzene; (ii) efficiently removed pollutants (80–90%), namely alachlor and chlorpyriphos; (iii) poorly removed (20%), namely mecoprop and simazine; (iv) pollutants that were recalcitrant to elimination (0%), namely clofibric and diuron.

Halogenated pollutants were eliminated at a higher rate than other pollutants (Table 3). This could be due to the system's prevailing anoxic conditions. Reductive dehalogenation may occur in such conditions (Van Pée and Unversucht, 2003). In fact, several studies report that the removal of lindane (Buser and Müller, 1995; Middeldorp et al., 1996; Bhat et al., 2005; Quintero et al., 2005), endosulfan (Aslan and Turkman, 2006), pentachlorophenol (Susarla et al., 1997), chlorpyriphos (Moore et al., 2002) and alachlor (Graham et al., 2000) exceeds 90% in anaerobic conditions. Nevertheless, as the matrix complexity hindered the identification of intermediate products in this study, we cannot ascertain the precise elimination pathway. Moreover, we cannot rule out plant uptake of some compounds with a log Kow in the range of 1–4, as reported previously (Burken, 2003).

Other chlorinated products showed low removal, e.g. mecoprop (phenoxycarboxylic acid herbicide). Mecoprop has a similar structure to clofibric acid, but it was only slightly eliminated, as previously observed in aerobic wastewater treatment plants (Bernhard et al., 2006). In fact, several studies have shown that mecoprop degrades aerobically but not anaerobically (Harison et al., 1998). Nevertheless, mecoprop degradation has been described recently in reductive denitrification conditions (Harrison et al., 2003). Diuron is generally considered as persistent in soil, water and ground water (Giacomazzi and Cochet, 2004). However, slight removal has been observed in ponds (Rose et al., 2006). Its removal was not observed in this study.

The behavior of the wetland was similar to that of anaerobic reactors (Table 3). The literature shows that removal is higher than 99% in anaerobic reactors. In fact, reductive dehalogenation has been described as the fastest removal pathway for highly chlorinated compounds (Armenante et al., 1999).

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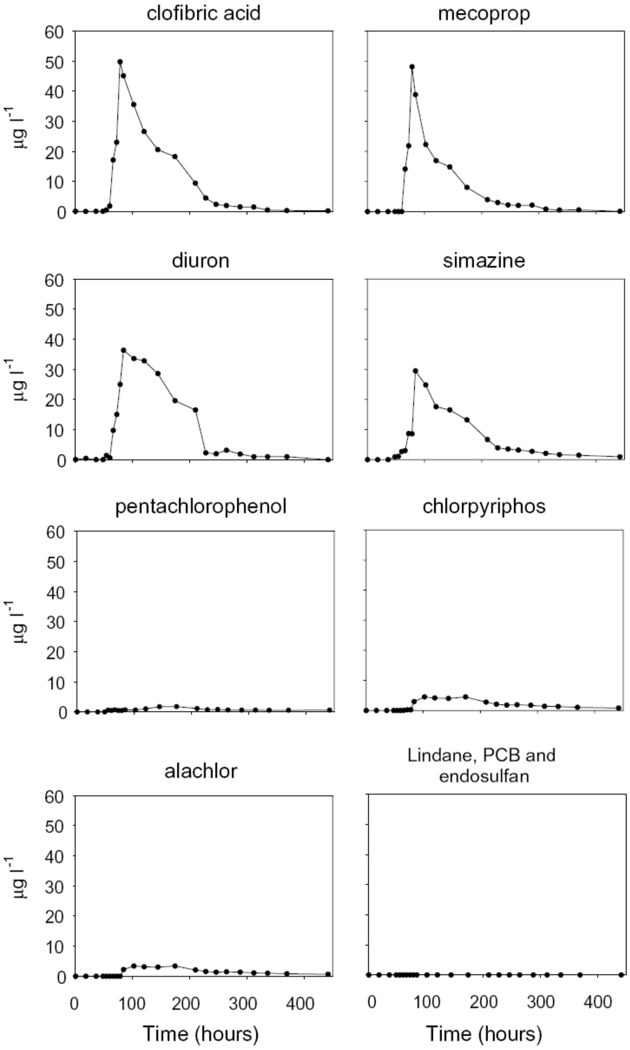

Fig. 1. Behavior of selected pollutants and clofibric acid used as a tracer. Pentachlorobenzene (PCB), endosulfan and lindane were always below the LOD.

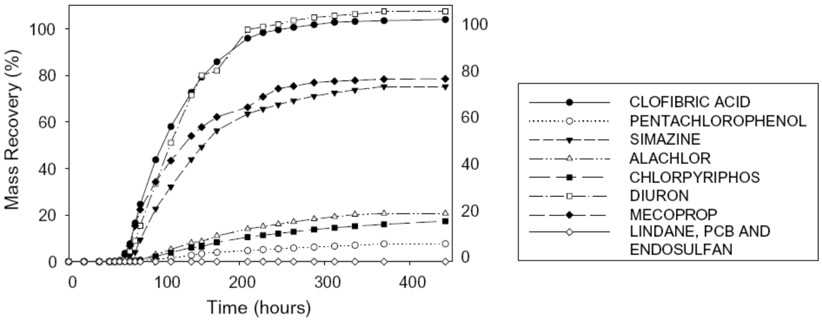

Fig. 2. Cumulative percent mass recovery for pollutants and tracer.

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Table 3
Removal efficiency (%) of pollutants after single-shot injection experiment obtained from mass recovery and comparison with other systems

| | SSF CW (%) | System | HRT (days) | Efficiency (%) | Reference |
|---|---|---|---|---|---|
| Simazine | 25 | SSF CW | 2.3 | 64 | Stearman et al. (2003) |
| | | SSF CW | 20 | 95 | |
| Alachlor | 80 | Biodenitrification reactor | 9.7[a] | 50[a] | Graham et al. (2000) |
| Chlorpyriphos | 83 | SSF CW | 3.0 | 98 | Moore et al. (2002) |
| Pentachlorobenzene | >99 | – | – | – | – |
| Pentachlorophenol | 94 | UASB reactor | 0.8–0.9 | >99 | Shen et al. (2006) |
| Endosulfan | >99 | Biodenitrification reactor | 0.2–0.3 | >95 | Aslan and Turkman (2006) |
| Lindane | >99 | UASB reactor | 2 | 98 | Bhat et al. (2005) |
| Diuron | 0 | Ponded wetland[b] | 7–13 | 27–55 | Rose et al. (2006) |
| Mecoprop | 22 | Complete WWTP | 0.9 | 13 | Bernhard et al. (2006) |
| | | MBR | 0.3–0.4 | 50 | |

[a] Obtained from half life assays; UASB reactor, upflow anaerobic sludge blanket reactor; MBR, membrane biological reactor; WWTP, wastewater treatment plant with activated sludge treatment.

[b] Open pond and vegetated pond in series from a cotton farm. SSF CW are referred in all cases to horizontal flow systems.

As anoxic reactions may have been of major importance at the time of the experiment, dehalogenation in this study could be explained by reductive pathways (Van Pée and Unversucht, 2003).

### 3.4. Pollutant distribution throughout the gravel compartment

Injected pollutants were analyzed and quantified in the organic matter that had accumulated on the gravel bed and in its associated biofilm (Table 4). Only compounds that remained 21 days after the injection process and that had accumulated in the first part of the bed (2 m from the inlet) were measured. Therefore, sorption to gravel could be neglected due to the biodegradation, plant uptake and desorption processes occurring between injection and gravel sampling. Nearly 20% of the spiked pentachlorobenzene was retained in the gravel, due its higher log $K_{ow}$ (Table 1). Pentachlorophenol showed the same behavior. However, it is not as hydrophobic as pentachlorobenzene. In fact, its presence could be explained by the degradation of pentachlorobenzene to pentachlorophenol (Engst et al., 1977). Therefore, the pollutant elimination percentage attributable to gravel bed accumulation was not a major removal pathway in the wetland. Removal through plant uptake and sorption through the root system of plants could be expected for a systemic herbicide like diuron (Hess and Warren, 2002). Nevertheless, no removal was observed for this herbicide. Other compounds need to be studied further in order to discern this herbicide's distribution in the wetland. In summary, the fact that a high amount of chlorinated compounds had been completely eliminated by anaerobic systems (Table 3), the prevalence of anaerobic pathways in horizontal flow constructed wetlands, the low recovery of pollutants in wetland effluent and their low concentration in the gravel bed after 21 days provide some evidence of biodegradation as the main removal source.

Table 4
Pollutant concentrations and its loading in gravel bed ($n = 2$) following the injection experiment

| | Gravel[a] | |
|---|---|---|
| | μg kg$^{-1}$ | (%) |
| Simazine | 0.46 ± 0.19 | 2.0 |
| Alachlor | <0.18 | – |
| Chlorpyriphos | 1.72 ± 0.42 | 7.6 |
| Pentachlorobenzene | 4.50 ± 0.51 | 20.0 |
| Pentachlorophenol | 5.18 ± 1.21 | 23.0 |
| Endosulfan | 0.25 ± 0.09 | 1.12 |
| Lindane | 3.23 ± 0.46 | 14.3 |
| Diuron | <0.21 | – |
| Mecoprop | <0.12 | – |
| Clofibric acid (tracer) | <0.12 | – |

[a] The percentage mass recovery from the gravel was calculated from the dry gravel concentration, density (1300 kg m$^{-3}$) and volume (3.42 m$^3$) of the first section of the wetland (from the inlet to 2 m in length) where the samples were taken and organic matter is mostly accumulated in this type of systems. The other sections of the bed do not affect to the total mass (Matamoros and Bayona, 2006). The percentage is referring to the injected mass.

Therefore, the presence of biofilm, gravel, roots and organic matter complexes in anaerobic conditions may enable the SSF CW to eliminate chlorinated pollutants efficiently by reductive dehalogenation, as reported recently in isotopic studies (Braeckevelt et al., 2007).

## 4. Conclusions

Under normal loading conditions (HLR of 36 mm and HRT of about 5–6 days), the reductive dehalogenation of highly chlorinated compounds (pentachlorophenol, lindane, pentachlorobenzene and endosulfan) has been postulated as a highly efficient removal pathway in this CW (>99%). Distinct behavior was observed for less or non-chlorinated pollutants. Approximately 20% of simazine and mecoprop was eliminated, while approximately 80% of alachlor and chlorpyriphos was removed. Diuron and clofibric were not eliminated from the effluent. Furthermore, whereas less than 20% of the pentachlorobenzene, lindane and pentachlorophenol elimination was attributable to interaction with organic matter in gravel, the other

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pollutants showed low or no interaction at all. Therefore, the main conclusion of this study is that it is feasible to use SSF CWs to reach complete or partial elimination of the selected chlorinated European Water Framework Pollutants.

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### 2.3.2.3. Discussió de les metodologies analítiques.

Per tal de reduir el nombre de passos del procés analític, es va analitzar simultàniament la fracció dissolta i particulada de l'aigua. Tal i com s'ha comentat en l'apartat 1.1.3., aquesta és una tècnica bastant comuna quan s'analitzen compostos que són presents en les dues fases.

**Taula 2.8.** Resultats de l'avaluació de la recuperació i la repetibilitat (RSD) d'una mostra d'efluent de l'aiguamoll fortificada amb 5 ppb de cada compost individual (n=3). Els LODs i LOQs foren calculats mitjançant una mostra de l'efluent de l'aiguamoll D2 abans de l'experiment d'injecció (veure *article 4*).

| **Substància** | **Recuperació (%)** | **RSD (%)** | **LOD** (μg $L^{-1}$) | **LOQ** (μg $L^{-1}$) |
|---|---|---|---|---|
| Diuron | 92 | 5.6 | 0.2 | 0.5 |
| Àcid clofíbric | 85 | 9.0 | 0.4 | 0.9 |
| Pentaclorobenzè | 86 | 6.5 | 0.4 | 0.9 |
| Mecoprop | 81 | 10.1 | 0.3 | 0.8 |
| Simazina | 107 | 5.0 | 0.4 | 0.9 |
| Pentaclorofenol | 82 | 6.5 | 0.2 | 0.3 |
| Alaclor | 88 | 5.6 | 0.1 | 0.2 |
| Lindà | 99 | 4.5 | 0.3 | 0.6 |
| Clorpirifos | 109 | 5.3 | 0.4 | 0.9 |
| Endosulfan | 89 | 8.8 | 0.3 | 0.7 |

Per tal de garantir la qualitat de les dades resultants, es va dur a terme la valoració de la recuperació i la precisió (repetibilitat) del mètode analític (Taula 2.8). Aquesta es va realitzar per triplicat a partir d'una mostra procedent de l'efluent de l'aiguamoll D2 abans de l'assaig d'injecció, fortificant-la amb 5 μg $L^{-1}$ de cada compost individual. La mostra fortificada es va deixar equilibrar durant 12 hores. Finalment les tres mostres es van processar tal i com s'indica a *l'article 4*. La traça iònica total i les específiques dels anàlits obtingudes per GC-MS es recullen a la Figura 2.6.

Abans d'iniciar l'experiment d'injecció, es va comprovar l'absència dels compostos a analitzar així com dels seus corresponents *surrogates*, tant en l'afluent com en l'efluent dels aiguamolls.

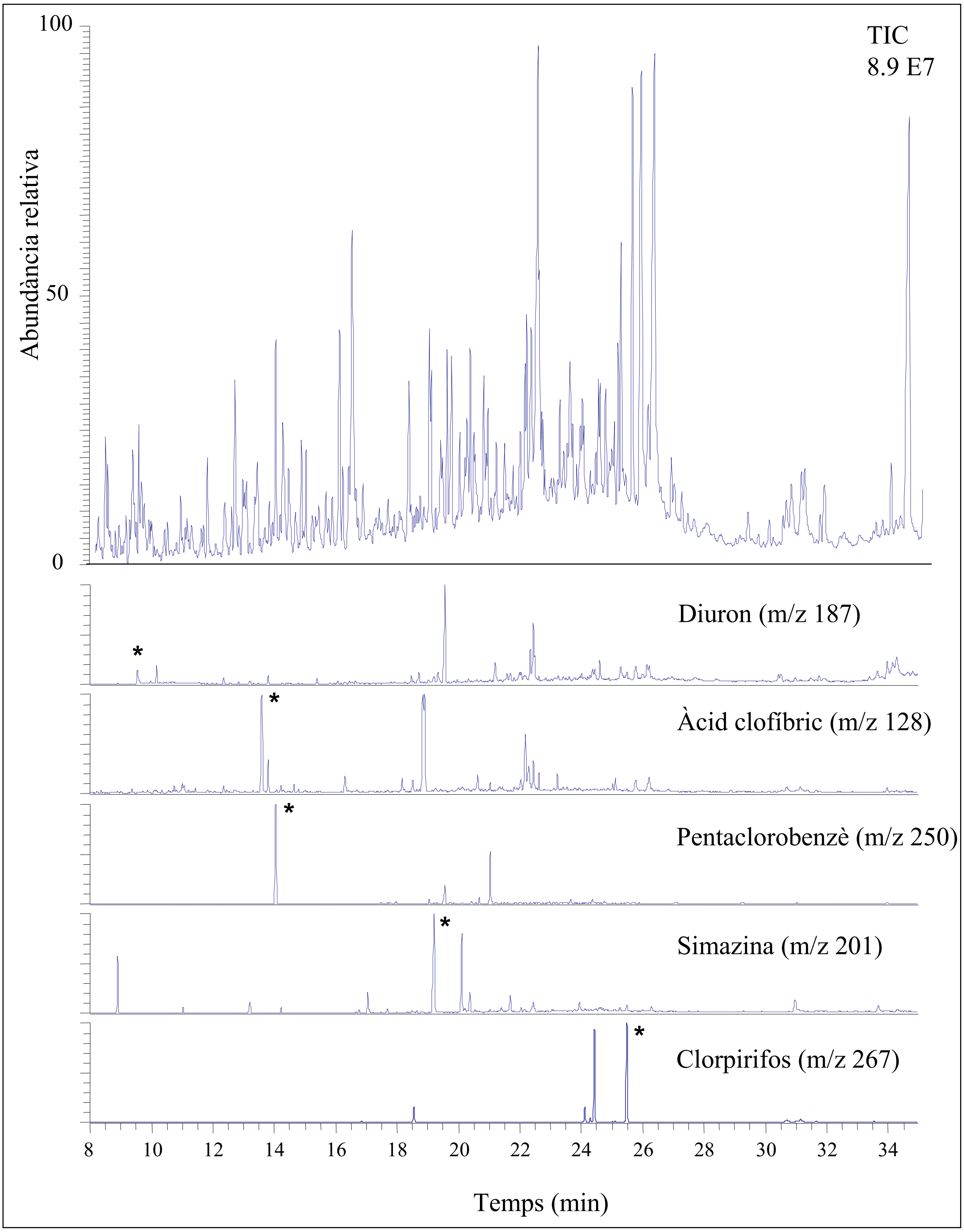

**Figura 2.6.** Registre dels ions totals i les traces iòniques corresponents als ions característics de cada compost on els pics característics són assenyalats amb un asterisc. Mostra: efluent de l'aiguamoll D2 dosificat amb 5 µg $L^{-1}$

Les recuperacions observades per a una mostra d'efluent fortificada amb 5 μg $L^{-1}$ de cada compost individual, es troben entre el 81 i el 109%, indicant una recuperació adequada dels mateixos.

Els LODs i LOQs van resultar ser bastant elevats en comparació a altres metodologies analítiques (Claver et al., 2006). Malgrat això, si es té present que no es va realitzar cap filtració ni purificació de la mostra, i que els LOQs es troben al voltant de 100 cops per sota de les concentracions màximes trobades en l'efluent de l'aiguamoll després de l'experiment d'injecció, es pot concloure que aquesta metodologia resulta ser adient per a l'estudi dut a terme en la present Tesi.

Pel que fa a l'anàlisi d'aquestes substàncies en les graves, aquesta es va dur a terme tot seguint la metodologia preestablerta per als PPCPs, amb unes recuperacions entre el 50 i 100%.

### 2.3.2.4. Discussió general dels resultats

Donat que la recuperació en massa total de contaminant injectat va ser per a la major part de substàncies molt baixa, i que les concentracions obtingudes de l'anàlisi de les graves no és suficient com per explicar aquesta eliminació, la biodegradació i l'assimilació per part de les plantes són dos de les vies més plausibles per explicar aquesta baixa recuperació màssica dels contaminants.

Així doncs, l'assimilació d'aquestes substàncies prioritàries per part de les plantes s'explica primerament per la transferència del contaminants orgànics cap a les arrels mitjançant transport passiu o per difusió, encara que hi ha compostos com els fenoxiàcids, que poden ser transportats activament. Briggs *et al.* (1983) van descriure que el factor de concentració entre l'arrel i l'aigua que l'envolta és linealment proporcional a la hidrofobicitat (Kow) dels compostos no ionitzables.

Malgrat això, la transferència d'aquests compostos des de les arrels fins altres llocs de la planta (translocació), generalment realitzada a través del xilema, encara que també es pot donar pel floema, requereix uns intervals d'hidrofobicitat dels compostos diferents (Figura 2.7). Així doncs, d'aquests resultats es suposaria que la major part dels contaminants orgànics injectats en el aiguamoll construït han sofert algun tipus d'interacció amb les plantes, primerament a través de les arrels i seguidament dintre de la planta, d'acord amb el seu log Kow.

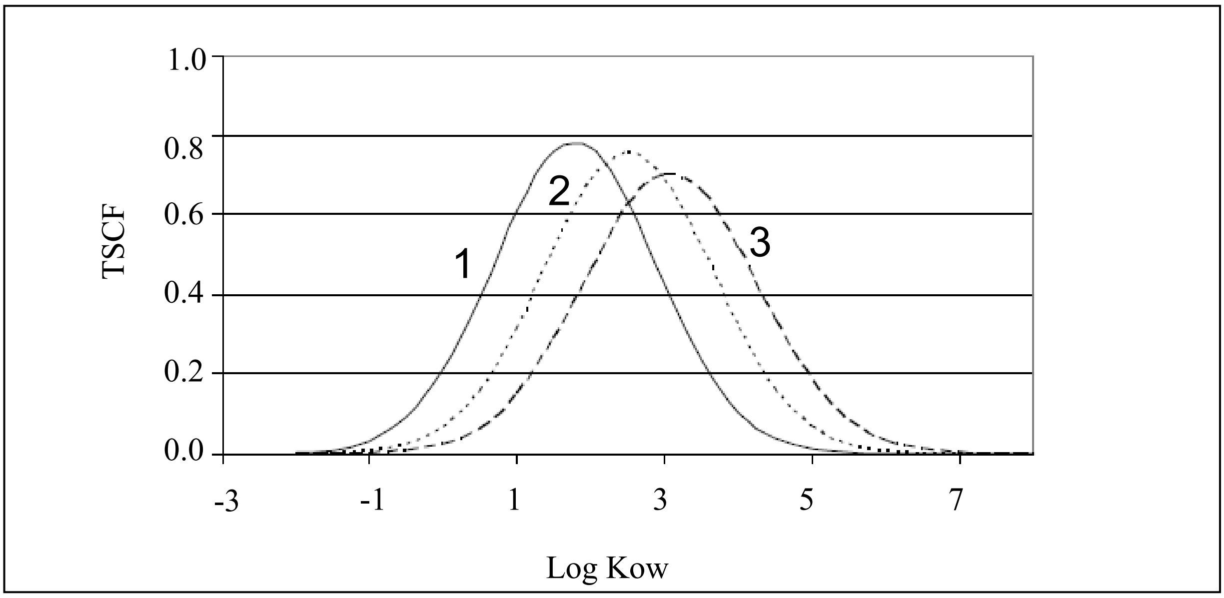

**Figura 2.7**. Variació del factor de concentració per corrent de flux de transpiració (*transpiration stream concentration factor*, TSCF) envers el log Kow dels contaminants 1.(Briggs et al., 1982); 2.(Burken i Schnoor, 1998) ; 3. (Hsu et al., 1990). Figura adaptada de Collins *et al.* (2006).

No obstant, tot i que l'assimilació i la distribució d'aquests contaminants orgànics s'ha demostrat ser dependent de les propietats físicoquímiques dels compostos, aquesta també ho és de les característiques del sòl i l'aigua, del tipus de plantes i la seva fisiologia (incloses propietats tals com el contingut de lípids i d'aigua o la velocitat de transpiració) (Paterson et al., 1994; Gao et al., 2005). Per exemple, l'assimilació del lindà és menor que la del trifluoralin degut a la seva menor hidrofobicitat i reactivitat (Li et al., 2002). Per altra banda, el canyís (*Phragmites australis)* ha estat designat com una planta adequada per tal de detoxificar efluents agrícoles amb alt contingut de plaguicides, degut a la seva elevada activitat de conjugació (Schroder et al., 2005). Per altra banda, els microambients propers a les arrels de les plantes (rizosfera), amb una microbiota característica, així com els exsudats enzimàtics d'aquesta, podrien jugar un rol molt important en l'eliminació de la major part d'aquestes substàncies. Per a alguns compostos com el pentaclorofenol ja s'ha demostrat (Dams et al., 2007). Finalment, l'elevat contingut de matèria orgànica present en l'aiguamoll pot actuar de manera competitiva amb les arrels sorbint els contaminants més hidrofòbics.

Per altra banda, quan els compostos presenten grups ionitzats, el comportament sembla ser diferent. Donat que la paret cel·lular de les plantes està carregada positivament, aquest compostos poden travessar-la i un cop dintre translocar-se pel floema, distribuint-se per tota la planta (Trapp, 2003). En aquest sentit, ha estat descrita l'assimilació del 48% del 2,4-D en cultius hidropònics després de 72 hores d'incubació (Paterson et al., 1994). Tot i això, durant la injecció de dos compostos d'estructura similar, com són el mecoprop i l'àcid clofíbric, aquesta assimilació per part de la vegetació present en l'aiguamoll no s'ha observat. Aquest procés diferencial sorgiria de la diferent configuració de la rizosfera que envolta els rizomes i les arrels de les plantes. En aquest sentit, l'elevada quantitat de biofilm adherit a la rizosfera li confereix a aquesta, una càrrega superficial negativa (Carlson i Silverstein, 1998), generant una repulsió electrostàtica entre la superfície del biofilm i els grups funcionals ionitzats presents tant en l'àcid clofíbric com en el mecoprop, impedint l'assimilació per part de les plantes. En conseqüència els compostos amb càrrega neta negativa presenten una baixa acumulació en el llit dels aiguamolls.

# **2.4.** Aiguamolls construïts de flux subsuperficial vertical

## 2.4.1 Introducció

Contrariament als HFCWs, els sistemes verticals es caracteritzen pel predomini de les condicions aeròbies, amb concentracions d'oxigen al voltant de la saturació. Donat que les rutes metabòliques aeròbies són generalment més eficients en l'eliminació de la matèria orgànica, les avantatges en front als sistemes anteriors a l'hora de tractar matèria orgànica així com realitzar la nitrificació estan extensament reportades (Brix i Arias, 2005).

En l'article que es presenta a continuació "*Removal of pharmaceuticals and personal care products (PPCPs) from urban wastewater in a pilot vertical flow constructed wetland and a sand filter*." es discuteix la influènica de l'existència de vegetació i l'increment de la càrrega hidràulica sobre l'eliminació dels paràmetres generals de depuració ($DBO_5$, TSS i amoni) i l'eliminació dels PPCPs existents a l'aigua residual.

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## 2.4.2. Article 5: Removal of pharmaceuticals and personal care products (PPCPs) from urban wastewater in a pilot vertical flow constructed wetland and a sand filter.

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# Removal of Pharmaceuticals and Personal Care Products (PPCPs) from Urban Wastewater in a Pilot Vertical Flow Constructed Wetland and a Sand Filter

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Removal efficiencies and elimination kinetics of 13 pharmaceuticals and personal care products (PPCPs) and $BOD_5$, TSS, and ammonium were evaluated in a pilot vertical subsurface-flow constructed wetland (VFCW) and compared with those obtained by a sand filter. On the basis of the observed removals, the PPCPs studied were grouped in relation to their removal efficiency into (i) PPCPs very efficiently removed, that is, >95% removal in one of the systems (caffeine, salicylic acid, methyl dihydrojasmonate, CA-ibuprofen, hydrocinnamic acid, oxybenzone, ibuprofen, OH-ibuprofen); (ii) PPCPs moderately removed, with removals between 70 and 90% in the two systems (naproxen, diclofenac, galaxolide, and tonalide); and finally (iii) PPCPs poorly removed, with elimination rates of <30% (carbamazepine). At design hydraulic loading rate (HLR) the planted VFCW and the nonplanted SF exhibited similar PPCP removal efficiencies, but the VFCW was less sensitive to removal decline in overloading conditions (up to 2 times the design HLR). Moreover, under a clogging simulation, the HLR and the presence of vegetation were some of the key aspects affecting the PPCPs, $BOD_5$, and ammonium removal from domestic wastewater. The VFCW evaluated was more efficient in terms of removal efficiency and loading rate for most of the PPCPs studied in comparison to constructed wetlands of other configurations (i.e., horizontal subsurface flow) and SF (nonplanted). The shorter hydraulic residence time (a few hours) in VFCW compared to that in other CW configurations ranging from days to weeks makes VFCWs a very appropriate wastewater treatment option in space-limited areas.

## Introduction

Pharmaceuticals and personal care products (PPCPs) have been continuously discharged into the aquatic environment for more than a century without any restrictions (*1*, *2*). PPCPs have been detected in effluents from a variety of wastewater treatment plants (WWTPs) in concentrations from parts per trillion to low parts per billion (*3*, *4*). Some compounds such as ibuprofen and salicylic acid are generally removed with high efficiencies in conventional WWTPs, but others, such as carbamazepine and clofibric acid, are not efficiently removed (*1*). Tertiary treatments such as ozonolysis and advanced oxidation techniques have been evaluated (*5*) and found to increase PPCP removal, but the additional treatment also increases the costs. Consequently, variable concentrations of these compounds are detected in surface, ground, and coastal waters receiving treated sewage effluents (*6*), and recent studies on the environmental risk assessment of PPCPs in Denmark have shown that their concentrations could exceed the predicted noneffect concentration (PNEC) (*7*).

Constructed wetlands (CW) are land-based wastewater treatment systems that consist of shallow ponds, beds, or trenches that contain floating or emergent, rooted wetland vegetation (*8*). The removal of PPCP in lagoons, free water surface CWs (FWSCWs), and horizontal subsurface-flow CWs (HFCWs) has been studied (*9*–*11*). A main limitation of these CWs is the large surface area generally required, which limits their application to the sanitation of small populations (<2000 inhabitants) or tertiary treatments. Vertical-flow constructed wetlands (VFCWs) are more efficient because they operate under aerobic conditions and require smaller surface area (*12*). The hydraulic residence time (HRT) in VFCWs is a few hours (*13*) instead of days as is typical for HFCWs. VFCWs thus have the potential to be used for larger populations because they are more area efficient. In this study, we present for the first time data on the elimination of PPCPs from urban wastewater in aerobic VFCWs. Moreover, a comparison between a planted VFCW and an unplanted system (a sand filter, SF) was carried out to evaluate the influence of vegetation on PPCP removal. Finally, the VFCW and SF were flooded with wastewater to simulate clogging and its effect on the treatment performance. A flooded system or clogged system will have a reduced oxygen supply. The microbial processes in the bed might then be oxygen limited and result in a rapid failure of the treatment performance as well as a reduction of the PPCP removal (*9*). Compounds selected in this study included a variety of chemical classes that originate from human use (i.e., analgesics, lipid regulators, antiepileptic drugs, sunscreens, and fragrances).

## Materials and Methods

**Chemicals.** GC grade (Suprasolv) hexane, methanol, ethyl acetate, acetone, and dichloromethane were obtained from Merck (Darmstadt, Germany). Analytical grade acetic acid and hydrochloric acid were obtained from Panreac (Barcelona, Spain). Hydrocinnamic acid, oxybenzone, carbamazepine, ibuprofen, caffeine, methyl dihydrojasmonate, galaxolide, tonalide, ketoprofen, salicylic acid, diclofenac, 2,2′-dinitrophenyl, and dihydrocarbamazepine were purchased from Sigma-Aldrich (Steinheim, Germany). 2,4,5-Trichlorophenoxypropionic acid (2,4,5-TPA) was obtained from Reidel-de-Haen (Seelze, Germany). Trimethylsulfonium hydroxide (TMSH) was purchased from Fluka (Buchs, Switzerland), and 0.45 $\mu$m glass fiber filters of 47 mm were purchased from Millipore (Bedford, MA).

**Pilot Plant.** The study took place at an experimental pilot plant located in the vicinity of Århus, Denmark (*14*). The plant was constructed during the spring of 2001 within the grounds of a municipal WWTP of a 30000 population equivalent (PE) town. The pilot plant consists of a prefabricated 2 $m^3$ three-chamber polyethylene sedimentation tank, a 5 $m^2$ VFCW, and a 10 $m^2$ SF bed. The two beds are constructed in a HDPE-lined metal container divided into

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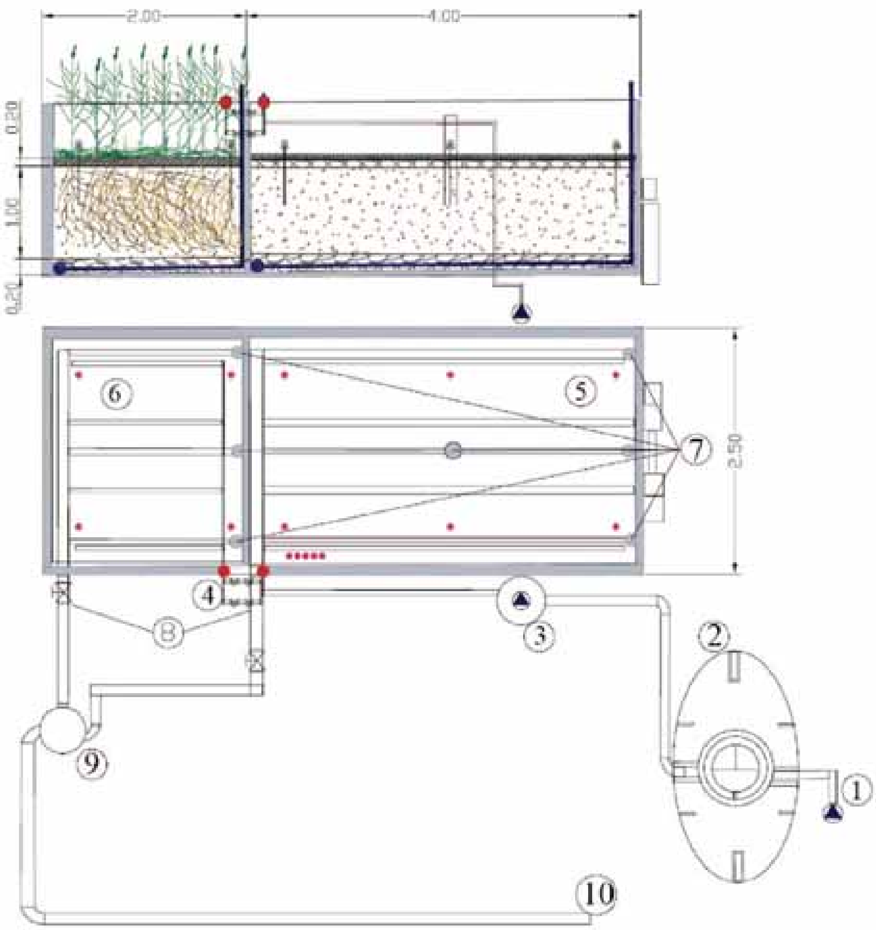

**FIGURE 1. Layout of the experimental pilot plant: (1) inlet pumping well; (2) sedimentation tank; (3) pumping well; (4) flow control manifold control; (5) biological sand filter (SF); (6) vertical flow constructed wetland (VFCW); (7) aeration pipes; (8) effluent from VFCW and SF; (9) sampling well; (10) final effluent.**

two sections. Each bed is filled with two layers of different diameter gravel. The bottom layer of 0.20 m depth contains coarse gravel (8–16 mm) and houses the drainage system. On top of this layer lies a 0.8 m deep gravel section of 0–4 mm ($D_{10}$ = 0.55 mm and $D_{60}$ = 3.1 mm). The wastewater distribution system is placed on top of this gravel layer and consists of four 40 mm perforated polyethylene pipes in each bed. The VFCW has a dense vegetation of *Phragmites australis*, and the SF is covered by a perforated plastic liner (0.2 m of 16–32 mm gravel to simulate conditions in buried sand filters). The *Phragmites* vegetation was 5 years old; hence, the plants were well developed and the roots penetrated to the bottom of the gravel filter at the time of the study. The pilot plant is fed with raw urban wastewater taken from a well located just before the WWTP influent. Raw wastewater is pumped by a timer-controlled pump into the sedimentation tank. From there, the water drains to a level-controlled pumping well from which the water is distributed through pressure pipes onto the surface of the two systems. The distribution of loading between the two systems was controlled and monitored by flow meters. After percolating vertically through the gravel filters (unsaturated flow), the effluent is collected in drainage pipes located in the bottom of the system and led by gravity to the outlets. Valved 4 mm diameter stainless steel piezometers were installed in the beds (four in the VFCW and six in the SF) for the collection of gas samples from the unsaturated filters.

**Experimental Design and Sampling Strategy.** Four different hydraulic loading rates (HLRs) were applied onto the systems to evaluate the dependency of treatment performance on loading rate (13, 30, 70, and 160 mm day$^{-1}$). The loading rates were controlled by increasing the frequency of pumping of settled wastewater from the inlet well. The systems were operated at a specific loading rate for 2 weeks, and inlet and outlet samples were collected daily during the last 3 days of the loading period when performance of the systems had stabilized under the particular loading rate. The inlet and outlet samples were collected in 1 L glass amber bottles and kept refrigerated during transportation to the laboratory, where they were stored at 4 °C until analysis (within 2 days). The samples were analyzed for general parameters and PPCPs as described below. Gas samples from the piezometers were taken by 50 mL glass syringes and analyzed for $CO_2$, $O_2$, and $CH_4$ by gas chromatography (GC) the same day. When the four loading rates had been tested, the outlet levels from the two beds were raised to the bed surface to saturate the beds completely with water and to simulate the effects of clogging. The performance of the water-saturated systems was measured at a HLR of 70 mm day$^{-1}$. The experiments were carried out during June and July 2006 when the vegetation was well developed.

**Analytical Methodology.** *Wastewater Analysis.* Conventional wastewater parameters including TSS, $BOD_5$, and $NH_4^+$ were analyzed following standard methods (*15*). The pH was measured in influent wastewater by a conventional pH-electrode, and dissolved oxygen (DO) and temperature were measured with an OxyGard DO meter. The composition of the gas samples ($CO_2$, $O_2$, and $CH_4$) was analyzed by a gas

**TABLE 1. Average (±1 SD) Influent and Effluent Water Quality of the Vertical Flow Constructed Wetland (VFCW) and the Sand Filter (SF) at Different Hydraulic Loading Rates (HLR)**[a]

| HLR | system | DO (mg L$^{-1}$) | pH | BOD$_5$ (mg L$^{-1}$) | TSS (mg L$^{-1}$) | NH$_4$-N (mg L$^{-1}$) |
|---|---|---|---|---|---|---|
| 13 mm day$^{-1}$ | inlet | 0.5 ± 0.2 | 7.3 ± 0.1 | 217 ± 15 | 60 ± 16 | 41.3 ± 1.3 |
| | VFCW | 8.4 ± 0.4 | 8.2 ± 0.1 | 5.7 ± 3.2 (97) | 2.8 ± 0.3 (80) | 0.1 ± 0.1 (99) |
| | SF | 5.1 ± 0.9 | 7.9 ± 0.4 | 4.0 ± 3.5 (98) | 2.8 ± 0.1 (62) | 0.1 ± 0.1 (99) |
| 30 mm day$^{-1}$ | inlet | 0.3 ± 0.1 | 7.2 ± 0.1 | 213 ± 6 | 59 ± 7 | 46.2 ± 2.3 |
| | VFCW | 9.2 ± 0.4 | 8.2 ± 0.1 | 1.0 ± 0.1 (99) | 0.7 ± 0.3 (99) | 0.1 ± 0.1 (99) |
| | SF | 8.9 ± 0.5 | 8.0 ± 0.2 | 1.0 ± 0.1 (99) | 2.4 ± 1.6 (96) | 0.1 ± 0.1 (99) |
| 70 mm day$^{-1}$ | inlet | 0.3 ± 0.1 | 7.2 ± 0.1 | 247 ± 3 | 84 ± 6 | 45.1 ± 0.5 |
| | VFCW | 9.0 ± 0.5 | 8.1 ± 0.1 | 0.7 ± 0.6 (99) | 1.7 ± 1.2 (98) | 0.1 ± 0.1 (99) |
| | SF | 5.9 ± 0.5 | 7.6 ± 0.1 | 1.7 ± 0.6 (99) | 1.3 ± 0.5 (98) | 0.9 ± 0.6 (98) |
| 160 mm day$^{-1}$ | inlet | 0.1 ± 0.1 | 7.3 ± 0.1 | 227 ± 21 | 79 ± 10 | 45.5 ± 2.0 |
| | VFCW | 7.5 ± 0.3 | 7.7 ± 0.1 | 3.0 ± 0.1 (98) | 2.1 ± 0.3 (97) | 0.1 ± 0.1 (99) |
| | SF | 2.3 ± 0.9 | 7.2 ± 0.1 | 12.3 ± 3.2 (95) | 4.6 ± 1.9 (94) | 14.9 ± 1.2 (67) |
| 70 mm day$^{-1}$ | inlet | 0.1 ± 0.1 | 6.7 ± 0.1 | 230 ± 18 | 97 ± 8 | 59.3 ± 3.1 |
| (water saturated) | VFCW | 4.8 ± 1.0 | 7.2 ± 0.1 | 10.7 ± 1.5 | 15.1 ± 5.1 (84) | 8.1 ± 0.8 (86) |
| | SF | 1.2 ± 0.26 | 7.1 ± 0.2 | 17.7 ± 2.1 | 21.3 ± 0.8 (78) | 14.0 ± 4.7 (76) |

[a] Values in parentheses are the removal percentages.

chromatograph equipped with a thermal conductivity detector (Shimadzu GC-8A, Kyoto, Japan) as described by Brix (*16*). The concentrations of PPCPs in wastewater samples were analyzed after the samples had been filtered and processed as reported by Matamoros et al. (*9*). Briefly, a sample volume of 500 mL was spiked with 1 μg of a surrogate standard mix (i.e., fenoprop for the acidic compounds, 2,2′-dinitrophenyl for musk, and dihydrocarbamazepine for neutral compounds). The spiked sample was percolated through a polymeric solid-phase extraction cartridge (100 mg Strata X) from Phenomenex (Torrance, CA). Activated cartridges were eluted with 10 mL of hexane/ethyl acetate (1:1). The extract was evaporated to ca. 20 μL under a gentle nitrogen stream, and 186 ng of triphenylamine was added as internal standard. Then the vial was reconstituted to 300 μL with ethyl acetate.

Methylation of the carboxylic acid group was performed online in a hot GC injector by adding 10 μL of TMSH (0.25 mol L$^{-1}$ in methanol) to a 50 μL sample before injection. Derivatized samples were injected onto a TRACE GC-MS (Thermo-Finnigan, Dreieich, Germany) in the electron impact mode (70 eV ionization energy) fitted with a 30 m × 0.25 mm i.d. × 0.25 μm DB-5 column (J&W Scientific, Folsom, CA). Temperature program and quantification details are described elsewhere (*9*). In addition, for sunscreen compounds the following diagnostic ions were monitored for time-scheduled acquisition for target analyte quantification (in bold) and identification: oxybenzone **227**/151/105; hydrocinnamic acid **104**/164/91.

The LOD and LOQ of the analytical procedure were determined (using Milli-Q water) from the mean background noise plus 3 or 10 times the standard deviation of the background noise, respectively. LOD and LOQ were 8 and 25 ng L$^{-1}$, respectively, for hydrocinnamic acid, 98 and 364 ng L$^{-1}$, respectively, for oxybenzone, and 22 and 67 ng L$^{-1}$, respectively, for carbamazepine. Filters were processed and analyzed as reported elsewhere with LOD and LOQ between 0.1 and 229 ng L$^{-1}$ and between 0.44 and 0.770 ng L$^{-1}$, respectively (*10*). The blank values of analytical procedure for water and filters remained always below the quantification limit (LOQ).

*Statistical Analysis.* SPSS v13 package (Chicago, IL) was used for statistical data analysis. The removal comparison between planted and nonplanted systems was analyzed by using the Mann–Whitney test for two independent samples (nonparametric statistics).

**TABLE 2. Content of Oxygen, Carbon Dioxide, and Methane (Average ± 1 SD) in the Interstitial Gases in the Vertical Flow Constructed Wetland (VFCW) and the Sand Filter (SF) at Different Hydraulic Loading Rates (HLR)**

| HLR | system | O$_2$ (vol %) | CO$_2$ (vol %) | CH$_4$ (vol %) |
|---|---|---|---|---|
| 13 mm day$^{-1}$ | VFCW | 20 ± 05 | 0.5 ± 0.5 | nd[a] |
| | SF | 20 ± 0.5 | 0.3 ± 0.1 | nd |
| 30 mm day$^{-1}$ | VFCW | 19 ± 0.3 | 0.4 ± 0.1 | nd |
| | SF | 19 ± 0.3 | 0.6 ± 0.1 | nd |
| 70 mm day$^{-1}$ | VFCW | 19 ± 0.6 | 1.4 ± 0.4 | nd |
| | SF | 18 ± 1.1 | 1.6 ± 0.8 | nd |
| 160 mm day$^{-1}$ | VFCW | 18 ± 0.9 | 2.5 ± 0.7 | 0.02 ± 0.01 |
| | SF | 13 ± 3.9 | 5.3 ± 3.3 | 0.03 ± 0.01 |
| 70 mm day$^{-1}$ | VFCW | ns[b] | ns | ns |
| (water saturated) | SF | ns | ns | ns |

[a] Not detected. [b] Not sampled.

## Results and Discussion

**General Parameters.** The surface organic loading rate varied between 3 and 37 g of BOD$_5$ m$^{-2}$ day$^{-1}$ during the experimental period. Table 1 shows TSS, BOD$_5$, and NH$_4^+$ concentrations, measured at each HLR checked, and shows efficient removal of all the parameters studied. The high BOD$_5$ and ammonium removal is consistent with the prevailing oxic conditions of the filters, suggesting that aerobic pathways are predominant to remove organic matter, as generally reported for VFCWs and unsaturated SFs (*12*). Moreover, the gas composition measured in each system confirmed the occurrence of high O$_2$ levels and the release of CO$_2$ as a result of organic matter mineralization (Table 2). As expected, an increase in the HLR led to a slight decrease in TSS, NH$_4^+$, and BOD$_5$ removal, particularly in the SF. The reduced removal occurred concurrently with a decrease in O$_2$ and an increase in CO$_2$. Also, at the highest HLR, methane production was detected in the SF and VFCW, indicating methanogenic processes in anaerobic microsites of the filters (*17*). The better BOD$_5$ and NH$_4^+$ removal in the VFCW compared to the SF is probably due to the positive plant influence. The presence of plants may increase oxygen transfer into the system, and roots of the plants may stimulate Biofilm development (*18*). Flooding of the systems at a 70 mm day$^{-1}$ loading rate resulted in lower O$_2$ levels in the effluent water and lower BOD$_5$ and NH$_4^+$ removals. The effects of flooding were more severe for SF than for the VFCW probably due, again, to the positive plant influence (*18*).

**TABLE 3. Minimum, Maximum (in Parentheses), and Average Concentrations of PPCPs in the Wastewater Influent for All Loading Rates and the Removal Efficiencies (Percent) Observed in the Vertical Flow Constructed Wetland (VFCW) and the Sand Filter (SF) at a Hydraulic Loading Rate of 70 mm Day$^{-1}$ and Working with either Unsaturated Water Flow or Saturated Flow[a]**

| | | unsaturated flow | | saturated flow | | | |
|---|---|---|---|---|---|---|---|
| | influent[b,c] ($\mu$g L$^{-1}$) | VFCW (% rem) | SF (% rem) | VFCW (% rem) | SF (% rem) | HFCW (*9*) (% rem) | WWTP (% rem) |
| Pharmaceuticals | | | | | | | |
| salicylic acid | (45.7–72.3) 53.9 | 98 ± 1 | 98 ± 1 | 85 ± 7 | 77 ± 7 | 96 | 99 (*3*) |
| ibuprofen | (8.3–17.2) 11.7 | 99 ± 1 | 90 ± 3*[d] | 55 ± 1 | 49 ± 1* | 71 | 60–70 (*33*)/ 90 (*1*) |
| OH-ibuprofen | (12.4 – 16.9) 3.7 | 99 ± 1 | 86 ± 3* | 51 ± 1 | 47 ± 2* | 62 | 95 (*34*) |
| CA-ibuprofen | (8.7–12.4) 10.6 | 99 ± 1 | 95 ± 3* | 71 ± 6 | 68 ± 8 | 87 | 95 (*34*) |
| naproxen | (0.96–2.15) 1.57 | 89 ± 5 | 80 ± 5 | 62 ± 3 | 66 ± 7 | 85 | 40–55 (*33*)/66 (*1*) |
| diclofenac | (0.48–1.28) 0.82 | 73 ± 3 | 76 ± 7 | 53 ± 2 | 39 ± 22 | 15 | 9–75 (*1*)/17 (*23*) |
| carbamazepine | (1.24–2.9) 2.06 | 26 ± 14 | 11 ± 7 | 20 ± 4 | 8 ± 15 | 16 (*35*) | 8 (*23*)/7 (*1*) |
| caffeine | (35.2–64.0) 48.4 | 99 ± 1 | 98 ± 1 | 82 ± 1 | 75 ± 6* | 97 | 99 (*23*) |
| Personal Care Products | | | | | | | |
| methyl-dihydrojasmonate | (18.8–31.8) 22.8 | 99 ± 1 | 98 ± 1* | 78 ± 4 | 76 ± 8 | 99 | 98 (*21*) |
| hydrocinnamic acid | (11.2–17.6) 15.4 | 99 ± 1 | 99 ± 1 | 82 ± 3 | 69 ± 11 | na[e] | na |
| oxybenzone | (8.58–22.1) 14.8 | 97 ± 1 | 95 ± 2 | 88 ± 3 | 64 ± 24 | na | 68–99 (*36*) |
| galaxolide | (3.05–12.4) 5.62 | 90 ± 1 | 92 ± 1 | 88 ± 2 | 88 ± 2 | 86 | 70–85 (*33*)/89 (*21*) |
| tonalide | (0.66–1.83) 0.99 | 82 ± 1 | 82 ± 1 | 75 ± 2 | 73 ± 4 | 88 | 75–90 (*33*)/88 (*21*) |
| HRT (h) | | 4–6 | 4–6 | 137 | 126 | 114 | 12–24 |

[a] Removals obtained in horizontal flow constructed wetlands (HFCW) and conventional wastewater treatment plants (WWTP) are listed for comparison. [b] $n = 15$. [c] Particulate and dissolved wastewater. [d] Statistically significant differences at a significance level of 0.05 (VFCW vs SF). [e] Not available.

**PPCP Removal at Design HLR.** PPCP levels detected in the influent (Table 3) are consistent with the levels found in other raw wastewaters from the EU and Denmark (*19, 20*). The set of PPCP was selected on the basis of their concentration and a high detection frequency. Analgesics, fragrances, and sunscreen products are major compounds in urban wastewater. Nevertheless, high PPCP variability in inlet concentration was observed between campaigns. When HLR increased, then HRT in the sedimentation tank decreased, which led to an increase in PPCP concentration in sedimentation tank outlet (i.e., the influent to SF and VFCW). This suggests that some removal took place in the sedimentation tank at low HLR. Contaminant removal was calculated by analyzing both dissolved and particulate phases from the influent and effluent. Whereas the major portion of PPCP occurred in the dissolved phase (*9*), galaxolide and tonalide were strongly bound to the particulate phase due their high hydrophobicity (log $K_{ow} = 5.7–5.9$) (*21*).

PPCP removal was higher for the most abundant compounds, which is in agreement with their high biodegradation reported elsewhere (*9*). Although an apparent difference in PPCP removal efficiency between the planted VFCW and the SF was observed at the design HLR of 70 mm day$^{-1}$, a statistically significant difference was observed for only some of them (Table 3). On the basis of the observed removals, the PPCPs studied can be grouped in relation to their removal efficiency into (i) PPCPs very efficiently removed, that is, >95% removal in one of the systems (caffeine, salicylic acid, methyl dihydrojasmonate, CA-ibuprofen, hydrocinnamic acid, oxybenzone, ibuprofen, OH-ibuprofen); (ii) PPCPs moderately removed, with removals between 70 and 90% in the two systems (naproxen, diclofenac, galaxolide, and tonalide); and finally (iii) PPCPs poorly removed, with elimination rates of <30% (carbamazepine). A similar difference in removals has been observed in treated wastewater irrigated on arable land. In this case, compounds were sorbed or transformed while percolating through the topsoil layer, and only some of them (i.e., carbamazepine) were detected in the lysimeter wells (*22*).

Comparing the obtained PPCP removal in the VFCW with the removals obtained in conventional WWTPs and HFCW, VFCWs appear to be more efficient for some PPCPs. Even diclofenac, a compound that has been extensively reported as recalcitrant in a variety of wastewater treatment technologies (*23, 24*), showed a removal efficiency of ca. 73% in the VFCW studied. Some of the highly efficiently removed compounds (i.e., salicylic acid, caffeine, methyl dihydrojasmonate, and oxybenzone) as well as the compounds that can be eliminated by sorption onto the organic matter (i.e., galaxolide and tonalide) (*9*) and carbamazepine, which have a low removal efficiency, seem to behave very similarly in the two systems studied. The better PPCP removal observed in the VFCW and the SF compared to HFCWs is probably caused by the fact that aerobic processes predominate in VFCWs and SFs, whereas HFCW are usually oxygen deficient because of the water-saturated conditions. This is consistent with previously reported removals of PPCPs in aerated microcosms (*25, 26*). Therefore, the two main removal mechanisms postulated in this study are biodegradation and sorption onto the substrate. Whereas sorption of galaxolide and tonalide onto the organic matter retained in the substrate is supported by their high log $K_{ow}$ values, plant uptake is not considered to be significant, at least for negatively charged compounds (*27*) or highly hydrophobic ones (galaxolide and tonalide) (*28*). Further research is needed to ascertain the role of plant uptake for other neutral compounds (i.e., caffeine, methyl dihydrojasmonate, and oxybenzone).

**Effect of HLR on PPCP Removal.** To compare the PPCP removal of the VFCW with that of the SF and the relationship to HLR, the total mass removal of each compound was plotted against its total mass loading rate (Figure 2). It is seen from the plots that (i) salicylic and hydrocinnamic acids in both systems are not affected by the mass loading rate and are nearly completely removed in both systems; (ii) oxybenzone, caffeine, methyl dihidrojasmonate, ibuprofen, OH-ibuprofen, and CA-ibuprofen are nearly completely removed in the VFCW at all loading rates but in the SF the removal decreased at the highest loading rate; (iii) galaxolide and tonalide are removed at similar rates <95% in both systems; (iv) naproxen is removed more efficiently in the VFCW compared to the SF, particularly at the highest loading rate; and (v) carbamazepine and diclofenac are poorly removed in both types of

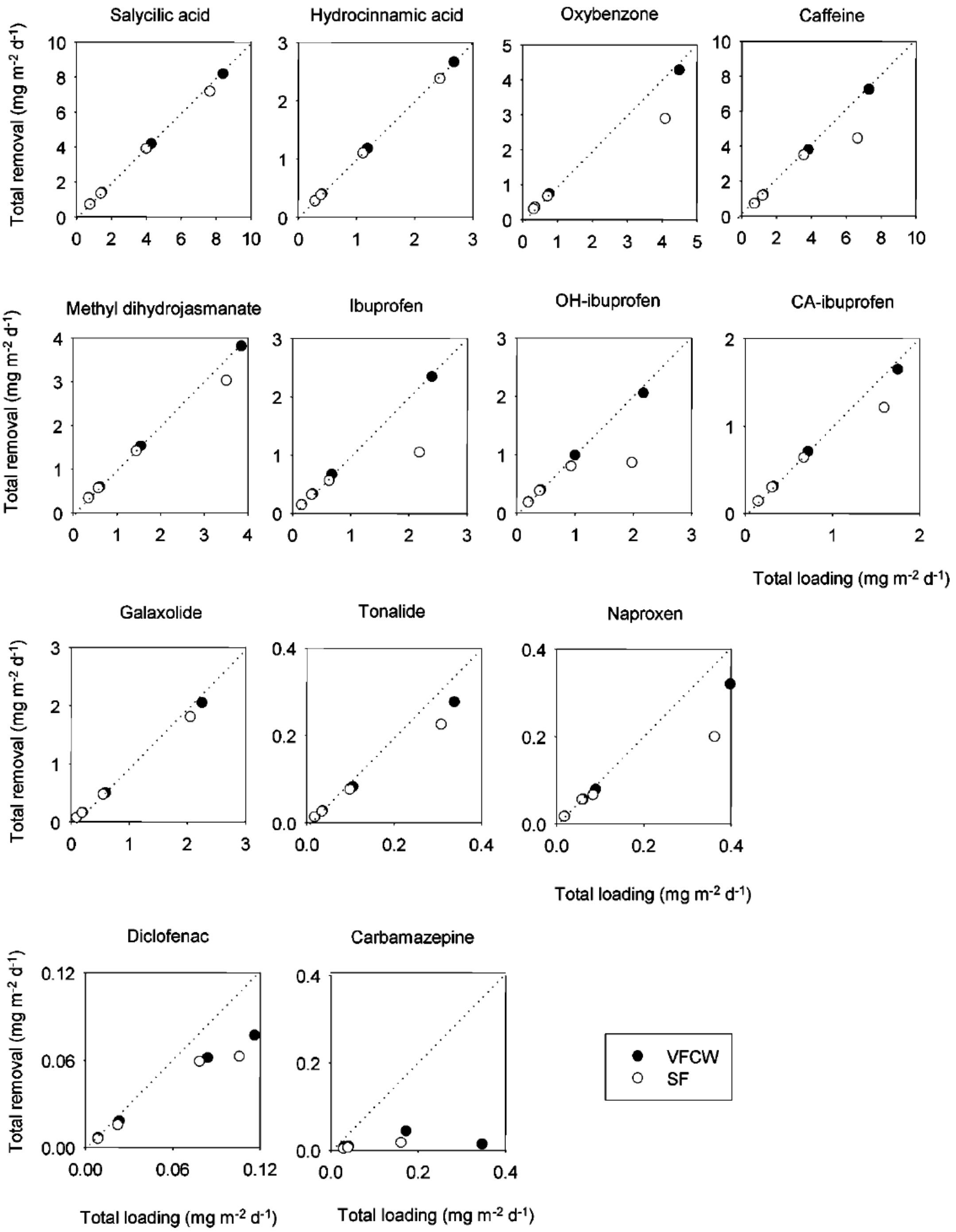

**FIGURE 2. PPCP average mass removal rate in the vertical flow constructed wetland (VFCW) and the sand filter (SF) against their mass loading rates. Discontinuous line represents 100% removal.**

systems. Overall, the VFCW generally removed PPCP more efficiently than the SF, probably due to a more oxygenated bed. Compounds such as carbamazepine and diclofenac do not follow this general rule due to their remarkable recalcitrance in comparison to other PPCPs (*23*). Galaxolide and tonalide also behave differently due to the fact that their removal is mainly attributable to the interaction and sorption onto the organic matter present in the system (*9*). Salicylic and hydrocinnamic acid were nearly completely removed in both systems, and therefore any effect of the plants could not be observed in the present study.

**Effects of Flooding.** Clogging of constructed wetland and sand filters affects many treatment processes and generally will lead to a decreased removal efficiency of many constituents of wastewaters (*29*). To simulate the clogging effect, both systems were filled with wastewater at the design HLR (70 mm day$^{-1}$) and their performances compared. As shown in Table 3, PPCP removals decreased as a consequence of flooding for all of the PPCPs studied, except for carbamazepine, and the fragrances galaxolide and tonalide [because their removal is closely related to sorption onto organic matter (*21*)]. Nevertheless, statistically significant differences were

**TABLE 4. Area-Based First-Order Removal Rate Constants and Mass Loading Rates in the Vertical Flow Constructed Wetland (VFCW) and the Sand Filter at a Hydraulic Loading Rate of 70 mm Day$^{-1}$[a]**

| | VFCW | | SF | | HFCW (*9*) | |
|---|---|---|---|---|---|---|
| | loading (mg m$^{-2}$ day$^{-1}$) | $k_{A1}$ (m day$^{-1}$) | $k_{A1}$ (m day$^{-1}$) | ratio | loading (mg m$^{-2}$ day$^{-1}$) | $k_{A1}$ (m day$^{-1}$) |
| General Parameters | | | | | | |
| $BOD_5$ | 16.9[b] | 0.43 | 0.34 | 1.22 ± 0.31 | 6.75[b] | 0.06 |
| TSS | 6.11[b] | 0.28 | 0.29 | 1.23 ± 0.25 | | |
| $NH_4^+$ | 3.30[b] | 0.56 | 0.27 | 2.83 ± 2.91 | 1.47[b] | 0.04 |
| Pharmaceuticals | | | | | | |
| salicylic acid | 4.14 | 0.29 | 0.27 | 1.12 ± 0.22 | | na[c] |
| ibuprofen | 0.65 | 0.40 | 0.16 | 2.78 ± 2.64 | 0.71 | 0.05 |
| OH-ibuprofen | 0.96 | 0.37 | 0.14 | 2.65 ± 2.13 | 0.77 | 0.04 |
| CA-ibuprofen | 0.65 | 0.31 | 0.21 | 1.37 ± 0.63 | 1.12 | 0.11 |
| naproxen | 0.08 | 0.16 | 0.11 | 1.50 ± 0.57 | 0.06 | 0.08 |
| diclofenac | 0.08 | 0.10 | 0.10 | 1.22 ± 0.15 | 0.07 | <0.01 |
| carbamazepine | 0.17 | 0.02 | 0.01 | 1.39 ± 0.61 | | na |
| caffeine | 3.67 | 0.37 | 0.29 | 1.90 ± 2.03 | 0.23 | 0.11 |
| Personal Care Products | | | | | | |
| methyl-dihydrojasmonate | 1.49 | 0.34 | 0.28 | 1.47 ± 0.74 | 0.61 | 0.19 |
| hydrocinnamic acid | 1.15 | 0.41 | 0.44 | 1.00 ± 0.44 | | na |
| oxybenzone | 0.72 | 0.26 | 0.21 | 1.48 ± 0.85 | | na |
| galaxolide | 0.59 | 0.14 | 0.14 | 1.05 ± 0.13 | 0.32 | 0.05 |
| tonalide | 0.11 | 0.11 | 0.11 | 1.15 ± 0.19 | 0.20 | 0.06 |

[a] Average ratio values ± 1 SD. Literature data for horizontal flow constructed wetlands (HFCW) are listed for comparison. [b] In g m$^{-2}$ day$^{-1}$. [c] Not available.

observed only for ibuprofen, OH-ibuprofen, and caffeine. This could be attributable to the relatively small sample size per each HLR ($n = 3$) evaluated. The PPCPs that were most efficiently removed in the systems were less affected by flooding. The planted bed (VFCW) generally performed better than the SF when flooded. In the VFCW oxygen levels were less affected than in the SF, indicating that aerobic processes were more predominant in that system. The contribution of aerobic pathways decreases (e.g., a decline in ammonium removal), and anaerobic pathways (denitrification, sulfate reduction, and methanogenesis) start to occur (*30*). Hence, the aerobic processes seem to be more efficient in the elimination of most of the PPCPs evaluated than anaerobic processes.

**Correlation between General Parameters and PPCP Removal.** $CO_2$, $O_2$, DO pH, $BOD_5$, TSS, and $NH_4^+$ were correlated with the PPCP removal using the complete data set from the VFCW and the SF study. Significant positive correlations were observed between removal of all PPCPs and the general wastewater parameters (Table 1 of the Supporting Information), which shows that under conditions where $BOD_5$, TSS, and $NH_4^+$ are efficiently removed, PPCPs will most likely also be removed. PPCP removal correlated positively with $O_2$ gas in the filter and negatively with $CO_2$ gas, indicating the importance of aerobic processes. Due to the high hydrophobicity of galaxolide and tonalide and their tendency to absorb into organic matter particles, positive correlations between the removal of these compounds and the total content of TSS were expected. However, these correlations were not observed, probably because the TSS breakthrough point was not reached at any time in this study. Therefore, low correlations were observed between PPCP removal and TSS. The two PPCP compounds that showed the lowest removals in this study (carbamazepine and diclofenac) presented the lowest correlation coefficients. The recalcitrance of these two compounds has been extensively reported (*23, 26*).

**Removal Kinetics.** The area-based first-order removal rate constants ($k_{A1}$), using the equation described by Kadlec and Knight (*31*), were calculated using the average inflow and outflow concentrations at the different loading rates Figure 1 of the Supporting Information shows the estimated area-based rate constants for all PPCPs plotted against loading rate. As can be inferred from these plots, the rate constants are dependent on the mass loading rates. Table 4 compares the rate constants estimated at design HLR of 70 mm day$^{-1}$ with rate constants observed in a HFCW at HLR of 36 mm day$^{-1}$. It can be seen that VFCWs are more efficient in terms of both $BOD_5$, TSS, and $NH_4^+$ removal and PPCP removal.

The ratios between the average rate constants for the PPCP removal in the two systems were calculated by using eq 1 to compare the performance of the two systems:

$$\text{ratio} = \frac{\sum_{n=1}^{n=i}\left(\frac{k_{A1,\text{VFCW}}}{k_{A1,\text{SF}}}\right)}{n} \qquad (1)$$

where $n$ is the number of HLR checked and $k_{A1}$ is the rate constant obtained at each loading rate and type of system, respectively. The high variability of this ratio is attributable to the wide range of HLR evaluated.

It can be seen from Table 4 that high ratio values (>2) corresponded to compounds that showed different behaviors between VFCW and SF and low ratio values were observed for compounds that were not affected by the type of system. In this regard, it become evident that $NH_4^+$ removal is highly affected by the oxygen occurrence, probably due to the requirements of the nitrification processes. Similarly, the removal of ibuprofen and OH-ibuprofen is highly oxygen dependent (*25*). On the other hand, $BOD_5$ and TSS removals were only slightly affected, as were PPCP compounds that were only slightly affected by HLR and flooding (i.e., hydroccinnamic and salycilic acid, galaxolide and tonalide, diclofenac and carbamazepine). Finally, the removal of caffeine, CA-ibuprofen, oxybenzone, methyl dihydrojamonate, and naproxen was almost independent of the system evaluated. Accordingly, bed aeration is a key aspect to promote the removal of most of the PPCPs analyzed. Moreover, the contribution of the rhizosphere Biofilm and root exudates to the degradation of some chemical compounds is unknown (*32*).

In summary, this study clearly documents that VFCWs achieve a better performance compared to HFCW and only slightly better when it is compared with SF and conventional WWTPs for most of the PPCPs studied. Nevertheless, the use of VFCW could be a feasible technology to remove most PPCPs from domestic wastewater from small populations, allowing the design of more compact systems.

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## Supporting Information Available

Pearson correlation coefficients and area-based removal rate constants. This information is available free of charge via the Internet at http://pubs.acs.org.

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# Removal of pharmaceuticals and personal care products (PPCPs) from urban wastewater in a pilot vertical flow constructed wetland and a sand filter.

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Summary of Suplemment Information
Numer of tables: 1 table SI
Number of figures: 1 figure SI

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**Table 1SI.** Pearson Correlation coefficients between general parameters (pH, $O_2$, $CO_2$, DO, $DBO_5$, TSS and $NH_4$-N) and PPCPs removal.

| | wastewater | | | | | Interstitial gases | |
|---|---|---|---|---|---|---|---|
| | DO (mg $L^{-1}$) | pH | $BOD_5$ (rem %) | TSS (rem %) | $NH_4$-N (rem %) | $O_2$ (Vol %) | $CO_2$ (Vol %) |
| General parameters | | | | | | | |
| $BOD_5$ | 0.809* | 0.757* | 1.000* | 0.705* | 0.889* | 0.845* | -0.812* |
| TSS | 0.593* | 0.573* | 0.705* | 1.000* | 0.664* | 0.625* | -0.608* |
| $NH_4^+$ | 0.683* | 0.684* | 0.778* | 0.569* | 1.000* | 0.881* | -0.869* |
| Pharmaceuticals | | | | | | | |
| Salicylic acid | 0.787* | 0.626* | 0.912* | 0.735* | 0.847* | 0.804* | -0.783* |
| Ibuprofen | 0.815* | 0.755* | 0.859* | 0.591* | 0.981* | 0.913* | -0.879* |
| OH-ibuprofen | 0.846* | 0.779* | 0.870* | 0.585* | 0.972* | 0.920* | -0.893* |
| CA-ibuprofen | 0.732* | 0.681* | 0.835* | 0.591* | 0.915* | 0.896* | -0.886* |
| Naproxen | 0.632* | 0.775* | 0.698* | 0.462* | 0.843* | 0.910* | -0.922* |
| Diclofenac | 0.595* | 0.594* | 0.585* | 0.538* | 0.631* | 0.606* | -0.640* |
| Carbamazepine | 0.572* | 0.724* | 0.496* | 0.403 | 0.488* | 0.629* | -0.586* |
| Caffeine | 0.755* | 0.678* | 0.890* | 0.675* | 0.987* | 0.917* | -0.895* |
| **Personal care products** | | | | | | | |
| Methyl-dihydrojasmonate | 0.777* | 0.669* | 0.882* | 0.746* | 0.958* | 0.887* | -0.866* |
| Hydrocinnamic acid | 0.763* | 0.743* | 0.870* | 0.717* | 0.966 | 0.945* | -0.930* |
| Oxybenzone | 0.605* | 0.528* | 0.827* | 0.622* | 0.916 | 0.945* | -0.791* |
| Galaxolide | -0.250 | -0.234 | -0.089 | -0.004 | 0.103 | 0.026 | -0.031 |
| Tonalide | 0.048 | 0.072 | 0.252 | 0.385 | 0.492* | 0.303 | -0.237 |

* significance value lower than 0.05, then correlation is significant;

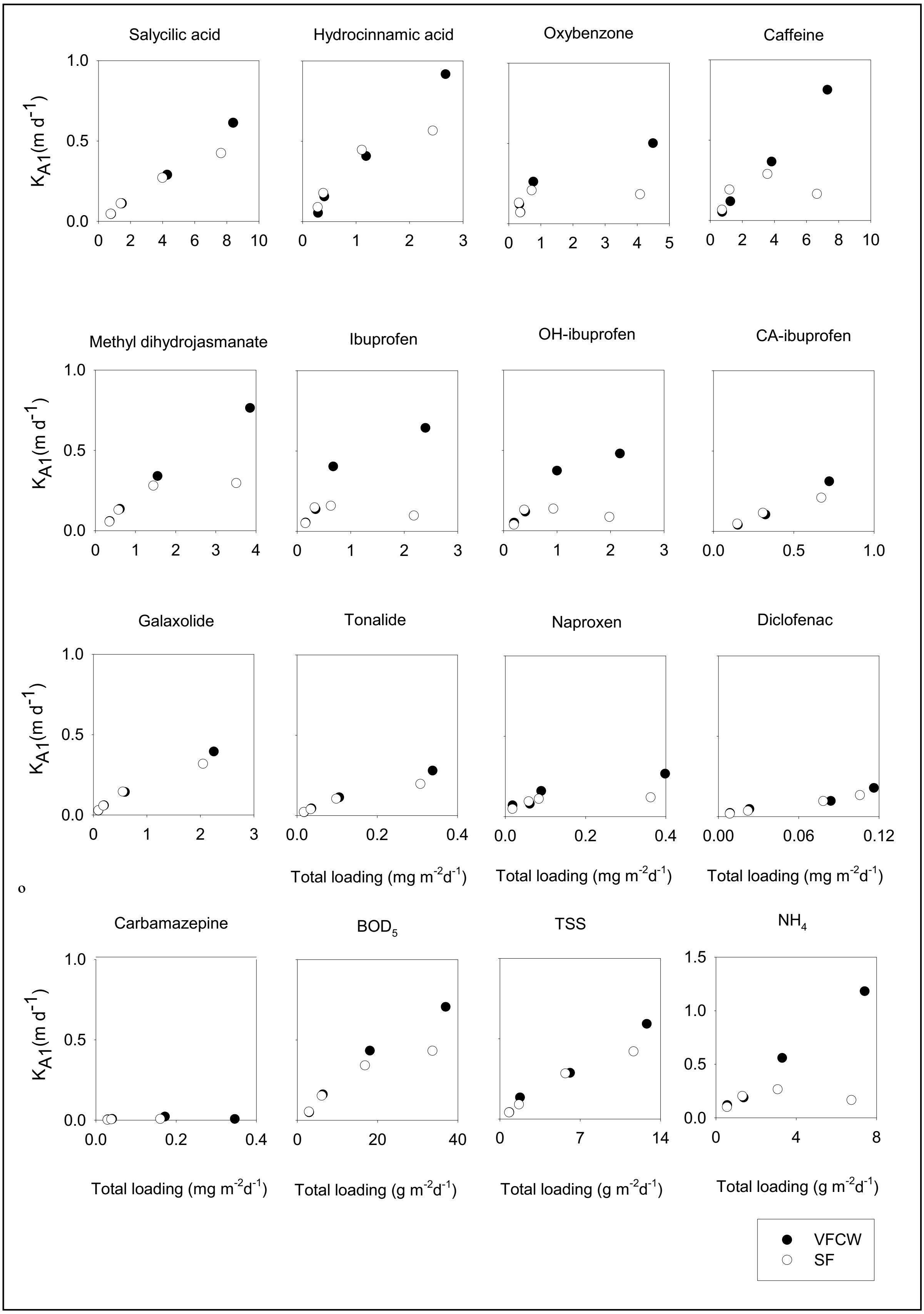

**Figure 1 SI**. Area-based rate constant (m $d^{-1}$) plotted against mass loading rates for $BOD_5$, TSS, $NH_4^+$ and PPCPs.

## 2.4.3.Discussió de les metodologies analítiques

Les metodologies analítiques utilitzades en aquest article per tal d'analitzar els PPCPs són les mateixes que es van emprar en l'*article 3*. Tanmateix, en aquest article s'han inclòs dos nous PPCPs, l'àcid hidrocinàmic i l'oxibenzona, ambdós són emprats com a protectors de la radiació UV en diferents tipus de cremes solars. En aquest apartat només es descriuen els paràmetres de qualitat referents als dos compostos nous.

A la Taula 2.9 s'hi podem observar tant els LODs i LOQs, com la repetibilitat i la recuperació (analitzades per triplicat) per a les dues matrius d'estudi, l'aigua i els sòlids suspesos. Mentre els LODs i LOQs es van calcular amb aigua Milli Q, la repetibilitat va ser calculada mitjançant una mostra d'aigua residual procedent de l'afluent del VFCW que contenia entre 13 i 15 µg $L^{-1}$ d'oxibenzona i d'àcid hidrocinàmic, respectivament. La recuperació es va realitzar tal i com s'ha assenyalat anteriorment en la secció 2.3.1.5., calculant-la a partir d'una mostra fortificada amb 6 µg $L^{-1}$ tant d'oxibenzona com d'àcid hidrocinàmic.

**Taula 2.9.** Repetibilitat (n=3) expressada com a percentatge de desviació estàndard relativa (RSD), la recuperació (n=3), el LOD i el LOQ per a l'oxibenzona i l'àcid hidrocinàmic.

| Mostra aquosa | | | | |
|---|---|---|---|---|
| | Repetibilitat (%) | Recuperació (%)* | LOD (ng $L^{-1}$) | LOQ(ng $L^{-1}$) |
| Oxibenzona | 10 | 76±7 | 98 | 364 |
| Àcid hidrocinàmic | 4.0 | 90±13 | 8 | 25 |
| **Sòlids suspesos** | | | | |
| | Repetibilitat (%) | Recuperació (%)* | LOD (ng $L^{-1}$) | LOQ(ng $L^{-1}$) |
| Oxibenzona | 18 | 89±18 | 229 | 770 |
| Àcid hidrocinàmic | 7.0 | 93±7 | 14 | 47 |

* mitjana ± RSD

Donat que l'àcid hidrocinàmic es va analitzar en la seva forma derivatitzada, aquest va mostrar uns LOD i LOQ menors que els corresponents a l'oxibenzona, ja que aquesta última no presenta cap grup reactiu amb l'agent derivatitzant (TMSH).

Finalment, tot i que la preconcentració en fase polimèrica és adient tant per compostos polars com apolars, els resultats mostrats a la Taula 2.9 semblen indicar que la presència d'un grup cetona és menys favorable que la presència d'un grup carboxílic protonat, obtenint millors recuperacions per a aquest últim.

Tots dos factors, la no derivatització, així com la menor preconcentració donen lloc a que l'oxibenzona presenti una repetibilitat pitjor que no pas l'àcid hidrocinàmic.

### 2.4.4. Discussió general dels resultats

Els resultats obtinguts confirmen que les vies aeròbies d'eliminació de la matèria orgànica són més favorables que les anaeròbies, tant per als paràmetres generals de qualitat (exemple: $DBO_5$, TSS), com per a l'eliminació de la major part dels PPCPs estudiats. Treballs similars duts a terme en columnes de sorra han trobat una major eliminació de PPCPs quan aquestes columnes treballen sota condicions de no saturació d'aigua, amb l'excepció de l'àcid clofíbric (Scheytt et al., 2007). Per altra banda, és el primer cop que l'efecte de la vegetació es considera en l'estudi de l'eliminació d'aquests productes. De tota manera, i tot i que l'assimilació de la major part dels PPCPs (aniònics) per part de les arrels es creu que és baixa degut a la repulsió electrostàtica generada per les càrregues electrostàtiques del biofilm, l'assimilació per part de les arrels no es pot descartar per als compostos no ionitzats. De fet, estudis duts a terme en enciams (Boxall et al., 2006) demostren que l'assimilació de fàrmacs veterinaris seguiria la corba gaussiana anteriorment descrita per Briggs *et al*. (veure secció 2.3.2.4).

Així doncs, sembla ser que l'efecte de les plantes sobre l'eliminació dels PPCPs és degut tant a l'increment de la transferència d'oxigen entre l'atmosfera i el sistema, així com per la creació d'una rizosfera que afavoreix les condicions d'eliminació de gran part dels PPCPs. Aquesta rizosfera comprèn la zona delimitada pels primers mil·límetres propers a l'arrel, on es desenvolupen diferents tipus de bacteris, fongs i microfauna característica que aprofiten aquestes condicions especials d'interrelació amb les plantes per tal de proliferar. Els exsudats, tant de les plantes com de la mateixa microbiota, ajuden a la cohesió de la matèria orgànica que envolta la rizosfera, tot creant unes condicions que afavoreixen l'eliminació de gran quantitat de contaminants. En aquest sentit, la modificació genètica de plantes per tal d'incrementar la producció exsudats enzimàtics capaços d'eliminar compostos orgànics clorats, ja ha estat descrita i emprada per al triclorophenol (Wang et al., 2004)

Així doncs, les eficiències en l'eliminació dels PPCPs trobades en aquests sistemes poden ser comparades fins i tot amb les que es troben en bioreactors de membranes (Kimura et al., 2005). De tal manera, sembla que aquest tipus de sistemes serien dels més eficient dintre dels aiguamolls construïts avaluats en aquesta Tesi. A part, aquests sistemes són els més compactes i econòmics, especialment degut a que requereixen una baixa àrea superficial.

# 2.5. Aiguamolls construïts de flux superficial

## 2.5.1. Introducció

Els aiguamolls de flux superficial tenen generalment una aplicació diferent a la dels aiguamolls subsuperficials. Aquesta rau en el tractament d'efluents secundaris, enlloc de tractar efluents primaris com s'ha presentat anteriorment en els HFCWs i VFCWs. Així doncs, s'empren com a sistemes d'atenuació de la descàrrega de contaminació orgànica al medi. En aquest sentit, poden tenir un paper rellevant si es considera que la descàrrega de microcontaminants a rius i rieres de la Mediterrània a través de les EDARs és un factor molt important a tenir en compte, més quant aquest rius són poc cabalosos i l'efecte dilució és mínim.

En l'article "*Organic micropollutant removal in a full-scale surface flow constructed wetland fed with secondary effluent*" es va realitzar un estudi de la descàrrega de microcontaminants a través de l'EDAR de Granollers al riu Congost, així com l'ús d'un aiguamoll de flux superficial per tal d'atenuar-la. En la Figura 2.8 es mostra una fotografia aèria de l'EDAR, el riu Congost així com de l'aiguamoll.

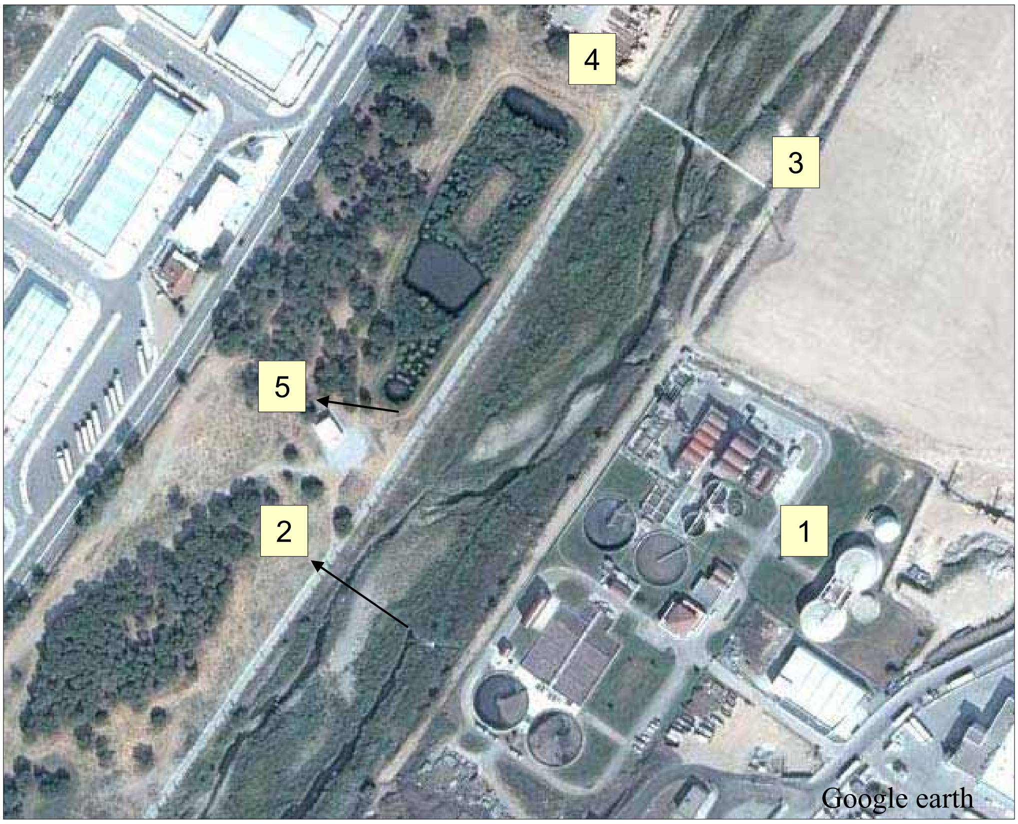

**Figura 2.8.** Fotografia aèria de la zona de mostreig, 1. EDAR; 2. Efluent de l'EDAR 3. Riu Congost; 4. Afluent de l'aiguamoll; 5. Efluent de l'aiguamoll (adaptada de Google Earth)

## 2.5.2. Article 6: Organic micropollutant removal in a full-scale surface flow constructed wetland fed with secondary effluent

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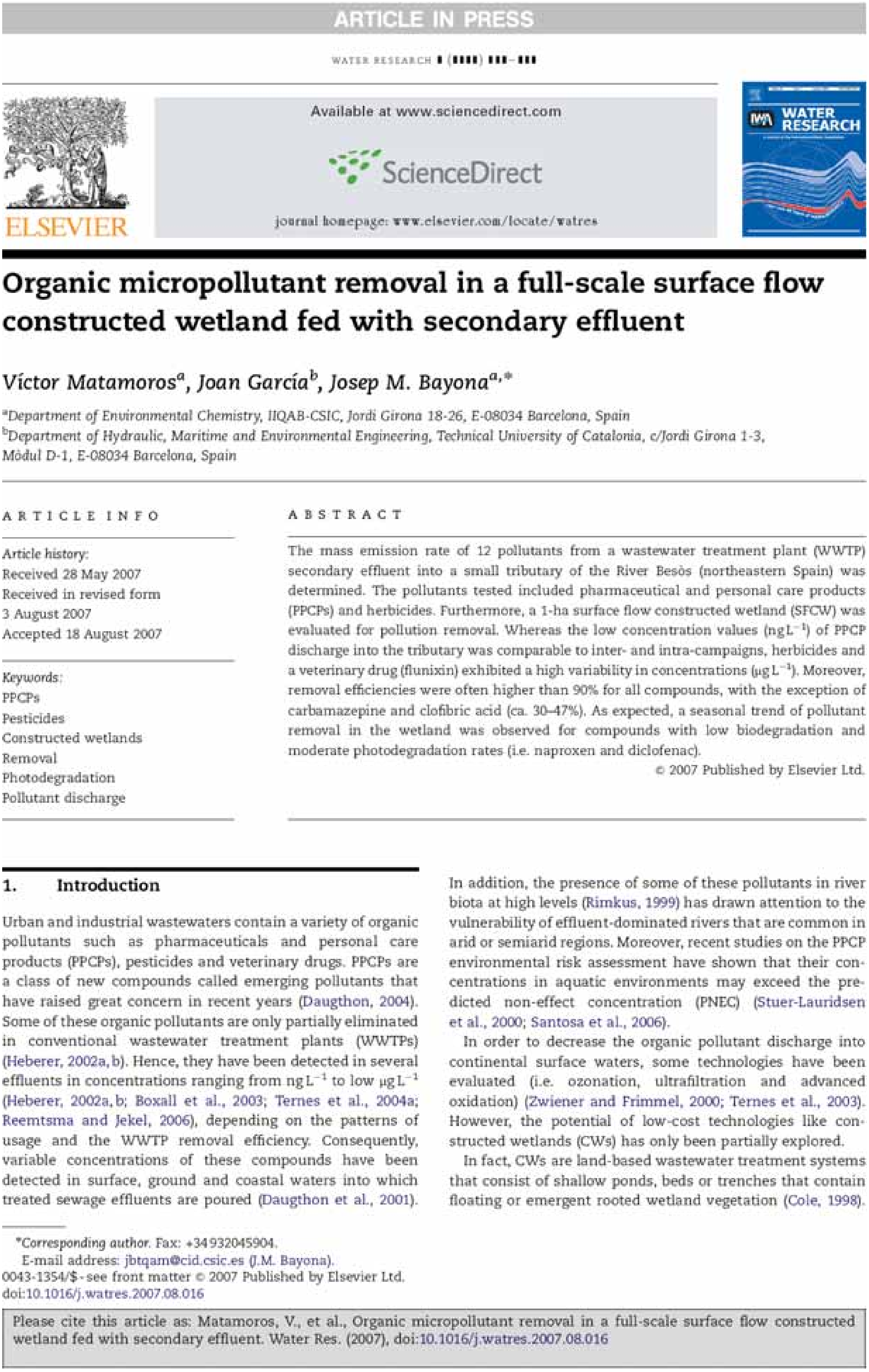
*Water Research*, in press 2007



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# Organic micropollutant removal in a full-scale surface flow constructed wetland fed with secondary effluent

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ABSTRACT

The mass emission rate of 12 pollutants from a wastewater treatment plant (WWTP) secondary effluent into a small tributary of the River Besòs (northeastern Spain) was determined. The pollutants tested included pharmaceutical and personal care products (PPCPs) and herbicides. Furthermore, a 1-ha surface flow constructed wetland (SFCW) was evaluated for pollution removal. Whereas the low concentration values ($\mathrm{ng\,L^{-1}}$) of PPCP discharge into the tributary was comparable to inter- and intra-campaigns, herbicides and a veterinary drug (flunixin) exhibited a high variability in concentrations ($\mathrm{\mu g\,L^{-1}}$). Moreover, removal efficiencies were often higher than 90% for all compounds, with the exception of carbamazepine and clofibric acid (ca. 30–47%). As expected, a seasonal trend of pollutant removal in the wetland was observed for compounds with low biodegradation and moderate photodegradation rates (i.e. naproxen and diclofenac).

## 1. Introduction

Urban and industrial wastewaters contain a variety of organic pollutants such as pharmaceuticals and personal care products (PPCPs), pesticides and veterinary drugs. PPCPs are a class of new compounds called emerging pollutants that have raised great concern in recent years (Daugthon, 2004). Some of these organic pollutants are only partially eliminated in conventional wastewater treatment plants (WWTPs) (Heberer, 2002a, b). Hence, they have been detected in several effluents in concentrations ranging from $\mathrm{ng\,L^{-1}}$ to low $\mathrm{\mu g\,L^{-1}}$ (Heberer, 2002a, b; Boxall et al., 2003; Ternes et al., 2004a; Reemtsma and Jekel, 2006), depending on the patterns of usage and the WWTP removal efficiency. Consequently, variable concentrations of these compounds have been detected in surface, ground and coastal waters into which treated sewage effluents are poured (Daugthon et al., 2001). In addition, the presence of some of these pollutants in river biota at high levels (Rimkus, 1999) has drawn attention to the vulnerability of effluent-dominated rivers that are common in arid or semiarid regions. Moreover, recent studies on the PPCP environmental risk assessment have shown that their concentrations in aquatic environments may exceed the predicted non-effect concentration (PNEC) (Stuer-Lauridsen et al., 2000; Santosa et al., 2006).

In order to decrease the organic pollutant discharge into continental surface waters, some technologies have been evaluated (i.e. ozonation, ultrafiltration and advanced oxidation) (Zwiener and Frimmel, 2000; Ternes et al., 2003). However, the potential of low-cost technologies like constructed wetlands (CWs) has only been partially explored.

In fact, CWs are land-based wastewater treatment systems that consist of shallow ponds, beds or trenches that contain floating or emergent rooted wetland vegetation (Cole, 1998).

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CWs can be classified as surface flow or subsurface flow wetlands (vertical or horizontal). Whereas horizontal and vertical subsurface flow constructed wetlands (SSFCWs) are used as a secondary treatment with comparatively high organic matter loading rates, SFCW are used as a tertiary treatment (Kadlec and Knight, 1996). CWs used for urban wastewater treatment have shown a certain capacity for removing several organic pollutants (Langergraber et al., 2003; Huang et al., 2004; Matamoros et al., 2005; Matamoros and Bayona, 2006). However, whereas most SFCW studies are mainly focused on pesticide removal from agricultural or urban runoff (Moore et al., 2002; Schulz et al., 2003), few studies exist on PPCPs (Gross et al., 2004) and none on veterinary product attenuation.

Therefore, the objective of this study was to evaluate the behaviour of a variety of organic pollutants in an SFCW fed with secondary effluents from a conventional WWTP. This wetland was constructed as a part of a series of restoration activities in a high-density population area around the River Congost, Granollers (Barcelona), in northeastern Spain. The emergent target pollutants included pharmaceuticals (clofibric acid, carbamazepine, caffeine, ketoprofen, diclofenac, ibuprofen and naproxen), fragrances (methyl dihydrojasmonate, galaxolide and tonalide), herbicides (mecoprop, MCPA and terbutylazine) and veterinary drugs (flunixin). Furthermore, the organic micropollutant mass loading rates discharged into the river were considered and the pollution attenuation obtained by the SFCW treatment of the secondary effluent was presented.

## 2. Experimental section

### 2.1. *Chemicals*

Gas chromatography (GC) grade (Suprasolv) hexane, methanol, ethyl acetate, acetone and dichloromethane were obtained from Merck (Darmstadt, Germany). Analytical-grade acetic acid and hydrogen chloride were obtained from Panreac (Barcelona, Spain); mecoprop, MCPA, terbutylazine, clofibric acid, carbamazepine, caffeine, methyl dihydrojasmonate, galaxolide, tonalide, ibuprofen, naproxen, ketoprofen, diclofenac, 2,2′-dinitrophenyl and dihydrocarbamazepine were purchased from Sigma-Aldrich (Steinheim, Germany). The 2,4,5-trichlorophenoxypropionic acid (2,4,5-TPA) was obtained from Reidel-de-Haen (Seelze, Germany). Trimethylsulfonium hydroxide (TMSH) was purchased from Fluka (Buchs, Switzerland) and the 0.45 μm glass fibre filters of $\phi = 47$ mm were purchased from Millipore (Bedford, MA).

### 2.2. *Sampling site description*

The SFCW is fed with a small part of the treated effluent (secondary effluent) from the Granollers urban WWTP, which serves 154,000 inhabitants. The WWTP influent flow rate consists of about 45% industrial wastewater and 55% domestic sewage with an average total flow of 23.700 $m^3 d^{-1}$. The WWTP carries out pre-treatment, primary clarification, activated sludge treatment and secondary clarification. The effluent is mainly diverted to the Congost river (only a small amount is conveyed to the wetland), which is a tributary of the Besòs river. In addition, the Granollers WWTP effluent discharge (0.27 $m^3 s^{-1}$) accounts for surges in the flow of the River Congost in drought periods.

The SFCW is made up of a single cell with a surface area of 1 ha (Fig. 1). It has shallow stretches of water (0.3–0.4 m deep) planted with *Typha* and *Phragmites*, unplanted deep stretches of water (1.5 m deep) and a small island. These different areas were created in order to increase the potential biodiversity of the system. The system started to operate in April 2003.

The wetland treats approximately 100 $m^3 d^{-1}$ (0.4% of the WWTP effluent) with a hydraulic retention time (HRT) of around 1 month. Average concentrations of total suspended solids (TSS) from the SFCW effluent in 2005 and 2006 were $35 \pm 26$ and $21 \pm 17$ $mg L^{-1}$, respectively. The average chemical oxygen demand in the SFCW effluent was $82 \pm 26$ and $59 \pm 17$ $mg L^{-1}$ in 2005 and 2006, respectively (data provided by the Granollers Municipal Office, from biweekly sampling campaigns).

### 2.3. *Sampling*

Influent and effluent wetland samples were collected daily over 1 week (5 days per week) in two different sampling campaigns (June 2005 and February 2006). Note that the influent is the secondary effluent of the WWTP. Moreover, in the second campaign, water samples from the middle area of the wetland (see Fig. 1, P1) were also collected daily at 1 m from the border of the wetland. All the samples were collected in 1 L amber glass bottles and kept refrigerated during transportation to the laboratory, where they were stored at 4 °C until analysis. The sample holding time was less than 2 days.

### 2.4. *Analytical methodology*

Conventional wastewater parameters including ammonium, biological oxygen demand ($BOD_5$) and TSS were analysed following the standard methods (APHA-AWWA-WPCF, 2001) procedure. The pH was determined using a selective electrode. The temperature was taken with a digital thermometer and the dissolved oxygen (DO) was measured with a digital oxymeter.

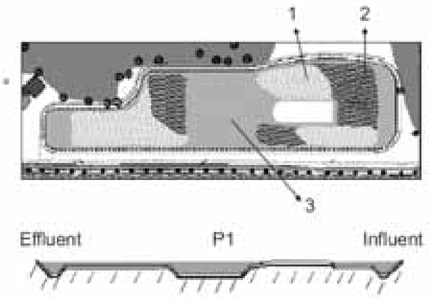

**Fig. 1 – Schematic diagram of SFCW plant. 1: *Typha*; 2: *Phragmites*; 3: unplanted deep zones.**

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Wastewater samples were filtered and processed as reported previously (Matamoros and Bayona, 2006). A sample volume of 500 mL was spiked for a short period with 1 μg per compound of a surrogate standard mix (i.e. fenoprop for the acidic compounds, 2,2′-dinitrophenyl for musk, and dihydrocarbamazepine for neutral compounds). The spiked sample was percolated through a polymeric solid-phase extraction cartridge (100 mg Strata X) from Phenomenex (Torrance, CA). Activated cartridges were eluted with 10 mL of hexane/ethyl acetate (1:1). The extract was evaporated until ca. 20 μL under a gentle nitrogen stream, and 186 ng of triphenylamine as an internal standard was added. The vial was then reconstituted to 300 μL with ethyl acetate.

Methylation of the carboxylic acid was performed in a GC injector port at 270 °C by adding 10 μL of TMSH solution (0.25 mol $L^{-1}$ in methanol) to a 50 μL sample before injection. Derivatized samples were injected into a Trace gas chromatography-mass spectrometry (Thermo-Finnigan, Dreieich, Germany) in the electron impact mode (70 eV ionization energy) fitted with a 30 m × 0.25 mm i.d. × 0.25 μm DB-5 (J&W Scientific, Folsom, CA). The temperature programme and quantification details for PPCPs are described elsewhere (Matamoros and Bayona, 2006). In addition, for herbicides and flunixin the following diagnostic ions were monitored for time-scheduled acquisition for target analyte quantification (in bold) and identification: mecoprop **169**/142/228; MCPA **214**/141/155; dicamba **203**/234/188; terbutylazine **214**/173/229; flunixin **295**/263/310. The rest of the analytical parameters were similar to those described previously for PPCPs. Filters were processed and analysed as reported elsewhere (Matamoros and Bayona, 2006).

## 3. Results and discussion

### 3.1. General parameters

During the experimental period, the surface organic loading rate was 0.11 g $BOD_5\,m^{-2}\,d^{-1}$. Table 1 shows DO, pH, $NH_4$-N, TSS and $BOD_5$ concentrations that were measured during each campaign and that obtained low TSS and $BOD_5$ removal (or even no removal) due to the proliferation of phytoplankton and zooplankton on the outer edges of the wetland. The high oxygen level in the effluent reveals the predominance of aerobic conditions. Furthermore, difference in ammonium removal (15%) between spring and summer was observed. This trend shows the temperature dependence of mechanisms involved in ammonium removal (i.e. nitrification and plant uptake). Finally, the removal of approximately three logarithmic colony forming units (CFU) of faecal coliforms may make possible the reuse of this water for agricultural purposes, in compliance with the WHO guidelines (3 log CFU/100 ml) (Mara and Cairncross, 1989).

### 3.2. Presence of PPCPs and pesticides in the WWTP effluent

The set of emerging pollutants was selected on the basis of their concentration and detection frequency in a previously screening campaign. Subsequently, the mass of pesticides and PPCPs discharged by the WWTP into the Congost river was calculated using the data taken from two different campaigns carried out in 2005 and 2006 (Fig. 2). The PPCP mass emission rate was lower than that for pesticides, which was probably due to the release of these compounds into the municipal sewer by industries. Furthermore, whereas PPCP discharge into the tributary was comparable to the inter- (same sampling period) and intra- (different sampling period) campaigns, herbicides and flunixin exhibited a high variability. These differences could be accounted for by the continual use of PPCPs, rather than by seasonal patterns in the use and production of these pesticides and flunixin. In fact, the strong correlation between the phenoxycarboxylic acids (i.e. mecoprop and MCPA) and the presence of another phenoxycarboxylic herbicide, called dicamba, suggests the application of a mixture of various phenoxyacarboxylic acids. Nevertheless, besides its application in agriculture, the phenoxy acid herbicide mecoprop is also used as a root

**Table 1 – Overall averages, removals (in brackets) and standard deviations of the general water quality parameters during the sampling period**

| | Temperature (°C) | DO ($mg\,L^{-1}$) | pH | $BOD_5$ ($mg\,L^{-1}$) | TSS ($mg\,L^{-1}$) | $NH_4$-N ($mg\,L^{-1}$) | Faecal coliforms[a] |
|---|---|---|---|---|---|---|---|
| | | | | May 2005 | | | |
| Influent | 19.3±0.2 | 1.1±0.4 | 7.6±0.1 | 10.8±3.0 | 17.8±10.9 | 37.8±9.2 | 5.1±4.5 |
| Effluent | 16.9±1.3 | 7.8±1.9 | 8.1±0.1 | 4.0±1.9 (63%) | 10.3±9.9 (42%) | 3.0±1.9 (94%) | 1.4±1.3 (3.7[b]) |
| | | | | February 2006 | | | |
| Influent | 16.6±0.9 | 1.3±0.4 | 6.9±0.1 | 11.2±2.3 | 16.3±4.9 | 61.0±8.9 | 4.0±3.4 |
| Effluent | 7.8±1.5 | 8.9±1.7 | 7.3±0.2 | 29.6±13.0 (n.r.) | 40.3±20.1 (n.r.) | 15.3±2.2 (79%) | 1.9±10.3 (2.1[b]) |

n.r., no removal observed.

[a] Expressed as log CFU/100 ml.

[b] Removal expressed as log units.

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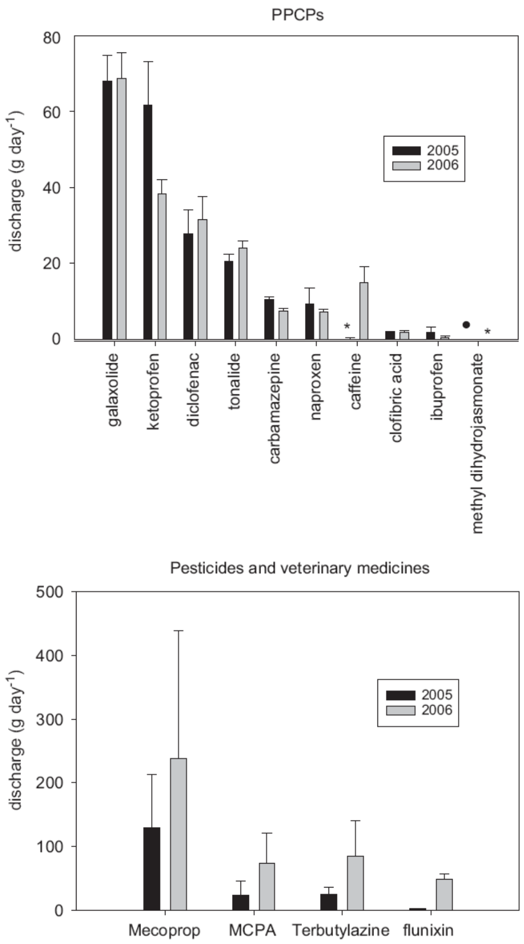

**Fig. 2 – Amount discharge of PPCPs, pesticides and veterinary medicines from the Granollers WWTP to the Congost River. Asterisk and black dots denote that samples were below limit of detection (LOD) and limit of quantification (LOQ), respectively.**

protection agent in bituminous roof sealing membranes (Foobe et al., 2006). In addition, the flunixin source may originate from a horse farm located in the WWTP drainage area.

In terms of concentrations, PPCPs were in the ng$\,L^{-1}$ instead of $\mu$g$\,L^{-1}$ for pesticides (Table 2) in the WWTP effluent (wetland influent). Whereas low PPCP concentrations are typical of a WWTP effluent (Ternes et al., 2004a), high pesticide concentrations are expected due to the inadequate treatment of these compounds by WWTPs (Reemtsma and Jekel, 2006).

Furthermore, the widely used PPCP compounds with high removal rates in WWTPs (i.e. ibuprofen, methyl drihydrojamonate) occur at low concentration levels in the WWTP effluent while the less used and more recalcitrant compounds are prevalent (i.e. ketoprofen, diclofenac and carbamazepine).

### 3.3. *Removal of PPCPs and pesticides in SFCWs*

Contaminant removal was studied by analysing both the dissolved and particulate phases from the influent and effluent. Whereas a major proportion of emerging pollutants occurred in the dissolved phase, galaxolide and tonalide were strongly associated with the particulate phase due their high hydrophobicity ($\log K_{\mathrm{ow}} = 5.7$–$5.9$).

According to their removal efficiency (Table 2), the emerging pollutants identified in this work can be classified as (i) highly efficiently removed when the elimination rate exceeded 95% (i.e. ibuprofen, ketoprofen,); (ii) efficiently removed when the elimination rate ranged from 80% to 90% (i.e. galaxolide, tonalide, mecoprop and MCPA); (iii) moderately removed, when the elimination rate ranged from 50% to 80% (i.e. naproxen, diclofenac) and, (iv) poorly removed compounds when the elimination rate was lower than 50% throughout the greater part of any one campaign (i.e. clofibric acid, carbamazepine, flunixin and terbutylazine).

It should be considered that this classification of emerging pollutant removal rates is strongly affected by the influent variability. Whereas flunixin and terbutylazine can reach high attenuation variability (i.e. 0–80%), PPCPs maintain relatively constant influent concentrations and removal rates.

As it is shown in Table 2, the high PPCP removal observed in this study is attributable to the high HRT of the wetland (i.e. 1 month) in comparison with the other treatment systems like HFCW or WWTP (a few days or hours). Furthermore, the prevalence of aerobic conditions in the wetland promotes biochemical pathways, such as aerobic respiration, that are more efficient in removing most emerging pollutants than anaerobic pathways (Zwiener and Frimmel, 2003; Yu et al., 2006). In addition, when this tertiary treatment is compared with advanced oxidation treatments like ozonation (Zwiener and Frimmel, 2000) or membrane bioreactors (MBR) (Kimura et al., 2005), the PPCP removal efficiency is similar. Furthermore, it has the advantage of low operational and maintenance costs, no external energy requirement and landscape integration; unfortunately, the SFCWs require a large surface area.

Furthermore, whereas photodegradation processes are not included in treatments like WWTPs or horizontal subsurface flow CWs, SFCWs allow it. In fact, photodegradation is reported as a feasible tool for eliminating certain PPCPs from aquatic environments (Andreozzi et al., 2003). Therefore, while ketoprofen, clofibric acid and carbamazepine seem to be recalcitrant in a variety of wastewater treatments (Beausse, 2004), ketoprofen was efficiently removed in the SFCW, which is probably linked to its fast photodegradation kinetics (Lin and Reinhard, 2005). Conversely, clofibric acid and cabamazepine behave as recalcitrants in SFCWs because of their low photodegradation, with described half-lives of about 100 days at high latitudes in winter (Andreozzi et al., 2003).

Pesticide and flunixin removal rates must be considered cautiously due to their high variability in their behaviour. Whereas a high removal for phenoxycarboxylic acids (mecoprop and MCPA) was obtained, clofibric acid,

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**Table 2 – Overall influent averages cold and warm season removal rates (in percentage) of the emerging micropollutants considered in this study**

| | Influent averages ($\mu g L^{-1}$) | Removal (%) | | | |
|---|---|---|---|---|---|
| | | June 2005 | February 2006 | HFCW(1) | WWTP |
| *Pharmaceuticals* | | | | | |
| Ibuprofen | 0.04 ± 0.03 | 96 ± 2 | 95 ± 1 | 71 | 60–70 (4)/90(5) |
| Naproxen | 0.34 ± 0.06 | 92 ± 1 | 52 ± 9 | 85 | 40–55 (4)/66(5) |
| Diclofenac | 1.25 ± 0.11 | 96 ± 1 | 73 ± 7 | n.r. | 24 (6)/17 (7) |
| Ketoprofen | 2.10 ± 0.70 | 99 ± 1 | 97 ± 1 | n.r. | 48–69(5) |
| Clofibric acid | 0.07 ± 0.01 | 36 ± 3 | 32 ± 8 | n.r. (2) | n.r.(7) |
| Carbamazepine | 0.37 ± 0.08 | 30 ± 10 | 47 ± 6 | 16 (2) | 8 (7)/7 (5) |
| *Veterinary medicines* | | | | | |
| Flunixin | 1.06 ± 1.36 | n.r. | 64 ± 3 | – | – |
| *Personal care products* | | | | | |
| Galaxolide | 2.86 ± 0.02 | 85 ± 2 | 88 ± 1 | 86 | 70–85 (4)/89(8) |
| Tonalide | 0.86 ± 0.10 | 88 ± 2 | 90 ± 1 | 88 | 75–90 (4)/8 (8) |
| *Pesticides* | | | | | |
| Mecoprop | 7.80 ± 3.24 | 79 ± 2 | 91 ± 1 | 22(3) | 13/50 (6) |
| MCPA | 2.01 ± 1.50 | 93 ± 1 | 79 ± 2 | – | – |
| terbutylazine | 2.30 ± 1.82 | 1 ± 14 | 80 ± 1 | – | – |
| HRT (hours) | – | 720 | 720 | 114 | 12–24 |

Removal values observed from other studies for horizontal subsurface flow constructed wetlands (HFCW) and conventional WWTP are shown for comparison. ($n = 10$) Sum of particulate and dissolved phase were considered for overall influent concentrations and removals.
n.r., no removal observed; 1. Matamoros and Bayona (2006) 2. Matamoros et al. (2005); 3. Matamoros et al. (2007);4. Carballa et al. (2004); 5. Daugthon and Ternes (1999); 6. Bernhard et al. (2006); 7. Heberer (2002b); 8. Simonich et al. (2002).

which is structurally related to mecoprop herbicides and clofibric acid exhibited a low removal rate. It is apparent that this may confer low metabolic and environmental stability to mecoprop. However, terbutylazine and flunixin removal were not evaluated due to their high influent variability.

### 3.4. *Seasonal removal variability*

Seasonal variability was examined when the two aforementioned campaigns were compared. Two physical conditions contribute to this variability, namely, solar radiation and water temperature. Low temperatures decrease the biodegradation kinetics and low sun irradiation decreases the photodegradation rates. As expected, compounds that require longer time for biodegradation and/or photodegradation were affected by seasonality (i.e. naproxen, diclofenac and MCPA). However, compounds that were eliminated by their interaction with organic matter and sediment (i.e. galaxolide and tonalide) (Simonich et al., 2002), recalcitrant compounds (carbamazepine and clofibric acid) and pharmaceuticals that were mostly eliminated (ibuprofen and ketoprofen) were not affected.

### 3.5. *Spatial trends*

Fig. 3 shows the spatial trends of the selected pollutants along the wetland in the last campaign. As it is shown, the first part of system made a greater contribution to elimination than the latter part of system. Conversely, clofibric acid and naproxen do not follow this trend, and behave similarly in both parts. In fact, clofibric acid has been proposed as a tracer compound in several studies, including constructed wetlands and sand filters (Drillia et al., 2005; Matamoros et al., 2005), with reported recalcitrant behaviour in a number of studies (Daugthon and Ternes, 1999). Although carbamazepine is also defined as a recalcitrant, its different removal behaviour could be attributed to its

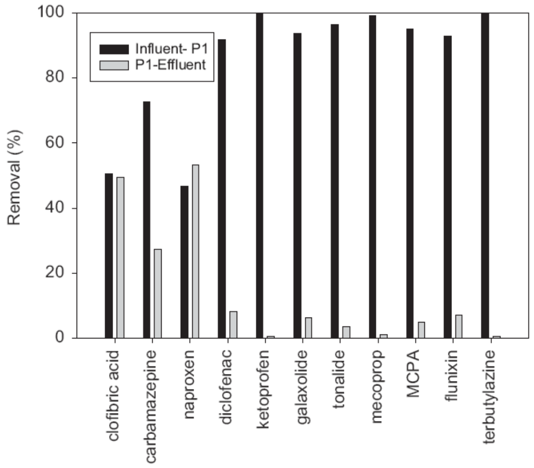

**Fig. 3 – Relative mass removal distributed in two SFCW sections. P1 represents an intermediate sample. Influent–P1 and P1–effluent are referred to the relative mass removal in the first and latter part, respectively.**

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Table 3 – Average and standard deviation of apparent $\log K'd$ values calculated from influent, P1 and effluent SFCW ($n = 6$)

| | $\log K'd$ | $\log K_{ow}$[a] | $\log D_{ow}$[b] | $\log K'_d$ | $\log K_{oc}$ |
|---|---|---|---|---|---|
| Galaxolide | $4.55 \pm 0.28$ | 5.6 | n.a. | 4.81 (Matamoros and Bayona, 2006); 3.3–3.7 (Ternes et al., 2004b) | 4.80 (Balk and Ford, 1999); 4.14 (Ternes et al., 2004b) |
| Tonalide | $4.76 \pm 0.27$ | 5.9 | n.a. | 5.00 (Matamoros and Bayona, 2006); 3.4–3.7(Ternes et al., 2004b) | 4.86 (Balk and Ford 1999); 4.17 (Ternes et al., 2004b) |
| Mecoprop | $1.91 \pm 0.24$ | 3.13 | 0.01 | – | 1.30 (Hansen, 2004) |
| MCPA | $2.21 \pm 0.09$ | 2.94 | −0.86 | – | 1.45 (Hansen, 2004) |
| Flunixin | $3.44 \pm 0.29$ | 4.90 | 3.78 | – | – |
| Terbutyazine | $3.33 \pm 0.32$ | 3.04 | n.a. | – | 2.55 (Hansen, 2004) |

n.a., not applicable.

[a] EPI suite (2007).

[b] Calculated at pH 7.

interaction with the organic matter present in the SFCW due to the higher $\log K_{ow}$ (2.25) than that for clofibric acid (−0.14) (Matamoros et al., 2005).

This high removal rate in the first part might be explained by the adaptation of the biofilm to the high pollutant burden in this section. However, the fact that first-order removal kinetics have a major influence on PPCPs in constructed wetlands—except for naproxen that was fitted to zero order (Matamoros and Bayona, 2006)—leads to the assumption that the low concentration of compounds leads to low removal rates. Therefore, whereas a high emerging pollutant removal difference was expected between SFCW sites, naproxen behaves similarly.

### 3.6. *Apparent sorption coefficients ($K'd$)*

Apparent distribution constants were obtained from the analysis of the particulate and dissolved phases of the influent, P1 and effluent. Table 3 shows the distribution constants of emerging pollutants (compounds that were up to LOQ in the particulate phase) and their comparison with those reported in other studies. Whereas fragrance compounds (galaxolide and tonalide) exhibited the highest interaction with particles, phenoxycarboxylic acids had the lowest interaction. Nevertheless, flunixin and terbutylazine showed a moderate interaction with suspended solids.

A decrease in the $K'd$ along the SFCW was expected due to the high proliferation of biomass from P1 to effluent and consequently increasing TSS concentration. However, the experimental $K'd$ was relatively constant (see standard deviation, Table 3). Therefore, biota uptake or higher removal efficiencies (sorption to the particulate phase, photodegradation and biodegradation) of these emerging pollutants in the dissolved phase are higher than those in the particulate phase are postulated as it was previously reported (Alexander, 1995; Schwarzenbach et al., 2003).

In addition, whereas fragrances and terbutylazine behave similarly in terms of $K_{ow}$, $K'd$ and $K_{oc}$, phenoxycarboxylic acids and flunixin have a lower $\log K'd$ than their $\log K_{ow}$. This different behaviour can be accounted for the ionization of carboxylic groups at neutral pH for flunixin and phenoxycarboxylic acids.

## 4. Conclusions

In summary, whereas PPCPs are discharged into the Congost River by the WWTP effluent at $\mathrm{ng\,L^{-1}}$ levels, herbicides and flunixin are at $\mu\mathrm{g\,L^{-1}}$. Emerging pollutant removal efficiency through the SFCW was compound dependant. In general, higher than 90% for the predominant PPCPs, except for the recalcitrant ones (i.e. carbamazepine and clofibric acid). Pesticides and veterinary drugs behave differently due to their high influent concentration variability leading to a wide removal efficiency range (0–90%). The higher removal efficiencies for emerging microcontaminants observed in this study than those reported for horizontal subsurface flow constructed wetlands and conventional WWTPs seem to be related to the high HRT (i.e. 1 month). This high HRT promotes biodegradation and photodegradation reactions that are involved in the removal of emerging contaminants. Furthermore, seasonal and spatial trends showed a high dependence on temperature (biodegradation) and sun irradiation (photodegradation) for the moderately removed compounds. Apparent distribution constants $K'd$ are strongly dependant on the compound ionization. On the other hand, the neutral compounds are correlated to their hydrophobicity ($\log K_{ow}$).

The results of this study clearly demonstrate that the studied wetland has a good capacity for removing a variety of emerging contaminants close to the ones obtained in high-cost tertiary treatments (ozonation or MBR). Therefore, the application of cost-effective technologies such as constructed wetlands should be considered as an efficient alternative for reducing the amount of emerging contaminants discharged into aquatic ecosystems.

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## 2.5.3. Discussió de les metodologies analítiques

Les metodologies analítiques emparades en aquest article per tal d'analitzar els PPCPs són les mateixes que es van emprar en els articles anteriors. La Figura 2.9 mostra un cromatograma corresponent a l'afluent del sistema, on s'assenyalen els nous compostos identificats a l'aiguamoll de Can Cabanes. Els nous compostos identificats són majoritàriament herbicides, possiblement emprats en la preservació d'edificis o bé en aplicacions agrícoles a la zona.

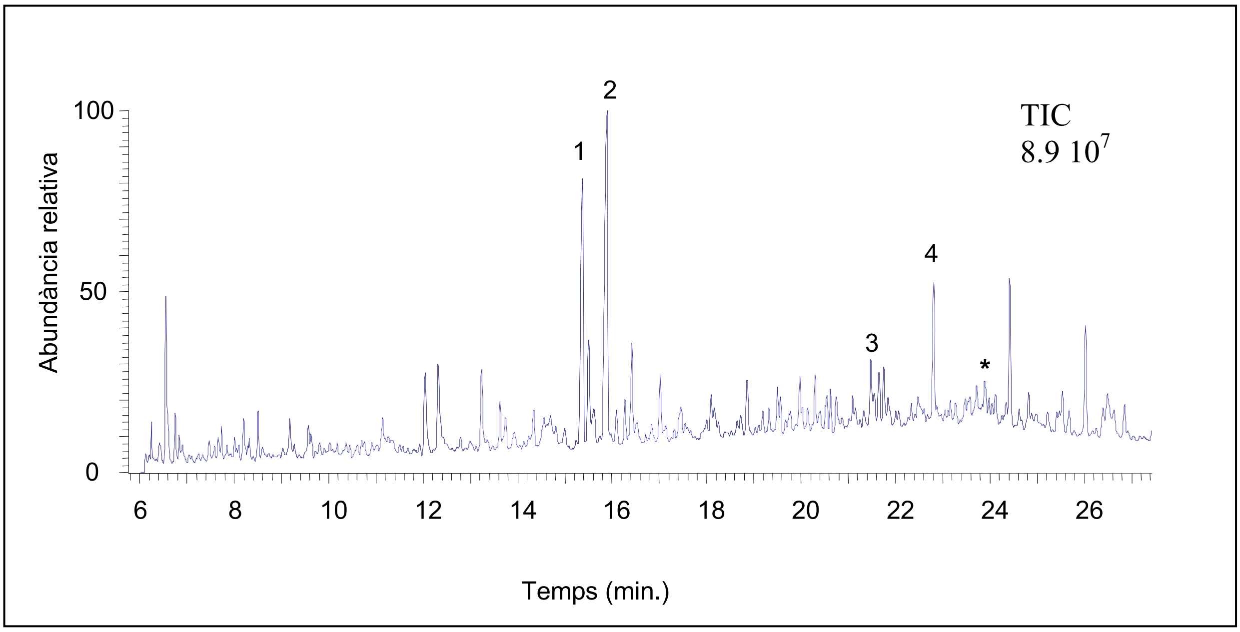

**Figura 2.9.** Registre del corrent iònic total en la modalitat d'escaneig d'una mostra de l'efluent de l'EDAR de Granollers on es mostren els nous compostos identificats. 1. Mecoprop; 2. MCPA; 3. Terbutilazina, 4. Flunixin, (*). Trifenilamina (*patró intern*).

**Paràmetres de qualitat analítica**

La repetibilitat es va avaluar analitzant per triplicat una mostra d'efluent de l'EDAR de Granollers. Per altra banda, els LODs i LOQs, es van calcular tal i com s'ha descrit anteriorment per als PPCPs (secció 2.3.1.5.). La recuperació es va calcular tal i com s'ha indicat anteriorment en la mateixa secció, en aquest cas es van fortificar 500 mL d'aigua residual amb 6 µg $L^{-1}$ de cada compost individual, mentre els filtres es fortificaren amb 1 µg $L^{-1}$. Els resultats es mostren en la Taula 2.10.

**Taula 2.10**. Repetibilitat (n=3), expressada com a percentatge de desviació estàndard relativa (RSD), la recuperació mitjana i la seva corresponent RSD (n=3) així com els LODs i LOQs dels mètodes analítics.

| Mostra aquosa | | | | |
|---|---|---|---|---|
| | Repetibilitat (%) | Recuperació (%) | LOD (ng L$^{-1}$) | LOQ (ng L$^{-1}$) |
| Mecoprop | 3.5 | 95±6 | 7 | 25 |
| MCPA | 2.8 | 96±6 | 2 | 10 |
| Terbutilazina | 4.2 | 89±9 | 6 | 21 |
| Flunixin | 3.0 | 94±9 | 9 | 29 |
| Sòlids suspesos | | | | |
| | Repetibilitat (%) | Recuperació (%) | LOD (ng L$^{-1}$) | LOQ (ng L$^{-1}$) |
| Mecoprop | 7.6 | 90±8 | 10 | 32 |
| MCPA | 3.8 | 85±2 | 20 | 66 |
| Terbutilazina | 6.1 | 93±6 | 10 | 33 |
| Flunixin | 6.2 | 94±6 | 23 | 78 |

Tots el paràmetres de qualitat van resultar ser satisfactoris, obtenint unes recuperacions individuals superiors al 80% en tots els casos. D'altra banda, tot i que els LODs i LOQs van resultar ser lleugerament superiors als descrits en la literatura (Comoretto et al., 2007), aquests van ser sufients per quantificar totes les mostres, tant de l'afluent com de l'efluent de l'aiguamoll de Can Cabanes.

### 2.5.4. Discussió dels resultats generals

En aquest nou sistema actuen tant les vies d'eliminació aeròbies i anaeròbies descrites anteriorment com la fotodegradació, nova via d'eliminació a tenir en compte. En aquest sentit, es descriu una moderada fotodegradació i biodegradació del diclofenac i el naproxèn, ja que si alguna de les dues vies, la fotodegradació (ketoprofèn) o la biodegradació (ibuprofèn), fos molt important no es podrien obervar diferències estacionals, observant-se elevades eficiències d'eliminació al llarg de tot l'any. Estudis recents en el camp de la fotodegradació de fàrmacs, atribueixen una moderada/alta fotodegradació al diclofenac (Buser et al., 1998) i al naproxèn (Lin i Reinhard, 2005), mentre que la seva biodegradació és baixa. D'aquesta manera es podria dir que la fotodegradació és clau en l'eliminació d'aquests fàrmacs en aquests tipus d'aiguamolls. Malgrat això, la generació de fotoproductes de degradació, fa que molts cops tot i que el producte de partida hagi desaparegut, els generats puguin ser molt més tòxics, aquest és el cas del diclofenac (Schmitt-Jansen et al., 2007).

Per altra banda, donat que l'aiguamoll tracta menys del 1% de l'aigua abocada per l'EDAR de Granollers, la descàrrega de gran nombre i quantitat de contaminants emergents, tant PPCPs com plaguicides al riu Congost, fa que aigües avall el possible efecte que aquests puguin causar sobre la biota sigui un problema a resoldre. Tot i això, la construcció d'una sèrie de HFCWs prop de la desembocadura del riu Besòs (Huertas et al., 2006), del qual el Congost n'és un afluent, ha fet millorar les condicions ecològiques del riu prop de la seva desembocadura.

En aquest sentit, l'ús d'aquest sistema després de l'EDAR va lligat a una disminució de la descàrrega de contaminants al medi així com a la creació, en aquest cas, d'una àrea de demostració i d'educació ambiental.