

ASSESSMENT OF WASTEWATER PHYTOTECHNOLOGIES FOR THE ATTENUATION OF EMERGING ORGANIC CONTAMINANTS AND DEVELOPMENT OF INNOVATIVE ANALYTICAL METHODS FOR THEIR DETERMINATION

Aida Garcia Rodríguez

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Universitat de Girona

Doctoral Thesis

**Assessment of wastewater phytotechnologies for
the attenuation of emerging organic contaminants
and development of innovative analytical methods
for their determination**

Aida Garcia Rodríguez

2016

Doctoral program in water science and technology

Supervised by Dr. Clàudia Fontàs and Dr. Víctor Matamoros
(Tutor: Dr. Clàudia Fontàs)

Thesis submitted in fulfilment of the requirements for the degree of Doctor from the
University of Girona



Dr. Clàudia Fontàs, of the University of Girona, and Dr. Víctor Matamoros, of the IDAEA-CSIC from Barcelona,

WE DECLARE:

That the thesis titled “Assessment of wastewater phytotechnologies for the attenuation of emerging organic contaminants and development of innovative analytical methods for their determination”, presented by Aida Garcia Rodríguez to obtain a doctoral degree, has been completed under our supervision and meets the requirements to opt for an International Doctorate.

For all intents and purposes, we hereby sign this document.

Dr. Clàudia Fontàs

Dr. Víctor Matamoros

Girona, 20 June of 2016

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Als meus pares,

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LIST OF PUBLICATIONS

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Garcia-Rodríguez, A., Sagristà, E., Matamoros, V., Fontàs, C., Hidalgo, M., Salvadó, V., 2014. Determination of pharmaceutical compounds in sewage sludge using a standard addition method approach. *Int. J. Environ. Anal. Chem.* 94, 1199-1209.

Garcia-Rodríguez, A., Matamoros, V., Fontàs, C., Salvadó, V., 2015. The influence of *Lemna* sp. and *Spirogyra* sp. on the removal of pharmaceuticals and endocrine disruptors in treated wastewaters. *Int. J. Environ. Sci. Technol.* 12, 2327-2338.

Garcia-Rodríguez, A., Matamoros, V., Kolev, S.D., Fontàs, C., 2015. Development of a polymer inclusion membrane (PIM) for the preconcentration of antibiotics in environmental water samples. *J. Membr. Sci.* 492, 32-39.

Garcia-Rodríguez, A., Fontàs, C., Matamoros, V., Almeida, M.I.G.S., Cattrall, R.W., Kolev, S.D., 2016. Monitoring of sulfamethoxazole in natural waters with a polymer inclusion membrane-based passive sampler. Minimizing the effect of the flow pattern of the aquatic system. *Microchem. J.* 124, 175-180.

LIST OF ABBREVIATIONS

2,4-D	2,4-dichlorophenoxyacetic acid
17-ET	17 α -ethinylestradiol
ACAPh	Acetaminophen
Al⁺Cl⁻	Aliquat 336
AOPs	Advanced oxidation processes
AP	Anaerobic pond
BBPA	Bis(1-butylpentyl)adipate
BLMs	Bulk liquid membranes
BPA	Bisphenol A
CAFF	Caffeine
CARB	Carbamazepine
CAS	Conventional activated sludge
CFMs	Chemically-functionalized membranes
CLF AC	Clofibric acid
CTA	Cellulose triacetate
CWs	Constructed wetlands
d	Days
DAD	Diode array detector
DBP	Dibutylphthalate
DBS	Dibutylsebacate
DCF	Diclofenac
DDD	Defined daily dose
DEHA	Bis(2-ethylhexyl)adipate
DGT	Diffusion gradient thin films
DOM	Dissolved organic matter

D_{ow}	Octanol-water distribution coefficient
dw	Dry weight
E2	17 β -estradiol
ECD	Electron capture detector
EDCs	Endocrine disrupting chemicals
EF	Extraction efficiency
ELMs	Emulsion liquid membranes
EOCs	Emerging organic contaminants
ESI	Electrospray ionisation
EU	European Union
FID	Flame ionization detector
FOD	Frequency of detection
FP	Facultative pond
FW	Fresh weight
GC	Gas chromatography
HF	Horizontal flow
HF-LPME	Hollow fiber liquid phase microextraction
HLRs	Hydraulic loading rates
HPLC	High performance liquid chromatography
HRAPs	High-rate algal ponds
HRT	Hydraulic retention time
IBP	Ibuprofen
ILCs	Isotope-labelled compounds
J₀	Initial flux
k	Pseudo-first order kinetic removal rate
k	Removal rate coefficient
K_a	Acid dissociation constant

K_H	Henry's law constant
K_{ow}	Octanol-water partitioning coefficient
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
LC-MS/MS	Liquid chromatography - tandem mass spectrometry
LDPE	Low density polyethylene
LECA	Light expanded clay aggregates
LLE	Liquid-liquid extraction
LMs	Liquid membranes
LOD	Limits of detection
MAE	Microwave assisted extraction
MBS	Membrane based systems
MBRs	Membrane biological reactors
MCPA	2-methyl-4-chlorophenoxyacetic acid
MP	Maturation pond
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MW	Molecular weight
NAP	Naproxen
NF	Nanofiltration
NI	Negative ionisation
NPD	Nitrogen-phosphorus detector
NPOE	2-nitrophenyloctyl ether
NPPE	2-nitrophenylpentyl ether
NSAIDs	Non-steroidal anti-inflammatory drugs
OTC	Oxytetracycline

PAHs	Polycyclic aromatic hydrocarbons
PC1	First component
PC2	Second components
PCA	Principle component analysis
PCBs	Polychlorinated biphenyls
PCDD	Polychlorinated dibenzo-p-dioxins
PCPs	Personal care products
PDA	Photodiode array detector
PF	Preconcentration factor
PI	Positive ionisation
PIM	Polymer inclusion membrane
PLE	Pressurised liquid extraction
POCIs	Polar organic chemical integrative samplers
POPs	Persistent organic pollutants
PPs	Polishing ponds
PPCPs	Pharmaceutical and personal care products
PROPR	Propranolol
PTFE	Polytetrafluoroethylene
PVC	Polyvinylchloride
PVDF	Polyvinylidene fluoride
R	Receiver
RBCs	Rotating biological contactors
RO	Reverse osmosis
RSD	Relative standard deviation
rt	Retention time
S	Source
SAs	Sulfonamides

SF	Surface flow
SLMs	Supported liquid membranes
SMX	Sulfamethoxazole
SMZ	Sulfamethazine
SPDM	Semi-permeable membrane device
SPE	Solid phase extraction
SPME	Solid phase microextraction
SPY	Sulfapyridie
SRT	Sludge retention time
SSF	Subsurface flow
STZ	Sulfathiazole
S_w	Water solubility
SX	Solvent extraction
t_{1/2}	Half-lives
TC	Tetracycline
TCD	Thermal conductivity detector
TCs	Tetracyclines
TE	Transport efficiency
THF	Tetrahydrofuran
TPs	Transformation products
TWA	Time weighted average
UF	Ultrafiltration
Unicef	United Nations Children's Fund
USE	Ultrasound assisted extraction
USEPA	US Environmental Protection Agency
UV	Ultraviolet
UW	Ultrapure water

VF	Vertical flow
VOCs	Volatile organic compounds
WHO	World Health Organization
WRF	White-rot fungi
WSPs	Waste stabilization ponds
WW	Wastewater
WWTPs	Wastewater treatment plants

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ABSTRACT

Water scarcity is an issue of great concern due to the increase of water demand and climate change. This problem can be solved by using reclaimed water. However, in addition to water quantity, reclaimed water needs to meet quality standards as well. Wastewater treatment plants (WWTPs) are designed to remove easily or moderately biodegradable compounds, but they are not able to completely remove emerging organic contaminants (EOCs). For instance, EOCs such as pharmaceuticals and endocrine disrupting compounds (EDCs) are continuously being introduced into the environment through different pathways, which makes some of them to be considered as pseudo-persistent. As consequence, these compounds have been found in water bodies where, they have been reported to pose adverse effects on both human health and ecosystems.

Therefore, it has become crucial to conduct research studies to develop methodologies for their monitoring and to evaluate the effectiveness of the processes occurring in wastewaters eco-technologies for removing them from WWTP effluents.

Within this thesis, studies focused on the development of simple-to-use and innovative analytical methods to determine EOCs in both waters and biosolid have been developed. It has been demonstrated that a fast, robust, and easy to use method can be applied for the determination of pharmaceuticals in sewage sludge. A method based on ultrasound assisted extraction (USE) with a standard addition calibration has allowed the simultaneous determination of some daily use pharmaceuticals in biosolid and sewage sludge samples from different WWTPs over an eight month period.

Moreover, due to the low concentration at which some EOCs are present in natural waters, it is important to develop reliable analytical preconcentration methods that ease their determination and routine monitoring. We have evaluated the use of polymer inclusion membranes (PIMs) as a new tool for the extraction, transport and preconcentration, in a single step, of antibiotics present in waters. Afterwards, a cheap and simple-to-use new passive sampling device based on PIMs has been tested to be used for the monitoring of a widely used antibiotic, named sulfamethoxazole (SMX), commonly found in WWTP effluents and environmental waters.

In addition, we also aimed to assess the effectiveness of the processes occurring in biologically-based WW reclamation systems, such as constructed wetlands (CWs) and polishing ponds (PPs), for removing EOCs from WWTP effluents. The processes were evaluated by assessing the following factors: .the presence of bacterial communities, organic matter, sunlight radiation, and aquatic plants. Our findings have highlighted that biodegradation and photodegradation are the most important removal processes occurring in natural systems. Furthermore, they suggest that PPs are a highly effective and cost efficient technology for treating polluted waters containing the studied EOCs.

RESUM

L'increment en la demanda hídrica i el canvi climàtic han comportat una disminució de l'accés a l'aigua a nivell global. L'ús d'aigua regenerada s'ha convertit en una eina indispensable per tal d'intentar pal·liar aquest problema. Malgrat això, les estacions de tractament d'aigües residuals (EDAR) no han estat dissenyades per eliminar compostos com els contaminants orgànics emergents (COE) i molts d'ells acaben abocant-se al medi aquàtic. A tall d'exemple, COE com els productes farmacèutics i els compostos disruptors endocrins (CDE) s'han identificat i quantificat tant en aigües superficials com biosòlids. Tot i les baixes concentracions, són diferents els estudis que han demostrat els efectes adversos de la presència de COE en la salut humana i en els ecosistemes.

Per tant, és essencial dur a terme estudis d'investigació per desenvolupar metodologies que permetin el monitoratge d'aquests contaminants en el medi. Així com avaluar l'efectivitat de sistemes naturals de tractament d'aigües residuals de baix cost i estudiar-ne els processos que dirigeixen l'atenuació de COE.

En la present tesi s'han desenvolupat mètodes analítics simples i innovadors que permeten determinar COE tant en aigües d'efluents d'EDAR com en biosòlids. S'ha desenvolupat un mètode ràpid, robust i fàcil d'utilitzar per a la determinació fàrmacs en fangs d'EDAR. Aquest mètode basat en l'extracció assistida per ultrasons amb calibratge per addició estàndard ha permès la determinació simultània d'alguns fàrmacs d'ús diari en mostres de fangs i biosòlids de diferents EDAR.

D'altra banda, a causa de la baixa concentració a la qual alguns EOC es troben en les aigües naturals, és important desenvolupar mètodes analítics que permetin preconcentrar-los per tal de facilitar-ne la seva determinació i monitoratge. Per això, hem avaluat l'ús de membranes d'inclusió polimèrica (MIP) com a nova eina per a l'extracció, el transport i la preconcentració, en un sol pas, de certs antibiòtics presents en aigües. Posteriorment, s'ha avaluat l'ús d'un nou dispositiu de mostreig passiu, barat i fàcil d'usar, basat MIPs, per al monitoratge d'un antibiòtic altament utilitzat i detectat freqüentment en efluents d'EDARs i aigües ambientals.

A més, la nostra investigació també ha tingut com a objectiu l'avaluació dels processos d'atenuació de COE que tenen lloc en sistemes de tractament d'aigües residuals basats en processos biològics i de baix cost, com ara les llacunes d'afinament (LLA). Hem avaluat l'efecte de la presència de microorganismes (comunitats bacterianes), matèria orgànica, radiació solar, i plantes aquàtiques en l'eliminació de diversos fàrmacs i CDE presents en efluents d'EDAR. Els nostres resultats han demostrat que la biodegradació i la fotodegradació són els processos més rellevants. A més, suggereixen que l'ús de LLA

podria ser de gran interès per atenuar la presència de COE, així com l'estrès hídric al qual està sotmès tant l'ecosistema aquàtic, com l'agricultura.

RESUMEN

La escasez de agua es un tema de preocupación global debido al incremento en la demanda hídrica y el cambio climático. Con la idea de solucionar este reto, el uso del agua regenerada se ha convertido en los últimos años en una herramienta indispensable para la gestión de este limitado recurso. Sin embargo, más que la cantidad del agua, el agua regenerada tiene que cumplir unos estándares de calidad. Las estaciones depuradoras de aguas residuales (EDARs) han sido diseñadas para eliminar compuestos fácilmente o moderadamente biodegradables, no son capaces de eliminar por completo compuestos como los contaminantes orgánicos emergentes (COEs). En este sentido, COEs como los fármacos y los compuestos disruptores endocrinos (CDEs) introducen de forma continua en el medio ambiente, hecho que convierte a algunos de ellos en pseudo-persistentes. Aún y la baja concentración en que éstos compuestos se encuentran en aguas y biosólidos, pueden producir efectos adversos en la salud humana y los ecosistemas.

Por este motivo, es relevante desarrollar metodologías para su seguimiento así como evaluar los procesos de eliminación que tienen lugar en las diferentes eco tecnologías de aguas residuales y que están involucrados en la atenuación de COEs procedentes de efluentes de EDARs.

En la presente tesis, los estudios se han centrado en el desarrollo de métodos analíticos simples e innovadores para la determinación de COEs tanto en aguas como en biosólidos.

Se ha demostrado que un método rápido, robusto y fácil de usar puede ser aplicado para la determinación de fármacos en lodos de depuradora. Un método basado en la extracción asistida por ultrasonido con una calibración de adición estándar ha permitido la determinación simultánea de determinados fármacos de uso diario en muestras de lodos y biosólidos de distintas EDARs durante un período de ocho meses.

A la vez, debido a la baja concentración en que se encuentran algunos COEs en aguas naturales, es importante desarrollar métodos para su preconcentración por tal de facilitar su determinación y monitoreo. Hemos evaluado el uso de membranas de inclusión

polimérica (MIP) como nueva herramienta para la extracción, el transporte y la preconcentración, en un solo paso, de distintos antibióticos presentes en las aguas. Posteriormente, un nuevo dispositivo de muestreo pasivo fácil de usar, basado en MIP, ha sido probado para el monitoreo de un antibiótico ampliamente utilizado y detectado en efluentes de EDAR y aguas ambientales.

Además, debido a la creciente presencia de los sistemas de tratamiento de agua residual basados en procesos biológicos, tales como humedales construidos (HCs) y lagunas de afinamiento (LA), nuestra investigación ha tenido también como objetivo evaluar los procesos que tienen lugar en este tipo de tratamientos con respecto a la eliminación de distintos COEs.

Hemos estudiado cómo la presencia de comunidades bacterianas, materia orgánica, la radiación solar, y plantas acuáticas están implicadas en la eliminación de fármacos y CDEs presentes en efluentes secundarios de EDARs. Nuestros resultados han puesto de manifiesto que los procesos de biodegradación y fotodegradación son los más relevantes y sugieren que las LA son una tecnología eficaz y rentable para el tratamiento de aguas residuales que contengan los COEs estudiados.

CHAPTER 1

General Introduction

Water is an essential resource for the sustainable socio-economic development of any country. As population grows, the demand for water from the domestic, agricultural and industrial sectors increases, and the pressure on water resources intensifies leading to tensions, conflicts among users, and excessive pressure on the environment. The increasing stress on freshwater resources brought about by rising water demand and its profligate use, as well as by growing pollution worldwide, is of serious concern (“Everylittledrop” <http://everylittledrop.com.au/>; Pedrouzo Lanuza, 2011; Heather et al., 2013). Indeed, the United Nations Children's Fund (UNICEF), the United Nations (UN), and the World Health Organization (WHO) have identified access to clean water as the greatest relevant global crisis of this century.

While nearly 70% of the Earth's surface is covered by water, only 2.5 % is freshwater, and only 1% of it is available for human consumption (Castro, 2007). In accordance with Directive 2006/118/EC of the European Parliament and of the Council on the protection of groundwater against pollution and deterioration, groundwater is the largest source of freshwater in the European Union (EU), and at the same time the most sensitive one (Jurado et al., 2012; Heather et al., 2013).

Climate change is having an influence on the decrease of available freshwater resources, but water scarcity is not only a natural phenomenon, it is also a human one. Water scarcity occurs when water demand approaches or exceeds the accessible water supply (Heather et al., 2013). Population growth, urbanization, economic and lifestyle changes, industrial processes, and the development of new technologies, among others, have led to increased demand for water. According to worldwide statistics, freshwater demand is increasing by 64 billion cubic metres per year (“Worldometers” <http://www.worldometers.info/water/>). This high water demand together with temporal and spatial variations in water availability, contributes to water scarcity, which has a significant effect on aquatic ecosystems (World water council, 2009; WBCSD, 2009).

World population growth is expected to increase by another 40-50% within the next 50 years (Stewart et al., 2014). Thus, both water demand and wastewater (WW) production due to the increase of agricultural and industrial activities will rise, creating more conflicts between water demand and supply, and originating challenges for the management and development of alternative water supply systems (World water council, 2009; Meffe and de Bustamante, 2014). Moreover, water resources will be much more exposed to sources of contamination (e.g. waste disposals, discharges of WW effluents, fertilizers used on farmland) and consequently, water will be a limiting

factor of the socio-economic development and urbanization (World water council, 2009; Department of Civil and Environmental Engineering, 2009; Jurado et al., 2012; Meffe and de Bustamante, 2014).

In order to minimize pressure on the environment and on water resources, and to guarantee economic and social security for human development, water management measures should be taken to study alternative water supplies by taking into consideration water end use, risks, and energy requirements (EPA <http://www.epa.vic.gov.au/>, 2014). Nowadays, water reclamation and reuse activities (e.g. land crop irrigation, golf course irrigation) have become indispensable for water management (EPA <http://www.epa.vic.gov.au/>, 2014; World water council, 2009).

Water security consists of more than water quantity; the quality of the water is remarkably important as well, for both human and ecological safety (i.e. biodiversity preservation and ecosystems maintenance) (World water council, 2009; Heather et al., 2013). Different treatments applied in wastewater treatment plants (WWTPs) are designed to remove easily or moderately biodegradable carbon, nitrogen, and phosphorous compounds, as well as microbial organisms (Borràs and Sala, 2006; Verlicchi et al., 2012). However, there are still hundreds of other chemicals in WW, such as emerging organic contaminants (EOCs), that are not removed by WWTPs and therefore can have potentially negative effects on both human health and ecosystems (Murray et al., 2010; Corada-Fernández et al., 2015).

1.1 Emerging organic contaminants in the environment

A great variety of synthetic organic chemicals are currently used by society in large quantities for many different purposes (e.g. human and veterinary healthcare, food preservation, agriculture) (Pal et al., 2010). Because of population growth, urbanization, and economic development, rising amounts of these anthropogenic pollutants are continuously introduced into the environment (Yan et al., 2014). The concern is that they will contaminate surface and drinking water supplies, where relatively low concentrations may exert adverse ecological effects (Pal et al., 2010).

In the last three decades, the impact of organic pollutants on the environment has focused almost exclusively on persistent organic pollutants (POPs). These are organic

chemical substances that possess a particular combination of physical and chemical properties such that, once released into the environment, they (1) remain intact for exceptionally long periods of time (many years); (2) become widely distributed throughout the environment as a result of natural processes involving soil, water and, most notably, air; (3) accumulate in the fatty tissue of living organisms including humans, (4) are found at higher concentrations at higher levels in the food chain; and (5) are toxic to both humans and wildlife (Stockholm convention, 2008). To address this global concern, the European Community joined forces with 91 other states to sign the Stockholm Convention in 2001 (in 2013, there are 178 signatory states plus EU). These signers agreed to reduce or eliminate the production, use, and/or release of 12 key POPs. Some of the compounds included in this list are pesticides (e.g. lindane), polychlorinated biphenyls (PCBs) or dioxins such as polychlorinated dibenzo-p-dioxins (PCDD) (Verenitch et al., 2006). The Stockholm Convention was adapted to EU legislation in regulation (EC) No 850/2004 (European Parliament and of the Council, 2004).

However, in recent years, attention has increasingly focused on the occurrence, environmental fate, and potential toxicity of another group of organic bioactive chemicals that are continuously released into water bodies in considerably large quantities (Garcia-Rodríguez et al., 2014; Zenker et al., 2014). Collectively named as EOCs, some of them are suspected of having similar harmful effects on the ecosystems as POPs, but they are still unregulated by environmental legislation (Lapworth et al., 2012; Stewart et al., 2014; Zenker et al., 2014). Although EOCs have engendered a new generation of water quality concerns (Watkinson et al., 2009; Yan et al., 2014), information about their distribution and long-term effects on the environment is still scarce (Pal et al., 2010; Garcia-Rodríguez et al., 2014; Stewart et al., 2014; Zenker et al., 2014).

Research on EOCs is complex, as it comprises a large number of diverse organic chemicals, with different structures and physicochemical properties. To deal with this, a network of reference laboratories, research centres and related organizations known as NORMAN promotes the exchange of information on emerging environmental substances. Table 1.1 shows the list of emerging substances identified by the NORMAN network (<http://www.norman-network.net/>).

Table 1.1. NORMAN list of emerging substances.

Emerging substances	
Algal toxins	Industrial chemicals
Biocide transformation products	Lubricants
Biocides	Moth repellent / Antimicrobial agents
Bio-terrorism / Sabotage agents	Perfluoroalkylated substances and their transformation products
Disinfection by-products (drinking water) / biocides	Personal care products
Drugs of abuse	Pharmaceuticals
Flame retardants	Plant protection products
Food additives	Plasticisers
Gasoline additives	Surfactants
	Trace metals and their compounds

Nonetheless, EOCs can also be classified according to their origin, environmental persistence, human toxicity and ecotoxicity. Currently, some of the best known and documented EOCs are pharmaceuticals, personal care products (PCPs), endocrine disrupting chemicals (EDCs), and lifestyle compounds (Pedrouzo Lanuza, 2011; Jurado et al., 2012; Lapworth et al., 2012; Garcia-Rodríguez et al., 2014). Moreover, new chemicals are continuously introduced into the market, leading to the appearance of possible new EOCs, discovered thanks to the ongoing progress in analytical techniques (Pal et al., 2010; Stewart et al., 2014). All of these compounds are displayed in the aquatic environment, but the presence of some of their transformation products (TPs) should also be taken into consideration, as in some cases they can have the same or even more harmful effects on the ecosystems (Zenker et al., 2014).

1.1.1 Source and fate of EOCs in the environment

EOCs are ubiquitous in the aquatic environment (Garcia-Rodríguez et al., 2014). These contaminants are released into environmental waters via a wide range of different point sources (Pal et al., 2010; Lapworth et al., 2012; Yan et al., 2014).

The main ones are summarised in Fig. 1.1:

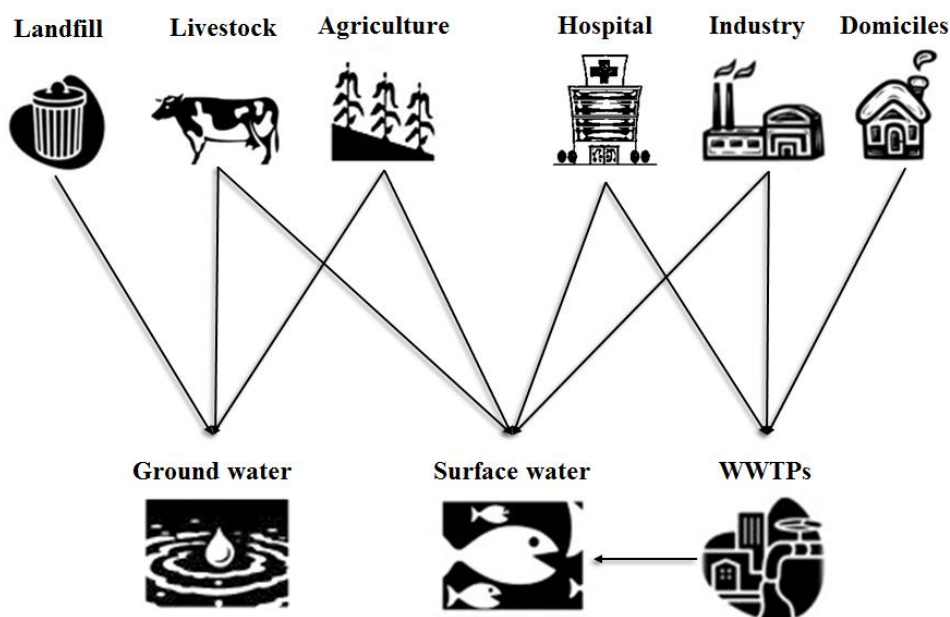


Figure 1.1. EOCs point sources (adapted from Moore et al. (2011))

Most of the EOCs are daily use compounds. For example, in Spain, the defined daily dose (DDD) of some pharmaceutical compounds such as carbamazepine (CARB) per 1,000 inhabitants per day was 1.23 in 2006, but up to 6.38 and 21.51 for diclofenac (DCF) and ibuprofen (IBP), respectively, in 2012 (AEMPS, 2014). On the other hand, the consumption of antibiotics (i.e. penicillin, beta-lactams, tetracyclines (TCs), sulfonamides (SAs), macrolides, quinolones, and others) in 2013 was of 25 DDD per 1,000 inhabitants per day (ECDC, 2013).

Therefore, some of the main point sources of these compounds are WWTPs, which treat WWs from different sources: domestic, livestock, and agricultural activities as well as effluents from hospitals and chemical manufacturing plants (Pal et al., 2010). Although PCPs and industrial products are discharged directly into WW after domestic or industrial use, pharmaceutical compounds are mainly discharged into the WW after human consumption. Human and veterinary pharmaceuticals are not completely metabolized after consumption, thus a percentage of the parent compounds and the associated metabolites are excreted via urine and faeces, which can end up in surface waters or WWTPs, in case of humans, or in the soil and, consequently, surface or groundwater, in the case of animal waste. Furthermore, expired or unused

pharmaceuticals can be disposed inappropriately in waters or landfill sites, facilitating their entrance into the water cycle (Heberer, 2002; Pal et al., 2010; Li, 2014; Meffe and de Bustamante, 2014). Once EOCs are present in surface waters, they are dispersed to other water bodies such as estuaries, aquifers, and marine systems (Pal et al., 2010).

The use of reclaimed water for agricultural irrigation has emerged as a new strategy for coping with water scarcity in semiarid countries such as ours. This leads to the human health concern that some EOCs present in WW effluents can be incorporated into the diet through crop uptake. As has already been published, this depends on the physicochemical properties of the different compounds as well as the environmental crop growing conditions (Calderón-Preciado et al., 2011; Calderón-Preciado et al., 2013).

The farmland application of biosolids generated in WWTPs is another important source of EOCs in agriculture. After treatment, the sewage sludge generated in WWTPs, known as biosolid, becomes an important source of organic matter, organic acids, nitrogen, phosphorus, and other nutrients that make it suitable as agricultural fertilizer, which is highly promoted by the EU as a good environmental option (Sagrìstà, 2012). However, during WW treatment, some EOCs, depending on their physicochemical properties, specifically those which are defined as hydrophobic ($\log K_{ow} > 4$, this concept will be defined in section 1.1.3), tend to interact with sewage sludge rather than remaining in the treated WW. This promotes the accumulation of certain EOCs in the biosolids, which are then used in agriculture as fertilizers. Thus, the use of biosolids in farming can promote the occurrence of EOCs in surface and groundwater systems after agricultural run-off or even their potential uptake by crops (Li, 2014).

The fate of EOCs in the environment involves very complex processes that depend on different factors (López de Alda and Barceló; Pal et al., 2010):

- Source points
- Physicochemical properties of each compound (e.g. polarity, acid-base properties, volatility, and polarity)
- Degree of natural attenuation of each compound (e.g. chemical-, photo-, and bio-degradation)
- Degree of sorption onto suspended solids, sediments, and vegetation, among others

- Environmental conditions (e.g. temperature, water pH, water flow rate, and organic matter)

1.1.2 Overview of the EOCs studied in this work

Among the broad range of existing EOCs, the studies conducted as part of this thesis are focused on pharmaceuticals, endocrine disrupting compounds (EDCs), and lifestyle compounds. These compounds were selected in accordance with their different physicochemical properties and their ubiquity in WWTP effluents.

- *Pharmaceuticals*

Pharmaceuticals are chemicals used universally for the diagnosis, mitigation, and treatment of human and animal diseases. Considered as the most important group of EOCs due to their impact on ecosystems, they are produced worldwide and have different physicochemical properties (Patrolecco et al., 2013; Zenker et al., 2014). In addition, because pharmaceuticals are incompletely absorbed after consumption, they are excreted as the parent compounds, metabolites or water soluble conjugates (Togola and Budzinski, 2007; Zenker et al., 2014).

Table 1.2 shows the different families of pharmaceuticals studied in this thesis in accordance with their end use (antibiotics, analgesics and non-steroidal anti-inflammatory drugs (NSAIDs), blood lipid regulators, β -blockers, and psychoactive drugs).

Table 1.2. Groups of pharmaceutical compounds studied in this thesis and their relevance.

Antibiotics
Antibiotics are compounds widely used for human and veterinary purposes, and in aquaculture. They are mainly applied to treat and prevent diseases, increase feed efficiency and enhance livestock growth rate (Kümmerer, 2009). They act by inhibiting or erasing the growth of microorganisms, such as viruses, fungi, or bacteria (Drlica and Perlin, 2011; Michael et al., 2013). Sulfonamides (SAs) and tetracyclines (TCs) were chosen for this thesis due to their broad reported use (Kümmerer, 2009; Li, 2014; Tang et al., 2015).

Table 1.2 Groups of pharmaceutical compounds studied in this thesis and their relevance (**continued**).**Analgesics and NSAIDs**

Analgesics are pain-relief and anti-inflammatory drugs that include non-narcotic analgesics (e.g. acetaminophen (ACAPh) and NSAIDs (e.g. DCF, NAP, and IBP), among others. They act in various ways on the peripheral and central nervous systems and are widely used to alleviate the pain present in almost all diseases (Li, 2014).

Large amounts of these pharmaceuticals are annually prescribed by medical professionals, but high quantities are frequently sold without prescriptions (Heberer, 2002; Ziylan and Ince, 2011). The ones studied in this thesis (those named above) are among the most consumed analgesics in Spain (Heberer, 2002; Martinez Bueno et al., 2012).

Blood lipid regulators

Clofibric acid (CLF AC) is an active metabolite of some widely used blood lipid regulators (i.e. clofibrate, theofibrate, and etofibrate) (Stumpf et al., 1999; Dietrich et al., 2005; Spellman, 2014) that have physicochemical properties similar to the NSAIDs described above, as they also have an acidic group in their molecular structure (Petrovic et al., 2013). Lipid regulators lower the concentration of lipoproteins, the agents responsible for the cholesterol and triglycerides in blood (Sullivan et al., 2005). These drugs are widely consumed by humans and are therefore likely to be found them in the environment, as along with their metabolites, mainly CLF AC, which has been reported to have a high persistence in the environment and also to possess biological activity that can be similar or completely different from its parent compound (Nunes et al., 2004).

 β -blockers

Anti-hypertensive drugs or β -blockers, such as propranolol (PROPR), are used for the treatment of cardiovascular disorders. They act by blocking the action of stress hormones, reducing the blood pressure in the arteries (Lichtfouse et al., 2011; Gros et al., 2012; Li, 2014).

Table 1.2 Groups of pharmaceutical compounds studied in this thesis and their relevance (**continued**).**Psychoactive drugs**

CARB is a well-known psychoactive drug used as an anticonvulsant and mood-stabilizer, mainly administered for the treatment of epilepsy and bipolar disorder. It acts by reducing nerve impulses that causes pain ([Drugs.com](#), 2000-2015).

According to Li et al. (2014), evidence shows that CARB is transformed to a variety of degradation intermediates, producing biologically active products through transformative reactions of the parent compound. Nevertheless, it has been classified as one of the most recalcitrant pharmaceutical compounds in the aquatic environment (Meffe and de Bustamante, 2014; Zhang et al., 2014).

- *Endocrine disrupting compounds*

The US Environmental Protection Agency (USEPA) defines an EDC as “an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behaviour” (Crisp et al., 1997). Various natural and synthetic chemical compounds that induce endocrine disrupting responses have been identified, including pharmaceuticals, pesticides, industrial chemicals, and heavy metals (Giesy et al., 2002). Therefore, EDCs can have serious impacts on human and animal health (Li, 2014).

Table 1.3 shows the two groups of EDCs studied in this thesis: a synthetic estrogen, 17 α -ethinylestradiol (17-ET), and a xenoestrogen, bisphenol A (BPA).

Table 1.3. EDCs studied in this thesis and their relevance.**Estrogens**

Estrogens are a group of steroid compounds known for their relevance in the estrous cycle and their function as the main female sex hormone (Jurado et al., 2012). Synthetic steroids, such as 17-ET, are usually prescribed as oral contraceptives and possess a high pharmacological potency (Heberer, 2002). Due to their hormonal properties, the presence of these compounds can have critical effects on the environment at very low concentrations (Li, 2014).

Table 1.3 EDCs studied in this thesis and their relevance (**continued**).**Xenoestrogens**

Xenoestrogens are nonsteroidal, synthetic chemicals that simulate the actions of natural estrogens such as such as 17β -estradiol (E2). They include substances such as pesticides and by-products that can be assimilated by the body through ingestion or adsorption (Steinmetz et al., 1998; Akingbemi et al., 2004). An example is BPA, a monomer of polycarbonate plastics, which is widely used in the food-packaging industry and in dentistry. Thus, there is a significant human exposure to this EDC (Steinmetz et al., 1998; Akingbemi et al., 2004).

- *Lifestyle compounds*

The term psychostimulants refers to a group of compounds that enhances cognitive and behavioural functions by stimulating the central nervous system. They elevate mood and improve performance and concentration, by relieving feelings of tiredness and languor (Wesensten, 2012; Gerlach, 2014). One of the best-known is caffeine (CAFF) (Table 1.4).

Table 1.4. Lifestyle compounds studied in this thesis and their relevance.**Caffeine (CAFF)**

CAFF is one of the most widely consumed substances in the world. Pure caffeine is included on the US EPA list of high production volume chemicals (Gasior et al., 2000; Buerge et al., 2003). It is contained in various beverages, food products, condiments, tobacco and medication (Chen et al., 2002). The average global consumption of CAFF is considered to be about $70 \text{ mg person}^{-1} \text{ day}^{-1}$, although it varies in different countries (Buerge et al., 2003). In pharmaceuticals, caffeine is remarkably important, as it improves the effect of certain analgesics in cough, cold, and headache medications. It is used as a cerebral, cardiac, and respiratory stimulant and as a diuretic. Because of its regular and constant consumption, CAFF is actually considered as a potential chemical marker for domestic WW contamination (Buerge et al., 2003).

1.1.3 Physicochemical properties of the EOCs studied in this work

The occurrence and fate of EOCs in the environment is dependent on diverse factors associated with the physicochemical parameters of each compound and on the characteristics of the environment (e.g. temperature, pH, redox potential, organic matter, and water and soil composition) (Meffe and de Bustamante, 2014). Therefore, this section will be devoted to explaining some of the physicochemical parameters that are considered to play an important role in the fate of EOCs in the environment and thus can be relevant to the understanding their fate and occurrence (Meffe and de Bustamante, 2014; Zhang et al., 2014). Among them, water solubility (S_w), the octanol-water partitioning coefficient (K_{ow}), the octanol-water distribution coefficient (D_{ow}), the acid dissociation constants (K_a), and Henry's law constant (K_H) will be explained because they have been demonstrated to be relevant for understanding the compound's mobility between environmental compartments (Jurado et al., 2012; Meffe and de Bustamante, 2014). Table 1.5 shows the physicochemical properties for each of the EOCs studied in this thesis.

Table 1.5. EOCs studied in this thesis. Chemical structures (obtained from ChemSpider) and physicochemical properties (data compiled from references: (Anderson et al., 2005; Kim et al., 2009; Shahab Shariati et al., 2009; Pal et al., 2010; Vazquez-Roig et al., 2010; Yudthavorasit et al., 2011; Ziylan and Ince, 2011; Jurado et al., 2012; Ratola et al., 2012; Meffe and de Bustamante, 2014; Zhang et al., 2014)).

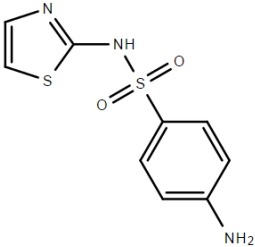
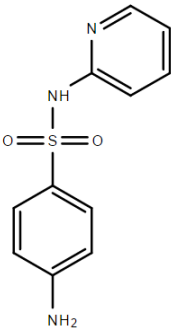
Category	Trade name (acronym)	Chemical structure	Physicochemical properties					
			MW (g mol ⁻¹)	S _w (mg L ⁻¹)	pKa	Log K _{ow}	Log D _{ow}	K _H (atm·m ³ ·mol ⁻¹)
Antibiotic	Sulfathiazole STZ		255.32	373	2.01/7.11	0.72	0.69	5.85 e-14
	Sulfapyridine SPY		249.29	268	2.90/8.40	0.35	0.46	1.08 e-13

Table 1.5 EOCs studied in this thesis. Chemical structures (obtained from ChemSpider) and physicochemical properties (data compiled from references: (Anderson et al., 2005; Kim et al., 2009; Shahab Shariati et al., 2009; Pal et al., 2010; Vazquez-Roig et al., 2010; Yudthavorasit et al., 2011; Ziylan and Ince, 2011; Jurado et al., 2012; Ratola et al., 2012; Meffe and de Bustamante, 2014; Zhang et al., 2014)) (**continued**).

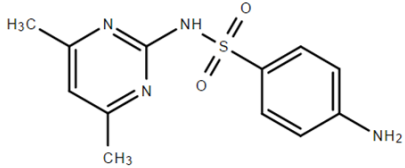
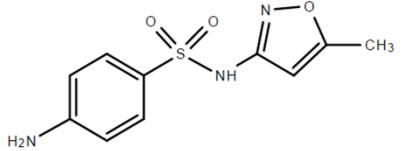
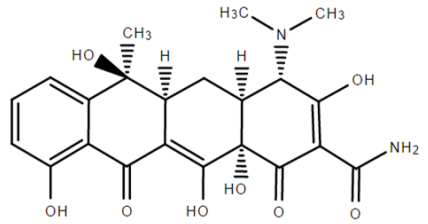
Sulfamethazine SMZ		278.33	1500	2.07/7.49	0.89	0.31	1.93 e-10
Sulfamethoxazole SMX		253.32	610	1.85/5.60	0.90	0.22	9.56 e-13
Tetracycline TC		444.43	231	3.22/7.78/9.58	-1.19	-2.66	4.66 e-24

Table 1.5 EOCs studied in this thesis. Chemical structures (obtained from ChemSpider) and physicochemical properties (data compiled from references: (Anderson et al., 2005; Kim et al., 2009; Shahab Shariati et al., 2009; Pal et al., 2010; Vazquez-Roig et al., 2010; Yudthavorasit et al., 2011; Ziylan and Ince, 2011; Jurado et al., 2012; Ratola et al., 2012; Meffe and de Bustamante, 2014; Zhang et al., 2014)) (**continued**).

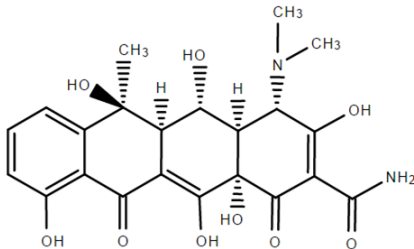
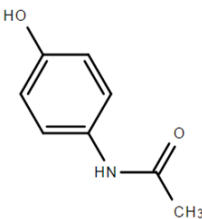
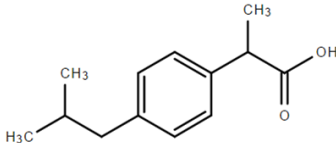
	Oxytetracycline OTC	460.43	313	3.22/7.46/8.94	-1.12	-3.84	1.7 e-25
							
Analgesics and NSAIDs	Acetaminophen ACAPh	151.16	1400-2400	1.72-2.3/9.38	0.46-2	0.85	6.42 e-13
							
	Ibuprofen IBP	206.29	21-41.1	4.91	3.97	1.68	1.52 e-7
							

Table 1.5 EOCs studied in this thesis. Chemical structures (obtained from ChemSpider) and physicochemical properties (data compiled from references: (Anderson et al., 2005; Kim et al., 2009; Shahab Shariati et al., 2009; Pal et al., 2010; Vazquez-Roig et al., 2010; Yudthavorasit et al., 2011; Ziylan and Ince, 2011; Jurado et al., 2012; Ratola et al., 2012; Meffe and de Bustamante, 2014; Zhang et al., 2014)) (**continued**).

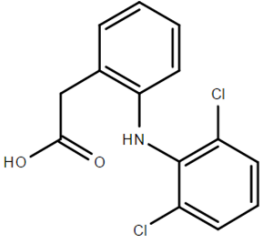
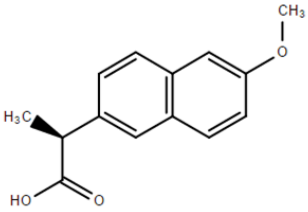
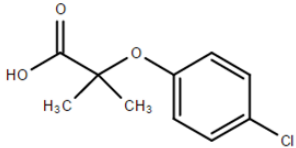
	Diclofenac		318.10	2.37-4.52	4.15	4.57	0.96	4.75 e-12
	DCF							
	Naproxen		230.3	15.9	5	3.18	0.18	3.39 e-10
	NAP							
Lipid regulator	Clofibric acid		214.65	582.50	3.00-3.46	2.57	-0.42	2.19 e-8
	CLF AC							

Table 1.5 EOCs studied in this thesis. Chemical structures (obtained from ChemSpider) and physicochemical properties (data compiled from references: (Anderson et al., 2005; Kim et al., 2009; Shahab Shariati et al., 2009; Pal et al., 2010; Vazquez-Roig et al., 2010; Yudthavorasit et al., 2011; Ziylan and Ince, 2011; Jurado et al., 2012; Ratola et al., 2012; Meffe and de Bustamante, 2014; Zhang et al., 2014)) **(continued)**.

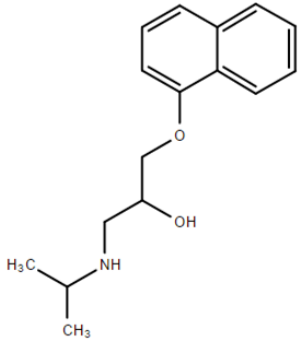
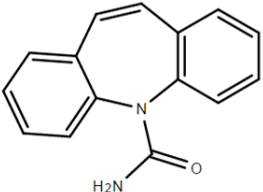
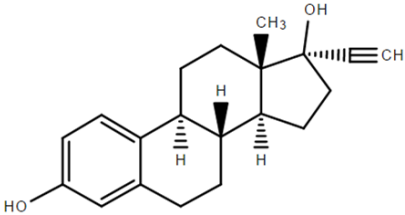
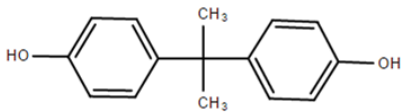
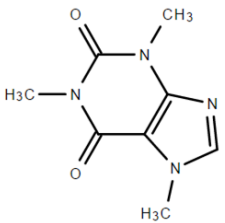
β-blocker	Propranolol PROPR		259.80	61.7-228	9.42-13.84	3.48	0.19	7.98 e-13
Antiepileptic	Carbamazepine CARB		236.27	17.70	7.00/13.94	2.45	2.77	1.08 e-10
Estrogen	17α-Ethinylestradiol 17-ET		296.41	4.80-11.3	10.20	3.67-4.20	3.92	7.94 e-12

Table 1.5 EOCs studied in this thesis. Chemical structures (obtained from ChemSpider) and physicochemical properties (data compiled from references: (Anderson et al., 2005; Kim et al., 2009; Shahab Shariati et al., 2009; Pal et al., 2010; Vazquez-Roig et al., 2010; Yudthavorasit et al., 2011; Ziylan and Ince, 2011; Jurado et al., 2012; Ratola et al., 2012; Meffe and de Bustamante, 2014; Zhang et al., 2014)) **(continued)**.

Xenoestrogen	Bisphenol A BPA		228.29	120-172.7	9.7-11.3	3.32	3.64	9.16 e-12
Life-style compound	Caffeine CAFF		194.19	21600-2163.51	10.4	-0.07	-0.55	3.58 e-11

- *Water solubility*

Water solubility refers to the maximum probable concentration of a compound that can be dissolved in water. It is a relevant parameter to determine the mobility of EOCs in the environment because it is a measure of their hydrophobicity (Essington, 2004; Leeuwen and Vermeire, 2007).

High S_w values imply a greater tendency of the EOC to remain dissolved in water (e.g. SAs, ACAPh, CAFF) and, consequently, to be transported through the water cycle, within which the compound can be exposed to a variety of degradation processes (e.g. biodegradation, photodegradation) (Leeuwen and Vermeire, 2007). In these cases, the tendency of the compound to be adsorbed into suspended or particular organic matter and to be bioconcentrated in aquatic organisms is low. Conversely, EOCs that have low water solubility values (e.g. DCF, NAP, 17-ET) tend to precipitate, to bioconcentrate or to volatilize more easily (Leeuwen and Vermeire, 2007). Water solubility is inversely related to Henry's law constant, which controls the volatilization of the compounds that move from the waterborne to the air (Leeuwen and Vermeire, 2007).

The general solubility equation only requires two parameters (i.e. melting point (MP) and $\log K_{ow}$) and is defined by Eq. (1.1) (Ran and Yalkowsky, 2001).

$$\log S_w = 0.5 - 0.01 (\text{MP}-25) - \log K_{ow} \quad (1.1)$$

- *Octanol-water partition coefficient*

The partition coefficient K_{ow} is considered to be a standard measure of the hydrophobicity of a chemical compound. It allows estimate the tendency of the compound to accumulate into the solid or particulate phase (Michael et al., 2013; Zhang et al., 2014). K_{ow} refers to the ratio of the concentration of a chemical compound in a known volume of octanol and in a known volume of water (Eq. (1.2)) (Ávila Martín, 2013).

$$K_{ow} = [\text{solute}]_{\text{octanol}}/[\text{solute}]_{\text{water}} \quad (1.2)$$

Many authors consider EOCs with $\log K_{ow} < 4$ to be hydrophilic (e.g. SAs, TCs), while those with $\log K_{ow} > 4$ (e.g. IBP, DCF, 17-ET) are reported as hydrophobic.

Nevertheless, this is not always true for ionisable compounds such as IBP or DCF (see next section). In general, hydrophobic compounds present a high adsorption affinity, especially onto organic matter, and hence tend to become more recalcitrant to biodegradation processes and consequently tend to bioaccumulate. Otherwise, hydrophilic compounds usually have low ($\log K_{ow} < 2.5$) or medium ($2.5 < \log K_{ow} < 4$) sorption potential and show a lower tendency to bioaccumulate (Jurado et al., 2012; Michael et al., 2013; Meffe and de Bustamante, 2014; Zhang et al., 2014).

This parameter is usually related to S_w : EOCs with low $\log K_{ow}$ values tend to present great S_w values, exhibiting low adsorption values onto solids and low bioaccumulation potential. Nevertheless, this correlation is not always correct, as in some cases high S_w does not imply a decrease in hydrophobicity in terms of $\log K_{ow}$. This latest parameter does not always correspond with adsorption capacity onto mineral sediments (Leeuwen and Vermeire, 2007; Jurado et al., 2012).

- *Octanol-water distribution coefficient*

The octanol-water distribution coefficient (D_{ow}) is nearly equivalent to K_{ow} . It is defined as the ratio of the sum of non-ionized plus ionized species in *n*-octanol to that in water (see Eq. (1.3)) (Wells, 2006).

$$D_{ow} = \frac{[\text{non-ionised+ionised species}]_{\text{octanol}}}{[\text{non-ionised+ionised species}]_{\text{water}}} \quad (1.3)$$

This parameter is representative of the hydrophobic character of ionizable organic compounds as a function of pH and thus shows the tendency of these compounds to sorb to soils, sediments or biomass and how they are distributed in the environment (Kümmerer, 2013; Zhang et al., 2014). According to Zhang et al. (2014), compounds with $\log D_{ow}$ ranging from -2.3 to 3 (e.g. CAFF, CLF AC, NAP, IBP, DCF, and CARB) are moderately hydrophilic and do not tend to bind significantly to organic matter. On the other hand, Wells (2006) considered compounds with $\log D_{ow} < 1$ (e.g. CAFF, CLF AC, and NAP) to be highly hydrophilic.

$\log D_{ow}$ has been used to estimate other chemical parameters used in environmental studies such as S_w , soil adsorption coefficients, and bioconcentration factors in aquatic

life (Kümmerer, 2013). Somehow $\log D_{ow}$ has been suggested as an adequate indicator of some EOCs removal in biologically-based WW treatment systems such as constructed wetlands (CWs), as different authors have found an inverse relationship between $\log D_{ow}$ and EOCs removal (Lee et al., 2011; Zhang et al., 2012). However, in a review conducted by Zhang et al. in 2014, where the $\log D_{ow}$ values of several EOCs were correlated with their respective removal efficiencies from a variety of studies conducted with the same type of treatment, the utility of this chemical parameter for predicting the removal of some EOCs was found to be unsatisfactory.

- *Acid dissociation constant*

Recent research has shown that the sorption affinity of EOCs to solids or organic matter, apart from being dependant on the S_w and $\log K_{ow}$ values, is also conditioned by the surface charge of the compound (Zhang et al., 2014). Depending on the environmental pH, the compounds can be positively or negatively charged or neutral. Therefore, the behaviour of a certain EOC in the environment depends on the pH and the mass fraction of each compound form. The acidic dissociation constant (K_a) measures the acidic strength of a compound and is expressed by the concentration of ionized compound molecules divided by the concentration of the non-ionized ones (Eq. (1.4)) (Ávila Martín, 2013).

$$pK_a = -\log ([H^+][A^-]/[HA]) \quad (1.4)$$

The lower the pK_a value, the higher the K_a of the compound and the higher is its acidic strength. Hence, this parameter determines the ionization capacity/degree of a compound, which affects its sorption tendency and its bioaccumulation (Kümmerer, 2009; Ávila Martín, 2013; Meffe and de Bustamante, 2014). For a given pK_a value, the higher the pH, higher the compound's ionisation would be, which would enhance its S_w . In accordance to Zhang et al. (2014), the sorption behaviour of compounds is dependent on different factors, among them the compound's chemical structure and also soil characteristics, as adsorption will occur because of the electrostatic interactions between negatively charged surfaces and positively charged groups of the compounds.

Most antibiotics, such as SAs and TCs, are usually complex molecules that are moderately water soluble and ionizable. Depending on the pH conditions they exist as

either neutral or charged species in different proportions, so sorption mechanisms are likely to occur for these EOCs (Kümmerer, 2009; Zhang et al., 2014). In the case of the NSAIDs IBP, DCF, and NAP, they are practically all anions in the pH range 5-8, and show a lower tendency to be adsorbed. CARB, as well, presents an almost negligible sorption affinity for sediments and organic matter, which is explained by its neutral form at the environmental pH and its moderately low hydrophobicity.

- *Henry's Law constant*

Henry's Law constant (K_H) is an indicative parameter of a compound's volatility. It is a partition coefficient that is commonly defined as the relation between the concentration of a compound in water and its concentration in air at equilibrium (Eq (1.5)). It estimates the volatilization of a compound from a dissolved phase to the air (Hemond and Fechner-Levy, 2000; Montgomery, 2007).

$$K_H = [\text{solute}]_{\text{air}}/[\text{solute}]_{\text{water}} \quad (1.5)$$

Compounds with high values of Henry's Law constants are considered to be readily aerated (e.g. cis-2-chloroethylene, a volatile organic compound, $K_H = 3.37 \times 10^{-3} \text{ atm} \cdot \text{m}^3/\text{mole}$), while those with low values are more difficult to volatilize (Moyer and Kostecki, 2003; Wang et al., 2007). The K_H value of a compound can be assessed from its vapour pressure, molecular weight (MW), and solubility (Moyer and Kostecki, 2003). According to the K_H values reported in Table 1.5, the EOCs studied in this thesis are classified as low volatile.

1.1.4 Occurrence of the studied EOCs in the environment

In recent years a growing number of studies have reported the occurrence of EOCs worldwide in different water bodies (i.e. WW, surface water, ground water, and drinking water) (Pal et al., 2010; Xu et al., 2011; Yan et al., 2014; Yan et al., 2015).

Several authors claim that the occurrence of EOCs is influenced by diverse regional and human activities (i.e. industrial, commercial and residential). Moreover, there is also a relevant seasonal and climate influence, as the consumption of some compounds varies

greatly depending on the time of the year. For example, IBP, ACAPh, DCF, NAP, and SMX consumption has been reported to increase considerably over winter (Castiglioni et al., 2006; Pal et al., 2010; Yu et al., 2013; Meffe and de Bustamante, 2014).

After consumption, EOCs can be excreted as active metabolites or, to some extent, parent compounds, which leads to their occurrence in WWs (in the range of few $\mu\text{g L}^{-1}$). Commonly, EOCs are found at lower concentrations in the WWTP effluents (ng L^{-1} – few $\mu\text{g L}^{-1}$) rather than in the influents ($\mu\text{g L}^{-1}$ – few mg L^{-1}) (see Table 1.6) (Yu et al., 2013). Nevertheless, as it can be seen in Table 1.6, in some cases the compounds' concentrations in the WWTP effluents are similar or even higher than those observed at the WWTP entrance (e.g. DCF, NAP, PROPR, CARB) (Gros et al., 2006; Santos et al., 2009; Collado et al., 2014). During metabolic processes in the human body and biochemical processes in WWTPs, parent compounds are transformed to metabolites and derivatives, and vice versa. Thus, during WW treatment, some metabolites and/or derivative products can convert back to the parent compound, resulting in a higher concentration of the parent compound in the effluent of the WWTP than in the influent (Jelic et al., 2011; Yu et al., 2013).

Generally, concentrations of EOCs decrease from the WWTP effluents to the surface and ground water bodies, which can be seen in Table 1.6 (Pal et al., 2010; Li, 2014). For example, Nödler et al. (2010) found concentration decreases for SMX (from 509 ng L^{-1} to 93 ng L^{-1}), CARB (from 1429 ng L^{-1} to 265 ng L^{-1}), DCF (from 1492 ng L^{-1} to 156 ng L^{-1}), and CLF AC (from 103 ng L^{-1} to 21 ng L^{-1}) from the WWTP effluent to the surface water body. Once WWTP effluents reach natural bodies, a dilution factor occurs, which depends on the natural water flow that is determined by the seasons of the year. Moreover, natural attenuation processes (e.g. biodegradation, photodegradation, sorption, and plant uptake) are also key factors in the concentration decline of the EOCs (Li, 2014).

To date, the occurrence of EOCs has been much better characterized in WW and surface water bodies than in groundwater. WWs and surface waters are commonly thought to contain a greater number of EOCs (Lapworth et al., 2012; Meffe and de Bustamante, 2014). In accordance with Li et al. (2014), some of the compounds studied in this thesis are the ones with the highest frequency of detection (FOD) in WWTP effluents, surface water, and groundwater (i.e. SMX, IBP, ACAPh, NAP, DCF, CARB, and CAFF). Table 1.6 shows that the concentration of the studied EOCs in groundwater ranges from the ng

L^{-1} to $\mu\text{g L}^{-1}$ (Yan et al., 2014; Zenker et al., 2014). Moreover, the table also includes data related to the concentration of EOCs in sewage sludge due to its relevance in the study of WW treatment performances and its application as a biosolid in agricultural lands (Jelic et al., 2011; Jones et al., 2014). Among the studied compounds, the ones found at highest concentrations in sewage sludge are SAs (see table 1.2), probably due to their low biodegradability in comparison with other compounds (Ingerslev and Halling-Sørensen, 2000).

Table 1.6. Occurrence of EOCs in the environment.

Compound	WWTP influent (ng L ⁻¹)	WWTP effluent (ng L ⁻¹)	Surface water (ng L ⁻¹)	Ground water (ng L ⁻¹)	Sewage sludge (ng g ⁻¹)	References
STZ	10.57·10 ³ - 11.16·10 ⁶	26 - 171	-	-	<LOQ - 63	1,13,16
SPY	68	16 - 167	-	-	N.D. - 28000	3, 13,16
SMZ	4010 – 97.23·10 ³	173	0.1 100	298 - 390	<LOQ - 26	1, 10, 13, 16, 22, 23
SMX	70 - 4090	10 - 548	<LOD - 1488	2 - 252	<LOQ – 6.8·10 ⁴	1, 2, 3, 4, 6, 10, 11, 13, 16, 18, 19, 22, 23,
TC	16.5 - 980	1.9	0.8 - 270	2.7 - 750	1914	1, 7, 15, 22, 23
OTC	72.5 – 23.62·10 ³	3.8	0.9 – 220	ND - 6870	87.5 - 7630	1, 7, 8, 15, 22, 23
ACAPh	370-21.80·10 ⁴	ND - 2102	42 - 1992	15142	ND - 419	2, 6, 10, 13, 16, 17, 18, 24
IBP	516 -11.50·10 ⁴	13 -10.16·10 ³	14 - 414	3 - 1491	ND - 548	2, 6, 8, 9, 10, 11, 13, 14, 15, 16, 17, 20, 21, 24
DCF	86 - 580	ND – 13.81·10 ³	29 - 156	121	ND - 209	2, 6, 8, 10, 13, 14, 16, 17, 18, 20, 24
NAP	99 - 2.1·10 ⁵	ND - 5433	33	-	ND - 930	2, 6, 13, 15, 16, 20, 21, 24
CLF AC	57 - 420	ND - 103	11 - 21	1113	ND - 135	6, 10, 16, 17, 18, 24
PROPR	168	13 - 290	<LOD - 40	-	<LOQ - 140	6, 8, 9, 13, 16, 17
CARB	27 - 860	ND - 1429	20 - 595	10 - 5312	ND - 258	2, 6, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 21, 24

Table 1.6 Occurrence of EOCs in the environment. (continued)

17-ET	8 - 95	2 - 8	-	-	<LOQ - 313	12, 16
BPA	60 - 600	ND - 44	5-106	79 - 2527	20 - 1860	5, 8, 10, 11, 14, 20, 24
CAFF	4870-7370	1620-2440	62-1048	13 - 9774	ND - 248	5, 10, 11, 14, 15, 16, 17, 18, 21

Data compiled from references: 1.(Choi et al., 2007) , 2.(Collado et al., 2014), 3.(Conkle et al., 2008), 4.(Díaz-Cruz et al., 2008), 5.(Esteban et al., 2014), 6.(Gros et al., 2006), 7.(Jia et al., 2009), 8.(Jones et al., 2014), 9.(Kim et al., 2009), 10.(Lapworth et al., 2012), 11.(Loos et al., 2010), 12.(Manickum and John, 2014), 13.(Martinez Bueno et al., 2012), 14.(Matamoros et al., 2012a), 15.(McClellan and Halden, 2010), 16.(Nieto et al., 2010a), 17.(Nieto et al., 2010b), 18.(Nödler et al., 2010), 19.(Rossmann et al., 2014), 20.(Samaras et al., 2013), 21.(Santos et al., 2009) , 22.(Tang et al., 2015), 23.(Wei et al., 2011), 24.(Yu et al., 2013).

ND, no detected, LOQ, limit of quantification, LOD, limit of detection.

1.1.5 The impact of the studied EOCs on the aquatic environment

The previous section stated that a variety of EOCs are found in different environmental media at low concentrations, from ng L^{-1} to low $\mu\text{g L}^{-1}$. Despite these trace level concentrations, these organic contaminants are continuously released into the environment, with harmful effects (Hernando et al., 2006). Consequently, there is an increasing concern about the presence of EOCs in the environment and their involved risks owing to:

- The different classes of EOCs detected in WWTPs effluents.
- The toxicity of the different EOCs and their metabolites.
- The generation of more harmful transformation by-products.
- The pseudo-persistence of these contaminants in the environmental media.
- The long-term effects of all the parent compounds, metabolites and by-products on ecosystems (animals and vegetation), and humans.
- Their possible bioaccumulation.
- The additive, synergistic or antagonistic effects of the occurrence of multiple compounds.

Nowadays, information on the environmental significance of most of these EOCs is still scarce. Therefore, the risk posed by their presence in terms of the above-stated concerns is poorly understood. Nevertheless, since the aquatic organisms are constantly exposed to EOCs (Pedrouzo Lanuza, 2011; Stewart et al., 2014; Yan et al., 2014; Yan et al., 2015), consideration must be given to the possible bioaccumulation of these compounds in aquatic organisms, enhancing chronic ecological risk and supposing a plausible human health risk across the food chain (McClellan and Halden, 2010; Collado Alsina, 2013).

In vitro and in vivo studies have found that many EOCs exhibit toxicity at ultratrace level concentrations (ng L^{-1}) for flora and fauna species, whether individually or in combination (Yan et al., 2015). For example, the occurrence of antibiotics at such low concentrations may harmfully affect soil microbial communities, could promote chronic toxicity, and can encourage the proliferation of antibiotic resistant bacteria (Marcela Seifrtová, 2009; Quoc Tuc Dinh, 2011; Michael et al., 2013). Likewise, antibiotics

belonging to the SA and TC classes may be taken up by crop plants (Hernando et al., 2006), so human health implications cannot be disregarded.

Also closely watched are steroid drugs such as the contraceptive 17-ET, as can have critical effects on the environment (e.g. feminization of fish species, DNA alterations) at very low concentrations (Li, 2014). In vivo studies have shown that fishes exposed to low ng L^{-1} levels of this microcontaminant may have feminizing effects in some males in wild fish species (Heberer, 2002). Indeed, Kidd et al. (2007) indicated that concentrations of 5–6 ng L^{-1} of 17-ET in a Canadian lake led to the feminization of male fathead minnows (*Pimephales promelas*), which may produce the collapse of the fish species population (Kidd et al., 2007).

The effect of other contaminants, such as CLF AC on fish species like mosquitofish (*Gambusia holbrooki*), has also been studied. Nunes et al. (2004) found that even though some of the studied enzymes were affected by the presence of CLF AC at the highest tested concentration (500 mg L^{-1}), chronic exposure at the different tested concentrations (32.25, 62.5, 125, 250 and 500 mg L^{-1}) did not significantly alter the enzymatic activities.

Harmful effects of EOCs have also been reported on terrestrial animals. The exposure of rats to low levels of BPA (low $\mu\text{g L}^{-1}$) appeared to have inhibitory effects on testicular steroidogenesis and exerted inhibitory effects on Leydig cells (Akingbemi et al., 2004). In addition, DCF has had harmful effects on the population level of Asian vultures, which are near extinction as a result of exposure to lethal residues of this pharmaceutical in livestock carcasses (Yan et al., 2014).

Additionally to parent compounds, metabolites should also be taken into account. Even though the metabolization of some compounds can exceed 90%, others are metabolized by only 10% or less (Kümmerer, 2009). The formed metabolites can occasionally be more toxic than the parent compounds and their water solubility is frequently higher, which enhances their continuous release and interaction with the aquatic environment. An example is acetyl-sulfamethoxazole, the major human metabolite of SMX, which represents more than 50% of the administered parent compound dose in human excretion and exhibits higher toxicity to humans than the parent compound (Kümmerer, 2009). The presence of this metabolite has been stated in WWTP influents at concentrations 2.5- 3.5 times higher than those of the parent compound (Michael et al., 2013). Besides the human metabolites, there are a lot of other compounds, the so- called

transformation products (TPs) (note that human metabolites can also be described as TPs), that can be generated inside the WWTPs or in the aquatic environment due to different attenuation processes (e.g. photodegradation, biodegradation). For example, it has been reported that the oxidation of DCF generates different metabolites, such as hydroxydiclofenac (Sein et al., 2008; Dobrin et al., 2013), which has been identified in the bile of female rainbow trout chronically exposed to waterborne DCF and leads to damage in the intestinal tract (Mehinto et al., 2010).

EOCs do not enter the environmental media individually, and synergistic effects between different classes of compounds should not be disregarded, as the combined exposure to various types of compounds can considerably intensify harmful effects (Pal et al., 2010). For example, a study conducted in 2012 evaluated the exposure of zebrafish to different pharmaceuticals previously found in the Duoro River (i.e. CARB, fenofibric acid, PROPR, SMX and trimethoprim), both individually and in mixtures. Alteration effects were found in male zebrafish livers after they were exposed to the pharmaceutical mixture, but also after individual exposure to PROPR (Madureira et al., 2012).

Nevertheless, the toxicity of EOCs has not yet been fully studied. Additional studies are required to know how compounds act (individually but also as a mixture) (Verlicchi et al., 2012; Collado Alsina, 2013; Stewart et al., 2014; Yan et al., 2014). Furthermore, besides the infinite number of possible interactions between EOCs, concentrations of these compounds are usually spatially and temporally varied in the environment. Consequently, reliable risk assessment studies to evaluate the toxic effects of long-term exposure to a combination of EOCs are necessary to monitor the chemical and ecological status of ecosystems (Lapworth et al., 2012; Collado Alsina, 2013).

As stated above, there is clear evidence that the presence of some EOCs has harmful environmental implications and therefore they may need to be treated as POPs and be included in comprehensive risk assessment studies (Hernando et al., 2006). Section 1.1.7 will address the present and future regulation of EOCs.

1.1.6 Wastewater treatment plants and their role in the removal of EOCs

As stated in the previous sections, EOCs occur in surface water bodies as a result of their discharge by WWTPs, among others (Watkinson et al., 2007; Matamoros et al., 2009; Onesios et al., 2009; Pal et al., 2010; Camacho-Muñoz et al., 2012).

The removal of these compounds in WWTPs is influenced by the WW characteristics, the treatment technology used, and the operational settings (e.g. sludge retention time, SRT), as well as by environmental parameters (e.g. temperature, dilution, natural attenuation) (Jelic et al., 2011). In addition, the physicochemical properties of EOCs play an essential role in their behaviour during WW treatment. As discussed in a preceding section, S_w , $\log K_{ow}$, $\log D_{ow}$, and pK_a values have a great influence on the tendency of a compound to remain in the water phase or to be absorbed by solids, such as the sewage sludge. Thus, due to the presence of a variety of compounds in WW, with different physicochemical properties, distinct removal rates are expected during the WW treatment, resulting in different EOC concentrations in the WWTP effluents and in the sewage sludge generated (Jelic et al., 2011). Table 1.7 shows reported removal efficiencies of different EOCs, present in WW, after being treated in WWTPs through different treatments.

The water crisis faced by many countries in the 21st century requires the reuse of WW, where possible, for irrigation purposes or industrial activities (Audrey D. Levine, 2004; McKenzie, 2005). Thus, it is important to frequently check the different processes occurring in WWTPs and to know how different WW treatments applied in WWTPs contribute to the removal of EOCs and hence to the improvement of the reclaimed water quality.

Conventional WWTPs have not been designed to remove EOCs (Sydney water, 2015; Pilon-Smits, 2005; Nuno Ratola, 2012; Verlicchi et al., 2012). Therefore, most of these compounds are often only partially eliminated (20-90%) during WW treatment (Watkinson et al., 2007; Murray et al., 2010). The attenuation of EOCs during WW treatment involves a variety of biological, chemical and physical processes (Wang et al., 2005; Zhang et al., 2014):

- Biodegradation: transformation of EOCs through the action of microbes present in the media.

- Photodegradation: degradation or transformation of compounds due to the effect of sunlight (i.e. oxidation).
- Sorption: adsorption or absorption of EOCs onto suspended particles, organic carbon or mineral surfaces.
- Oxidation: besides disinfection, physicochemical oxidation is also used in WW treatment to remove organic chemicals. The choice of the oxidant used (e.g. TiO_2 or ozone) is dictated by its benefits for the targeted pollution.
- Volatilization: compound transformation from the solid or liquid phase to the vapour phase.
- Chemical precipitation: change in form of compounds dissolved in water into solid particles. Counter ions are added to the WW to reduce the solubility of ionic compounds (i.e. coagulation).

These removal and transformation processes are dependent on the physicochemical properties of the different EOCs and also are influenced by several other factors (e.g. WW composition, seasonal variation, water depth) (Nuno Ratola, 2012; Garcia-Rodríguez et al., 2014; Zhang et al., 2014). All these mechanisms will be further described and discussed in Chapter 6 of this thesis.

WW treatment generally involves three treatment stages: primary, secondary and tertiary.

- *Pre-treatment and primary treatment*

The first step for WW treatment consists of a pre-treatment and a primary treatment, relatively simple mechanical processes whose main purpose is removing suspended solids and organic matter (Libhaber and Orozco-Jaramillo, 2012). Screening to trap solid objects and sedimentation by gravity is used to remove suspended solids (Group; Camacho-Muñoz et al., 2012). Moreover, coagulation-flocculation processes enhance the removal of suspended solids and colloids; by adding metal salts or polymers, a chemical precipitation is produced, causing these particles to agglomerate and allowing their removal by decantation or filtration (Spellman, 2008). Carballa et al. (2005) observed that after a coagulation-flocculation process, DCF ($\log K_{ow} < 5$) was removed by 50-70%. Conversely, CARB and IBP were not affected by this primary treatment

(Carballa et al., 2005). In this first treatment step, the main removal mechanism was sorption. Carballa et al. (2005) observed that musk fragrances such as galaxoldie and tonalide ($\log K_{ow} > 5$) were removed by 50–70% during the primary sewage treatment.

- *Secondary treatment*

WW is then treated, at least, by a secondary treatment designed to remove, through bacterial decomposition, any dissolved organic matter and suspended solids that got past the primary treatment screening, and nutrients (Sydney water, 2015; Camacho-Muñoz et al., 2012).

The most widely used secondary treatment technology is conventional activated sludge (CAS) (Verlicchi et al., 2012), which consists of an aeration reactor in which air or oxygen is introduced into a sewage liquor to develop a biological floc (Camacho-Muñoz et al., 2012). It has a lower capital cost than more advanced methods and limited operational requirements, but it consumes a lot of energy, has operating problems (e.g. odour and foaming) and produces considerable amounts of sludge (A.M. Deegan, 2011).

Various studies present removal efficiencies for different EOCs after CAS treatment (Table 1.7), showing how effectively it removes pharmaceuticals such as IBP (91-94%) and ACAPh (99%), but not others such as DCF (21-22%), CARB (not eliminated), or CLF AC (16%) (Radjenović et al., 2009; Sipma et al., 2010). Watkinson et al. (2007) found that SAs (i.e. STZ and SMX) were also poorly removed (25%) after CAS treatment, which is in agreement with the mean removal rate of 33% for SMX in a study by Sipma et al. (2010). Radjenovic et al. (2009), however, have shown an acceptable removal rate (i.e. 74%) for this compound.

Table1.7. Removal efficiencies reported for different EOCs present in WW after being treated in full scale WWTPs through different processes (corresponding references in brackets).

	% Removal efficiency			
	CAS	MBR	AOPs	CWs
STZ	88(13); 55(3)	59-73(3)	>95(2)	-
SPY	51(13)	56(13); 21-39(3)	>99(8)	>99(1)
SMZ	83(13); 22(3)	46-49(3)	>95(2)	100(14, 15); 68-73(7)
SMX	52(13); 33(11); 46(3)	54(2); 73(11); 52-55(3)	88->99(5); 97(2)	92(1); 16-53(6); 16-24(14)
TC	56(13)	-	-	69->99(4)
OTC	44(13)	-	-	93->99(4); 91-95(7)
ACAPh	93(13); 99(11)	99(13); >99(11)	-	>99(1)/45(6,14)
IBP	65-90(9); 87(13); 94(11)	98(13); 97(11)	12->99(2)	>99(1); 7-96(15); 5-97(6); 14-52(14)
DCF	69-75(9); 29(13); 21(11)	60(13); 34(11)	85->99(5); 97->99(2)	38-96(15); 3-98(6); 8-79(14)
NAP	45-66(9); 73(13); 66(11)	91(13); 85(11)	60->99(8)	>99(1); 34-96(6)
CLF AC	34-51(9); 40(13); 16(11)	65(13); 67(11)	90->99(5); 10-98(2)	32-75(15,6)
PROPR	39(13); 59(11)	72(13); 72(11)		38(15,6)
CARB	7(9); 18(13); 0-60(15)	15(13); 0(11)	75->99(5); 97->99(2); 10-80(8)	51(1); 11-97(15); 3-97(6)
17-ET	78(13); 71(10)	60(13)	97->99(2)	-
BPA	88(10)	-	>99(2); 50-80(8)	62-73(12)
CAFF	93->99(15)	-	-	>99(1); 3-86(6); 14-47(14)

Notes.

1.(Conkle et al., 2008), 2.(Esplugas et al., 2007), 3.(García Galán et al., 2012), 4. (Huang et al., 2015), 5. (Klavarioti et al., 2009), 6. (Li, 2014), 7. (Liu et al., 2013), 8.(Nakada et al., 2007), 9. (Petrović et al., 2003), 10. (Qiang et al., 2013), 11.(Sipma et al., 2010), 12.(Toro-Vélez et al., 2016), 13. (Verlicchi et al., 2012), 14. (Verlicchi and Zambello, 2014), 15. (Zhang et al., 2014).

The EOCs-removal efficiency of this technology depends on the hydraulic retention time (HRT), SRT, pH, temperature, microbial community, dissolved oxygen, the presence of recalcitrant or toxic substances, and the compounds' characteristics (Deegan et al., 2011).

Nowadays, in addition to CAS, many other secondary treatment technologies, such as membrane biological reactors (MBRs), rotating biological contactors (RBCs) or trickling filters, are available. However, the current use of biological-based WW treatment technologies such as CWs and waste stabilization ponds (WSPs) has proven to be a good alternative, at least for small communities (<2000 PE) (Matamoros et al. 2016). Chapter 4 (section 4.3) will explain the different available biological-based WW technologies for removing EOCs from WW.

- *Tertiary treatment*

Tertiary treatment is the final cleaning process that enhances WW quality before its reuse or discharge into the aquatic environment. In this stage, bacteria, viruses and parasites not removed during the secondary treatment and harmful to public health are eliminated (Sydney water, 2015).

Over the last decade, in addition to conventional tertiary treatment technologies such as chlorination or ultraviolet (UV) irradiation, advanced treatment technologies with very efficient EOCs removal rates (up to 99%), such as membrane separation or advanced oxidation processes (AOPs), have emerged (Klavarioti et al., 2008; Meneses et al., 2010; A.M. Deegan, 2011). Some of the most widely employed tertiary treatment technologies are described in this section.

Chlorination

Chlorination is by far one of the most common disinfection methods for WW treatment. It can be applied as a solid (calcium hypochlorite), liquid (sodium hypochlorite) or gas (elemental chlorine, Cl₂) (Chlorine Chemistry, 1997; Drinan and Spellman, 2012). This method is effective in destroying pathogens before the effluent is discharged into receiving streams, rivers or oceans (Chlorine Chemistry, 1997).

Chlorination is reportedly effective for the removal of some EOCs. The normal chlorine dose in a standard tertiary treatment is about 3 mg L^{-1} (Matamoros and Salvadó, 2013). However, studies have obtained removal rates greater than 99% for 17-ET and BPA with chlorine doses ranging from 0.25 to 2 mg L^{-1} (Alum et al., 2004), and rates greater than 80% for DCF with chlorine doses ranging from 5 to 30 mg L^{-1} (Noutsopoulos et al., 2015). Otherwise, low removal efficiencies ($<10\%$) have been reported for IBP after chlorination treatment (i.e. $5\text{-}30 \text{ mg Cl}_2 \text{ L}^{-1}$) (Noutsopoulos et al., 2015). Despite the effectiveness of this EOC-removal method, recent studies have shown that hazardous by-products are formed during this WW oxidation treatment, which represents a potential risk for humans and the environment (Souto, 1999; Drinan and Spellman, 2012).

Ultraviolet irradiation

WW irradiation with UV light is considered a highly effective disinfection method. This treatment improves disinfection efficacy and reduces health and environmental risks by preventing the formation of disinfection by-products (Parvez, 2014). The efficiency of UV light irradiation is dependent on the WW turbidity, the contact time with the UV light and the UV light intensity (Drinan et al., 2000). It is mainly used to inactivate waterborne pathogens without chemicals and to remove organic and inorganic chemicals including chlorine, chloramines, ozone and total organic carbon (Stockholm convention, 2008). Moreover, various studies have shown that UV light is effective for the complete removal of photodegradable EOCs (e.g. DCF), but is an ineffective way to eliminate other EOCs such as CLF AC and CARB (Giri et al., 2010; Matamoros and Salvadó, 2013). To improve its efficiency, UV light is commonly applied in combination with oxidants such as ozone, hydrogen peroxide, and chlorine, or semiconductors such as titanium dioxide (Giri et al., 2010; Matamoros and Salvadó, 2013; Parvez, 2014).

Advanced oxidation processes

Advanced oxidation processes (AOPs) have been implemented to complement conventional systems and to enhance WW disinfection and the treatment of refractory

organic pollutants (A.M. Deegan, 2011). This method requires a chemical agent such as ozone, hydrogen peroxide or the catalytic agent titanium dioxide (Fig. 1.2). Moreover, an energy source such as ultraviolet light is usually required (Deegan et al., 2011). This type of process produces free hydroxyl radicals that facilitate the transformation of contaminants to more biodegradable and less harmful compounds (Huber et al., 2003; Alum et al., 2004).

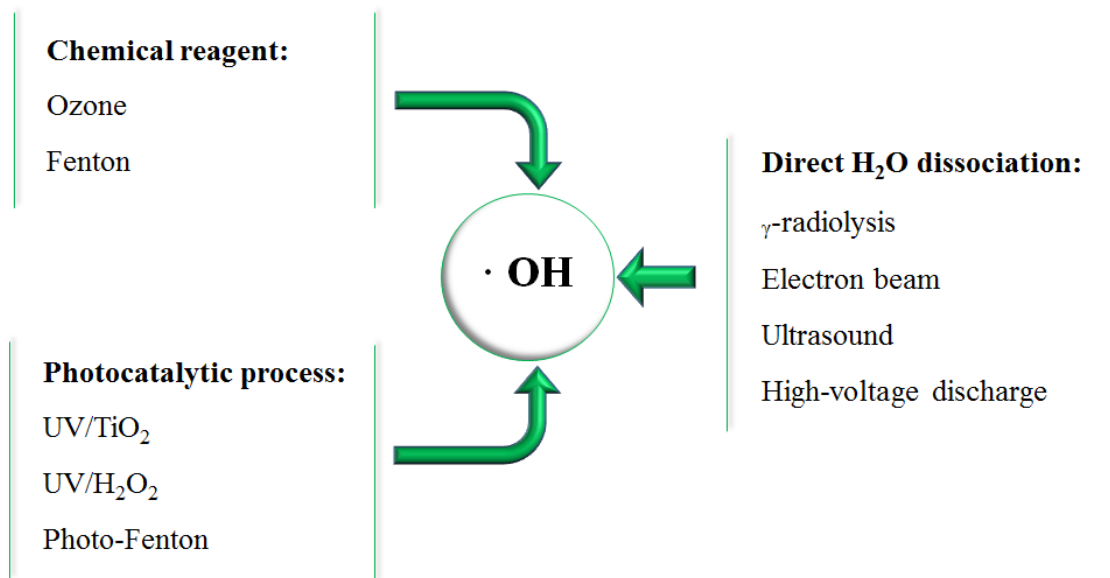


Figure 1.2. Most commonly applied AOPs (Giri et al., 2010; Deegan et al., 2011).

Numerous publications have looked at the removal of EOCs (e.g. antibiotics, lipid regulators, EDCs, and psychoactive drugs) from WW using AOPs (A.M. Deegan, 2011). For example, ozone effectively removes SMX (>92%), DCF (>96%), and CARB (>98%) (Ternes et al., 2003). Combinations of different oxidizing agents and UV light enhance the removal of some EOCs (i.e. CLF AC, CARB, DCF, IBP, and NAP), almost reaching complete elimination after a reaction time of 60 min (Giri et al., 2010). However, EOCs removal rates can vary greatly depending on the type and concentration of oxidizing agent used, on the characteristics of the applied UV light, and on the reaction time with the WW (Ternes et al., 2003).

Membrane separation processes

Membranes are increasingly used to treat WW intended for reuse as membrane technology has been demonstrated to contribute greatly to the removal of EOCs (Snyder et al., 2007). Among several membrane technologies, various studies have evaluated both the pilot and full-scale removal of these organic contaminants by nanofiltration (NF), ultrafiltration (UF) and reverse osmosis (RO) (Yoon et al., 2007; Deegan et al., 2011; Moore et al., 2011). Experiments conducted by Snyder et al. (2007) demonstrated that UF membranes had little relevance for the removal (<10%) of most of the studied EOCs (e.g. ACAPh, CAFF, DCF, IBP, and SMX). Otherwise, RO membranes showed good performances for the total removal of most of the studied EOCs (e.g. removal rates greater than 80% were observed for CAFF and IBP). Some studies have also evaluated NF/RO methods, reporting greater removal efficiencies for polar and charged compounds, which has been attributed to the interactions of the compounds with the membrane surfaces (Deegan et al., 2011). However, pharmaceuticals can be refused on NF and RO membranes by one or a combination of three basic mechanisms: charge exclusion, size exclusion, and physicochemical interactions between membrane, solvent, and solute (Deegan et al., 2011).

In general terms, advanced tertiary treatments are effective for the complete removal of some EOCs even though it depends on the type of treatment applied and on the WWTP operational characteristics. Nonetheless, membrane-based and advanced oxidation technologies are very expensive. They require large amounts of energy and chemicals, as well as technically knowledgeable and well trained plant operators (Deegan et al., 2011; Ávila et al., 2013; Zhang et al., 2014). In addition, harmful by-products can be generated due to the oxidation treatments (Drinan and Spellman, 2012). Consequently, alternative processes that provide high EOCs removal at a reasonable cost should be considered.

Natural biological-based WW treatment systems can be an appropriate cost-effective alternative to the advanced technologies described above. They are eco-efficient and low-cost treatment systems that require low operational maintenance and provide water with a better biological quality to be reused or released into the environment (Ortiz et

al., 2011; Matamoros and Salvadó, 2012). These treatment techniques remove EOCs using a variety of biological, physical and chemical processes (the most relevant being biodegradation, photodegradation or sorption mechanisms) that take place simultaneously. Otherwise, plant uptake and phytodegradation are also reported to contribute to the removal of contaminants (Zhang et al., 2014).

In fact, many studies have shown the effectiveness of aquatic plant-based systems such as WSPs and CWs for removing EOCs from WW (Hijosa-Valsero et al., 2010; Matamoros et al., 2012c; Zhang et al., 2014). More details about this type of WW treatment are given in Chapter 4 (section 4.3).

1.1.7 Regulations

Water policy exist to protect the environment from adverse effects of pollution by controlling and monitoring priority pollutants, for example pesticides, volatile organic compounds (VOCs), chlorinated solvents, water disinfection by-products, and polycyclic aromatic hydrocarbons (PAHs). However, most EOCs, despite their proved adverse effects on the environment, are not subject to any degree of regulation (Lapworth et al., 2012).

In 2009, the USEPA released a new contaminant candidate list (List 3) for drinking water, including some unregulated EOCs with potentially harmful health risks (e.g. 17-ET) (Pedrouzo Lanuza, 2011; Lapworth et al., 2012; Stewart et al., 2014).

The EU Water Framework Directive (WFD-2000/60/EC) on the evaluation of priority substances in surface water bodies includes the widely used DCF and 17-ET on the European monitoring list, the so-called watch list of EOCs that could be placed on the priority list in the future (Stewart et al., 2014; Zenker et al., 2014). Both WFD and the Groundwater Directive (GWD-2006/118/EC) establish environmental goals to protect groundwater, surface water and ecosystems. Nevertheless, the behaviour, distribution, toxicity and impact of many EOCs in water bodies are not well known enough to set threshold values. Therefore, due to the growing research and regulatory interest in this topic, increased EOCs regulations are expected in the coming decades (Lapworth et al., 2012)

1.2 Sampling strategies to monitor EOCs in water

Current regulations require the routine monitoring of conventional parameters (i.e. organic matter, suspended solids, nutrients, microbiological organisms, and phosphorus, nitrogen, and carbon compounds) to control the quality of water bodies (Köck-Schulmeyer et al., 2011). However, little attention is paid to the monitoring of EOCs, which is of concern because of their harmful effect on ecosystems and their possible accumulation in crops and soils irrigated with reclaimed water (Watkinson et al., 2007), before ending up in the groundwater system.

Moreover, monitoring the concentrations of EOCs in the environment requires an adequate sampling strategy (Söderström et al., 2009). To solve this problem, environmental chemists have created cost-effective and user-friendly sampling strategies, the most common of which are grab or composite sampling and passive sampling.

1.2.1 Grab and composite sampling

Most of the monitoring campaigns currently used to control concentrations of EOCs in the aquatic environment are performed by grab sampling, which consists of collecting discrete grab samples at specific points of the study area (Togola and Budzinski, 2007). This approach is fairly popular because it is efficient, cheap, and easy to perform (Facchi et al., 2007; Söderström et al., 2009). However, some shortcomings are also associated with grab sampling (Kot et al., 2000; Togola and Budzinski, 2007; Söderström et al., 2009):

- The sample only represents the concentrations of EOCs at the specific time and sampling point, giving an incomplete picture of the pollutants concentration that doesn't consider inter- and intra-day variations.
- A high sampling frequency is required to overcome the previous problem, especially in water bodies where EOCs concentrations are susceptible to fluctuation over relatively short periods of time.

- Large sample volumes are needed to obtain enriched extracts during sample treatment and thus, adequate concentrations prior to analysis, which is required due to the low concentrations at which EOCs are usually found.

Consequently, obtaining representative data on EOCs concentrations over time using the grab sampling approach requires a large number of samples, which increases cost and time of both sampling and analysis.

Automatic water samplers to collect composite samples are an alternative to grab sampling. Composite sampling consists of collecting a number of high frequency grab samples (of a given volume) over a certain period of time and then combining and homogenizing them for treatment as a single sample (Facchi et al., 2007; Söderström et al., 2009). The main advantage is that it allows the acquisition of representative EOCs concentrations by reducing the number of analyses and, consequently, reducing the overall time and cost compared to grab sampling (Facchi et al., 2007).

However, the limitations of traditional water monitoring methods cannot be underestimated and thus many statutory monitoring programmes are now evaluating alternative methods to monitor EOCs in the aquatic environment. Among them is the use of passive sampler devices that fulfil the requirements of long-term monitoring (Kot et al., 2000; Emelogu et al., 2013).

1.2.2 Passive sampling

Passive sampling is a promising alternative to the traditional grab sampling method. It is based on the free flow of contaminants from the water source to a receiving medium contained in a passive sampling device, which results from the differences between the two media in terms of compounds concentration (Kot et al., 2000; Emelogu et al., 2013). The compounds are exchanged until equilibrium is reached in the system or the sampling is interrupted (Emelogu et al., 2013).

This sampling method presents some advantages in comparison to grab sampling (Kot et al., 2000; Togola and Budzinski, 2007; Söderström et al., 2009):

- Passive sampling devices can be deployed over long periods of time (e.g. weeks or months).

- The concentration of EOCs is measured as a time weighted average (TWA), so the concentration of the compounds is integrated over the sampling time, which provides an overall view of the compounds concentration over the exposure sampling time.
- It detects episodic events more easily.
- Much lower LODs are achieved by sampling great volumes of water over the deployment period.
- It is simple to perform and less sample treatment is usually required prior to analysis as during device deployment the EOCs are already concentrating in the receiving medium.
- Only one device is needed for each sampling location.
- Fewer analyses are required during the monitoring period, meaning a reduction in analytical time and costs.
- It is based on diffusion so a power supply and maintenance are not required and the necessary equipment is not complicated.

However, the use of passive sampling to monitor EOCs also has some drawbacks. Before the deployment of the passive sampling devices, calibrations for the different target EOCs are needed, as the sampling rate of the device is dependent on the characteristics of each compound as well as on the sampler design. Moreover, environmental conditions such as water flow and turbulence, temperature, pH, and salinity significantly affect the uptake of the compounds by the passive sampler. Hence, it is also relevant to measure and adjust these external parameters (Söderström et al., 2009).

There are different types of passive samplers, such as semi-permeable membrane devices (SPMD), silicone rubber, low density polyethylene (LDPE), and polar organic chemical integrative samplers (POCIS) (Emelogu et al., 2013). Among them, POCIS are widely used to monitor the EOCs studied in this thesis (Togola and Budzinski, 2007; Söderström et al., 2009; Li et al., 2010; Vermeirssen et al., 2012).

In this thesis, we have developed an alternative passive sampling device based on the use of polymer inclusion membranes (PIMs). Further details about this new approach are given in Chapter 4 (section 4.2).

1.3 Analytical methods for the determination of EOCs

Over the last few decades, newly developed methods have revealed the occurrence of a large number of EOCs at trace concentrations in environmental samples (Onesios et al., 2009). However, further improvement is still needed in terms of sensitivity and selectivity, especially for complex matrices such as soils, sludge or WW, as well as to minimize the time and costs involved (Petrović et al., 2003).

The whole analytical procedure generally consists of four steps (Fig. 1.3): sampling, sample preparation, sample analysis (analyte separation and detection), and data analysis. Each step must be completed to obtain correct/consistent results at the end. As the four steps are consecutive, it is important to conduct them properly, because any errors produced at each step will accumulate over the whole analytical procedure. However, due to the time spent on sampling and sample preparation (about 80% of the total analytical procedure), these two stages are considered key components of the analytical process (Bones, 2007; Pavlović et al., 2007).

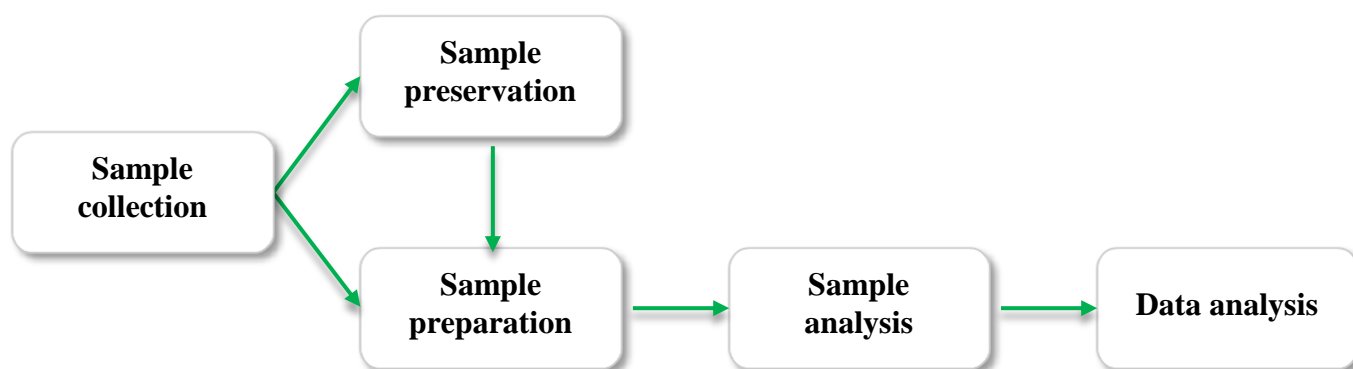


Figure 1.3. Steps in the analytical procedure for the EOCs determination in environmental samples (Sample preservation is included in the figure due to its importance).

1.3.1 Sample preparation

Sample preparation is the analytical step between sample collection and sample analysis. The function of sample preparation methods is to transform the real sample matrix into a suitable sample for analysis (Pavlović et al., 2007). It is not common to directly inject environmental samples into the instrument without applying any previous

treatment (Kot et al., 2000; Zhang, 2007). As stated previously, this stage is very important and is usually the more laborious and time consuming (Zhang, 2007).

Table 1.8 presents three considerations that highlight the need to conduct a sample preparation step on the analytical method prior to sample analysis (Kot et al., 2000; Bones, 2007; Pavlović et al., 2007; Comerton et al., 2009).

Table 1.8. Main considerations before sample preparation.

Low EOCs concentrations

Section 1.1.4 revealed that EOCs are present in environmental media at very low levels (ng L^{-1} - $\mu\text{g L}^{-1}$). These concentrations are usually below or near LOD of the instrumental techniques used for sample analysis. Hence, to ensure confidence in the analytical measurement and trustworthy results that reflect the EOCs concentrations in the original samples, and to increase the analytical sensitivity, a pre-concentration step of the analytes is usually mandatory.

Matrix interferences

Environmental sample matrices are complex and, as a result of natural processes, usually contain elements (e.g. dissolved organic matter, DOM) that can interfere or mask the analytes of interest. Matrix components can negatively influence the efficiency of the selected sample preparation method, such as decreasing the analytes' recovery efficiency and extraction quantity, thereby complicating analytes determination by the selected analytical technique. This phenomenon is defined as the matrix effect. Therefore, prior to sample analysis, it is necessary to remove matrix interferences and consequently increase the selectivity of the analytical method. This process is called clean-up and is dependent on the complexity of the sample matrix and on the detection method used afterwards.

Type of EOCs

During sample preparation, consideration must be given to the chemical form of the EOCs (e.g. ionized or non-ionized) and the instrument used for analysis, bearing in mind the analytical quality parameters required.

Therefore, even though sample preparation can be a time-consuming and laborious step, it is essential to determine of EOCs in environmental samples. Current analytical methods are commonly based on four main goals (i.e. to clean up, to extract, to pre-concentrate, and to adequate the analytes and sample form to the instrumental technique used), and aim to provide a robust and reproducible method that is independent of the sample matrix variations (Pavlović et al., 2007; Comerton et al., 2009).

The selection of the sample preparation method depends on the physicochemical properties of the EOCs to be extracted (e.g. $\log K_{ow}$, pK_a) as well as on the characteristics of the initial state of the sample matrix (i.e. solid, liquid, gas) (Pavlović et al., 2007). To ensure a viable and efficient sample preparation process, some conditions must be fulfilled: simplicity, relatively low costs, low sample and analytes loss, maximum selectivity for the analytes of interest, and the creation of proper conditions for the sample to be analysed (Bones, 2007).

Recently published analytical methods reduce sample sizes and avoid organic solvents, to achieve greater selectivity in extraction and increase the potential for automation (Pavlović et al., 2007). Currently, several methods for sample preparation allow the extraction and pre-concentration of the analytes from both aqueous and solid samples. Some examples are liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase microextraction (SPME), membrane based systems (MBS), ultrasonic extraction (USE), pressurized liquid extraction (PLE), and microwave assisted extraction (MAE). Among them, SPE and SPME are widely used and have been well studied for the analysis of EOCs in environmental samples (Tanwar et al., 2015). The former is mainly used to extract, pre-concentrate, and clean-up non-volatile or semi-volatile EOCs in liquid samples or pre-extracted solid samples. The latter, SPME, is commonly employed for volatile or semi-volatile EOCs (although it can also be used for the non-volatile ones) such as musk fragrances (Pavlović et al., 2007). Both SPE and SPME present advantages and disadvantages. Specifically, the main drawbacks of SPE are the time needed for sample preparation and the laborious optimization steps involved. On the other hand, SPME is simple and fast and avoids the use of organic solvents, but it presents problems related to method robustness, reproducibility, and low extraction efficiencies (Tanwar et al., 2015).

In this thesis we have used USE as a sample preparation method for sewage sludge and biosolid samples. This method is often used to extract EOCs from solid samples. The

sample, with an added solvent, is immersed in an ultrasonic bath, where it is exposed to ultrasonic radiation for a few minutes. Then, the extracted compounds are separated from the matrix by centrifugation or vacuum filtration. In order to achieve higher extraction efficiency, the same process is repeated two or three times. Afterwards, the obtained extracts are combined for analysis (Pavlović et al., 2007). This sample preparation method requires a relatively short extraction time in comparison with classic liquid extraction methods. However, due to the irregular distribution of ultrasound energy, some authors believe that USE is not very reproducible (Pavlović et al., 2007).

1.3.2 EOCs determination

Due to the matrix complexity of samples and the lack of detection mechanisms to confidently identify and quantify the analytes of interest when other matrix components are present, efficient chemical separation is greatly needed (Bones, 2007). Chromatographic techniques are widely used for environmental analysis, with gas chromatography (GC) and liquid chromatography (LC) most frequently used to separate EOCs (Jones et al., 2003; Martinez Bueno et al., 2012). Because these two chromatographic techniques are complementary, one or the other will be used depending on the EOCs polarity and/or volatility (Martinez Bueno et al., 2012). GC is used for non-polar and volatile or volatizable EOCs, while LC is used for the polar and non-volatile or thermally unstable ones. However, the two latest classes, with a previous derivatization, can also be analysed by GC (Zhang, 2007; Matamoros et al., 2012a). The most frequently reported stationary phases for chromatographic columns are DB-5 (5% diphenyl polydimethylsiloxane) and C-18 (Octadecyl silane) for GC and LC respectively (Nödler et al., 2010; Dinh et al., 2011; Gros et al., 2012; Martinez Bueno et al., 2012; Matamoros et al., 2012a; Matamoros et al., 2012b).

In this study LC has been chosen because it is useful for the separation of polar compounds, such as NSAIDs, and high molecular weight compounds, such as antibiotics.

On their own, these separation techniques (i.e. GC and LC) only provide one type of information to help examine the compound's identity: the retention time (rt) of the compounds in the stationary phase contained inside the chromatographic column

(Zhang, 2007), which is not enough for compounds identification and/or quantification. Therefore, an appropriate detector must be selected for use with the chromatographic technique.

The determination of EOCs in environmental samples currently involves different types of detectors for both GC and LC. Prior to their selection, several parameters, in addition to the characteristics of the compound to be determined, should be considered, such as detector sensitivity, selectivity, and linear range, among others (Cziczo, 2003). Table 1.9 summarizes some of the most common detectors together with their main characteristics (Cziczo, 2003; Zhang, 2007).

In this thesis, the studied EOCs have been determined by two different techniques, LC connected to a photodiode array detector (PDA) or LC coupled to a mass spectrometer (MS).

PDA functions as a multi-wavelength UV/Vis absorbance detector able to provide UV spectra of eluting peaks at multiple wavelengths. It facilitates peak identification and therefore is commonly used for method development. Earlier models presented lower sensitivity than those of today, as this parameter has been significantly improved over the years (Dong, 2006). However, this detector still lacks specificity and MS is widely used as a result of this drawback. MS determines the mass to charge ratio of individual compounds in a sample. This information permits determination of the nature, composition, and even structure of the compounds (Rouessac and Rouessac, 2013). MS can be used in a single mode, through which a selected ion can be monitored for each compound, or in tandem (MS/MS), which allows the monitoring of a mass transition (Rouessac and Rouessac, 2013). MS/MS provides greater performance than single MS, in terms of sensitivity and selectivity.

Table 1.9. Comparison of major GC and LC detectors.

	Type	Applications	Sensitivity	Selectivity	Linearity
GC	Thermal conductivity detector (TCD)	Universal	Fair	Low	Good (10 ³ -10 ⁴)
	Flame ionization detector (FID)	Universal for hydrocarbons	Very good	Low	Excellent (10 ⁶ -10 ⁷)
	Electron capture detector (ECD)	Selective for halogens; No response to hydrocarbons	Excellent	Fair	Poor (10 ² -10 ³)
	Nitrogen-phosphorus detector (NPD)	Selective for N and P containing compounds	Excellent	Good	Excellent (10 ⁵ -10 ⁷)
LC	UV-Vis and Photodiode array detector (PDA)	Specific for UV-VIS light absorbing compounds	Good Excellent	Fair-Good Very good	Good (10 ⁴)
	Fluorescence	Specific for compounds able to fluoresce	Excellent	Very good	Good (10 ⁵)
	Conductivity	Specific for ion species	Excellent	Good	Good (10 ⁵)
GC and LC	Mass spectrometry (MS)	Universal detector	Excellent	Excellent	*

* Varies, depending on the type of mass spectrometer and on the compounds being analysed.

Currently, LC-MS/MS is the most referenced technique for the determination of non-volatile polar and semi-polar organic compounds in environmental samples (Gracia-Lor et al., 2012; Martinez Bueno et al., 2012; Loos et al., 2013; Padhye et al., 2014; Rossmann et al., 2014; Yan et al., 2015). Otherwise, for the determination of non-polar and volatile or volatizable compounds, GC-MS or GC-MS/MS is the technique applied (Jones et al., 2003; Tixier et al., 2003; Comerton et al., 2009; Martinez Bueno et al., 2012).

Although LC-MS/MS is currently the most used technique, it has a remarkable drawback: an important influence of the matrix effect. GC-MS is less prone to water matrix interferences and generally shows lower LODs (Comerton et al., 2009). However, more complicated and time-consuming sample preparation is needed, which

can lead to sample loss during its manipulation, which increases when derivatization is needed (Comerton et al., 2009).

In LC-MS/MS, the presence of co-extracted matrix components affects compounds ionization, suppressing or enhancing their signal and leading to erroneous results during the analysis. This can hinder the identification, and seriously affect the quantification of the compounds (Comerton et al., 2009; Jones-Lepp et al., 2009; Gracia-Lor et al., 2012).

Matrix effects can be compensated by the addition of a surrogate compound or an isotopically labelled internal standard, by matrix matched calibration or by the use of the standard addition method (Comerton et al., 2009; Jones-Lepp et al., 2009). The use of internal standards to assess the loss of signal intensity is relatively simple, efficient and less time-consuming than the matrix matched calibration and the standard addition method (Matamoros et al., 2012b). However, it is not easy to find structurally similar, isotopically labelled compounds to be used as internal standards because they are not always commercially available and their cost is high. In addition, more than one internal standard may be required in multi-class methods. The matrix match method is used to compensate matrix effects that influence the analytical response. A solution that closely corresponds to the composition of the samples is used to prepare the matrix matched calibration standards, by adding the target compounds to blank extracts. However, matrix matched calibration shows some drawbacks: difficulty obtaining a closely matching blank matrix for every sample type, high instrumentation maintenance, and additional work (Matamoros et al., 2012b). Although more labour-intensive and time-consuming, the standard addition method is considered a reliable and efficient way to reduce matrix effects (Comerton et al., 2009). In this thesis, we have used both the external calibration curve and the standard addition method.

CHAPTER 2

Objectives

The overall aim of this study is to develop innovative analytical methods to determine EOCs and to understand the processes involved in their removal in biologically-based wastewater treatment plants. Therefore this general aim will be met by achieving the following specific objectives:

- To develop analytical methods to determine pharmaceutical compounds in sewage sludge using a standard addition method.
- To develop a preconcentration system based on a PIM to determine antibiotics in water samples.
- To design an innovative passive sampler including a PIM to monitor SMX in natural waters.
- To study the role of photodegradation in the removal of EOCs in secondary treated wastewaters.
- To assess the effect of microbiological activity (biodegradation) and aquatic plants from polishing ponds (PPs) on the mitigation of EOCs in wastewaters.

CHAPTER 3

Methods

3.1 Determination of pharmaceutical compounds in sewage sludge using a standard addition method approach

Chemicals and reagents

Liquid chromatography grade methanol was obtained from Carlo Erba (Milan, Italy). Formic acid, STZ, SMZ, SMX, SPY, CLF AC, NAP, DCF, and IBP were purchased from Sigma Aldrich (Steinheim, Germany). Millex- GN nylon filters (0.20 μm i.d. –13 mm) were from Merck Millipore (Billerica, Massachusetts).

Description of the wastewater treatment plant

We collected sewage sludge samples at three urban WWTPs in the north-east of Catalonia (Castell- Platja d'Aro, Palamós, and Blanes) that treat WW using conventional activated sludge technology. The sludge treatment at Castell-Platja d'Aro WWTP consists of aerobic digestion and a dehydration step. At Palamós, aerobic-anaerobic digestion and dehydration is used. At Blanes, the treatment consists of anaerobic digestion, dehydration, and composting. We took biosolid samples at the final dehydration step in Castell-Platja d'Aro and Palamós WWTPs, and after composting in Blanes. Biosolid samples of roughly 1 Kg each were collected from the three plants in 1 L clean plastic bottles in May, July, October, and December 2011 ($n = 12$), and transported under refrigeration to the laboratory.

Sample preparation

Samples were frozen at -20°C and later lyophilised in order to completely remove water from the sludge samples, grounded using a pestle and mortar, and passed through an 0.2 mm sieve as the efficiency of extraction in USE is dependent on the homogeneity of the sample matrix (Luque García and Luque de Castro, 2002; Koel and Kaljurand, 2010). Afterwards, samples were stored at -20°C until analysis. In order to develop the standard addition calibration method, we introduced 200 mg of sieved sludge sample into glass extraction tubes and spiked them with different amounts of pharmaceuticals (5–250 ng) using a mixture of the eight compounds in methanol (2 mL). The dried

sludge and the compounds were mixed thoroughly with a spatula and left uncovered for 24 hours to promote the evaporation of the solvent and good interaction between the analyte and the matrix.

Extraction optimization

We evaluated different v/v ratios (1:1, 1:2, and 1:3) of methanol and ultrapure water (UW) mixtures by adding 2 mL of this solution to 200 mg of sieved sludge spiked with 250 ng g⁻¹ of pharmaceuticals. The mixture was then sonicated for 15 min and centrifuged at 4000 rpm for 10 min. Three consecutive extractions were performed separating the extract on each occasion. The three extracts (2 mL each) were collected in the same vial and then filtered through an 0.2 µm nylon syringe filter. An aliquot of 5 µL, for anti-inflammatories and the lipid-regulator, and 10 µL, for antibiotics, was then taken from the resulting solution and injected into the LC-MS/MS system. All the experiments were performed in triplicate.

After selecting the most efficient composition of the extraction solution, we then analysed each individual extract separately to evaluate the contribution made by each to the total recovery.

Sample analysis

LC-MS/MS analysis was performed using a 1290 Infinity LC System coupled to a 6430 Triple Quad (Agilent Technologies, USA).

Chromatographic separation of antibiotics was performed with an Ascentis C18 Express fused-core column (50 mm × 4.6 mm × 2.7 µm) preceded by a guard column, using a mobile phase composed of UW containing 0.1% v/v of formic acid (component A) and methanol (component B). The separation was carried out applying the following elution profile: gradient elution from 15% B to 35% B in 5 min, gradient elution from 35% B to 100% B in 5 min, maintained at 100% B for 2 min and returned to the initial conditions in 3 min. The flow rate was set at 0.6 mL min⁻¹ and the injection volume was 10 µL. Chromatographic separation of anti-inflammatories and the lipid-regulator was performed using a Synergi Fusion-RP100A column (50 mm × 2 mm × 2.5 µm) preceded by a guard column with a mobile phase composed of 10 mM NH₄Ac

(component A) and methanol (component B). The separation of compounds was conducted by applying a gradient from 30% B to 70% B over 9 min, maintaining the 70% of B for 1 min before returning to the initial conditions over 1 min. The flow rate was set at 0.2 mL min⁻¹ and the injection volume was 5 µL. For both antibiotics and anti-inflammatory drugs, MS data acquisition was performed with the ESI operating in positive (PI) or negative (NI) ionisation mode under time-scheduled multiple reaction monitoring (MRM) detecting two transitions (quantifier/qualifier ions) per precursor ion (Table 3.1).

Table 3.1. Retention time, precursor, product ions used to identify and quantify pharmaceuticals.

	Retention time (min)	Precursor ion (m/z)	Product ion 1 (m/z) ^a	Product ion 2 (m/z) ^b	Collision energy (V)	Ionization mode
STZ	2.1	256	156	92	10	PI
SPY	2.3	250	156	92	15	PI
SMZ	3.4	279	186	124	15	PI
SMX	4.5	254	156	108	10	PI
CLF AC	4.5	213	127	85	12	NI
NAP	5.1	229	169	170	28	NI
DCF	7.7	294	250	214	0	NI
IBP	7.8	205	161	-	0	NI

Note: ^aquantification ion; ^bconfirmation ion

Method validation

Owing to the lack of sludge reference materials, the method was validated by analysing spiked samples. As we were unable to find blank biosolid samples and the organic matter content of sludge depended on the sludge treatment applied in the WWTP (aerobic digestion: 60–70%; anaerobic digestion: 40–50% and composting: 50–85%), we chose rabbit excrement as a blank matrix given that it is wholly composed of organic matter and has no content of any of the studied pharmaceuticals. This type of matrix represents the most complicated scenario.

Recoveries were evaluated by analysing 200 mg of rabbit excrement spiked at three concentration levels: 50 ng g⁻¹, 200 ng g⁻¹ (except for IBP, 500 ng g⁻¹), and 1000 ng g⁻¹. Different amounts of each compound ranging from 5 to 1250 ng were added to the

rabbit excrement to perform a six-point standard addition calibration per compound, which was performed in triplicate for each concentration level. The precision of the method was calculated as the relative standard deviation (%RSD, $n = 3$) of the standard addition calibration for each individual compound. The method detection limits (MDLs) were calculated by analysing 200 mg of rabbit excrement and adding different amounts of each compound ranging from 0 to 1250 ng in a six point standard addition calibration per compound, which was performed in triplicate for each concentration level. Finally, the MDLs were calculated using the Hubaux and Vos method calculator from Chemiasoft (Hubaux and Vos, 1970; Vanatta and Coleman, 1997).

3.2 Innovative analytical technologies based on PIMs for the determination of antibiotics

3.2.1 Development of a polymer inclusion membrane for the preconcentration of antibiotics in environmental water samples

Chemicals and reagents

Stock solutions (250 mg L^{-1}) of STZ, SPY, SMZ, SMX, OTC, and TC, all purchased from Sigma Aldrich (Steinheim, Germany), were prepared in methanol. Working solutions were prepared daily by appropriate dilution of the stock solutions with UW (Milli-Q Plus system, Millipore). Sodium chloride was used to prepare the stripping solution (Carlo Erba, Italy).

The carrier (Aliquat 336), polymers (cellulose triacetate (CTA) and polyvinylchloride (PVC)), and plasticizers nitrophenyl octylether (NPOE, 99%), dibutyl sebacate (DBS, 99%), and bisbutylpentyl adipate (BBPA)) were purchased from Fluka Chemie (Spain). The organic solvents chloroform and tetrahydrofuran (THF) (Panreac, Spain) were used to dissolve CTA and PVC, respectively.

LC grade methanol was obtained from Carlo Erba (Sabadell, Spain) and formic acid was purchased from Sigma Aldrich (Steinheim, Germany).

All reagents and solvents were of analytical reagent grade and used as received. UW was used for solution preparation.

PIMs preparation

PIMs based on CTA were prepared by dissolving 200 mg of the polymer in 20 mL of chloroform. After at least 5 h of agitation, the corresponding amount of Aliquat 336 was added. In some cases a plasticizer (i.e. NPOE, DBS or BBPA) was added to the casting solution. The same procedure was followed for the preparation of the PVC-based PIMs, but using 400 mg of the polymer and THF as the solvent. Each solution was stirred by a magnetic stirrer until all membrane components had dissolved and then poured into a 9.0 cm diameter flat bottom glass Petri dish which was set horizontally and covered loosely. The solvent was allowed to evaporate over 24 h at room temperature (Fig. 3.1 (a)), and the resulting transparent, flexible and homogeneous film, the PIM, was then carefully peeled off the bottom of the Petri dish (Fig. 3.1 (b)).

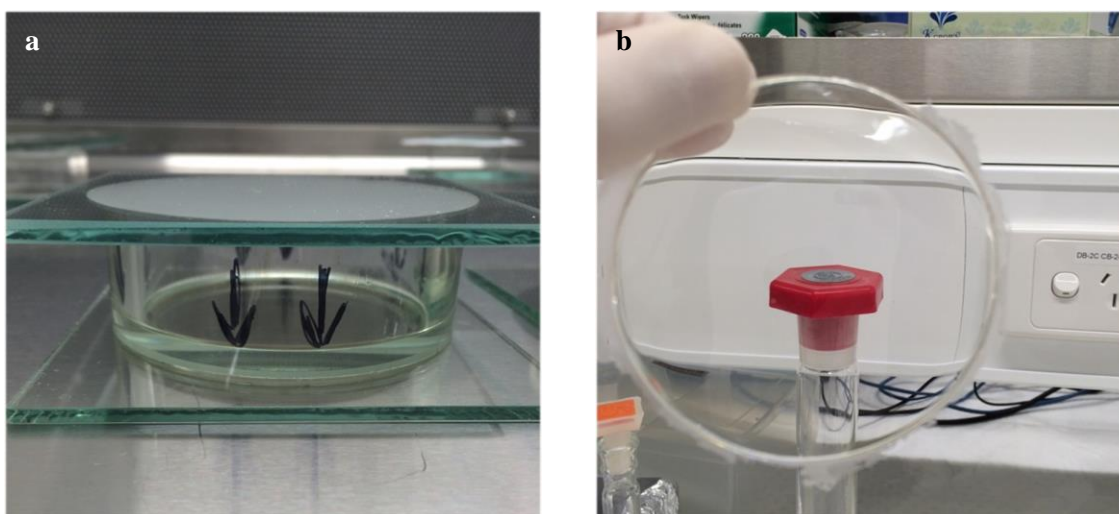


Figure 3.1. a) Solvent evaporation during the PIM casting process. b) Flexible, transparent and homogeneous film obtained after the complete solvent evaporation, PIM.

Circular segments were cut from the centre of the membranes and placed in a membrane permeation cell (for transport experiments), or in the proposed membrane-based device (preconcentration experiments). All PIM compositions are given in mass percentages in Chapter 4, section 4.2.

Transport experiments for PIM optimization

Transport experiments were carried out in a permeation cell (Fig. 3.2) similar to the one described by Fontàs et al. (2003) in which the mechanically stirred (800 rpm) source (S) and receiving (R) solutions (150 mL each) were separated by a PIM with an exposed membrane surface area of 11.5 cm². All experiments were conducted at ambient temperature of 22±1 °C.

The initial S solution contained 5 mg L⁻¹ of antibiotics in UW, at the studied pH, whereas 1 mol L⁻¹ NaCl was used as the R solution. Samples were taken from the S and R solutions at regular time intervals during the 24 h duration of the experiments.

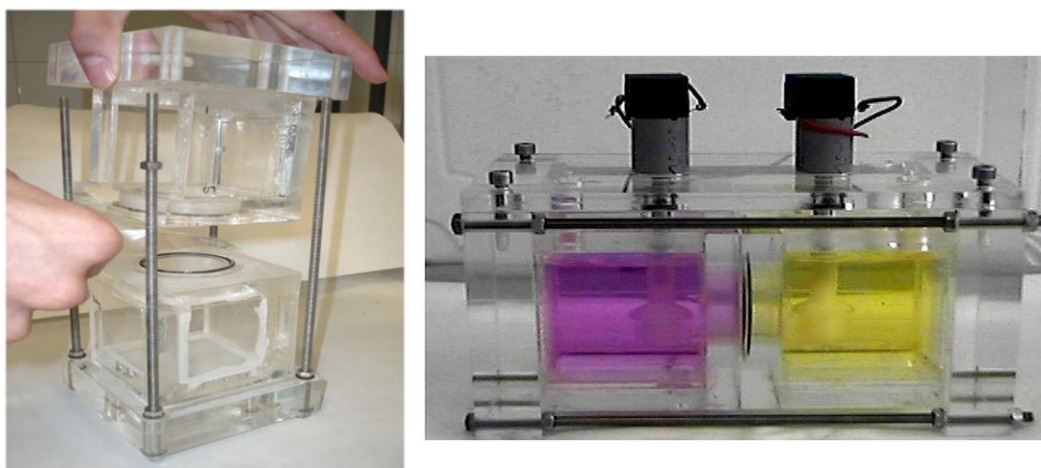


Figure 3.2 Permeation cell used for transport experiments.

The performance of the system was studied in terms of its antibiotic transport efficiency (TE), defined by Eq. (3.1) and its initial flux (J_0), calculated by Eq. (3.2).

$$\text{TE (\%)} = \frac{[\text{Antibiotic}]_{r,t}}{[\text{Antibiotic}]_{s,0}} 100 \quad (3.1)$$

where $[\text{Antibiotic}]_{r,t}$ refers to the antibiotic concentration in the R solution at time t and $[\text{Antibiotic}]_{s,0}$ refers to the initial concentration of the antibiotic in the S solution.

$$J_0 = \left(\frac{V}{A}\right) \left(\frac{\Delta [\text{Antibiotic}]_s}{\Delta t}\right) \quad (3.2)$$

where V is the volume of the S solution, A the exposed surface area of the membrane, and $\Delta[\text{Antibiotic}]_s/\Delta t$ is the depletion rate of the antibiotic in the S solution over time.

Antibiotics preconcentration using a membrane-based device

Preconcentration experiments were carried out by using a membrane-based device developed as part of this study. It consists of a glass tube with two openings, one at its top and another at its bottom. As shown in Fig. 3.3, the PIM was placed at the bottom opening and fixed with a screw cap specially designed to allow 1.5 cm² of the membrane surface to be exposed to the sample solution. The device contained the R solution (4 mL) and it was partially submerged into the mechanically stirred S solution (120 mL), located in a beaker. All preconcentration experiments were conducted at ambient temperature of 22±1 °C.

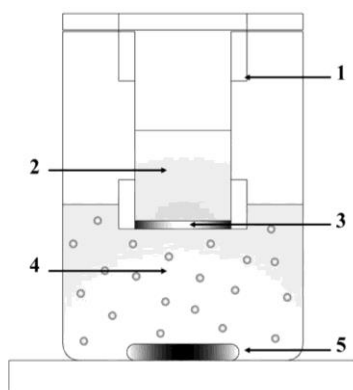


Figure 3.3. PIM-based device used for antibiotics preconcentration (1- device, 2- R solution, 3- PIM, 4- S solution , 5- magnetic stirrer).

The initial S solution contained 100 µg L⁻¹ of antibiotics in different water samples at the appropriate pH, whereas the R solution contained 0.5-1.0 mol L⁻¹ NaCl.

Samples were taken from both the S and R solutions ($V = 200 \mu\text{L}$) at fixed time periods.

Similarly to the transport experiments, the performance of the system was studied in terms of its antibiotics TE calculated by Eq. (3.3) which takes into account the difference in volumes of the S (V_s) and R (V_r) solutions:

$$TE (\%) = \left(\frac{V_r}{V_s} \right) \left(\frac{[Antibiotic]_{r,t}}{[Antibiotic]_{s,0}} \right) \cdot 100 \quad (3.3)$$

The preconcentration factor (PF) of this system was calculated by Eq. (3.4):

$$PF = \frac{[Antibiotic]_{r,t}}{[Antibiotic]_{s,0}} \quad (3.4)$$

Sample analysis

The antibiotics composition of the S and R solutions during the transport and preconcentration studies were analysed by an HPLC system (1200 Series Agilent Technologies, USA) with a DAD (1290 Infinity DAD Agilent Technologies, USA). The absorbance was measured at 274 nm. The analytical procedure involved the use of UW with a 0.1 v/v% of formic acid (component A) and methanol (component B) as the mobile phase. The antibiotics separation was carried out by applying the following elution profile: gradient elution from 15% B to 30% B in 5 min, then increasing the concentration of B up to 100% in 6 min and maintaining it for 2 min, then decreasing the concentration of B to its initial value of 15% B in 2 min and maintaining this concentration for 3 min. The flow rate was set at 0.5 mL min^{-1} and the injection volume was 8 μL . Chromatographic separation was performed with a C_{18} Ascentis Express fused-core column (0.5 cm x 0.46 cm x 2.7 μm) preceded by a guard column. The pH and conductivity of the real water samples were measured by a pH meter (Model GLP 22, Crison) and a portable conductivity meter (Ecoscan, Entech Instruments), respectively. An ion chromatograph (Model IC5000, Dionex) equipped with an autosampler AS-AP, a conductivity detector and an IonPac® AS18 anion-exchange column (4 x 250mm) with the AG guard column (4 x 50mm) was used for analysing the anionic composition of the water samples.

Real water samples

Well water (Sant Hilari, Girona, Spain) and river water (River Ter, Girona, Spain) samples were collected in 1 L clean amber glass bottles and transported refrigerated to the laboratory. The chemical parameters of the water samples are summarized in Table 3.2.

Table 3.2. Chemical parameters of well and river water samples.

Chemical parameter	Well water	River water
pH	7.50	8.00
Conductivity (μS)	275.00	462.00
Carbonate (mg L^{-1})	172.00	253.00
Chloride (mg L^{-1})	11.40	25.90
Nitrate (mg L^{-1})	21.90	5.30
Phosphate (mg L^{-1})	0.06	0.07
Sulphate (mg L^{-1})	8.70	15.00

3.2.2 Monitoring of sulfamethoxazole in natural waters with a polymer inclusion membrane-based passive sampler. Minimizing the effect of the flow pattern of the aquatic system

Chemicals and reagents

All chemicals were of analytical reagent grade and used as received. UW (18.2 M Ω cm, Millipore, France) was used for the preparation of all solutions.

SMX was purchased from Sigma Aldrich. A 250 mg L⁻¹ stock solution was prepared in methanol. Working solutions were prepared daily by appropriate dilution of the stock solution with environmental waters (groundwater, river water, and WW) or UW. Sodium chloride (Sigma Aldrich) was used to prepare the R solution.

The PIM components: Aliquat 336, CTA, PVC, and NPOE (99%), were purchased from Sigma Aldrich. Chloroform (Panreac, Spain) was used for dissolving CTA whereas THF (Panreac, Spain) was used for dissolving PVC.

LC grade methanol was obtained from Carlo Erba (Sabadell, Spain) and Gillman S.A. (Australia) and formic acid was purchased from Sigma Aldrich.

Membrane preparation

PIMs with four different compositions (M1: 30% CTA + 26% Aliquat 336 + 44% NPOE; M2: 30% CTA + 44% Aliquat 336 + 26% NPOE; M3: 30% PVC + 26% Aliquat 336 + 44% NPOE; M4: 30% PVC + 44% Aliquat 336 + 26% NPOE) were prepared following the procedures described in section 3.2.1.2. It should be noted that all PIM compositions are quoted in mass percentages. Once all the membrane components with a total mass of 1.222 - 1.316 g for the PVC-based PIMs and 0.643 - 0.682 g for the CTA-based PIMs were completely dissolved in THF and chloroform, respectively, the resulting solution was poured into a flat bottom glass Petri dish which was set horizontally and covered loosely. The solvent was allowed to evaporate over 24 - 48 h at room temperature, and the resulting film was then carefully peeled off the bottom of the Petri dish. Circular segments were cut from the centre of the membranes to ensure uniform thickness (i.e. M1: 94 ± 5 μm ; M2: 87 ± 5 μm ; M3: 145 ± 5 μm ; and M4: 125 ± 5 μm) and placed in the proposed membrane-based device.

Passive sampling configurations

- *Effect of the chemical parameters on SMX enrichment in the receiving solution*

The passive sampler and passive sampling configuration used in this study are similar to those used earlier by us (Garcia-Rodríguez et al., 2015) and are shown in Fig. 3.4 (a) and 3.4 (b), respectively.

The PIM-based passive sampler, which contained 4 mL of NaCl R solution, was partially submerged in 120 mL of mechanically stirred S solution ($100 \mu\text{g L}^{-1}$ SMX in UW or environmental water). All experiments were conducted in triplicate or duplicate at ambient temperature of 22 ± 1 °C.

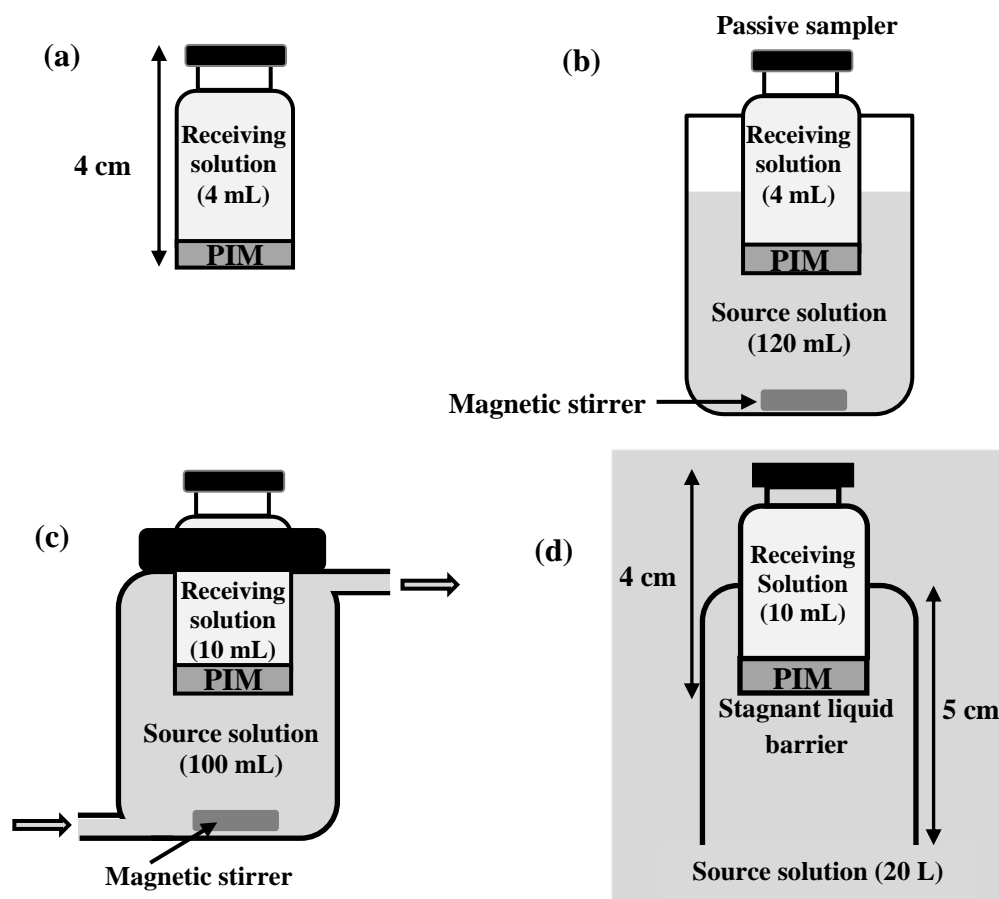


Figure 3.4. Schematics of the passive sampler (a), passive sampler configuration used in the SMX transport experiments (b), “flow-through” passive sampling configuration (c), and passive sampler with a stagnant liquid barrier (d).

Samples (200 μL each) were taken from both the S and R solutions at fixed time periods and replaced with the same volume of the corresponding original solutions.

The SMX enrichment factor (EF) of the PIM-based passive sampler was calculated by Eq. (3.5).

$$EF = \frac{[SMX]_{r,t}}{[SMX]_{s,0}} \quad (3.5)$$

Where $[SMX]_{r,t}$ is the transient SMX concentration in the R solution and $[SMX]_{s,0}$ is its initial concentration in the S solution.

The chemical parameters varied in these experiments were the membrane composition (type of base polymer, i.e. PVC and CTA and the concentrations of the carrier (Aliquat 336) and plasticizer (NPOE)), the concentration of NaCl in the R solution and the concentration of SMX in the S solution and the S solution matrix.

- Effect of the flow pattern of the source solution and the passive sampler design

Experiments utilizing a “flow-through” passive sampling configuration (Fig. 3.4 (c)) were carried out using a passive sampler identical to the one shown in Fig. 3.4 (a) but with 10 mL R solution. However, in the “flow through” case, a $500 \mu\text{g L}^{-1}$ SMX S solution in UW was pumped using a peristaltic pump (Miniplus 3, Gilson, USA) equipped with Tygon pump tubing (TACS, Australia) through the 100 mL flow-through glass cell attached to the passive sampler. The S solution in the glass cell was continuously stirred using a magnetic stirring bar. To check if the “flow-through” approach ensured constant SMX concentration at the membrane/S solution interface, experiments at different flow rates were conducted ($0.75\text{-}10 \text{ mL min}^{-1}$) in triplicate and the SMX concentration in the effluent S solution was measured at fixed periods of time to check if it was significantly lower than the influent S solution concentration of $500 \mu\text{g L}^{-1}$ SMX. The passive sampler incorporated a CTA/Aliquat 336/NPOE membrane and contained 10 mL of 2 mol L^{-1} NaCl R solution. The SMX concentration in the R solution was measured 1 and 2 h after the start of the experiment to evaluate the passive sampler EF (Eq (3.5)). The R solution samples were replaced with the same volume of the original R solution.

In the experiments using the “dip-in” approach, passive sampling in two different configurations was carried out. In the first configuration, the passive sampler (Fig. 3.3(a)) with 10 mL R solution was placed at the bottom of a 20 L tank containing a $500 \mu\text{g L}^{-1}$ SMX solution in UW. In the second configuration, the same passive sampler was first inserted into a plastic cylindrical container through a hole in its base as shown in Fig. 3.3 (d) which provided a stagnant liquid barrier between the S solution and the membrane.

Samples from the S and R solutions were taken at fixed time periods and replaced with the same volume of the corresponding original solutions. The experiments outlined above were conducted in triplicate.

Sample analysis

Samples were analysed in equal conditions as described in section 3.2.1. For these studies, the chromatographic separation was performed in either a C₁₈ Ascentis Express fused-core column (0.5 cm x 0.46 cm x 2.7 µm, Sigma-Aldrich) or in an Atlantis dC18 (3 µm, 2.1 x 150 mm, Waters, USA) column preceded in both cases by a guard column (Phenomenex, USA).

Water samples

In this study environmental waters of three different types were sampled and used in preparing spiked with SMX S solutions. The chemical composition and conductivity of these waters are summarized in Table 3.3.

Table 3.3. Chemical composition and conductivity of the ground, river and WW used in this study.

Chemical parameter	Groundwater 1	Groundwater 2	River water	Wastewater
pH	8.37	7.98	7.59	7.12
Conductivity (µS cm⁻¹)	527	275	462	530
Carbonate (mg L⁻¹)	268.5	172.0	253.0	Not determined
Chloride (mg L⁻¹)	15.4	11.4	25.9	56.3
Nitrate (mg L⁻¹)	1.2	21.9	5.3	30.9
Phosphate (mg L⁻¹)	<0.05	0.06	0.07	4.69
Sulphate (mg L⁻¹)	63.8	8.70	15.0	12.0

Both types of groundwater were collected from two different wells in the region of Girona (Catalunya, in the northeast of Spain). River water was collected from Ter River which flows through the city of Girona. WW was collected from the effluent of La Bisbal d'Empordà WWTP, which serves a population of 12000 inhabitants in the region of Girona.

3.3 The ability of phytotechnologies to remove EOCs from wastewater

3.3.1 The influence of light exposure, water quality and vegetation on the removal of sulfonamides and tetracyclines: A laboratory-scale study

Chemicals and reagents

Liquid chromatography grade methanol was obtained from Carlo Erba (Sabadell, Spain) and formic acid from Sigma Aldrich (Steinheim, Germany). OTC, TC, STZ, SMZ, SMX and SPY were purchased from Sigma Aldrich (Steinheim, Germany). Ciprofloxacin, which was used as the internal standard, was obtained from Sigma Aldrich (Steinheim, Germany). Millex-GN Nylon filters (0.20 μm i.d. - 13 mm) were from Merck Millipore (Billerica, Massachusetts).

Description of the wastewater treatment plant

Biological samples (algae and aquatic plants) and secondary-treated WW samples were obtained from the Empuriabrava WWTP, which is located in the northeast of Spain and serves a mostly residential area with ca 67,000 equivalent inhabitants. The WWTP undertakes pre-treatment, primary clarification, activated sludge treatment, and secondary clarification. The water is then pumped to the reclamation plant, which is composed of two parallel polishing ponds and a surface flow CW. The secondary effluent water presented a conductivity of 3000 $\mu\text{S cm}^{-1}$ and a concentration of total suspended solids of 2 mg L^{-1} .

Experimental design

Treated WW samples were collected at the secondary effluent from the Empuriabrava WWTP. Algae (*Spyrogira* sp.) and aquatic plants (*Z. palustris*) were collected from the polishing pond at the same time (Fig. 3.5). Before initiating the experiment, the algae

and aquatic plants were pre-acclimated to laboratory conditions by being placed in a fish tank for a month.



Figure 3.5. Sampling site.

The set-up of the laboratory-scale study included a series of different aquatic plant and control reactors fed with either treated WW or reagent UW. The aquatic plant reactors were planted with *Z. palustris* and *Spyrogira* sp., and there were both covered (aluminum foil was used) and uncovered control reactors. A total of 18 reactors systems were used (three of each type) (see Fig. 3.6). All systems consisted of glass reactors with 2.5 L of treated WW from the effluent of the secondary treatment. These reactors, which were randomly distributed, were left at room temperature (20 °C) and exposed to light from 36 W cool white fluorescent tubes with a photon flux of $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ in a 12 h light/darkness cycle.



Figure 3.6. Experimental setup.

A mixture of the six antibiotics in methanol was added to each reactor to obtain a final concentration of $200 \mu\text{g L}^{-1}$ (1 mL of spiking solution at 500 mg L^{-1} of each compound in methanol). 2 g fresh weight (FW) of algae and plants were added to the corresponding containers. The experiments were run for 20 d. The fresh mass of the aquatic vegetation at the end of the experiment was about 7 g FW for *Spyrogira* sp. and 8 g FW for *Z. palustris*. The conductivity and pH of the secondary-treated wastewater were $3000 \mu\text{S cm}^{-1}$ and 8 respectively.

Sampling strategy

Aqueous samples of 1 mL were taken regularly up to 20 d. All samples were collected in clean brown glass bottles and frozen until analysis. In order to keep the same water level in the reactors and correct the loss due to evaporation, the reactors were refilled with secondary-treated WW to a specific depth mark before each sampling.

Sample analysis

All water samples were filtered with $0.20 \mu\text{m}$ porous nylon filters and settled in brown glass vials. A sample volume of 0.75 mL of each sample was spiked with 190 ng of ciprofloxacin as the internal standard. In order to determine concentration of antibiotics, the samples were analyzed as described in section 3.1.5. In this case the injection volume was $20 \mu\text{L}$. MS data was acquired operating in PI mode under the time-scheduled MRM conditions listed in Table 3.3. The LOD of the analytical methodology determined using the Hubaux and Vos method (1970), ranged from 5.7 to $10.4 \mu\text{g L}^{-1}$ and repeatability was calculated as the variation coefficient, which was always lower than 6 % for both SAs and TCs (Table 3.4).

Table 3.4. Analytical parameters of selected antibiotics.

	Retention time (min)	MRM transitions (m/z)	Collision energy (eV)	LOD ($\mu\text{g L}^{-1}$)	RSD (%)
STZ	2.1	256/156	10	5.9	4.6
SPY	2.3	250/156	15	7.3	4.3
SMZ	3.4	279/186	15	5.7	4.3
SMX	4.5	254/156	10	7.5	5.6
OTC	3.7	461/426	20	10.4	4.7
TC	3.5	445/410	15	9.1	5.2

Data analysis

Experimental results were analyzed using the SPSS v.15 statistical package (Chicago, IL, USA). The correlation coefficients of the concentration depletion of the antibiotics as a function of time were calculated using parametric statistics (Pearson coefficient).

3.3.2 The influence of *Lemna* sp. and *Spirogyra* sp. on the removal of pharmaceuticals and endocrine disruptors in treated wastewaters

Chemicals and reagents

LC grade methanol was obtained from Carlo Erba (Sabadell, Spain) and formic acid from Sigma Aldrich (Steinheim, Germany). CAFF was from PANREAC (Barcelona, Spain). ACAPh, 17-ET, IBP, DCF, BPA, ROPR, and CARB were purchased from Sigma Aldrich (Steinheim, Germany). Millex-GN polytetrafluoroethylene (PTFE) filters (0.2 μm i.d. – 13 mm) were from Merck Millipore (Billerica, Massachusetts).

Description of the treatment plant

WW and vegetal samples were obtained from the same WWTP described in 3.3.1.

Experimental design

Secondary-treated WW samples, algae (*Spirogyra* sp.) and duckweed (*Lemna* sp.) (Fig. 3.7), were collected from the same WWTP described in section 3.3.1 (see Fig. 3.5). Before initiating the experiment, the two plants were preacclimated to laboratory conditions in a 70 L fish tank for a month.



Figure 3.7. Algae and duckweed used for the experiments.

The set-up of the laboratory-scale study included a series of planted and control reactors, some covered with aluminium foil and others uncovered, which were fed with either secondary-treated WW or reagent UW. A total of 18 reactors were used to allow three reactors for each set of conditions (see Fig. 3.8). Each system consisted of a glass reactor with 2.5 L of secondary-treated WW or UW. These reactors, which were randomly distributed, were left at room temperature (20 °C) and exposed to light from 36 W cool, white fluorescent tubes with a photon flux of $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ in a 12 h light/darkness cycle.

A mixture of six pharmaceuticals, two EDCs, and one stimulant was added to each reactor to obtain a final concentration of $100 \mu\text{g L}^{-1}$ (1 mL of spiking solution at 250 mg L^{-1} of each compound in methanol). Ten milligrams FW of algae and duckweeds were added to the corresponding containers. The experiments were run for 20 d. The fresh mass of the aquatic vegetation at the end of the experiment was about 20 g for *Lemna* sp. and 28 g for *Spirogyra* sp.

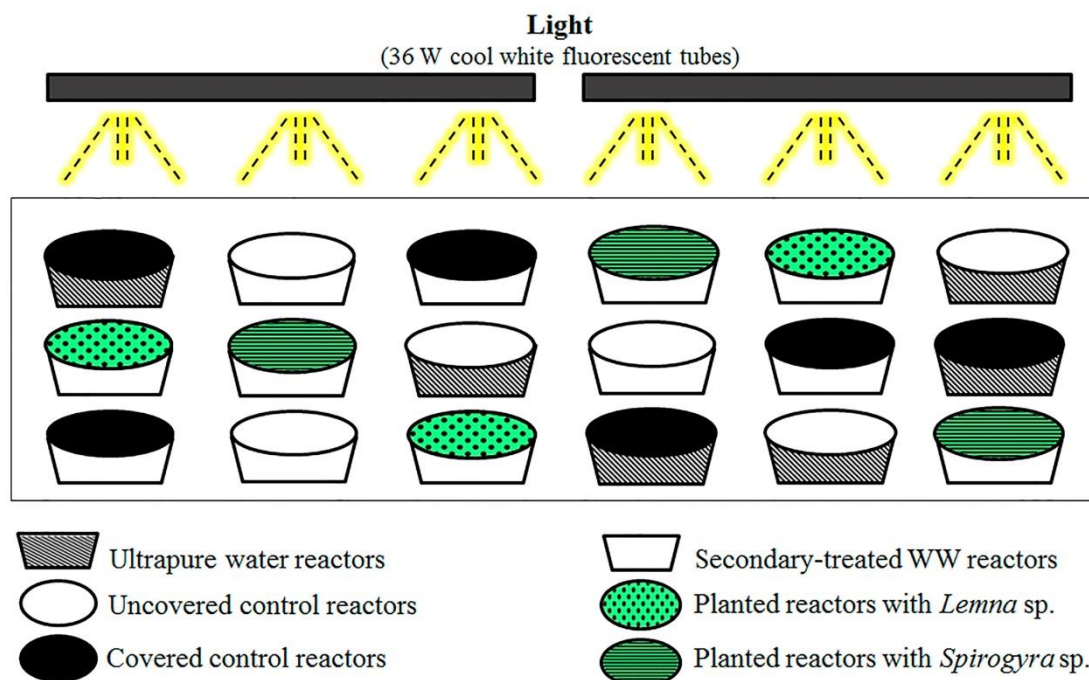


Figure 3.8. Scheme of the experimental setup.

Sampling strategy

Aqueous samples of 1 mL were taken regularly during the 20 d that the experiment lasted. All samples were collected in clean brown glass bottles, filtered with PTFE filters and frozen until analysis. In order to keep the same depth of water in the reactors and correct for any losses, the reactors were refilled with secondary-treated WW to a pre-set mark before each sampling.

Analytical Methodology

All water samples were filtered with 0.45 μm porous PTFE filters and settled in amber glass vials. To determine the pharmaceuticals and EDC concentration, samples were analyzed by an Agilent TM 1290 Infinity UPLC system (Palo Alto, CA, USA) coupled to a 6430 triple quadrupole mass spectrometer from Agilent Technologies (Palo Alto,

CA, USA). The LC column employed was a Kinetex C18 100A (50 mm x 2.10 mm x 2.6 μ m) preceded by a guard column.

Separation was achieved using a mobile phase composed of UW containing 0.1% v/v of formic acid (component A) and methanol (component B) for most of the compounds (DCF, CLF AC, IBP, ACAPh, CAFF, PROPR and CARB). For 17-ET and BPA, UW was used as component A. The separation was carried out applying the following elution profile: 20% B was kept constant during 2 min and then in 8 min. was increased to 85% B, gradient elution from 85% B to 100% B in 3 min, kept at 100% B for 5 min and returned to the initial conditions in 3 min. The flow rate was set at 0.4 mL min⁻¹ and the injection volume was of 10 μ L. MS data acquisition was performed with the ESI operating in both positive and negative ionization (PI and NI, respectively) mode under the time-scheduled MRM conditions listed in Table 3.5. Analytical quality parameters were studied (see Table 3.6). The repeatability in terms of the variation coefficient was always lower than 11%.

Table 3.5. Chromatographic parameters.

	Retention time (min)	MRM transitions (m/z)	Fragmentor voltage (V)	Collision energy (V)	Ionization mode
ACAPh	0.69	110 \rightarrow 65.1	135	20	PI
CAFF	1.32	195 \rightarrow 138	135	20	PI
PROPR	4.77	260 \rightarrow 183.1	135	15	PI
CARB	6.14	237 \rightarrow 194.1	135	20	PI
BPA	6.99	227 \rightarrow 212.1	135	15	NI
CLF AC	7.22	213 \rightarrow 127	80	10	NI
17-ET	7.85	295 \rightarrow 144.9	135	35	NI

Table 3.5 Chromatographic parameters (**continued**).

DCF	8.69	294 → 250	100	10	NI
IBP	8.81	205 → 161	60	0	NI

Table 3.6. Analytical quality parameters.

	Slope	Intercept	r²	LOD (µg /L)*	RSD (%)**
ACAPh	1086	5474	0.9978	9	2
CAFF	664	196	0.9998	5	5
PROPR	761	-3231	0.9999	4	4
CARB	4635	10916	0.9995	6	3
BPA	193	-362	0.9999	5	1
CLF.AC	319	-1095.95	0.9993	6	5
17-ET	31	-93	0.9999	5	5
DCF	76	-250	0.9983	6	6
IBP	8	-52	0.9971	7	11

* LOD = Limits of detection; **30 µg L⁻¹ (n=5)

Data analysis

Experimental results were analysed using SPSS V. 15 software (Chicago, IL, USA). The correlation coefficients of the concentration depletion of the polar microcontaminants as a function of time were calculated using parametric statistics (Pearson correlation coefficient). A principal component analysis (PCA) was conducted to treat the kinetic values obtained from the experimental data set. Once the data matrix was completed, it was autoscaled to have zero as the mean and unit variance (correlation matrix) in order to avoid problems arising from the different measurement scales and numerical ranges of the original variables. Varimax rotation was also used in the analysis.

CHAPTER 4

Results

CHAPTER 4

Section 1

Determination of pharmaceutical compounds in sewage sludge using a standard addition method approach

This section is based on the article:

Garcia-Rodríguez, A., Sagristà, E., Matamoros, V., Fontàs, C., Hidalgo, M., Salvadó, V., 2014. Determination of pharmaceutical compounds in sewage sludge using a standard addition method approach. *International Journal of Environmental Analytical Chemistry* 94, 1199-1209.

Overview

The design of WWTPs has yet to take into account the need to remove pharmaceuticals and so these plants can in fact contribute to the spread of these chemicals in the environment through effluent discharges and the disposal of sewage sludge. Recent studies have found treated sewage sludge to contain antibiotics and other pharmaceuticals at trace concentration levels and, given that the solid fraction generated in WWTPs is often used as a fertilizer, it is important to assess the environmental and health risk that this practice represents (Lillenberg et al., 2009). The EU has not established trigger values for pharmaceutical residues in biosolids, although the draft of a future EU directive recommends that treated sludge be disposed of in soils in such a way that health and environmental risks are minimised (EuropeanWaterAssociation, 2000). As a first step to deal with these concerns, it will be necessary to develop robust and sensitive analytical methodologies to monitor pharmaceutical concentration levels in biosolid and sewage samples before they are applied to soils. Several analytical methods consisting of the extraction of the pharmaceuticals from the solid matrix and the subsequent clean-up and pre-concentration of the obtained extracts, followed by liquid chromatography-mass spectrometry (LC-MS) analysis, have been developed to this end (Haller et al., 2002; Nieto et al., 2010; Sagristà et al., 2010; Pamreddy et al., 2013). The performance of the sample preparation, which normally accounts for 70–90% of the analysis time, significantly affects the reliability and quality of the data. Hence, laboratories routinely handling large amounts of samples are interested in the development of simple, robust and low cost methodologies.

Ultrasound assisted extraction (USE) (Shafrir and Avisar, 2012; Yu and Wu, 2012), microwave-assisted extraction (MAE) (Azzouz and Ballesteros, 2012) and pressurised liquid extraction (PLE) (Nieto et al., 2010; Pamreddy et al., 2013) have all been used in extracting pharmaceuticals from sludge. Although it might be expected that PLE and MAE would give better results than conventional methods (Soxhlet and USE), in terms of recoveries and repeatabilities, their efficiency is often heavily dependent on the characteristics of the matrices and analytes. In USE, the creation of microscopic bubbles in the liquid phase by high frequency acoustic waves in the liquid phase, known as the cavitation phenomena, increases the polarity of the extraction solution and, hence, improves the extraction efficiency (Luque García and Luque de Castro, 2002; Koel and Kaljurand, 2010). Therefore, USE allows the extraction of a wide variety of compounds

independently of their polarity. This technique can also be used in the extraction of thermolabile analytes as the temperature does not increase significantly (Luque García and Luque de Castro, 2002). Several methods based on USE followed by a clean-up step to eliminate organic matter and interfering compounds co-extracted with the analytes have recently been found to be highly efficient in extracting pharmaceuticals from biosolids (Martín et al., 2010; Shafirir and Avisar, 2012; Yu and Wu, 2012). The presence of matrix components in complex environmental samples, such as sludge extract, can interfere with the identification and quantification of the analytes by LC-MS and can produce signal suppression or enhancement when electrospray ionisation (ESI) is used (Hao et al., 2007). In this regard, several analytical approaches can be employed to reduce matrix interferences. The application of a thorough clean-up procedure removes a large part of the endogenous compounds present in samples although exhaustive sample clean-up is time-consuming and can lead to significant analyte loss.

Others strategies are serial dilution of the final extract, which minimises the amount of matrix components injected in the LC-MS, and the improvement of the analytical separation by using two dimensional LC or longer gradients (Gómez et al., 2006). Matrix effects can also be compensated by using isotope-labelled compounds (ILCs) as internal standards (Cappiello et al., 2008) and by applying a laborious but effective matrix-matched calibration method, which requires the availability of uncontaminated sample matrix, or a standard addition calibration method (Gomes et al., 2004; Quintana and Reemtsma, 2004; Poulsen and Bester, 2010). The main limitation of ILC is that it is difficult to find stable compounds for all the analytes and so compounds with similar molecular structures but whose ionisation behaviour can differ significantly from that of the analytes are used as internal standards. Additionally, costs may increase owing to the need to use more than one ILC in multi-compound analysis (Hao et al., 2007).

The main aim of the present study is to develop a simple and robust method without the need for a clean-up step and the use of ILCs based on USE, LC-MS/MS and standard addition calibration for the determination of non-steroidal anti-inflammatory drugs (NAP, DCF, and IBP), lipid regulators (CLF AC), and antibiotics (STZ, SMZ, SMX, and SPY) in biosolids. The pharmaceutical compounds, whose structures and physico-chemical characteristics (pKa and log Kow) are given in Table 1.5, were

selected on the basis of their concentrations and the high frequency with which they are detected in raw wastewaters and biosolids (Miège et al., 2009; McClellan and Halden, 2010).

Once developed and validated, the analytical methodology was applied to the analysis of biosolids collected at three Catalanian WWTPs.

Results and discussion

Evaluation of the extraction conditions

Three different methanol-water ratios (v/v) (1:1, 1:2, and 1:3) were evaluated as extraction solutions, given that they are reported as being highly efficient in extracting anti-inflammatory drugs and sulfonamides from biosolids (Martín et al., 2010; Nieto et al., 2010), to treat 200 mg of sludge samples spiked at 250 ng g^{-1} . In order to reduce the concentration levels of interfering compounds, we chose to use small amounts of sludge so requiring smaller volumes of organic solvents. Fig. 4.1.1 shows the recoveries corrected after analysing the pharmaceutical compounds in the sludge sample. The best results, recoveries ranging from 85 to 132% and repeatabilities between 6 and 15% (%RSD), were obtained with the solvent mixture containing the highest percentage of methanol (1:1) and without changing the solvent pH (pH = 6.5–7.5). This finding agrees with Ding et al. (2011), who found that the higher the ratio of methanol the greater the extraction of pharmaceuticals.

However, some other studies have reported that an acidic pH is required for quantitative recoveries of these compounds (Nieto et al., 2010; Zuloaga et al., 2012). Nevertheless, the recoveries obtained in this study are similar to those achieved in the extraction of sulfonamides and NAP, DCF and IBP from sludge samples using methanol or methanol: water mixtures without pH adjustment (Lindberg et al., 2005; Ternes et al., 2005; Okuda et al., 2009). The high recoveries were obtained as there is a reduction in the extraction of matrix interferences at neutral or basic pH which permit an improved chromatographic response (Haller et al., 2002).

A methanol-water mixture (1:1) was then selected as the extraction solution and we proceeded to study the appropriate solvent volume. Three consecutive extractions using 2 mL aliquots of the methanol-water mixture (1:1) were applied to the same sample. The first 2 mL extracted between 45% and 96% of the pharmaceuticals present in the spiked biosolids (Fig. 4.1.1). This efficiency could be improved by applying a second extraction as can be seen in Fig. 4.1.2.

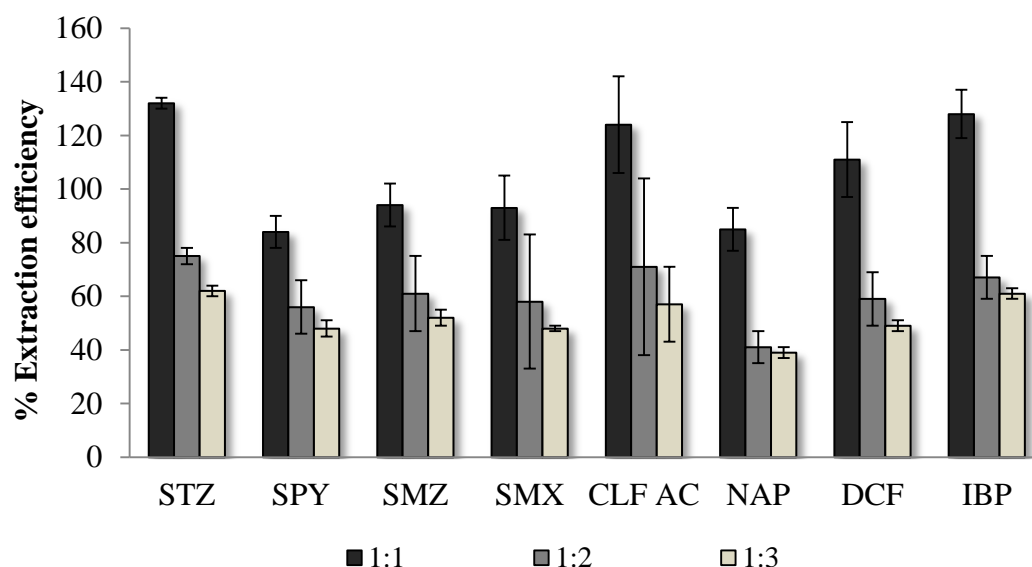


Figure 4.1.1. Extraction efficiency average and RSD (%) obtained for the three extraction solvent mixtures (methanol:water) evaluated (n=3).

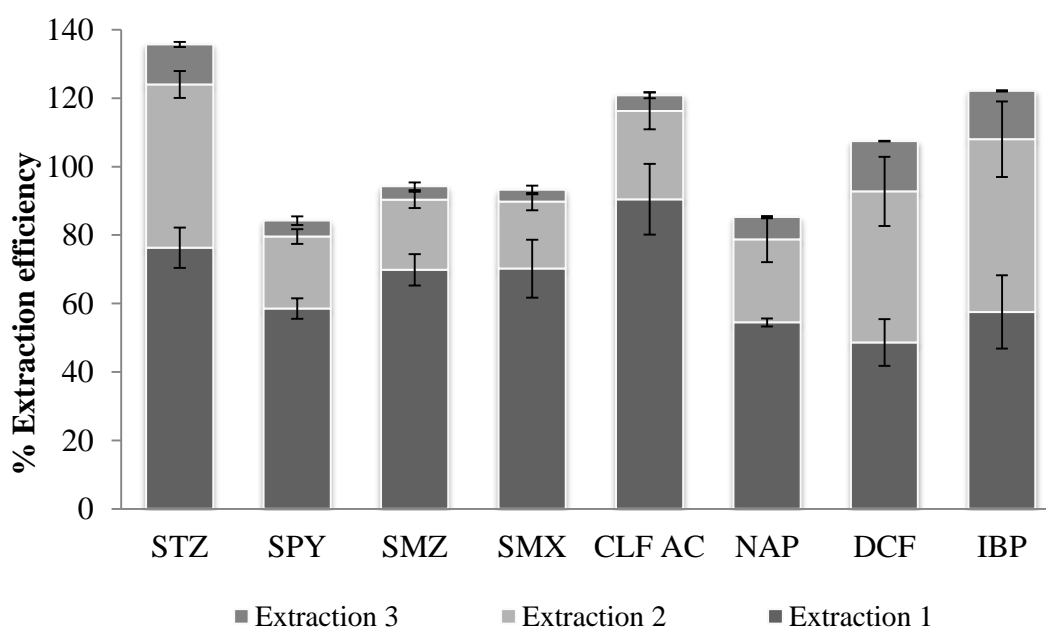


Figure 4.1.2. Influence of the solvent extraction mixture volume (when working with a 1:1 methanol-water ratio) in extraction efficiencies from biosolid samples.

However, as this would lead to the co-extraction of interfering organic matter, which would increase the signal suppression in the mass spectrometry analysis (Miao and Metcalfe, 2003; Hao et al., 2007), and good recoveries were obtained in the first extraction, we decided just to perform a single step. In order to develop a quick, easy and low cost extraction methodology, we decided not to purify the extract but rather to filter it and inject it directly into the LC-MS/MS instrument assuming that the concentration levels of interfering compounds are low. A standard addition calibration approach was applied to circumvent matrix effect problems in the quantification of pharmaceuticals in biosolids. Furthermore, taking into account the reliability and robustness of the LC-MS/MS analysis (%RSD < 10%, n = 5), no instrumental standard such as ILC was used.

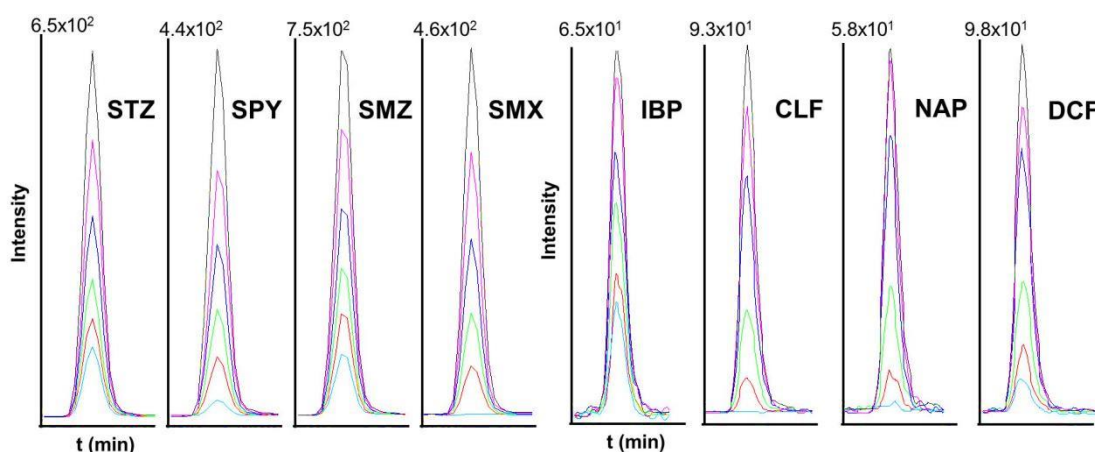


Figure 4.1.3. Chromatograms of the pharmaceuticals using standard addition calibration. Sample corresponding to biosolid sample from Blanes WWTP (October 2011). Colours illustrate the different amounts of pharmaceuticals spiked (from 0 ng in red to 250 ng in orange).

As can be seen in Fig. 4.1.3, chromatographic peaks for each compound were clearly defined and identified. In most cases the expected peak area relation between the six addition standard calibration points was obtained.

Method validation

Recoveries and precision of the method, based on the analyses of rabbit excrement samples spiked at 50 ng g⁻¹, 200 ng g⁻¹ (except for IBP, 500 ng g⁻¹) and 1000 ng g⁻¹

levels are shown in Table 4.1.1. Recoveries were calculated by comparing the concentration (ng g^{-1}) of each compound in the extracted samples with the initial concentration of compound added before the extraction procedure (above stated values for each compound). Recoveries are good for the three evaluated compound concentrations ranging from 76% (CLF AC) to 100% (SMZ) for the lowest concentration level, from 88% (NAP) to 122% (SMX) for the medium concentration level, and from 81% (NAP) to 131% (DCF) for the highest concentration level. The results obtained demonstrate the good performance of the method when dealing with high organic matter content samples with different compound concentrations. The SAs recoveries obtained are higher than those reported in extracting these compounds from animal manure by LLE (64–89%) (Haller et al., 2002), similar to those for the extraction of anti-inflammatory pharmaceuticals from sewage sludge by USE (Yu and Wu, 2012), and slightly higher than those achieved (68–87%) using PLE (Nieto et al., 2007a; Nieto et al., 2007b).

Table 4.1.1. Analytical calibration and quality parameters for the developed standard addition calibration method (n=6).

	Slope	Intercept	r ²	Recoveries (%)			MDL
				(RSD, %)			(ng/g dw)
				50 ng g ⁻¹	200 ng g ⁻¹	1000 ng g ⁻¹	
STZ	12.52	8.2	0.993	97 (15)	106 (15)	97 (11)	11
SPY	15.73	5.6	0.998	93 (10)	115 (21)	94 (5)	2
SMZ	26.41	12.4	0.999	100 (5)	101 (20)	92 (2)	2
SMX	10.9	5.6	0.996	93 (14)	122 (15)	99 (8)	7
CLF	27.12	18.3	0.999	76 (24)	89 (9)	121 (4)	12
NAP	6.24	4.05	0.999	-	88 (10)	81 (2)	12
DCF	16.17	15.15	0.997	96 (24)	91 (6)	131 (10)	8
IBP	10.58	23.9	0.998	114 (27)	93 (5)*	122 (3)	5

* 500 ng g^{-1} instead of 200 ng g^{-1}

The high recoveries obtained suggested that matrix effect during the LC-MS/MS analysis was not an issue due to the use of the standard addition calibration procedure. Method precision, expressed as the relative standard deviation of recoveries (%RSD, $n = 3$), ranged from 2% (SMZ and NAP at the highest concentration level) to 24% (CLF AC and DCF at the lowest concentration level). These RSD values are comparable to those (1–23%) obtained when USE was used to extract pharmaceuticals from sludge (Martín et al., 2010).

The linear response of the standard addition calibration curves covered an interval of two orders of magnitude ($25\text{--}1250\text{ ng g}^{-1}$) with determination coefficients (r^2) higher than 0.993. These results agree with the figures of merit of other methodologies used for the determination of pharmaceuticals ($0.5\text{--}1000\text{ ng g}^{-1}$ or $5\text{--}500\text{ ng g}^{-1}$) (Nieto et al., 2007a; Nieto et al., 2007b). With regards to MDLs, values ranging from 2 ng g^{-1} (SPY and SMZ) to 12 ng g^{-1} (CLF AC and NAP) dry weight (dw) were found. Those methodologies that are able to achieve MDLs lower than 2 ng g^{-1} (Díaz-Cruz et al., 2006; Vazquez-Roig et al., 2010) required further analytical steps (e.g. a PLE followed by a clean-up normally with SPE and the addition of ILCs as surrogate/internal standards). Furthermore, these methodologies need more time to perform and are not as simple and cost effective as the methodology developed here.

Application to the analysis of sludge samples

The methodology developed was applied to the monitoring of pharmaceuticals in sludge samples collected from different WWTPs located in the north-east of Spain. IBP, DCF and STZ were the pharmaceutical compounds that were most frequently detected (FOD > 90%) (Table 4.1.2). The concentration of pharmaceutical compounds in biosolids ranged from not detected to 1125 ng g^{-1} dw. These results are in agreement with reported concentrations (McClellan and Halden, 2010) of these compounds in biosolids. SMZ and IBP were the most abundant ($>200\text{ ng g}^{-1}$ dw on average), partially agreeing with earlier reports that found these compounds to be the most widely present in biosolid samples (Martín et al., 2010; McClellan and Halden, 2010; Ding et al., 2011) and raw wastewater (Miège et al., 2009). The abundance of pharmaceuticals in biosolids from Palamós WWTP was higher than in the other WWTPs. This may be explained by

the fact that Palamós WWTP digests sludge aerobically and anaerobically whereas Castell-Platja d'Aro WWTP only uses aerobic digestion.

These findings are in line with those of Conkle (2012) and Zwiener and Frimmel (2003) in confirming that aerobic conditions increase the efficiency of the removal of some pharmaceutical compounds. Finally, biosolids from Blanes WWTP presented the lowest concentration levels as they were sampled after composting. This sludge dewatering step involves aerobic conditions which, as mentioned above, enhance the degradation of pharmaceutical compounds although they still remain in dehydrated and composted urban sludge to a certain extent.

Table 4.1.2. FOD of selected pharmaceuticals in biosolids from three Catalanian WWTPs. Minimum and maximum concentrations are given for each compound in each WWTP. Average concentrations are given in brackets.

	FOD* (%)	Blanes WWTP (ng g⁻¹ dw)	Palamós WWTP (ng g⁻¹ dw)	Castell-Platja d'Aro WWTP (ng g⁻¹ dw)
STZ	8/12 (67)	2-10 (6)	<LOD-17 (9)	3-58 (18)
SPY	11/12 (92)	<LOD	<LOD-28 (12)	<LOD-225 (117)
SMZ	8/12 (67)	<LOD	53-249 (113)	35-620 (247)
SMX	4/12 (33)	<LOD-5 (4)	<LOD-15 (7)	2-85 (23)
CLF AC	5/12 (42)	<LOD-42(11)	<LOD-91 (35)	<LOD-28 (12)
NAP	7/12 (58)	<LOD-52 (13)	14-41 (23)	<LOD-22 (13)
DCF	12/12 (100)	9-140 (72)	26-86 (64)	45-113 (75)
IBP	11/12 (92)	50-473 (308)	164-1125 (479)	<LOD-108 (57)

Note: *Number of samples with concentrations greater than LOD/total number of samples.

From our research, a method based on USE and LC-MS/MS with standard addition calibration has been successfully developed for the determination of eight pharmaceuticals in biosolid samples from WWTPs. The application of the standard addition calibration can be considered to be reliable when plotted against conventional calibration methodologies based on ILC. The method yielded MDLs in the low ng g^{-1} range for most of the analytes; providing a reliable, simple-to-use and robust tool which may prove usefulness for routine analysis of pharmaceutical compounds in biosolid samples.

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CHAPTER 4

Section 2

*Innovative analytical technologies based on PIMs
for the determination of antibiotics*

This section is based on the articles:

Garcia-Rodríguez, A., Matamoros, V., Kolev, S.D., Fontàs, C., 2015. Development of a polymer inclusion membrane (PIM) for the preconcentration of antibiotics in environmental water samples. *Journal of Membrane Science* 492, 32-39.

Garcia-Rodríguez, A., Fontàs, C., Matamoros, V., Almeida, M.I.G.S., Catrall, R.W., Kolev, S.D., 2016. Monitoring of sulfamethoxazole in natural waters with a polymer inclusion membrane-based passive sampler. Minimizing the effect of the flow pattern of the aquatic system. *Microchemical Journal* 124, 175-180.

State of the art

Owing to the presence of EOCs in the environment, the interest on developing reliable sampling and analytical methods to monitor their presence is increasing.

Among the different options, such as adsorption or extraction processes, membrane systems bearing specific ligands or chemical groups can also be used to separate and concentrate these compounds.

Liquid membrane (LM) systems combine solvent extraction (SX) and membrane-based technologies, enabling extraction and back-extraction in one step and reducing the consumption of extractants and diluents. Hence, LM-based separation can be considered as an alternative to traditional SX.

LMs, also called chemically functionalized membranes (CFMs), are semi-permeable barriers that allow mass transfer of a target compound, usually between two liquid phases (i.e. source, S, and receiving, R, solutions/phases) that are separated by an immiscible LM.

Of the various types of LMs, the main ones are bulk liquid membranes (BLMs), emulsion liquid membranes (ELMs), supported liquid membranes (SLMs), and PIMs.

SLMs are composed of a porous hydrophobic membrane that acts as a solid support for an organic liquid phase. Thanks to capillary forces, the liquid phase is kept inside the membrane pores.

Studies based on the use of SLMs have been conducted to extract and subsequently determine antibiotics from aqueous samples, including SAs and TCs (Shariati et al., 2009; Msagati and Mamba, 2011; Yudtgavorasit et al., 2011). However, although they possess relatively high mass transfer, SLMs are prone to organic liquid phase loss, as it slowly leaches into the F and R aqueous solutions (Nghiem et al., 2006). This lack of long-term stability can have a negative impact on the system extraction performance and prevent the widespread adoption of SLMs in the industry.

In recent years, PIMs have emerged as promising alternatives to SLMs, as they retain most of the advantages of SLMs while exhibiting greater stability and versatility.

PIMs are commonly composed of an extractant (carrier), a base polymer (usually cellulose triacetate (CTA) or polyvinyl chloride (PVC)), and a plasticizer. Occasionally, the extractant also acts as plasticizer, so an additional one is not necessary. Moreover, to

enhance the solubility of the extracted species in the membrane liquid phase, a modifier is occasionally added to the PIM composition.

All the components are mixed and entrapped within the base polymer matrix. They are clutched inside the polymer matrix by a combination of physicochemical interactions (Pereira et al., 2009) and the process of polymer entanglement (Wool, 1993). Therefore, carrier losses to the aqueous solutions in contact with the membrane (i.e. F and R solutions) significantly diminish in comparison with SLMs. PIMs operate in a mode similar to SLMs, but the transport route through the membrane is still not well defined. Unlike SLMs, PIMs move relatively slowly through the membrane, because the extracted compounds must 'diffuse' through the polymer matrix. However, due to their mechanical stability, PIMs can be made into very thin films that somehow lessen the diffusive resistance effects (Nghiem et al., 2006).

PIMs usually appear as flexible, thin and stable films that are simple and cheap to prepare (Nghiem et al., 2006). Moreover, they possess good mechanical properties (e.g. strength and flexibility) and, unlike other separation processes, the technology required to use PIMs for the extraction of target compounds from aqueous solutions is fairly simple and could be easily adapted to a range of applications. Considering the diversity of target compounds that they can extract, these membranes are also versatile (Almeida et al., 2012).

So far, PIMs have exhibited potential to extract a number of metallic and non-metallic species (Nghiem et al., 2006; O'Rourke et al., 2009; Almeida et al., 2012). Target solutes for PIM separation have included a range of chemical species of metals/metalloids such as Au, Pb, Cd, Cu, Co, U, Zn, and As; non-metals such as halide, thiocyanate and nitrate anions; and small organic compounds such as thiourea, glyphosphate and 2,4-dichlorophenoxyacetic acid (2,4-D). However, little is known about the capacity of this type of membranes to extract antibiotics such as SAs and TCs. Research into PIMs progress, and the performance gap between PIMs and other liquid membranes, such as SLMs, is decreasing as their advantages are becoming more attractive.

PIMs composition

The components of the PIM (base polymer, carrier, and plasticizer and/or modifier) play an important role in its overall extraction efficiency and must be carefully chosen, as the membrane composition is usually tailored to a particular application.

As described below, each PIM component has a different function.

Polymers are structures formed by repetitions of basic units called monomers that form a long-chain polymer network of high molecular weight that provides the membrane with mechanical strength, while presenting as little diffusive resistance as possible (Güell, 2011). Ideally, the membrane should have enough strength to withstand simple mechanical stress without damage; fragile membranes are difficult to handle and can easily break.

The base polymer provides the membrane with mechanical strength. PVC and CTA have been the most widely used base polymers in PIMs. They provide membranes with high mechanical strength and are compatible with a large range of carriers, plasticizers, and modifiers.

CTA is a polar polymer with a number of hydroxyl and acetyl groups, capable of forming highly oriented hydrogen bonding, which gives CTA a crystalline structure. The polarity and crystalline nature of the CTA may make it incompatible with high concentrations of hydrophobic nonpolar carriers. Moreover, CTA can be slightly hydrated and, thus, susceptible to acid hydrolysis.

PVC is a non-flammable and durable polymer formed from a vinyl chloride monomer. The C-Cl functional group in PVC is relatively polar and nonspecific dispersion forces dominate the intermolecular interactions. Hence, PVC has an amorphous structure with a small degree of crystallinity. It is used as the polymer backbone in membranes because of its strength, inertness, and compatibility with a variety of carriers and plasticizers. Unlike CTA, PVC is resistant to acid solutions since it is not prone to acid hydrolysis.

Moreover, the polymer must entrap the carrier, and minimize any loss of it to the surrounding solutions. It should also be relatively inert to any chemical species that can come into contact with it (e.g. acids and bases).

Polymers possess different properties and should be selected considering the membrane

applications and the need for chemical, mechanical, and thermal stability and flexibility, among others. Factors such as polymer chain flexibility, molecular weight, chain interactions, and chemical and thermal stability of the polymer highly influence the final PIM properties (Güell, 2011). Although the number of polymers is vast, the literature on PIMs is mainly focused on two: CTA and PVC, although polyvinylidene fluoride (PVDF) is also studied. Both CTA and PVC (see Table 4.2.1) are relatively cheap polymers, combine well with active PIM components and are moderately inert under a wide range of aqueous conditions. In this work both have been used.

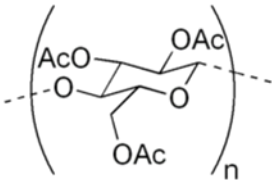
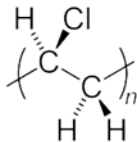
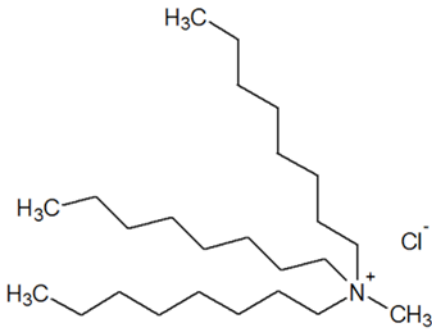
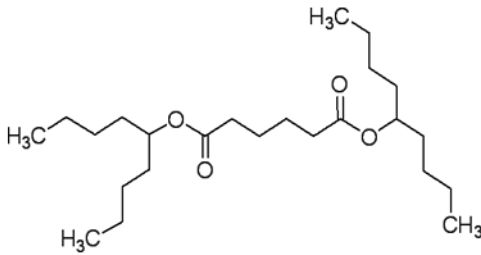
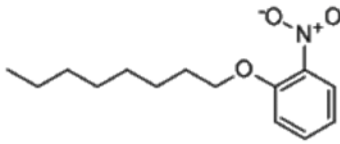
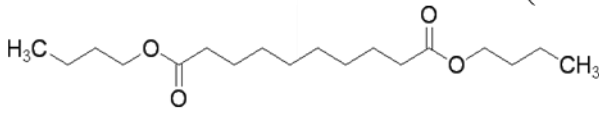
The *carrier* has the central role in the PIM extraction process, as its action is the most important factor in the performance of any PIM. It reactively complexes with the compound of interest, acting as the phase-transfer agent and thus extracting the compound from the aqueous phase into the membrane phase by forming a hydrophobic ion-pair or a complex, which afterwards diffuses within the membrane.

The carrier is usually a complexing agent or an ion-exchanger responsible for binding the species of interest and thus facilitating their extraction into the PIM. Hence the chemical and physical behaviour of the carrier is of great importance in the separation process and usually determines the suitability of a particular PIM for a given application. Carriers are frequently synthesized to specifically extract a compound or group of compounds, minimizing co-extraction of the non-target ones. Preferably, carriers have low water solubility and react quickly with the compound of interest. In addition, this important component of the PIM should be relatively inert to other species in the extraction system, although this is fully achieved in practice only rarely. A wide range of carriers have been incorporated into PIMs. They are often grouped by their mode of extraction: basic, acidic, and chelating, neutral or solvating, and macrocyclic and macromolecular.

Basic carriers are those whose structure is generally designed around a basic nitrogen centre. Some examples include the extensively used trioctylamine and quaternary ammonium salts, together with their corresponding commercial preparations: Alamine 336 and Aliquat 336, respectively, with the latter the one being used in our studies (see Table 4.2.1). It is a commercially available extractant, with plasticizing properties, extensively applied as a carrier in PIMs.

Transport by basic carriers rely on the formation of a complex or ion-pair with the aqueous target anionic compound and extracts it into the membrane, most frequently by an ion-exchange mechanism, as it will be shown over this section.

Table 4.2.1. Chemical structures of PIMs' components.

PIM components	
Polymers	
	
Cellulose triacetate (CTA)	Polyvinyl chloride (PVC)
Carrier	
	
Aliquat 336	
Plasticizers	
	
Bis(1-butylpentyl) adipate (BBPA)	2-Nitrophenil octyl ether (NPOE)
	
Dibutyl sebacate (DBS)	

Plasticizers often play an essential role in the extractive efficiency of a PIM. They are used to improve the PIM's flexibility and to provide better separation between polymer chains inside the membrane structure. The polymer chains of the base polymer are under the influence of weak and nonspecific van der Waals forces and stronger polar interactions resulting in the formation of a rigid membrane structure, which produces very poor diffusive fluxes for species introduced into the polymer. Hence, a plasticizer may have to be added to the PIM composition to penetrate between the polymer chains and reduce the intermolecular forces. Subsequently, compound diffusion through the membrane is easier thanks to a reduction of steric constriction.

Examples include 2-nitrophenyl octyl ether (NPOE), dibutyl sebacate (DBS) and bis(1-butylpentyl) adipate (BBPA) (Table 4.2.1), which have been used in this thesis. These plasticizers contain a hydrophobic alkyl backbone and one or more polar groups that neutralize the polar groups of the polymer. A balance between the nonpolar and polar groups is necessary. A longer alkyl chain results in higher hydrophobicity and viscosity and lower polarity of the plasticizer. On the other hand, an increase in the number of polar groups usually decreases viscosity and increases the hydrophilicity, thus leading to loss of the plasticizer to the aqueous phase(s).

Higher initial flux values for the extracted species are usually obtained when PIMs incorporate high-polarity and low viscosity plasticizers, such as NPOE. This has led to the conclusion that the initial flux values increase when increasing dielectric constant and decreasing viscosity of the plasticizer. However, most of the plasticizers used in PIMs have similar viscosity values, and moreover, the dielectric constant of the membrane liquid phase is also dependent in the dielectric constants of the carrier and the base polymer.

The compatibility of the PIM components relies on two principal factors: the successful entanglement of the polymer chains and the physicochemical interactions between the membrane components. The full range of intermolecular forces contributes to the stability of a PIM. If intermolecular interactions between components are attractive on average, the membrane components will remain miscible and the resulting PIM will be homogeneous and stable. Otherwise, if these interactions are repulsive on average, the resulting PIM will not be properly formed or will suffer from poor stability.

PIM preparation

The simplicity of PIM fabrication makes it particularly attractive for developing low-cost separation/analytical technologies.

In order to successfully cast a PIM, a good miscibility between the membrane components is imperative. Poorly miscible combinations will phase-separate upon drying, resulting in a brittle and inhomogeneous membrane, that will consequently contribute to the poor extractive performance of the PIM-based system. PIM components may only be miscible in specific or limited formulations, or exclusively in the presence of particular plasticizers or modifiers. If the PIM composition has been well designed, the resulting film should be mechanically stable, hydrophobic and safe to handle.

Transport mechanism

The PIM mass transfer process is usually referred to as facilitated transport and it allows transport of the extracted species from a S solution with a lower concentration to a R solution with a higher concentration. Thus, it is possible to transfer quantitatively the extracted species.

The overall transport mechanism across a typical PIM consists of the following three steps:

1. The extracted species diffuses across the stagnant diffusion layer at the membrane/S solution interface where it reacts with the carrier, and the reacted carrier species are replaced by another carrier species from the bulk of the membrane.
2. The product of the reaction between the extracted species and the carrier, which is usually a complex or an ion-pair, is transported through the membrane/R phase interface along the corresponding concentration gradient.
3. At the membrane/R phase interface, the extracted species are released into the R solution, which can be facilitated by a suitable R solution reagent. The carrier species liberated in this process are transported back to the membrane/S solution interface.

4.2.1 Development of a polymer inclusion membrane for the preconcentration of antibiotics in environmental water samples

Overview

The presence of antibiotics in the environment is of concern because there is an increasing body of evidence about their adverse impact on the ecosystem and in particular their ability to induce bacterial resistance. The adverse impact caused by the presence of antibiotics on ecosystems (Hylton et al., 2009; Dinh et al., 2011; Gao et al., 2012; Garcia-Rodríguez et al., 2014; Richardson and Ternes, 2014) and the concerns about their adverse influence on the biodegradation of plant tissues, the products of which are the main food source for aquatic life in freshwater systems (e.g. rivers and streams) (Richardson and Ternes, 2014), has led to the development of reliable sampling and analytical methods capable of determining the concentrations of these compounds in water samples. As previously stated, CFMs can be viewed as an attractive tool for separation and preconcentration processes.

Among the different types of CFMs, SLMs have recently acquired significant importance for the extraction and preconcentration of organic pollutants from water samples (Msagati and Mamba, 2011). As an example, three-phase hollow fiber liquid phase microextraction (HF-LPME), a class of SLM-based techniques, has been successfully applied for the determination of antibiotics in water samples (Shariati et al., 2009; Yudtgavorasit et al., 2011).

PIMs have emerged in recent years as an attractive alternative to SLMs due to their better stability (Nghiem et al., 2006; Fontàs et al., 2007).

However, only few studies deal with the use of PIMs for the extraction and transport of organic compounds. For example, saccharide transport has been studied through CTA-based PIMs by Riggs et al. (Riggs and Smith, 1997) and White et al. (2001). Also, this type of membranes has been used for the separation of lactic acid (Matsumoto et al., 1998). A more complex organic molecule, OTC, has also been successfully transported from milk samples using a PIM consisting of cellulose acetate as the base polymer,

trialkylphosphine oxide (Cyanex 923) as the carrier, and NPOE as the plasticizer. The R solution in this case contained HCl. (Pérez-Silva et al., 2012),

In the present study we have developed different PIMs for the extraction and transport of six organic compounds from aqueous samples. These compounds belong to two widely used antibiotic families: SAs (STZ, SPY, SMZ, and SMX) and TCs (TC and OTC). The chemistry of these compounds in environmental waters is very complex and pH-dependent. For example, TCs tend to form chelation complexes with multivalent cations, normally found in natural waters. Thus, the development of a specific PIM able to simultaneously transport and preconcentrate the 6 antibiotics mentioned above is a challenge. The present study has been focused on identifying a PIM composition to be incorporated in a device capable of antibiotics preconcentration and is based on preliminary results reported by us at Euromembrane 2012 conference (Garcia et al., 2012). The performance of the proposed device has been assessed using both synthetic and real aqueous samples, and the possibility of using it as a passive sampler has also been explored.

Results and discussion

Antibiotics extraction into Aliquat 336 containing PIMs

The interaction of both SAs and TCs with any ligand used as carrier is strongly pH-dependant. In the case of TCs, the overall charge of the molecules varies between +1 and -2 depending on solution pH (Anderson et al., 2005). The SAs, molecules become neutral at their isoelectric points at pH values between pK_{a1} and pK_{a2} , whereas the overall charge is +1 at $pH < pK_{a1}$ or -1 at $pH > pK_{a2}$. Thus, if the pH is high enough, all SAs and TCs will be present as anions. Then, it is expected that they will be able to interact with anion-exchangers such as Aliquat 336, which is a mixture of quaternary alkylammonium chlorides. This carrier exhibits good compatibility with both CTA and PVC, and due to its plasticizing properties, it has been used successfully without additional plasticizers to form successful PIMs (Pereira et al., 2009; Kagaya et al., 2012; Vázquez et al., 2014).

The transport of an antibiotic anion (Ant^-) from a S solution with basic pH across an Aliquat 336 (Al^+Cl^-) containing PIM into a R solution containing excess of Cl^- ions (NaCl) is illustrated in Fig. 4.2.1. The Ant^- forms an ion pair (Al^+Ant^-) with the carrier cation (Al^+) which diffuses towards the PIM/R solution interface where the antibiotic anion is back-extracted into the R solution by replacing it with a chloride anion. Thus, the original carrier ion-pair (Al^+Cl^-) is restored and diffuses towards the PIM/S solution interface to transport another antibiotic anion. This is a typical example of facilitated transport which is driven by the relatively high concentration of the R solution reagent (NaCl) and it allows the transport of the target chemical species (Ant^-) from a solution of lower concentration to a solution of higher concentration (Almeida et al., 2012). In this particular case the protonation of the antibiotic anion can further enhance the rate of the antibiotic transport from the S solution to the R solution.

To ensure that all antibiotics studied are present in their anionic forms in the S solution, all experiments were conducted at $\text{pH}=9$, except those where pH effects were studied.

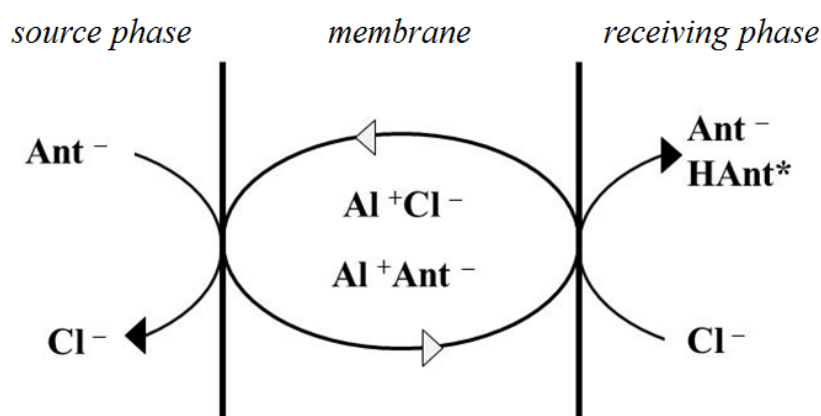


Figure 4.2.1. Facilitated transport mechanism of an Ant^- through a membrane containing Aliquat 336 (Al^+Cl^-) as carrier. HAnt^* corresponds to the protonated form of the antibiotic, which will depend on its acid-base properties and on the pH of the R phase.

Optimization of the PIM composition

The components of a PIM (base polymer, carrier and plasticizer) determine its extraction and transport performance. Even though the carrier determines the main extraction and transport characteristics of a PIM, it is also important to consider the nature of the other membrane components, i.e. base polymer and plasticizer/modifier,

since they can also affect significantly the chemical and physical characteristics of PIMs (Pereira et al., 2009; Vázquez et al., 2014). In this work we have tested both PVC and CTA as base polymers since in our preliminary study a significant difference was observed between PIM based on these polymers (Garcia et al., 2012). To better evaluate the effect of the membrane composition we have also investigated the effect of the carrier concentration in the PIM as well as the addition of a plasticizer.

The influence of the polymer was tested by preparing PIMs containing similar Aliquat 336 concentrations and CTA or PVC as the base polymer. CTA is a polar polymer with crystalline structure while PVC is less polar polymer than CTA and has a predominantly amorphous structure (Nghiem et al., 2006). Both provide membranes with a high mechanical strength. Table 4.2.2 shows the antibiotics TE after 24 h. As can be observed, when PIMs contained 25-30% Aliquat 336 a similar trend in the transport of antibiotics was observed, and it was independent of the base polymer. Higher TE values were obtained for STZ and SMX, whereas all other antibiotics were not transported in the case of PVC-based PIMs or were poorly transported across CTA-based PIMs. In order to check the effect of the carrier concentration in the membrane on the antibiotic TE, a PIM consisting of 55% CTA and 45% Aliquat 336 was tested. Although this PIM showed better TE, quantitative transport was not achieved. Thus, in order to improve these results other membrane compositions were studied.

Table 4.2.2. Antibiotic transport efficiencies achieved over a 24 h period with the use of different membrane compositions. (S solution 5 mg L⁻¹ of antibiotics at pH 9, R solution = 1 mol L⁻¹ NaCl).

Membrane components			Transport efficiency (%) (24 h)					
Polymer	Carrier	Plasticizer	STZ	SPY	SMZ	SMX	TC	OTC
CTA (75%)	Aliquat 336 (25%)	-	30	8	6	35	2	1
PVC (61%)	Aliquat 336 (31%)	-	36	-	-	33	-	-
CTA (55%)	Aliquat 336 (45%)	-	54	36	49	84	40	43
CTA (30%)	Aliquat 336 (26%)	NPOE (44%)	74	55	79	91	100	100
PVC (30%)	Aliquat 336 (26%)	NPOE (44%)	53	23	45	71	50	47

The addition of a plasticizer increases the fluidity/plasticity of a PIM and can improve its permeability due to the plasticization effect. This effect turns the PIM into a less viscous medium thus facilitating mass transport within it (Kebiche-Senhadji et al.,

2010), which often leads to substantial improvement in the membrane TE. Therefore, the addition of NPOE as a plasticizer was tested to check its effect on the transport of SAs and TCs. CTA and PVC-based PIMs were prepared with a composition consisting of 30% polymer, 26% Aliquat 336 and 44% NPOE. Table 4.2.2 shows that the antibiotic transport efficiencies were increased to 55-100% in the presence of NPOE in the CTA-based PIM, which allowed the quantitative transport of the two TCs and an almost quantitative transport of SMX. Transport efficiencies ranging from 55 to 79% were obtained for the remaining three antibiotics. However, the PVC-based PIM showed much lower transport efficiencies for TCs (about 50%) and SAs (23% to 71%). Thus, CTA-based membranes were investigated in the subsequent experiments.

Fig. 4.2.2 (pH=9) shows the transient concentration profiles for each of the six antibiotics obtained with the CTA-based PIM containing NPOE (Table 4.2.2). Different behaviour can be observed depending on the physicochemical characteristics of the antibiotics. STZ (with a thiazole functional group) shows a high extraction rate within the first 2 h, but the compound is only detected in the R phase after 3 h. This means that there is accumulation of the compound in the PIM, but this problem is overcome over time, since after 24 h 74% of STZ is transported into the R phase. In the case of SPY, the transport is very slow, which is in accordance with its high pK_{a2} value (8.4), that corresponds to the formation of 50-60% of the anionic form of this compound at the working pH. The transient concentration profiles of the two TCs and SMZ are quite similar, resulting in 50% transport after 5 h, and a quantitative transport after 24 h. The transport of SMX, which is the most acidic antibiotic in this study, exhibited the highest efficiency, since in 5 h almost 80% of the SMX is transported.

In our preliminary study (Garcia et al., 2012) we observed that the plasticizer used to form the PIM affected the transport of antibiotics, and that NPOE (viscosity, $\eta = 12.8 \times 10^{-3}$ Pa s (cP); dielectric constant, $\epsilon_r = 23.1$), and DBS ($\eta = 9.5 \times 10^{-3}$ Pa s (cP); $\epsilon_r = 4.5$) provided better transport efficiencies than BBPA ($\eta = 14 \times 10^{-3}$ Pa s (cP); $\epsilon_r = 4$). The fact that the PIMs containing BBPA exhibited the lowest TE can be explained by the highest viscosity of BBPA in comparison with those of the other two plasticizers. In accordance with research on PIMs conducted earlier by us (Fontàs et al., 2007), the rate of transport of compounds across PIMs decreases when high viscosity plasticizers are used, most likely due to lowering the membrane diffusion coefficients. On the other hand the dielectric constant of the plasticizer was found to affect substantially

membrane transport characteristics (Nghiem et al., 2006; Güell et al., 2011). Taking into account these results, we decided to investigate if it were possible to manipulate both viscosity and dielectric constant of the PIMs studied by incorporating more than one plasticizer in a PIM. Therefore, the combination of NPOE (for its high dielectric constant) and DBS (for its low viscosity) was used in the preparation of an Aliquat 336/CTA PIM containing 22% of each plasticizer. Table 4.2.3 compares the antibiotics' initial transport flux values (at $t = 5$ h) for PIMs containing a single plasticizer and PIMs containing both NPOE and DBS. Similar transport flux values were obtained for PIMs containing NPOE or DBS. However, the combination of NPOE and DBS led to deterioration in the PIM transport characteristics compared to PIMs containing only either NPOE or DBS. In the case of OTC the flux decreased by about 80% when using a PIM containing both NPOE and DBS. These results illustrate the complex character of the interactions between all PIM components (CTA, Aliquat 336 and plasticizers) which makes it difficult to predict what the combined effect of several components on the membrane transport characteristics will be. It is worth to note that the flux values of PIMs with NPOE or DBS are similar to those reported for metals such as Pt(IV) and Cd(II) when transported across PIMs containing the same components (Fontàs et al., 2007; Pont et al., 2008).

Since PIMs containing NPOE as a plasticizer showed the highest initial transport flux values, PIMs composed of 30% CTA, 26% Aliquat 336 and 44% NPOE were used in subsequent experiments.

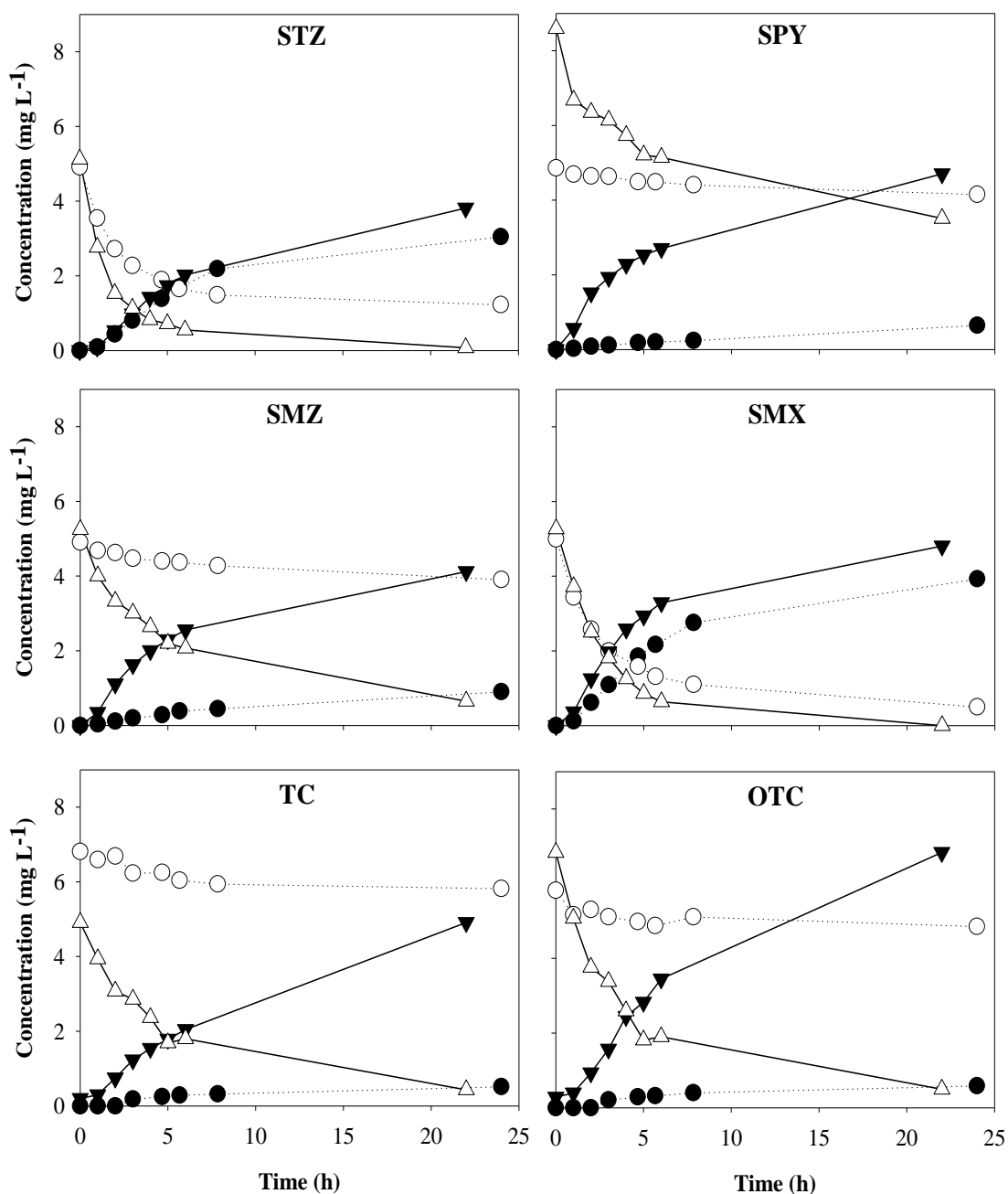


Figure 4.2.2. Comparison of the transient concentration profiles of antibiotics in transport experiments carried out with S solutions (5-10 mg L⁻¹ of antibiotics) at pH 7 (O) or 9 (Δ) and using a 1 mol L⁻¹ NaCl solution as the R phase (R solution corresponding to ● S pH 7 experiments, ▼ S pH 9 experiments). A PIM composed of 30% CTA, 26% Aliquat 336, and 44% NPOE was used.

Table 4.2.3. Influence of the plasticizer on the initial flux ($t = 5$ h) of antibiotics across a PIM composed of 30% CTA, 26% Aliquat 336 and 44% plasticizer. (S solution: 5 mg L^{-1} of antibiotics at pH 9, R solution: 1 mol L^{-1} NaCl).

Plasticizer	Antibiotics flux ($\times 10^{-7} \text{ mol m}^{-2} \text{ s}^{-1}$)			
	NPOE	DBS	BBPA	NPOE + DBS
STZ	7.23	5.92	5.75	4.83
SPY	4.98	4.09	1.73	1.73
SMZ	5.37	4.00	1.54	2.98
SMX	9.58	8.58	7.96	6.97
TC	4.08	4.54	0.79	1.20
OTC	7.13	7.47	1.80	1.47

Effect of the pH of the source phase

Even though it is necessary to work at basic pH to ensure an effective transport using Aliquat 336 as carrier, the TE of the CTA-based PIM discussed above was also tested with S solutions at a neutral pH. This was done with the aim of exploring possible future applications of the designed membrane when dealing with natural waters without the need of changing their pH, for example the incorporation of a PIM in a passive sampler. Fig. 4.2.2 shows the transient concentration profiles of the antibiotics in a S solution at pH 7. As can be noticed, TCs were not transported at this pH, whereas the TE for STZ, SPY and SMZ dramatically dropped in accordance with their acidity constants. It is worth noticing the good TE showed by SMX ($\text{pK}_{\text{a}1}=1.9$ and $\text{pK}_{\text{a}2}=5.6$), for which similar results were obtained for both neutral (pH 7) and basic (pH 9) S solutions. Taking into consideration that SMX is an extensively used sulfonamide, commonly detected in streams and groundwater (Chen et al., 2011), and in view of the good results obtained at neutral pH, the proposed PIM can be viewed as a suitable semi-permeable barrier to be used in a passive sampler for this antibiotic. Future studies will be conducted to test this possibility.

Preconcentration studies

As stated in the introduction, SAs and TCs are present at very low concentrations in environmental waters and, for that reason, prior to their determination it is usually mandatory to conduct a preconcentration step. Therefore, we have evaluated the possibility of using the proposed PIM in a specially designed device for the preconcentration of the six antibiotics studied prior to their determination by HPLC.

Thus, an experiment was conducted using the device shown in Fig. 3.3 which incorporated the CTA-based PIM, studied in the transport experiments outlined above.

The membrane separated 4 mL of 1 mol L⁻¹ NaCl R solution from 120 mL of a S solution at pH 9 which contained 100 µg L⁻¹ of each of the antibiotics studied.

Only the S solution was stirred, and after 24 h the R solution was analysed by HPLC. The results obtained are shown in Fig. 4.2.3 as initial concentrations of antibiotics in the feed solution and their final concentrations in the R phase. It can be seen that the device successfully preconcentrated all six antibiotics studied and in particular SMX. The good performance of the device incorporating the PIM prompted further studies on the effect of its parameters.

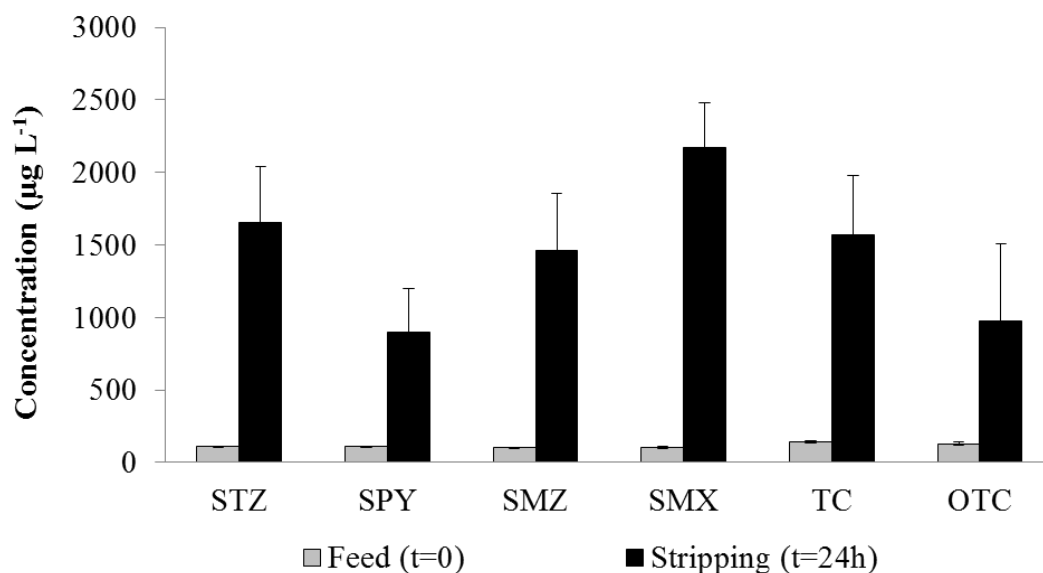


Figure 4.2.3. Initial concentrations (100 µg L⁻¹) of antibiotics studied in the S solution (pH 9) and their final concentrations in the receiving solution (1 mol L⁻¹ NaCl) after 24 h. PIM was composed of 30% CTA, 26% Aliquat 336, and 44% NPOE. Error bars are ± standard deviation (n = 4).

Influence of the composition of the receiving solution

Different NaCl solutions (0.1, 0.5 and 1.0 mol L⁻¹) as well as an acetic acid solution at pH 4 were tested as R solutions for the preconcentration of SAs and TCs. At this pH value all antibiotics are present predominantly in their molecular form. These experiments were aimed at determining the effect of acidity and the possibility to work with a less saline R solution, which is more compatible with the subsequent HPLC analysis. As shown in Fig. 4.2.4, the use of an acetic acid solution resulted in poorer TE. Moreover, among the different NaCl concentrations tested, similar results were obtained for SAs, but not for TCs, which were better transported when the R solution contained 0.1 or 0.5 mol L⁻¹ NaCl. Therefore, on the basis of these results, and to facilitate the sample analysis, 0.5 mol L⁻¹ NaCl solution was selected as the R solution in the subsequent studies.

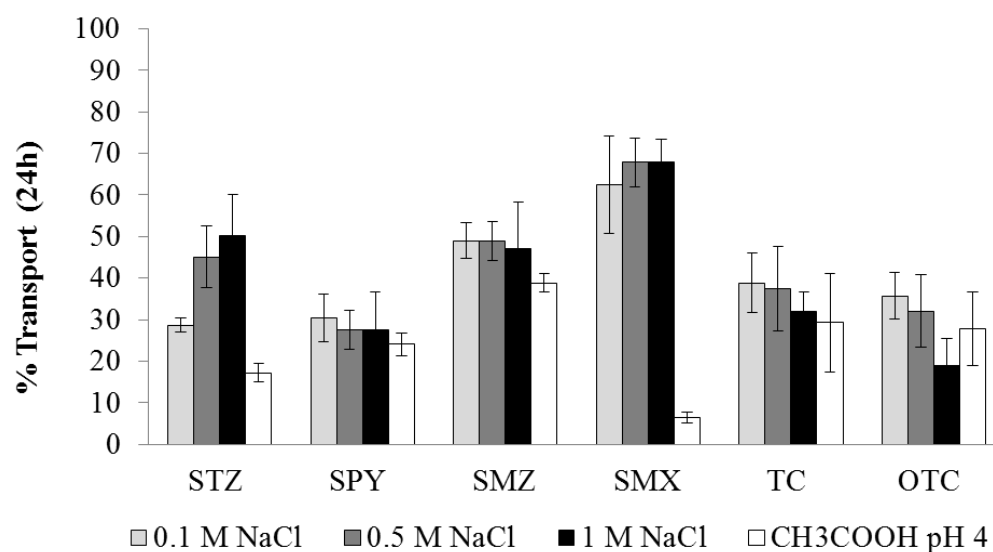


Figure 4.2.4. Evaluation of different receiving solutions for the transport of the 6 antibiotics studied in 24 h. The membrane was composed of 30% CTA, 26% Aliquat 336, and 44% NPOE. The S solution was at pH 9 and contained 100 µg L⁻¹ of each antibiotic. Error bars are \pm standard deviation ($n = 4$).

Influence of transport time

Fig. 4.2.5 shows the dependence of the antibiotics transport efficiencies over 3 different periods of transport time (5, 24, and 48 h). As expected, after 5 h of transport lower transport efficiencies were obtained, whereas much better transport efficiencies were achieved after 24 h of transport. A further 24 h of transport improved more significantly the TE for only SMZ, SMX, and TC. Therefore, a transport time of 48 h was selected. There are many other factors that can improve the TE without the need of using long transport times. One such factor is temperature which when increased accelerates diffusion through PIMs (Fontàs et al., 2005; Zhang et al., 2012). A larger contact area of the PIM would also allow improve the rate of transport.

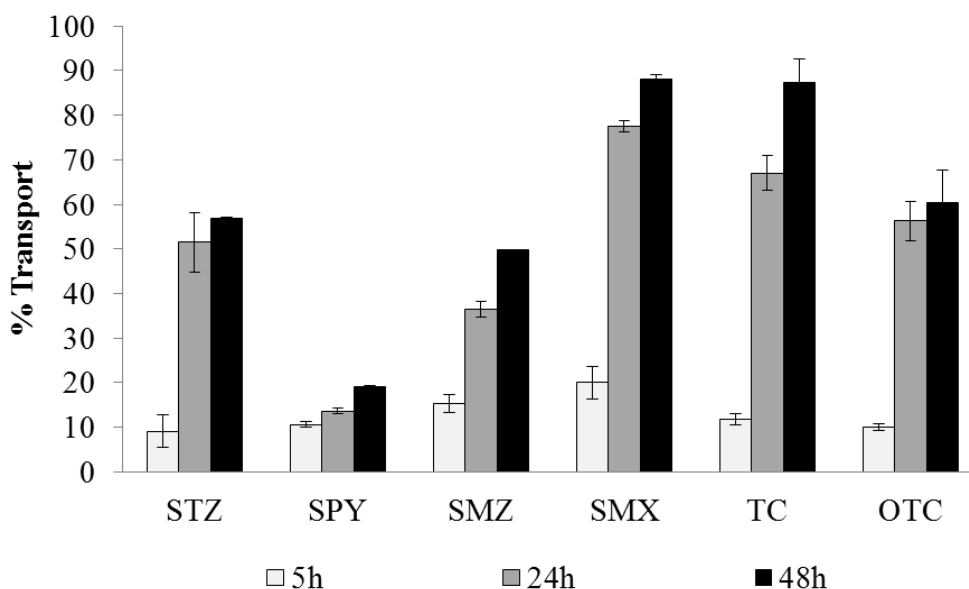


Figure 4.2.5. Influence of the duration of transport on the TE of the antibiotics studied (n=3). The S solutions contained $100 \mu\text{g L}^{-1}$ of the individual antibiotics studied at pH 9. The receiving solution contained 0.5 mol L^{-1} NaCl. The PIM was composed of 30% CTA, 26% Aliquat 336, and 44% NPOE.

Environmental application

The proposed PIM-based device for preconcentration of antibiotics was applied to environmental water samples (well and river water), spiked with a known concentration of antibiotics ($100 \mu\text{g L}^{-1}$).

Table 3.2 shows that the anionic compositions of the well and river waters differed significantly. It could be expected that the sample matrix and especially its anions would affect the transport of both SAs and TCs because of competition in the extraction process.

Table 4.2.4 summarises the PFs obtained for each antibiotic after 48 h of contact, for both well and river water samples. As it can be seen, similar PFs values were obtained for the antibiotics in the two types of water samples, which indicated that the difference in these sample matrices did not significantly affect the PIM transport. This result suggests that the extraction constants of the antibiotics studied are substantially higher than those of the anionic species present in the water samples (Table 3.2), making the proposed PIM-based device suitable for antibiotics preconcentration. The preconcentration achieved using the PIM-based device facilitated the determination of the six compounds by HPLC (Fig. 4.2.6).

Table 4.2.4. Evaluation of the sample matrix influence on the PIM PFs for the antibiotics studied in 48 h (n=3).

	Preconcentration factor (48h)					
	STZ	SPY	SMZ	SMX	TC	OTC
Well water (St. Hilari well)	11	18	21	21	11	11
River water (Ter river)	14	21	22	23	12	10

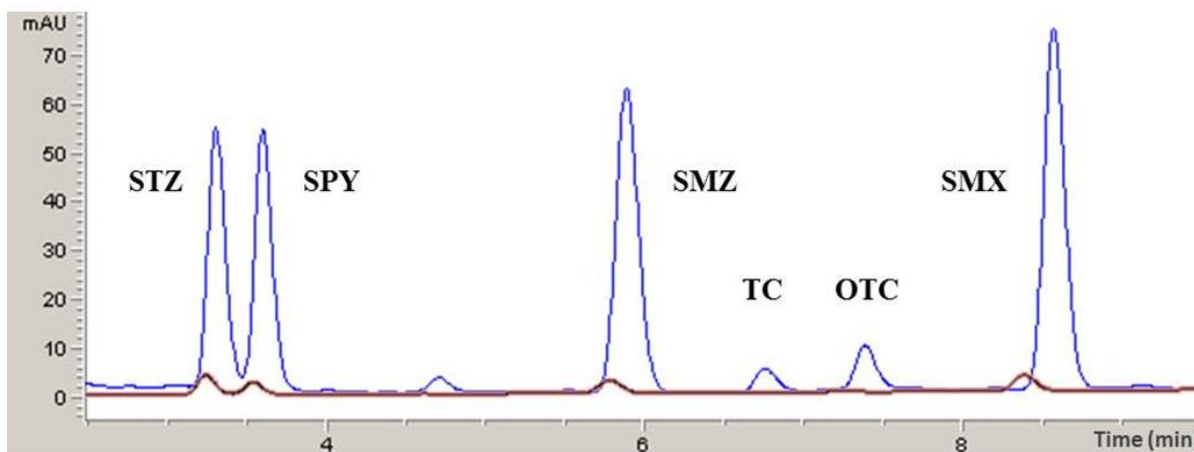


Figure 4.2.6. Chromatograms corresponding to a river water sample spiked with $100 \mu\text{g L}^{-1}$ of the antibiotics studied (original sample (red line) and receiving solution after a 48 h preconcentration step (blue line)).

From our work it could be extracted that a PIM suitable for transporting six organic compounds belonging to two widely used families of antibiotics, SAs and TCs, has been developed and its composition optimized for achieving maximum TE. The optimal composition includes 30% CTA, 26% Aliquat 336, and 44% NPOE.

A membrane-based separation device, incorporating this PIM, has been constructed for the simultaneous preconcentration of the six antibiotics mentioned above.

As expected, the TE of the PIM-device was found to be strongly dependent on sample pH. While high transport efficiencies for all 6 antibiotics studied were observed at pH 9, when these compounds were present predominantly in their anionic forms, substantially lower transport efficiencies (except for SMX) were obtained at pH 7. Despite this fact, a PIM-based preconcentration device was successfully applied to substantially increase the sensitivity (i.e. from 10 to 70 times) of the chromatographic determination of the six antibiotics studied in spiked river and well water samples of different compositions after a 48 h preconcentration step. These results demonstrate the suitability of the newly developed PIM-based device for environmental analysis of antibiotics and the potential of the proposed PIM for passive sampling applications involving the environmental monitoring of antibiotics.

4.2.2 Monitoring of sulfamethoxazole in natural waters with a polymer inclusion membrane-based passive sampler. Minimizing the effect of the flow pattern of the aquatic system

Overview

Because of the continuous release of antibiotics and their constant presence in the environment, these compounds are considered as “pseudopersistent” contaminants (Martinez Bueno et al., 2012).

SMX is a low reactive antibiotic that belongs to the family of SAs and is among one of the most frequently detected antibiotics in streams and groundwater (Chen et al., 2011). Its high FOD and relative persistence in aquatic systems indicates that it poses a potential risk to the ecosystem balance, as it exhibits biotoxicity for some fish and algae (Niu et al., 2013). A number of studies have demonstrated that the elimination of SMX through sewage treatment is incomplete. Residual SMX with concentrations varying from 0.01 to 2.0 $\mu\text{g L}^{-1}$ have been detected in municipal sewage treatment plant effluents and levels from 30 to nearly 500 ng L^{-1} have been observed in surface waters (Tamtam et al., 2008; Niu et al., 2013). Hence, in order to identify the presence, fate and behaviour of SMX in aquatic environments it is important to conduct continuous monitoring studies. However, currently, most of the published studies on antibiotics monitoring are based on discrete grab sampling with a subsequent SPE for the antibiotics followed by their determination by LC-MS/MS (Tong et al., 2009; Zhou et al., 2012; Panditi et al., 2013). This procedure relies on multiple analytical steps that result in lengthy and costly analysis, and tends to increase the magnitude of the experimental errors (Cahill et al., 2004; Hylton et al., 2009; Shariati et al., 2009). However, to overcome these drawbacks and acquire TWA concentrations passive sampling is usually applied. POCIS, which consist of a sorbent positioned between two microporous membranes or diffusion gradient thin films (DGT), where the analytes diffuse through a stagnant water boundary layer and a diffusion layer that comprises a filter membrane and hydrogel which separates the solid R phase from the aquatic system, are commonly used for the monitoring of organic species. Chen et al. (2012) evaluated for the first time the use of DGTs under laboratory conditions, involving the use of UW, for the monitoring of the antibiotic SMX, They observed an increase in the

SMX uptake over time. The tested system was applied for the monitoring of SMX in a UK river over a two week period. For both POCIS and DGT based passive sampling, it is necessary to elute the organic species accumulated in the solid sorbent with a suitable organic solvent prior to their determination, similarly as described above for the SPE step (Bailly et al., 2013; Almeida et al., 2014).

The complex and time-consuming extraction procedure can be omitted if SPMDs incorporating a PIM as the semi-permeable barrier and a suitable aqueous solution as the R phase are used instead. The target analytes could then be directly measured in the R solution at the end of the passive sampling period.

Currently, the above described passive sampling methodology based on PIMs has only been described by Almeida et al. (2014) for the sampling of Zn(II). To the best of our knowledge there is no study based on this alternative passive sampling methodology for the monitoring of organic compounds.

This paper describes the development of the first PIM-based passive sampler for SMX in environmental waters incorporating Aliquat 336 as the membrane carrier. Its performance as a function of the compositions of the membrane and the Sand R solutions, the passive sampler design, and the flow pattern of the source solution was studied.

Results and Discussion

Chemical parameters

- *Influence of the PIM composition*

Membrane composition is a key factor for a good PIM performance (Nagul et al., 2013). The choice of an appropriate carrier plays an essential role on PIM's extraction and TE. In this study the anion-exchanger Aliquat 336, which is a mixture of quaternary alkylammonium salts, was used as the carrier. This compound exhibits good compatibility with both CTA and PVC (Pereira et al., 2009; Kagaya et al., 2012;

Vázquez et al., 2014) and successful results with these membranes were obtained earlier by us in the extraction of different antibiotics (Garcia-Rodríguez et al., 2015).

However, the other membrane components (*i.e.* base polymer and plasticizer) are also crucial for the chemical and physical characteristics of the corresponding PIM. The effect of the membrane composition was assessed by comparing the EF (Eq. (3.5)) of the PIM-based passive sampler incorporating PIMs M1, M2, M3 and M4. Unlike the CTA-based PIMs which were found to be sufficiently mechanically strong, the PVC-based membranes exhibited poorer mechanical stability and appeared sticky. To overcome mechanical stability issues thicker PVC-based PIMs were prepared. Fig. 4.2.7 shows the effect of PIM composition and thickness on SMX transport across the membrane into the receiving phase for three different transport durations (*i.e.*, 2, 5 and 24 h). Though, as expected, the amount of SMX transported increased with time for all four membranes, significant differences in the SMX enrichment were observed between the CTA and PVC-based membranes with the same concentration of Aliquat 336 and NPOE. To some extent the higher EF values for the CTA-based PIMs can be explained by the fact that the PVC-based PIMs were between 33 and 66% thicker than the CTA-based membranes.

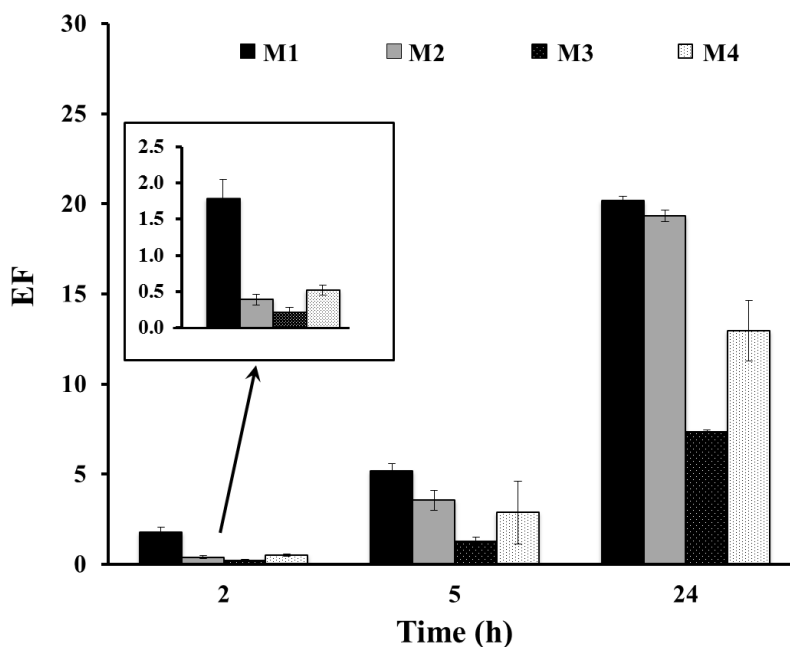


Figure 4.2.7. SMX enrichment in the receiving solution of the PIM-based passive sampler for 3 different transport durations and 4 PIM's compositions and thicknesses (M1: 30% CTA + 26% Aliquat 336 + 44% NPOE, 94 ± 5 μm ; M2: 30% CTA + 44% Aliquat 336 + 26% NPOE, 87 ± 5 μm ; M3: 30% PVC + 26% Aliquat 336 + 44% NPOE, 145 ± 5 μm ; M4: 30% PVC + 44% Aliquat 336 + 26% NPOE, 125 ± 5 μm ; initial SMX S solution concentration $100 \mu\text{g L}^{-1}$). Error bars are \pm standard deviation for $1\sigma_{n-1}$ ($n = 3$).

On the basis of the results presented in Fig. 4.2.7 the CTA-based membrane containing 26% Aliquat 336 and 44% NPOE (M1) was selected for use in all subsequent experiments.

- Influence of the receiving solution composition

The effect of the NaCl concentration in the receiving solution was studied at 2 different levels, i.e. 0.5 and 2 mol L⁻¹, in transport experiments where the source solution consisted of 100 µg L⁻¹ SMX in Groundwater 1. The transient concentrations of SMX in the source solution are presented in Fig. 4.2.8, where it can be seen that 2 mol L⁻¹ NaCl concentration produced better results due to a highest concentration gradient of chloride. Thus, this concentration was used in the subsequent experiments.

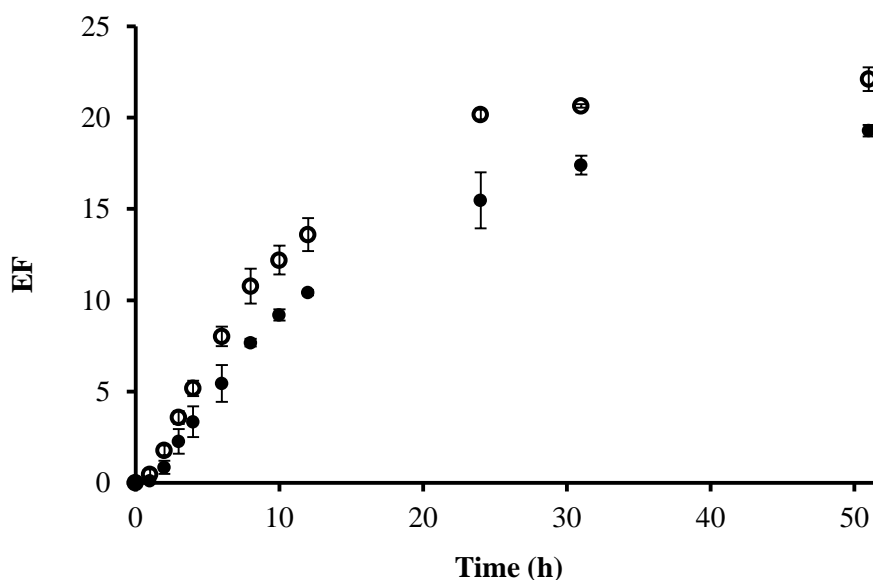


Figure 4.2.8. EF for SMX *versus* time in the case of R solutions containing (●) 0.5 or (○) 2.0 mol L⁻¹ NaCl, S solution containing initial SMX concentration of 100 µg L⁻¹ and a membrane composed of 30% CTA, 26% Aliquat 336 and 44% NPOE and with a thickness of 94±5 µm. Error bars are ± standard deviation for 1σ_{n-1} (n = 3).

- Influence of the water matrix

Since Aliquat 336 is an anion-exchanger, it can be expected that other anions present in the source solution will be able to compete with the extraction and transport of SMX. To evaluate this potential interference, transport experiments involving the natural and waste waters, listed in Table 3.3, spiked with $100 \mu\text{g L}^{-1}$ SMX, were used. Fig. 4.2.9 shows that there are no differences between the transient SMX concentrations in the receiving solution for the groundwater, river water and WW used despite their divergent anionic compositions (Table 3.3). These results indicate that the presence of other anionic compounds in the water matrices used did not affect the SMX transport into the R solution of the PIM-based passive sampler.

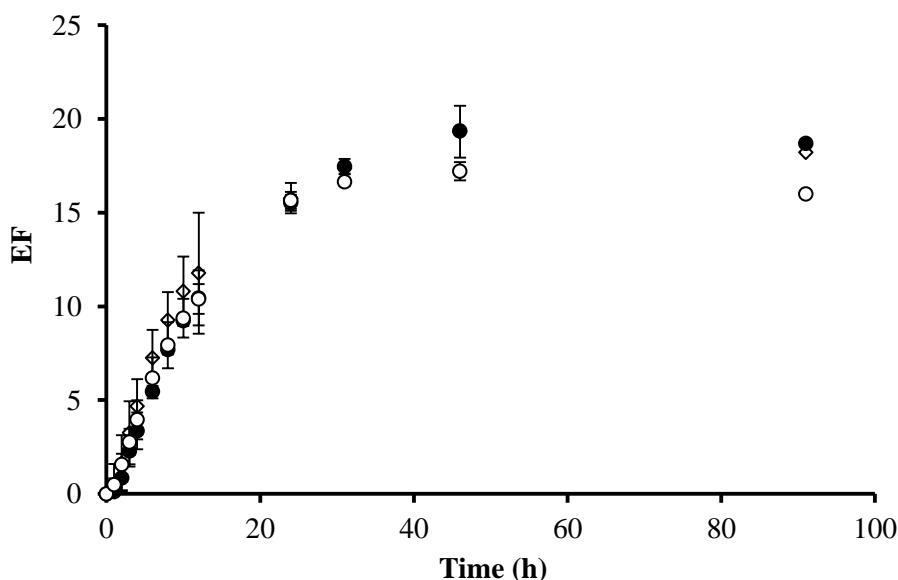


Figure 4.2.9. Kinetics of the accumulation of SMX in the R solution in transport experiments involving S solutions consisting of $100 \mu\text{g L}^{-1}$ SMX in (●) Groundwater 1, (◇) river water or (○) WW and a membrane composed of 30% CTA, 26% Aliquat 336 and 44% NPOE and with a thickness of $94 \pm 5 \mu\text{m}$. Error bars are \pm standard deviation for $1\sigma_{n-1}$ ($n = 3$).

The passive sampling performance can vary depending on the concentration of SMX in the source solution. In order to study the applicability of the passive sampler proposed in this study for monitoring SMX in natural waters, the two types of groundwater listed in Table 3.3 were spiked with SMX to prepare source solutions with SMX in the $0 - 80 \mu\text{g L}^{-1}$ concentration range. The duration of transport in the subsequent experiments was 24 h. Linear relationships between the concentration of SMX in the source and receiving solutions for the two types of groundwater with slopes within 3.5% of each

other were obtained, thus indicating that the water matrix did not affect significantly the transport of SMX. These results suggest that a calibration curve constructed for a particular natural water matrix is likely to be applicable to similar types of natural waters.

Influence of the flow pattern of the source solution

The effect of flow pattern of the S solution (i.e., flow rate and mixing) was evaluated by comparing two passive sampling calibration approaches, i.e., “flow-through” and “dip-in”.

The aim of the “flow-through” approach was to ensure that only fresh source solution with the selected SMX concentration was in contact with the membrane. Hence, the source solution was pumped through the inlet of the “flow-through” passive sampling configuration (Fig. 3.4 (c)) and then discharged to waste through its outlet (Fig. 4.2.10).

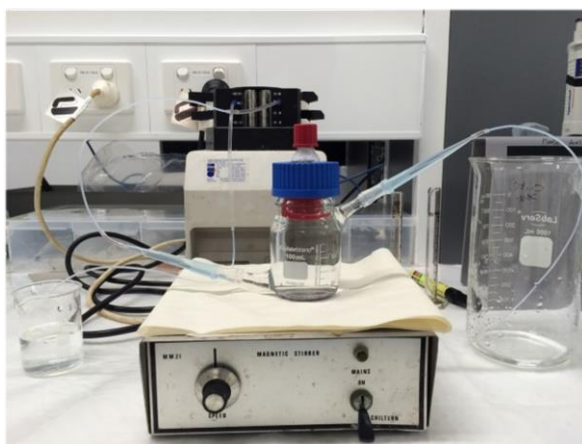


Figure 4.2.10. Experimental setup for the evaluation of the S solution' flow pattern.

Experiments with different source solution flow rates were conducted (i.e. 0.75, 2, 5, and 10 mL min⁻¹). At low flow rate values, differences between the influent and effluent SMX concentrations were observed, but at a flow rate of 10 mL min⁻¹ only a decrease of 2-3% of the initial SMX concentration was recorded.

Thus, the transient accumulation of SMX in the receiving solution using the “flow-through” approach at 10 mL min⁻¹ was compared with the “dip-in” approach (Fig. 4.2.11). Fig. 4.2.12 shows the significant effect of the flow pattern and associated

mixing and mass transport in the vicinity of the membrane/source solution interface on the SMX transport.



Figure 4.2.11. Experimental setup for the comparison of the “flow-through” and “dip-in” approach effectiveness to accumulate SMX in the PIM-based device.

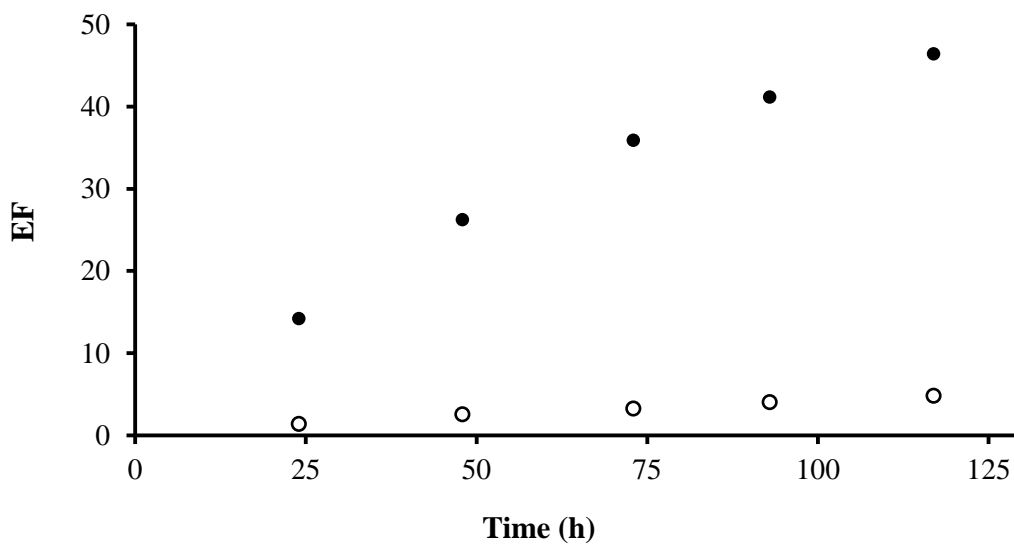


Figure 4.2.12. Transient SMX EF during 5 days of (●) “flow-through” (10 mL min^{-1} , Fig. 3.4 (c)) and (○) “dip-in” (Fig. 3.4 (b)) passive sampling using a S solution with initial SMX concentration of $500 \mu\text{g L}^{-1}$ and a membrane composed of 30% CTA, 26% Aliquat 336 and 44% NPOE, and with a thickness of $94 \pm 5 \mu\text{m}$.

On the basis of these results it was decided to develop a passive sampler with a design that minimized the effect of the flow pattern by ensuring that the transport mechanism of the analyte from the bulk of the source solution towards the membrane of the passive sampler was only based on diffusion.

Influence of the design of the passive sampler

A new configuration for the passive sampler with a stagnant liquid barrier between the source solution (aquatic system) and the membrane was constructed (see Fig. 3.4 (d)) with the aim of minimizing any flow rate or turbulence effects on the rate of accumulation of SMX in the receiving solution. It was expected that the flow rate effect on the overall mass transport rate of SMX from the bulk of the source solution into the receiving solution would be dampened significantly and the overall mass transfer rate of SMX would be dominated by the diffusion mass transfer across the membrane/stagnant layer within the open cylindrical section of the passive sampler. The performance of the proposed passive sampler was compared with that of the traditional passive sampler shown in Fig. 3.4 (a). Four different “dip-in” passive sampling experiments were conducted by using a $500 \mu\text{g L}^{-1}$ SMX source solution, two with the traditional passive sampler and two with the proposed passive sampler. In these experiments passive samplers each type were exposed to a quiet and mechanically stirred solutions. Fig. 4.2.13 shows that the proposed passive sampler accumulated less SMX for both sampling periods of 24 h and 48 h, but it was much less sensitive to the flow pattern than the traditional passive sampler as shown by the data for the cases with and without stirring of the source solution. Thus, it was decided to conduct a more detailed study to evaluate the influence of the flow pattern on the accumulation of SMX in the receiving solution when using the proposed passive sampler.

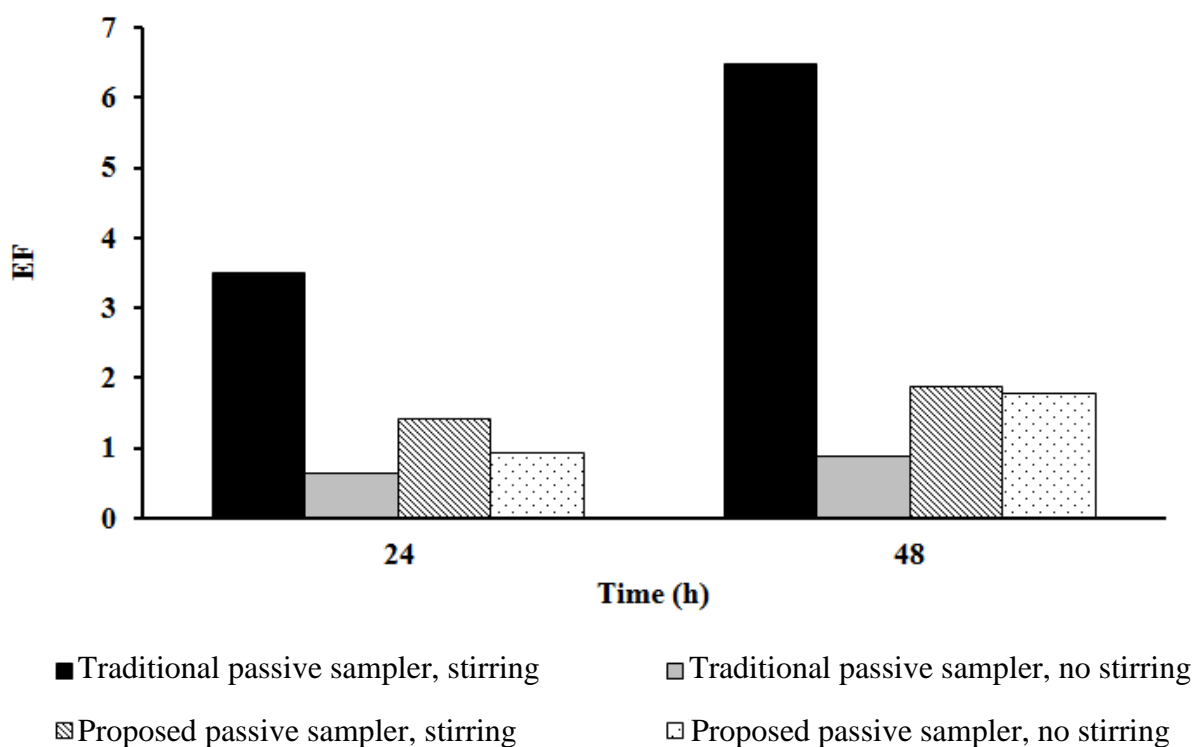


Figure 4.2.13. Effect of the flow pattern and passive sampler design on the transport of SMX from the source solution ($500 \mu\text{g SMX L}^{-1}$) to the R solution ($2 \text{ mol L}^{-1} \text{ NaCl}$). The membrane was composed of 30% CTA, 26% Aliquat 336 and 44% NPOE and with a thickness of $94 \pm 5 \mu\text{m}$.

The corresponding experiments were conducted in triplicate and the passive samplers were located in 20 L tanks containing a $50 \mu\text{g L}^{-1}$ SMX source solution (Fig 4.2.14).

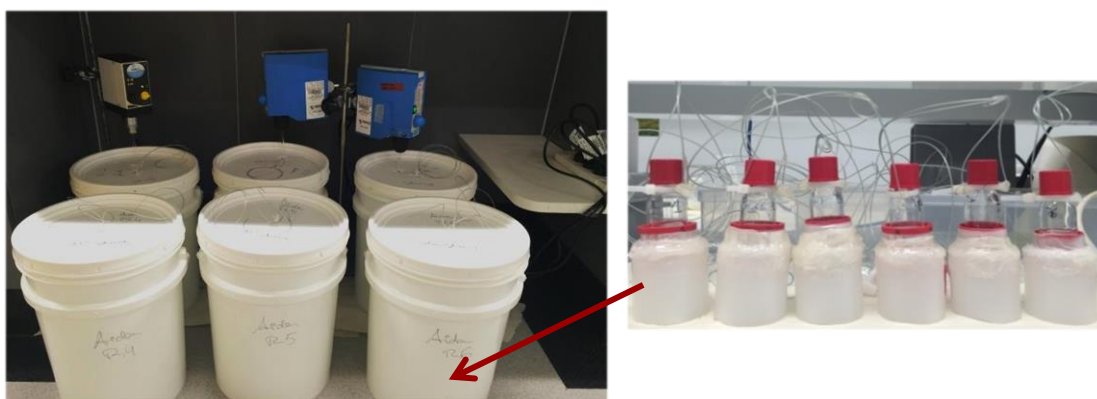


Figure 4.2.14. Experimental setup to evaluate the flow influence in the “dip-in” approach with the new passive sampler design.

Fig. 4.2.15 shows that similar results were achieved with and without stirring the source solution which is a strong indication for the promise of the proposed approach in minimizing the influence of the flow pattern during passive sampling.

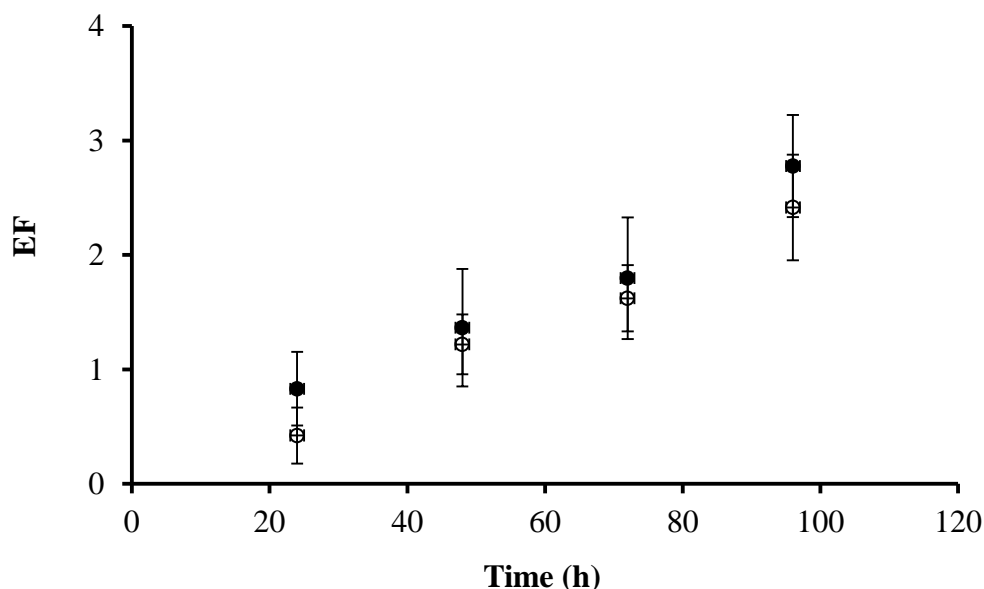


Figure 4.2.15. Influence of the flow pattern (● - stirring and ○ - no stirring) on the transport of SMX from the F ($50 \mu\text{g SMX L}^{-1}$) to the R ($2 \text{ mol L}^{-1} \text{ NaCl}$) solution when using the proposed passive sampler with a PIM composed of 30% CTA, 26% Aliquat 336 and 44% NPOE and with a thickness of $94 \pm 5 \mu\text{m}$.

In our research we have compared both “dip-in” and “flow-through” approaches in optimizing the chemical parameters and design of a passive sampler for SMX utilizing a PIM as its semi-permeable barrier. The optimal membrane composition consisted of 30% CTA, 26% Aliquat 336 and 44% NPOE. The R solution contained $2 \text{ mol L}^{-1} \text{ NaCl}$ as the R solution reagent. It was established that these chemical compositions of the PIM and the R solution minimized the effect of the S solution matrix on the accumulation of SMX in the R solution. However, the flow pattern of the S solution influenced significantly the performance of the passive sampler thus endangering the reliability of the passive sampling data. This problem was overcome successfully by inserting the membrane part of a traditional PIM-based passive sampler into a plastic cylindrical container through a hole in its base so that the S solution in contact with the membrane remained static irrespective of the flow pattern of the bulk source solution.

The SMX diffusional mass transfer across the static source solution within this cylindrical attachment towards the PIM was found to eliminate the effect of mixing in the bulk S solution on the accumulation rate of SMX in the R solution thus removing a major uncertainty in passive sampling caused by flow pattern of the S solution (aquatic system).

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CHAPTER 4

Section 3

*The ability of phytotechnologies to remove EOCs
from wastewater*

This section is based on the articles:

Garcia-Rodríguez, A., Matamoros, V., Fontàs, C., Salvadó, V., 2014. The ability of biologically based wastewater treatment systems to remove emerging organic contaminants—a review. *Environ. Sci. Pollut. Res.* 21, 11708–11728.

Garcia-Rodríguez, A., Matamoros, V., Fontàs, C., Salvadó, V., 2013. The influence of light exposure, water quality and vegetation on the removal of sulfonamides and tetracyclines: A laboratory-scale study. *Chemosphere* 90, 2297–2302.

Garcia-Rodríguez, A., Matamoros, V., Fontàs, C., Salvadó, V., 2015. The influence of *Lemna* sp. and *Spirogyra* sp. on the removal of pharmaceuticals and endocrine disruptors in treated wastewaters. *Int. J. Environ. Sci. Technol.* 12, 2327-2338.

State of the art

Chapter 1 has stated the presence of EOCs in the aquatic environment mainly due to their incomplete removal in conventional WWTPs (Pilon-Smits, 2005; Murray et al., 2010). Biologically based WW treatment systems are actually being considered as an attractive cost-effective and sustainable alternative to the advanced tertiary treatments that take place in WWTPs, as they also simulate the ability of natural ecosystems to attenuate pollution from water (Haberl et al., 2003; Imfeld et al., 2009). These systems are eco-engineered filters providing different micro-environments where physical, chemical, and biological removal processes can take place. Such processes facilitate the removal, recycling, transformation, or immobilization of sediment, nutrients, and other water pollutants (Gersberg et al., 1986; White et al., 2006; Imfeld et al., 2009). Moreover, as it will be seen in this chapter, biologically based systems have been proved to effectively remove certain EOCs such as pharmaceuticals and personal care products (Hijosa-Valsero et al., 2010a; Hijosa-Valsero et al., 2010b; Matamoros et al., 2012c; Petrović et al., 2014) and to provide ecologically rich effluents (Ortiz et al., 2011; Matamoros and Salvadó, 2012). Elimination mechanisms can be physical (sorption, photodegradation, volatilization, and sedimentation), chemical (degradation and hydrolysis), and biological (biodegradation and phytoremediation). Of these, biodegradation, photodegradation, phytoremediation, and sorption are the most predominant (Matamoros et al., 2008b; Matamoros et al., 2012a). These mechanisms can be influenced or modified by factors such as plant activity, wastewater composition, the accumulation of organic matter, and environmental changes.

The upcoming pages demonstrate and discuss the ability of different biologically based wastewater treatment systems, also known as phytotechnologies, such as CWs, WSPs, high-rate algal ponds (HRAPs), and *Daphnia* and fungal reactors, to remove EOCs, comparing the different and elimination processes occurring in these systems.

Removal processes occurring in biologically based WW treatment systems

A brief description of the basis of the main processes involved in the removal of EOCs in biologically based WW treatment systems is given in this section. Fig. 4.3.1 also illustrates these processes.

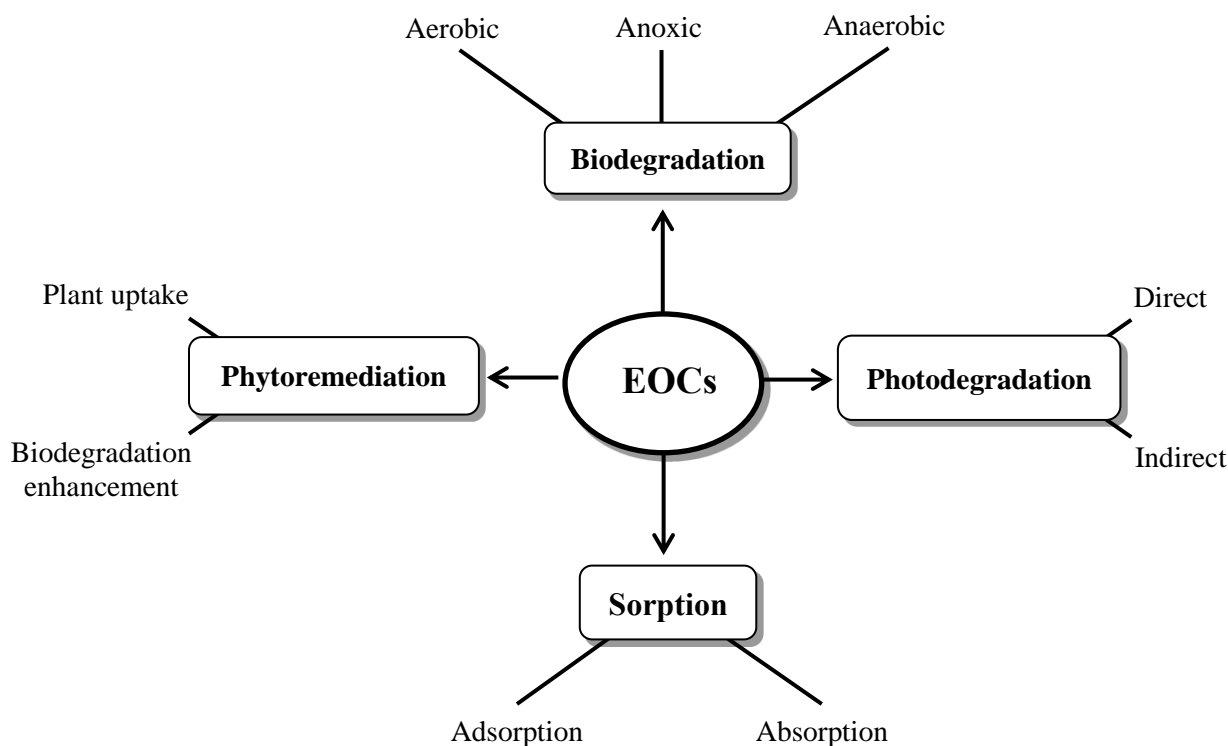


Figure 4.3.1. Processes occurring in biologically-based WW treatment systems that are involved on the removal of EOCs.

Biodegradation

Biodegradation is the process by which organic substances are decomposed by microorganisms (bacteria and fungi) into simpler chemical substances, resulting in some cases in the complete mineralization. Biodegradation is the most extended process occurring in biologically based WW treatment systems for the removal of pollutants (van Agteren et al., 1998; Alexander, 1999) and can be performed under aerobic and anaerobic conditions. EOCs decomposition processes in biologically based WW treatment systems are often considerably faster in aerobic than in anaerobic conditions (Zwiener and Frimmel, 2003; Hijosa-Valsero et al., 2010b; Lapworth et al., 2012).

Photodegradation

Some EOCs such as DCF or triclosan are photodegraded when they are exposed to sunlight either by direct or indirect mechanisms (Andreozzi et al., 2003). Direct photodegradation consists of absorption of solar light by the aquatic contaminants, followed by a chemical reaction. In the case of indirect photolysis, EOCs are degraded by strong oxidant species such as hydroxyl radicals (Zepp et al., 1981; Andreozzi et al., 2003) generated by natural photosensitizers (e.g., nitrate and humic acids) under solar radiation (Zepp and Cline, 1977). Photodegradation processes are largely influenced either by the dissolved or suspended solids contained in WW. The results of the competition between direct and indirect photodegradation reactions will determine the rate at which this degradation takes place and the photoproducts that are generated (Zepp et al., 1981).

Phytoremediation

Phytoremediation is a removal process that uses both higher plant species and algae for the environmental clean-up of surface waters, ground waters, soils, and sediments (Tsao, 2003). Most of the biologically based WW treatment systems discussed in this review contain vegetation, and consequently phytoremediation plays an important role in the removal of EOCs. Phytoremediation of organic pollutants can take place through plant uptake, plant exudates or enzymes, or with the participation of rhizosphere organisms (Susarla et al., 2002; Pilon-Smits, 2005). The removal processes depend on the compounds' properties and their interaction with the plant system (Susarla et al., 2002; Suresh and Ravishankar, 2004; Pilon-Smits, 2005; Zhang et al., 2010). These processes, which can be classified as phytodegradation, phytostabilization, phytovolatilization, rhizodegradation, and rhizofiltration, can occur simultaneously in biologically based WW treatment systems (Macek et al., 2000; Susarla et al., 2002; Suresh and Ravishankar, 2004; Pilon-Smits, 2005; Zhang et al., 2010).

The uptake and translocation of organic contaminants by the whole plant system are dependent on the contaminant concentration, the physicochemical properties, and on the type of plants (Kirakosyan and Kaufman, 2009). In order to be taken up by a plant, an organic compound must have appropriate chemical characteristics (i.e., solubility,

polarity, and partitioning coefficients) to be transported across the root membrane (Tsao, 2003). Contaminants with a $\log K_{ow}$ between 0.5–1 and 3–3.5 are able to move into the plant, whereas compounds with $\log K_{ow}$ out of this range are not (Tsao, 2003; Pilon-Smits, 2005). As an example, compounds which present a moderate value of hydrophobicity, such as CARB ($\log K_{ow}$ of 2.45), are more likely to be taken up by plants, as they can move across the root membranes and are sufficient soluble to travel through cell fluids. Moreover, non-charged compounds (e.g., CAFF) are easily incorporated by aquatic plants, whereas some negatively charged compounds such as DCF and NAP are not. In the case of negatively charged compounds, the effect of pKa and pH seems to be more relevant than lipophilicity (Zhang et al., 2013b), as the electrical repulsions between the negative charge of the compounds and the negative charge of the plants' biomembrane do not permit the uptake of the compound (Matamoros et al., 2009b; Trapp, 2009).

Sorption

Sorption can be both through adsorption, the physical adherence of molecules or ions of the pollutant onto the surface of a sorbent, or absorption, the incorporation of the contaminant into the sorbent (e.g. plant uptake) (Crini and Badot, 2010). Some biologically based WW treatment systems consist of a gravel bed where the organic matter is accumulated and afterwards biodegraded. This enables the removal of highly hydrophobic compounds such as galaxolide and tonalide ($\log K_{ow} > 5$) by adsorption (Matamoros and Bayona, 2006).

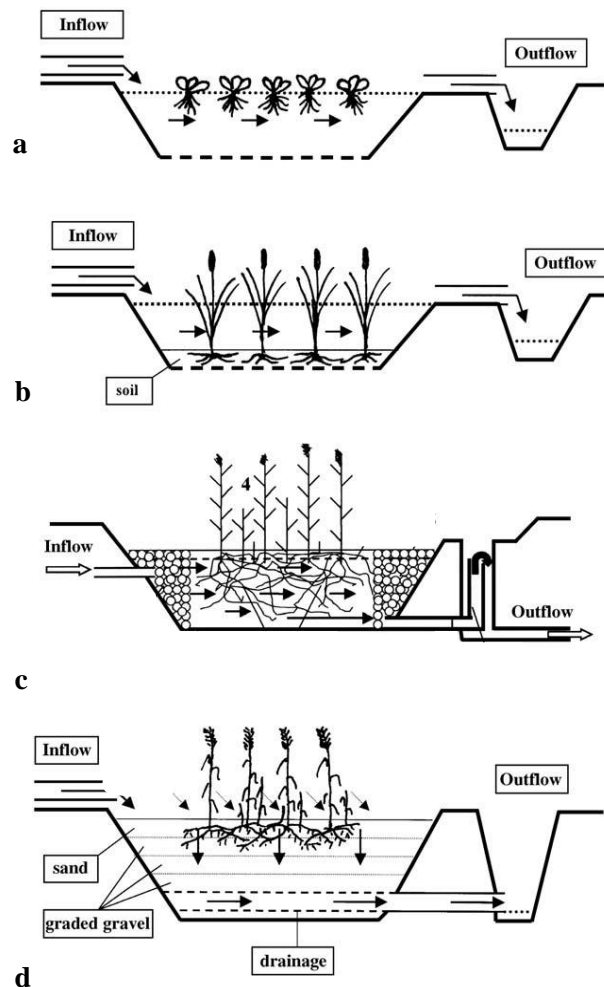
In the following section, the removal of EOCs in different types of biologically based WW treatment systems will be discussed in accordance with the removal processes described above. As it will be seen, more than one of these processes can occur simultaneously, enhancing contaminant removal.

Biologically based WW treatment systems

Constructed wetlands

CWs are biologically based WW treatment systems that are based on shallow ponds, beds, or trenches that contain floating or emergent rooted wetland vegetation (Cole, 1998). In these treatment systems, polluted water passes through a vegetated bed and then is percolated by gravity inducement. CWs allow to achieve the enough quality of water to be reused in a cost-effective way due to either by biodegradation, photodegradation, sorption, or/and phytoremediation processes (Randerson, 2006; Kadlec and Wallace, 2009). CWs are classified as subsurface flow (SSF) systems or surface flow (SF) systems according to their WW flow regime (subsurface and surface) (Vymazal, 2011). Moreover, CWs can be combined (hybrid systems) to take advantage of the characteristics of each different system (Vymazal, 2011). Figure 4.3.2 shows some of the aforementioned CWs configurations, based on Vymazal (2007).

Figure 4.3.2. Different CWs configurations: **a.** CW with free floating plants, **b.** Surface flow CW with emergent macrophytes, **c.** CW with horizontal subsurface flow, **d.** CW with vertical subsurface flow. Reprinted from Vymazal J. (2007). Removal of nutrients in various types of constructed wetlands. *Science of the Total Environment* 380 (1-3): 48-65.



In the following sections, studies developed with different types of CWs at laboratory, pilot, and full scale will be discussed.

Subsurface flow constructed wetlands

The use of SSFCWs as a cost-effective technology for urban WW treatment has gained growing interest over the last decades in small communities with less than 2,000 population equivalent (Puigagut et al., 2007). In this type of treatment, the improvement of water quality is achieved through the combined action of the solid matrix, plants, and microorganisms (Dordio et al., 2010). Hence, the next sections will be dedicated to study the role of aerobic and anaerobic microorganisms, effect of the solid matrix, the vegetation, and the temperature/seasonality on the removal of EOCs.

- Anaerobic, anoxic, and aerobic biodegradation processes

SSFCWs are generally divided in two types, based on the WW flow direction inside the wetland bed: horizontal flow (HF) and vertical flow (VF). HFCWs are continuously fed and the WW flows slowly under the surface of the gravel wetland bed, planted with macrophytes. Therefore, in HFCWs generally anaerobic environment prevails due to they work under saturated water conditions. Otherwise, VFCWs are intermittently fed from the wetland surface with large amounts of WW, having both feeding and resting periods (Vymazal, 2011). Then, in VF systems there is a greater oxygen transfer into the bed, meaning that they work under unsaturated water conditions, allowing a predominant aerobic environment (Matamoros and Bayona, 2008; Vymazal, 2011). As stated by several authors (Zwiener and Frimmel, 2003; Hijosa-Valsero et al., 2010b; Conkle et al., 2012; Zhang et al., 2012b), aerobic pathways are generally more efficient for the biodegradation of EOCs (e.g., pharmaceuticals), rather than anaerobic conditions, as in HFCWs (Matamoros et al., 2008b; Hijosa-Valsero et al., 2010a; Zhang et al., 2011). However, Park et al. (2009) conducted a study under anoxic conditions and found that some micropollutants can be biologically degraded under these conditions. For example, amide and urea functional groups from compounds such as CARB and atenolol were found to be biologically transformed through mediated hydrolysis reactions.

Matamoros et al. (2005) and Matamoros and Bayona (2006) compared the removal efficiency of pharmaceuticals and personal care products (PPCPs) in HFCWs with different water depth (0.5 vs. 0.27 m). The results indicated that the shallow system, with a less negative redox potential, was more efficient than the deep one at removing biodegradable compounds such as IBP, NAP, and methyl dihydrojasmonate (see Table 4.3.1). Conversely, Song et al. (2009) evaluated the effectiveness of VFCWs at the polishing step in conventional WW treatment and studied the removals of estrogens at different sand layer depths (i.e., 7.5, 30, and 60 cm filter layer depth). The highest removal efficiencies were achieved in the shallowest wetland (i.e., $68 \pm 28\%$, $84 \pm 15\%$, and $75 \pm 18\%$ for estrone, 17β -estradiol, and 17-ET, respectively). The authors suggested that the highest efficiency achieved in the extremely shallow wetland was not only the result of the presumably enhancement of aerobic conditions but also likely is due to the high root density, which increases the surface area available for sorption processes enhancing the effect of root exudates on estrogen removal.

Ávila et al. (2010) conducted an injection study to evaluate the capacity of HFCWs for removing IBP, NAP, DCF, tonalide, and BPA, achieving removals ranging from 85 to 99 % (see Table 4.3.1). The authors, based on laboratory degradation tests carried out by Quintana et al. (2005), suggested that NAP was removed by aerobic biodegradation, whereas DCF removal was by anaerobic biodegradation. This manifested the co-existence of aerobic and anaerobic removal pathways in HFCWs as it has been previously reported in the case of nutrients (García et al., 2005). For the musk fragrance tonalide and the plasticizer BPA, as they are highly hydrophobic ($\log K_{ow} > 3.5$), the key removal mechanism is adsorption onto solid particles. Similar results were found by Matamoros and Bayona (2008) in studying the removal of galaxolide and tonalide ($\log K_{ow} > 5$) in VFCWs and HFCWs planted with the same type of macrophytes (*Phragmites australis*).

- Effect of solid matrix

Although coarse granite gravel, crushed rock, or sand media are the most used solid matrix for CWs, Dordio et al. (2009, 2011) have demonstrated, at laboratory scale, that light expanded clay aggregates (LECA) or biosorbents such as cork can improve the removal efficiency of some EOCs. This increase was of up to 88 and 98 % for CARB

and IBP, respectively. Moreover, removal efficiencies up to 100 % for 2-methyl-4-chlorophenoxyacetic acid (MCPA) and CLF AC can be achieved using LECA in sterile conditions (Dordio et al., 2007). Nevertheless, as the adsorption capacity is limited by the saturation of the sorbent active sites, due in part to the development of biofilm, further field studies are needed to fully understand the effect of the solid matrix on the removal processes.

- *The role of vegetation*

Macrophytes existing in SSFCWs can positively contribute to the removal of EOCs by creating aerobic paths into the substrate with the oxygen released by plant roots and their corresponding microenvironments in the rhizosphere (Brix, 1997; Matamoros and Bayona, 2008). Moreover, macrophytes have many other properties (e.g., stabilize bed surface, act as an anchoring surface for biofilm, and release of exudates), making them a key design factor (Brix, 1997; Kyambadde et al., 2004; Vymazal, 2011); their presence also promotes the removal of EOCs by several biodegradation or phytoremediation mechanisms (White et al., 2006; Imfeld et al., 2009; Dordio et al., 2010). SSFCWs were reported to successfully remove organic contaminants such as pesticides, PAHs, or explosives from WW (Dordio et al., 2010).

The presence of *Typha angustifolia* in HFCWs increases the average removal of IBP and NAP by up to 20 and 40 %, respectively (see Table 4.3.1) (Zhang et al., 2011; Zhang et al., 2012b). These results are in agreement with those reported by Dordio et al. (2010), who observed that the removal of IBP was generally improved by the presence of *Typha* sp. (see Table 4.3.1). This macrophyte contributes to increase the aerobic water conditions, favoring the biodegradation of the most biodegradable compounds such as IBP (Matamoros et al., 2005; Matamoros et al., 2008a; Ávila et al., 2010). However, in the case of other compounds such as CAFF, salicylic acid, and ketoprofen, which presented removal efficiencies higher than 85 %, no remarkable differences between planted and unplanted beds were observed (Zhang et al., 2012b). These results agree with the reported easy degradability of salicylic acid in CWs (Matamoros and Bayona, 2006; Hijosa-Valsero et al., 2010a) and the potential of CAFF and ketoprofen to be biodegraded (Lin et al., 2010; Zhang et al., 2012a; Zhang et al., 2012b). Using *Typha angustifolia*-planted beds, no differences were observed for CARB, CLF

AC, and DCF removals (Zhang et al., 2012a; Zhang et al., 2012b), which is in agreement with the reported recalcitrance of these compounds (Matamoros et al., 2005; Matamoros et al., 2008a; Matamoros and Salvadó, 2012; Zhang et al., 2012b). However, in a laboratory-scale study, Dordio et al. (2010) found out that CARB and CLF AC were usually highly removed in beds planted with *Typha* sp. (88–97 % and 48–75 %, respectively) rather than in those unplanted (usually <12 % for both compounds). Moreover, in a later study, Dordio et al. (2011) quantified the concentration of CARB, after being removed from an aqueous solution, in leaf tissue of *Typha* sp., corroborating that this pharmaceutical is taken up by plants' roots and then translocated to leaves. Similar results were obtained by Zhang et al. (2012c, 2013b, c), who observed that non-charged compounds such as CAFF and CARB are easily incorporated by aquatic plants (*Scirpus validus*), whereas negatively charged compounds such as DCF and NAP are not, due to electrical repulsions, as it has been previously described in “Phytoremediation” section. Nevertheless, since all these studies have been performed at laboratory scale with enough contact time of the EOCs with the plants to permit their uptake, it is difficult to make extrapolations to the field situation where the contact time is lower and plant uptake is reduced due to the presence of biofilm and organic matter.

- Seasonal and temperature dependence

Hijosa-Valsero et al. (2010b) suggested that besides physicochemical properties of the compound and plant characteristics, some other factors such as microbial communities related to plant species, climate conditions, and WW composition should be considered as relevant parameters on the removal of EOCs by HFCWs. In this sense, several authors have stated the effect of temperature on the removal of biodegradable EOCs (e.g., CAFF, IBP, NAP), as it has a direct influence on the plant productivity and on the microbial and bacterial communities present in the aqueous media (Zhang et al., 2011). As reported by Truu et al. (2009), microorganisms living in CWs normally achieve their optimal activity at warm temperatures (15–25 °C), producing a beneficial effect on the removal of EOCs. This was demonstrated by Ávila et al. (2010, 2013) for the removal of IBP, DCF, tonalide, and BPA in a HFCW since greater removal efficiencies were found in spring (temperatures of 23 °C) when compared with those obtained in winter (temperatures of 15 °C).

Surface flow constructed wetlands

SFCWs are CWs densely vegetated by different plant species and consist of shallow basins of soil, or another medium able to support the rooted macrophytes, in which secondary-treated WW flows slowly over a compacted clay, to prevent groundwater pollution, at a relatively shallow depth (i.e., typically water depths are lower than 0.4 m) (Haberl et al., 2003; Halverson, 2004; Vymazal and Kröpfelova, 2008; Llorens et al., 2009; Vymazal, 2011) (see Fig. 4.3.2). In addition to phytoremediation, biodegradation, and sorption, photodegradation plays a significant role in the removal of EOCs by SFCWs due to the direct exposure of the water to sunlight.

In this regard, Hussain et al. (2012) studied the removal of ionophoric antibiotics (i.e., monensin, salinomycin, and narasin) in SFCWs, which had a shallow zone vegetated with alternate bands of *Phalaris arundinaceae* and *Typha latifolia*. Although these compounds have reported to be recalcitrant, the authors obtained removals ranging from 21 to 47 % of the initial compounds' concentration (see Table 4.3.1). These ionophoric antibiotics are very hydrophobic ($\log K_{ow}$ values near 6), so they are more likely to be adsorbed to the organic matter than to be taken up by plants (Suresh and Ravishankar, 2004; Paspaliaris et al., 2010). Therefore, the authors stated that compounds' adsorption is expected to be one of the main removal mechanisms, which could be either to the SFCW solid matrix or to the plants' root surface (Suresh and Ravishankar, 2004). Xian et al. (2010) studied the capacity of free-floating macrophytes (i.e., *Lolium multiflorum* Lam.) in CWs for the removal of SAs from swine WW and found removals ranging from 88 to 99 %. The authors proved that the presence of macrophytes enhanced the removal of poorly and moderately biodegradable SAs (91–99 %, 73–92 %, and 95–99 % for sulfadiazine, SMZ, and SMX, respectively) and that to some extent it may be explained due to their presence in the plant roots (percentages accumulated in the plant root of 20–30 %, 60–65 %, and 10–15 % for sulfadiazine, sulfamethazine, and sulfamethoxazole, respectively) (Al-Ahmad et al., 1999). Hence, they concluded that macrophytes had a relevant role in the removal of SAs.

Zarate et al. (2012) evaluated the capacity of a pilot-scale SFCW planted with macrophytes (i.e., *T. latifolia*, *Pontederia cordata*, and *Sagittaria graminea*) for the bioconcentration of triclosan and triclocarban. The analysis of roots and shoots of different macrophytes revealed concentrations of triclosan between 15 and 40 ng g⁻¹ in

roots and of near 20 ng g^{-1} in shoots, and concentrations between 28 and 35 ng g^{-1} in roots and between 10 and 23 ng g^{-1} in shoots for triclocarban. Then, although it has been stated that plant uptake is limited to compounds with $\log K_{ow}$ ranging from 0.5–1 to 3–3.5, bioconcentration patterns were observed for triclosan ($\log K_{ow} = 4.8$) and triclocarban ($\log K_{ow} = 4.9$).

Kiran Kumar et al. (2011) evaluated the natural attenuation of two EDCs (i.e., 17-ET and estradiol) by a microcosm of SFCWs containing floating, submerged, and emergent macrophytes. Both estrogens were removed with efficiencies higher than 90 %, which could be attributed to a combination of biodegradation and adsorption processes (Gaulke et al., 2008).

In another full-scale study, Matamoros et al. (2008b) and Matamoros and Salvadó (2012) found that emerging macrophyte SFCWs (one with a hydraulic retention time (HRT) of 8 days (d) and the other with a HRT of 30 d) were able to remove some pharmaceuticals (i.e., IBP, NAP, ketoprofen, and DCF), PCPs (i.e., galaxolide and tonalide), and pesticides (i.e., mecoprop and MCPA) up to 70 % (see Table 4.3.1). Temperature and sunlight irradiation changes in SFCWs were more relevant for photolabile compounds (e.g., NAP, DCF, and ketoprofen) than biodegradable compounds (e.g., IBP, CAFF, and NAP) (Fig. 4.3.3). They also highlighted that the seasonal development of aquatic plants, algae, and *Lemna* sp. in the SFCW system (mainly in summer and spring) may also have a high impact on the removal efficiency of EOCs. This agrees with other research studies reporting that the presence of vegetation (*Lemna* sp. and a developed rhizosphere from *Typha* sp. and *Phragmites* sp.) enhances biodegradation processes and the uptake of these contaminants (Reinhold et al., 2010; Dordio et al., 2011). Furthermore, the authors stated that hydrophobic compounds such as triclosan, galaxolide, and tonalide may be removed by sorption to the organic matter or root surface. Conversely, CARB and CLF AC were poorly removed (30–47 %), which agrees with their recalcitrance (see “Constructed wetlands” section). However, it is worth mentioning that based on previous laboratory-scale findings (Dordio et al., 2010; Zhang et al., 2013a; Zhang et al., 2013b), some removal of these compounds may be expected due to plant uptake process. Hence, further studies are needed to check it out.

As stated in the previous section, compounds' characteristics, SFCWs design parameters as well as environmental factors are relevant parameters that have significant influence on the removal of EOCs. To sum up, it can be said that during summer, aerobic-mediated biodegradation combined with photodegradation (due to direct sunlight exposure and volatilization) are the predominant removal process occurring in SFCWs.

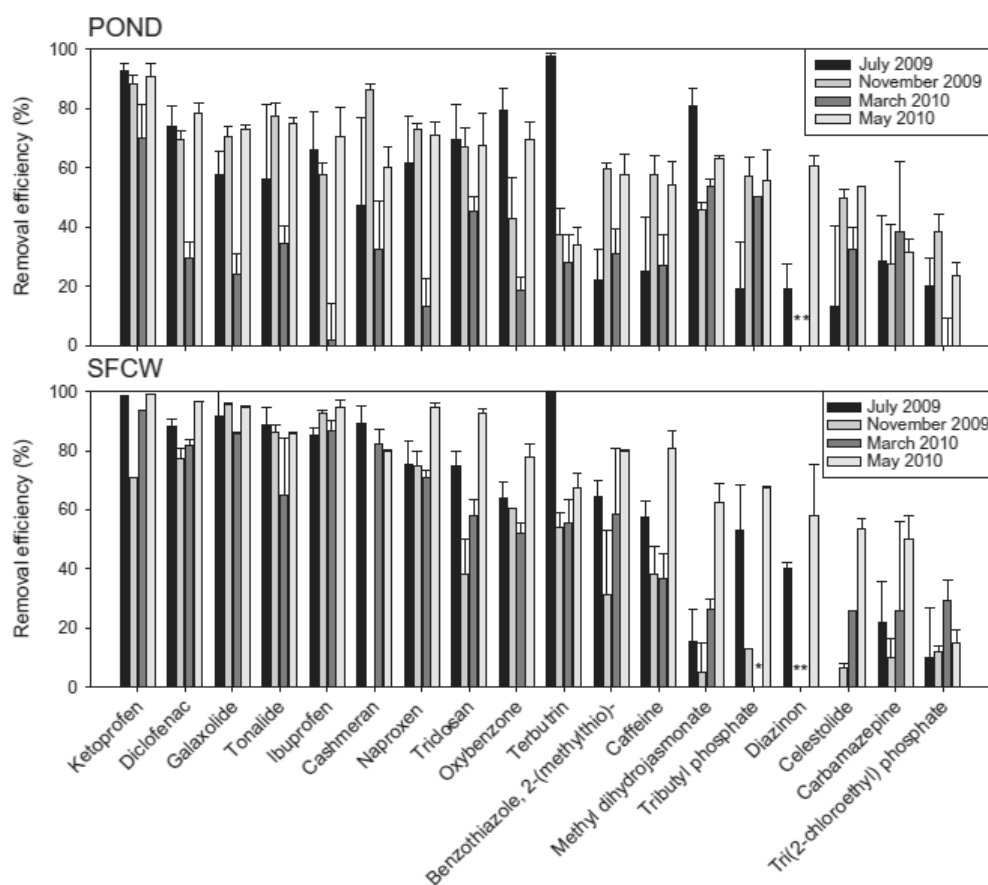


Figure 4.3.3. Removal efficiency of 27 EOCs observed by Matamoros and Salvadó (2012) in a full-scale biologically-based wastewater treatment plant consisting on a polishing pond (PP) followed by a SFCW. Mean removal rates obtained in different months are represented for each part of the treatment. (*) <LOD. Reprinted from Matamoros V. and Salvadó V. (2012). Evaluation of the seasonal performance of a water reclamation pond-constructed wetland system for removing emerging contaminants. *Chemosphere* 86:111-117.

Hybrid CW systems

Due to the difficulty in removing contaminants from WWs in a single-stage system, hybrid systems, which consist of various types of CWs staged in series, have been

introduced to exploit the different degradation pathways between systems (Vymazal, 2005). For example, the use of a VFCW located as a first step would be able to nitrify the ammonia species, whereas a HFCW afterwards is able to denitrificate the previously produced nitrates. Consequently, the combination of different redox potentials in CWs can be a suitable solution for removing compounds with certain recalcitrance. For example, Masi et al. (2004) reported a removal efficiency of estrogens up to 90 % in a CW composed of a first-stage HFCW and a second-stage VFCW. Reyes-Contreras et al. (2011) found that a hybrid system consisting of an upflow anaerobic sludge blanket reactor, a SFCW, and a HFCW in series led to an improvement in the removal of various EOCs. Ávila et al. (2014) studied the capacity of a hybrid CW system consisting of two VFCWs working in parallel, one HFCW, and one SFCW in series to eliminate 8 EOCs under three different hydraulic loading rates (HLRs) (0.06, 0.13, and 0.18 m day⁻¹ considering the area of the two VF beds) and found that the hybrid system was capable of removing EOCs up to 80 % at all three HLRs (Fig. 4.3.4). This demonstrated for the first time that the use of hybrid CW systems permits to increase HLRs without decreasing the removal of EOCs which makes this technology highly competitive in comparison with conventional activated sludge WWTPs.

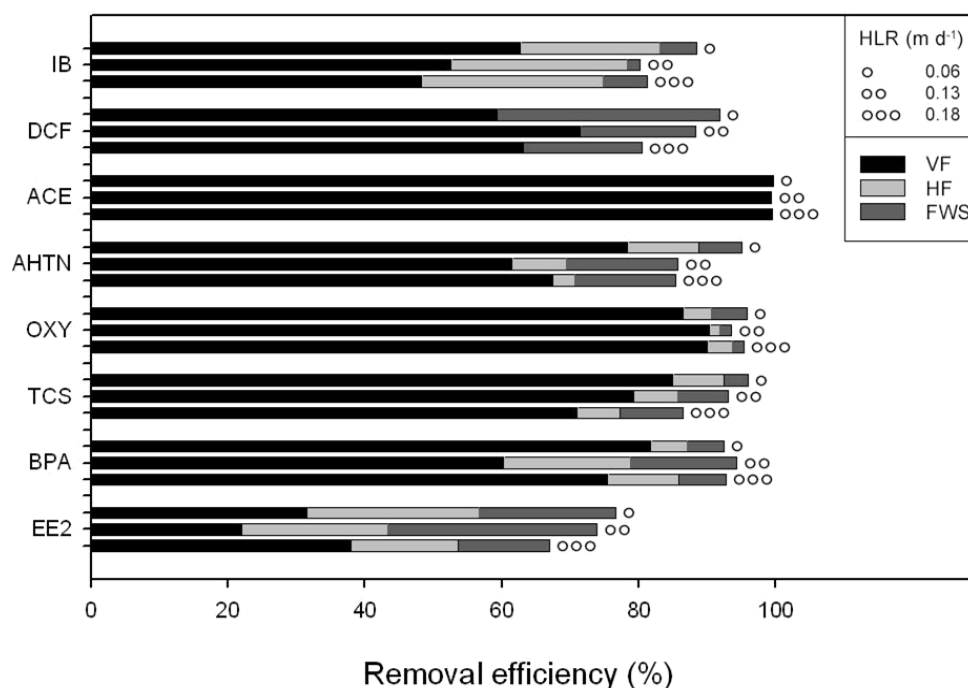


Figure 4.3.4. Accumulated average removal efficiencies of studied EOCs (IB: ibuprofen, DCF: diclofenac, ACE: acetaminophen, AHTN: tonalide, OXY: oxybenzone, TCS: triclosan, BPA: bisphenol A, EE2: 17 α -ethinylestradiol) along the different units of the hybrid CW system (VF: vertical flow CW, HF: horizontal flow CW, FWS: free water surface flow CW). Reprinted from Ávila C, Matamoros V, Reyes-Contreras C, Piña B, Casado M, Mita L, Rivetti C, Barata C, Garcia J, Bayona JM. (2014). Attenuation of emerging contaminants in a hybrid constructed wetland system under different hydraulic loading rates and their associated toxicological effects in wastewater. *Science of the Total Environment*.

Waste stabilization ponds

WSPs consist of large, shallow, or deep basins in which WW is completely treated by natural processes. They contain both algae and bacteria, and due to their presence, there are single or several series of anaerobic (AP), facultative (FP), or maturation ponds (MP) (Kayombo et al., 2003; Norton et al., 2012). In comparison with SFCWs, ponds present a higher water depth and lower amounts of plants (Norton et al., 2012). The main removal processes occurring in these systems are photodegradation, biodegradation, and sorption onto organic matter, which can be enhanced by the presence of algae or some aquatic plants (Garcia-Rodríguez et al., 2013).

Qiang et al. (2013) investigated the performance of a WSP in comparison with other available WW treatment technologies (SSFCW and activated sludge system) on the removal of six EDCs (BPA, 4-nonylphenol, estrone, 17 β -estradiol, estriol, and 17-ET) from raw WW and found that WSP was the least effective in removing target EDCs (18–46 % in winter). This can be explained by the low organic matter content in which the compounds can be adsorbed or the low microbial activity due to the anaerobic predominant conditions of this system. Hijosa-Valsero et al. (2010a) reported that the removal of PPCPs (i.e., NAP, IBP, DCF, salicylic acid, CAFF, and methyl dihydrojasmonate) in AP and FP systems ranged between 70 and 90 % (see Table 4.3.2). They found out that most of that removal (more than 80 %) was achieved in the AP. This fact was explained by the high algal proliferation and consequent aerobic conditions (3 mg L⁻¹ of dissolved oxygen) reached in the pond. Authors suggested biodegradation, photodegradation, plant exudates, and uptake to be the main removal processes. Conkle et al. (2008) observed that three aerated lagoons in series treating raw WW were capable of removing up to 90 % of pharmaceutical compounds, except CARB, nadolol, sotalol, SPY, and gemfibrozil (see Table 4.3.2), which demonstrates that aerobic conditions are the most adequate for removing EOCs by WSPs. A similar study was conducted by Li et al. (2013), who found out that, except for CARB, gemfibrozil, and trimethoprim, other EOCs (e.g., NAP, IBP, SMX, CAFF, and triclosan) were efficiently removed (88–100 %) by a lagoon system (see Table 4.3.2), obtaining maximum removal values in warm season. To sum up, it can be postulated that WSP systems treating raw WW can promote the removal of EOCs by different mechanisms (i.e., photodegradation, sorption, hydrolysis, biodegradation, and plant

uptake), but biologically mediated degradation by aeration could be considered the most relevant.

Maturation or PPs are ponds located after secondary WW treatment systems, which can be either a facultative pond or an activated sludge WWTP. Garcia-Rodríguez et al. (2013) assessed the capacity of algae (*Spirogyra* sp.) and aquatic plants (*Zannichellia palustris*) present in PPs for removing SAs and TCs through a laboratory-scale study. They concluded that TC was mainly removed by photodegradation, OTC was removed by biodegradation or hydrolysis, and SAs were mainly eliminated by biodegradation or indirect photodegradation. Although Gujarathi et al. (2005) pointed out that root exudates produced by the aquatic plant roots such as *Myriophyllum aquaticum* and *Pistia stratiotes* can oxidize antibiotics such as TC and OTC, the authors stated that the presence of *Spirogyra* sp. or *Zannichellia palustris* slightly affected the removal of both TCs and SAs. Results of another laboratory-scale study conducted by Garcia-Rodríguez et al. (2015) found out that when comparing planted and unplanted reactors filled with secondary-treated WW, those reactors planted (with *Lemna* sp. or *Spirogyra* sp.) presented faster elimination rates for ACAPh, BPA, 17-ET, PROPR, and IBP (Garcia-Rodríguez et al., 2015). Furthermore, the authors found that the elimination rates of these compounds were much lower in containers filled with ultrapure water than in those filled with secondary-treated WW. Therefore, it was concluded that biodegradation and plant uptake were the main removal factors. Since the low $\log D_{ow}$ of IBP and ACAPh (calculated at pH = 7), they would probably be uptaken by plants (Pilon-Smits, 2005). However, considering ionisation and ease of biodegradation of IBP and ACAPh, respectively, the authors pointed out that they are not likely to be uptaken by plants and neither to be adsorbed by organic matter (Lin et al., 2010), whereas BPA, 17-ET, and PROPR with a $\log K_{ow}$ between 2 and 4 are consistently taken up by plants (Deegan et al., 2011). These results were in accordance with those found by Shi et al. (2010) in a laboratory-scale study where they observed that 17 β -estradiol and 17-ET can be greatly removed from water by sorption when they are in contact with duckweed or algae, achieving removals higher than 80 % (see Table 4.3.2). In the study of Garcia-Rodríguez et al. (2015), no remarkable differences were observed between planted and unplanted reactors for CLF AC and DCF, which agrees with their recalcitrance (Hijosa-Valsero et al., 2010a; Hijosa-Valsero et al., 2010b). However, the greatest CARB removal rates were obtained in reactors planted

with *Lemna* sp., that could be explained because of its suitability to be taken up by plants ($\log K_{ow} = 2.45$), which would agree with the study reported by Dordio et al. (2011), who observed that rooted plants (i.e., Typhasp.) effectively contributed on the removal of CARB. However, in a full-scale study, Matamoros and Salvadó (2012) found out that the presence of *Lemna* sp. may reduce the removal efficiency of pharmaceutical compounds, as it covers the PP surface in summer and do not allow the sunlight to enter into the system, resulting in a decrease of the photodegradation and/or biodegradation processes. Moreover, during spring, due the lack of photosynthesis, algae growth stopped, resulting in algae degradation. This consumed all the available oxygen from the PP and it caused a decline of the aerobic biodegradation processes, which, as stated in previous sections, are favourable for most of the EOCs removal (Fig. 4.3.3).

Then, although the presence of aquatic plants is normally beneficial for the removal of EOCs, the type of plant species, WW composition, sunlight radiation, and the compounds' physicochemical characteristics are factors that should be considered.

High-rate algal ponds

Oswald et al. (1960) described HRAPs for the first time to be shallow, open raceway ponds that have been used for treating industrial, agricultural, or municipal WW with the purpose of producing algal biofuel (Park et al., 2011). The use of HRAPs has been open for deliberation, and, due to the increasing prices of fuels in the recent times, it seems that the application of this promising technology is being considered, which may simultaneously meet the requirements of cleaning WW and producing energy. Nevertheless, little attention has been paid on the effectiveness of this technology for removing EOCs. First results demonstrate that the shallow geometry of HRAPs is an advantage since it allows the photodegradation of antibiotics such as TCs in the course of the WW biological treatment (de Godos et al., 2012). Furthermore, Wang et al. (2013) found that microalgae (*Chorella pyrenoidosa*) exposed to a series concentration of triclosan from 100 to 800 $\mu\text{g L}^{-1}$ were able to uptake more than 50 % of triclosan from culture medium during the first 1 h of exposure. Therefore, this technology based

on microalgae seems to be a promising treatment for the removal of EOC from WW, but further studies are still needed.

Daphnia reactors

During recent years, the concept of improving the ecological and chemical water quality gained importance. Bearing this in mind, a new strategy, named “waterharmonica”, has recently been proposed by Claassen (2007), building on the idea formulated by Mitsch (1998) by which ecology and engineering are integrated. This technology is based on the presence of *Daphnia* sp. and its capacity for removing particles (Pau et al., 2013). Matamoros et al. (2012c) showed that biological filtration based on macroinvertebrates (e.g., Chydoridae, *Daphnia*, and Ostracoda) and algae is a reliable technology for removing some EOCs from treated WW. Fig. 4.3.5 compares the removal efficiency of EOCs at different mass loading rates in summer and winter. Authors found that removal efficiencies of DCF, triclosan, ketoprofen, cashmeran, galaxolide, tonalide, and NAP were affected by photodegradation, the extent of which varies considerably between winter and summer. IBP, methyl dihydrojasmonate, CAFF, NAP, and oxybenzone were affected by biodegradation, which was greater in summer due to its temperature dependence.

Fungal reactors

White-rot fungi (WRF) have the potential to be useful biocatalysts due to their abroad specificity to attack substrates through the action of intracellular (i.e., cytochrome P450 system) and extracellular (i.e., laccases and peroxidases) enzymes (Cerniglia, 1997). This versatility in degrading a wide variety of xenobiotics makes these microorganisms potentially useful in bioremediation applications. In this regard, Yang et al. (2013) compiled a review in which they pointed out that trace organic contaminants bearing strong electron-donating groups (e.g., hydroxyl and amines) such as BPA or nonylphenol are well removed by WRF, while compounds with electron-withdrawing groups (e.g., halogen, nitro, azepine, and triazine) such as DCF or triclosan are biodegraded mainly by WRF secreting all three major lignin-modifying enzymes (lignin

peroxidases, manganese-dependent peroxidases, and laccase). For example, Cabana et al. (2007) pointed out that the use of laccase and extracts from the white-rot fungus *Coriopsis polyzona* aids the complete removal of BPA, triclosan, and nonylphenol after four incubation hours at 40 °C and a pH of 4. Nevertheless, the few available studies on EOCs removal by WRF also report somewhat poorer removal by fungal reactors operated in non-sterile conditions and/or in continuous flow mode.

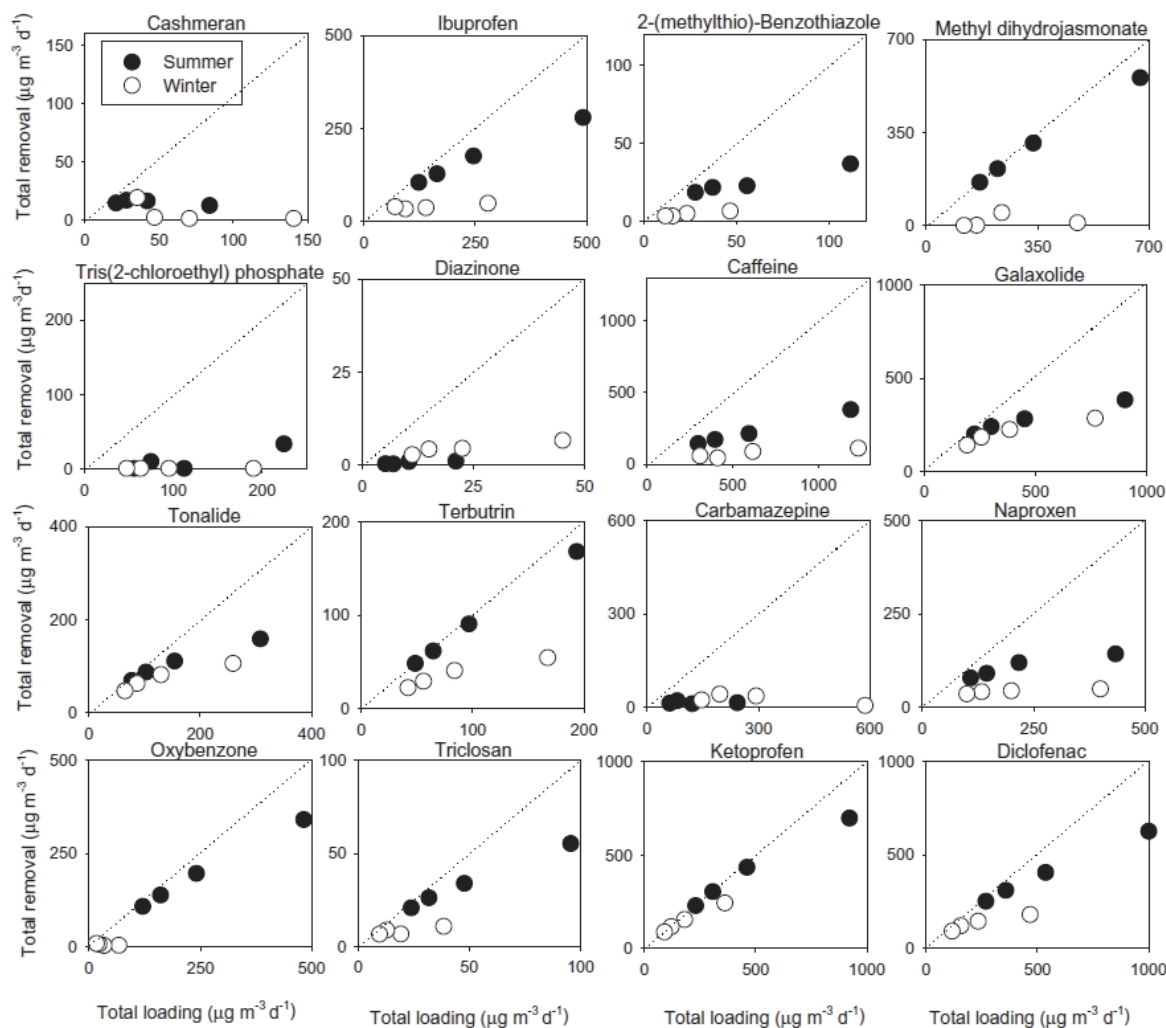


Figure 4.3.5. Average removal efficiency of EOCs observed by Matamoros et al. (2012c) in summer and winter, at different mass loading rates in a pilot scale study. Circles represent reactors (black: summer removal, white: winter removal). Dotted lines represent 100% removal. Reprinted from Matamoros V, Sala L, Salvadó V. (2012c). Evaluation of a biologically-based filtration water reclamation plant for removing emerging contaminants: A pilot plant study. *Bioresource Technology* 104:243-249.

Rodríguez-Rodríguez et al. (2012) assessed the capability of fungus *Trametes versicolor* in a sludge–bioslurry reactor operated in batch mode for 26 d to remove several groups of EOCs and observed that 15 out of 24 detected pharmaceuticals were

removed at efficiencies over 50 % after the treatment, including eight completely degraded, whereas removal ranged between 16–53 % and 22–100 % for the brominated flame retardants and the UV filters, respectively. Furthermore, Cruz-Morató et al. (2013) found that *Trametes versicolor* in a batch fluidized bed bioreactor treatment was capable of removing several pharmaceuticals in real urban WW under non-sterile conditions. The authors pointed out that after eight incubation d, six out of ten pharmaceutical compounds were capable to be completely removed, whereas CARB, ciprofloxacin, cefalexin, and acridone were less removed (<30 %). Nevertheless, the same reactor operated in batch mode and fed with synthetic fungal medium was capable of removing CARB up to 96 % within 2 d, whereas only half (54 %) of CARB was removed when operated in continuous mode (HRT 3 d) (Jelic et al., 2012). Although this seems to be a promising technology, which is already working for recalcitrant azo-dyes (Hai et al., 2009), further research is needed to be competitive with activated sludge WWTPs or some of the other biologically based WW treatment technologies reviewed in this article.

Table 4.3.1. Examples of occurrence and removal of EOCs in different studies conducted with distinct types of CWs at various scales.

CWs					
Type	Scale	System characteristics	Influent concentration ($\mu\text{g L}^{-1}$)	Removal efficiency (%)	Reference
HFCW	Microcosm	Dimension (m): 0.6 x 0.5 x 0.4 Type of plants: <i>Typha angustifolia</i> Operation mode: batch Experiment duration (d): 7	1000	CLF AC (48-75), CARB (88-97), IBP (82-96)	Dordio et al. (2010)
		Dimension (m): 0.93 x 0.59 x 0.52 Type of plants: <i>Phragmites australis</i> Experiment duration (d): 30	25	CLF AC (NR), CARB (5), IBP (51-52)	Matamoros et al. (2008a)
		Dimension (m): 1.2 x 0.6 x 0.6 Type of plants: <i>Typha angustifolia</i> Experiment duration (d): 4	4-18	CARB (27), NAP (91), DCF (55), IBP (78)	Zhang et al. (2011)
	Mesocosm		0.37-67.34	Ketoprofen (22-55), NAP (43-90), IBP (38-75), DCF (NR-40), salicylic acid (62-90), CARB (22-50), CAFF (57-99), methyl dihydrojasmonate (75-98), galaxolide (10-75), tonalide (20-70)	Hijosa-Valsero et al. (2010b)
		Dimension (m): 0.8 x 1.3 x 0.5 Surface area (m^2): 1 HRT (d): 2.1-5.1 Type of plants: <i>Phragmites australis</i> / <i>Typha angustifolia</i> Experiment duration (d): 10	0.06-46	Doxycycline (71-75), amoxicillin (21-45), ampicillin (NR), clarithromycin (31-39), erythromycin (NR-64), lincomycin (NR), SMX (80-87), sulfadimethoxine (99), trimethoprim (92-99)	Hijosa-Valsero et al. (2011)

Table 4.3.1 Examples of occurrence and removal of EOCs in different studies conducted with distinct types of CWs at various scales (**continued**).

	Dimension: 0.8m x 1.3m x 0.5m Surface area (m ²): 1 HRT (d): 0.5-2.21 Type of plants: <i>Phragmites australis</i> / <i>Typha angustifolia</i> Experiment duration (d): 90	0.20-64.90	Ketoprofen (NR-83), NAP (NR-82), IBP (NR-95), DCF (NR-75), salicylic acid (60-99), CAFF (45-99), CARB (NR-70), methyl dihydrojasmonate (40-97), galaxolide (10-82), tonalide (NR-85)	Reyes-Contreras et al. (2012)
	Dimension (m): 1.2 x 0.6 x 0.6 Type of plants: <i>Typha angustifolia</i> Operation mode: batch Experiment duration (d): 4	25	CARB (27), NAP (91), DCF (55), IBP (83), CAFF (93), salicylic acid (94), ketoprofen (95), CLF AC (34)	Zhang et al. (2012a)
	Dimension (m): 1.2 x 0.6 x 0.6 Type of plants: <i>Typha angustifolia</i> Operation mode: continuous Experiment duration (d): 4	25	CARB (28), NAP (93), DCF (44), IBP (72), CAFF (90), salicylic acid (90), ketorpofen (96), CLF AC (39)	Zhang et al. (2012b)
	Surface area (m ²): 0.62 + 1.65 HRT (d): 3.5 Type of plants: <i>Phragmites australis</i> Experiment duration (d): 24	0.1-56	IBP (98-99), NAP (99), DCF (99), tonalide (97-98), BPA (85-99)	Ávila et al. (2010)
	Dimension –big (m): 1.5 x 1.1 x 0.5 Dimension –small (m): 0.95 x 0.7 x 0.45 HRT (d): 3.5 Type of plants: <i>Phragmites australis</i> Experiment duration (d): 26	2-75	IBP (52-85), DCF (32-70), ACAPh (95), tonalide (88-91), oxybenzone (87-97), BPA (65-89)	Ávila et al. (2013)
	Surface area (m ²): 54-56 HLR (mm d ⁻¹): 36 Type of plants: <i>Phragmites australis</i> Experiment duration (d): 21	25	CLF AC (NR), CARB (16-26), IBP (48-81)	Matamoros et al. (2005)

Table 4.3.1 Examples of occurrence and removal of EOCs in different studies conducted with distinct types of CWs at various scales (**continued**).

		Surface area (m ²): 54-56 HLR (mm d ⁻¹): 36 Type of plants: <i>Phragmites australis</i> Experiment duration (d): 7	0.8-30	0.27m water depth: Salicylic acid (92-98), IBP (62-80), NAP (50-69), DCF (NR-45), ketoprofen (NR-69), CAFF (94-99), methyl dihydrojasmonate (99), galaxolide (45-61), tonalide (44-65) 0.5m water depth: Salicylic acid (77-97), IBP (17-52), NAP (NR-47), DCF (NR-11), ketoprofen (NR), CAFF (85-94), methyl dihydrojasmonate (61-94), galaxolide (31-44), tonalide (32-53)	Matamoros and Bayona (2006)
		Surface area (m ²): n.a. HRT (d): 0.3-3 Type of plants: <i>Phragmites australis</i> / <i>Typha Latifolia</i> Experiment duration (d): 30	0.75	ACAPh (47-99)	Ranieri et al. (2011)
	Full	Surface area (m ²): 470-4000 HRT (d): n.a. Type of plants: <i>Phragmites australis</i> Experiment duration (d): 60	0.09-70.8	Salicylic acid (95), IBP (65), NAP (45), DCF (21), CAFF (97), methyl dihydrojasmonate (99), ketoprofen (90), CARB (38), hydrocinnamic acid (95), oxybenzone (98), furosemide (71)	Matamoros et al. (2009)
VFCW	Microcosm	Surface area: (m ²): 0.15 HRT (h): 3.1-24.8 Type of plants: <i>Phragmites australis</i> Experiment duration (d): 30	0.39·10 ⁻³ -0.01	Estrone (68), 17β-estradiol (84), 17α-ET (75)	Song et al. (2009)
	Pilot	Surface area (m ²): 5 HLR (mm d ⁻¹): 13-160 Type of plants: <i>Phragmites australis</i> Experiment duration (d): 14	0.48-64.00	Salicylic acid (85-98), IBP (55-99), NAP (62-89), DCF (53-73), CAFF (82-99), methyl dihydrojasmonate (78-99), galaxolide (88-90), tonalide (75-82), CARB (20-26), hydrocinnamic acid (82-99), oxybenzone (88-97)	Matamoros et al. (2007)

Table 4.3.1 Examples of occurrence and removal of EOCs in different studies conducted with distinct types of CWs at various scales (**continued**).

	Full	Surface area (m ²): 15-16 HRT (h): n.a. Type of plants: <i>Phragmites australis</i> Experiment duration (d): 60	0.09-70.8	Salicylic acid (87), IBP (89), NAP (92), DCF (NR), CAFF (99), methyl dihydrojasmonate (99), ketoprofen (NR), CARB (NR), hydrocinnamic acid (98), oxybenzone (90), furosemide (NR)	Matamoros et al. (2009)
SFCW	Microcosm	Dimension (L): 5 Type of plants: <i>Salvinia molesta</i> , <i>Lemna minor</i> , <i>Ceratophyllum</i> <i>demersum</i> , <i>Elodea canadensis</i> Experiment duration (d): 38	10	DCF (99), triclosan (96-99), NAP (40-53), IBP (44- 77), CAFF (81-99), CLF AC (16-23), MCPA (7-17)	Matamoros et al. (2012b)
	Flask	Dimension (L): 0.1 Type of plants: <i>Lemna minor</i> or <i>Lemna punctata</i> Experiment duration (d): 2-9	2-3	IBP (47), fluoxetine (44), triclosan (95)	Reinhold et al. (2010)
	Mesocosm	Dimension (L): 3 Type of plants: <i>Typha</i> spp. Experiment duration (d): 21	500-2000	CARB (56-82)	Dordio et al. (2011a)
		Dimension (m): 0.8 x 1.3 x 0.5 Surface area (m ²): 1 HRT (d): 2.1-5.1 Type of plants: <i>Phragmites</i> <i>australis</i> / <i>Typha angustifolia</i> Experiment duration (d): 10	0.37-67.34	Ketoprofen (36-50), NAP (30-70), IBP (54-98), DCF (NR-55), salicylic acid (35-90), CARB (10-45), CAFF (20-99), methyl dihydrojasmonate (35-95), galaxolide (5-80), tonalide (3-75)	Hijosa-Valsero et al. (2010b)
			0.06-46	Doxycycline (62-65), amoxicillin (27-42), ampicillin (NR-32), clarithromycin (11-32), erythromycin (NR), lincomycin (NR), SMX (59-71), sulfadimethoxine (67-99), trimethoprim (65-88)	Hijosa-Valsero et al. (2011)

Table 4.3.1 Examples of occurrence and removal of EOCs in different studies conducted with distinct types of CWs at various scales (**continued**).

	Dimension (m): 0.8 x 1.3 x 0.5 Surface area (m ²): 1 HRT (d): 0.5-2.21 Type of plants: <i>Phragmites australis</i> / <i>Typha angustifolia</i> Experiment duration (d): 90	0.20-64.90	Ketoprofen (NR-95), NAP (22-92), IBP (NR-98), DCF (NR-90), salicylic acid (55-99), CAFF (20-99), CARB (NR-95), methyl dihydrojasmonate (40-99), galaxolide (5-95), tonalide (3-92)	Reyes-Contreras et al. (2012)
	Dimension (L): 3 Type of plants: <i>Scirpus validus</i> Experiment duration (d): 21	500-2000	DCF (93)	Zhang et al. (2012c)
	Dimension (L): 4 Type of plants: <i>Scirpus validus</i> Experiment duration (d): 21	500-2000	CLF AC (80)	Zhang et al. (2013a)
		500-2000	CARB (74), NAP (98)	Zhang et al. (2013b)
		500-2000	CAFF (>99)	Zhang et al. (2013c)
Pilot	Surface area (m ²)=9.29 HRT (d): 2.2 Type of plants: <i>Phalaris arundinaceae</i> L., <i>Typha latifolia</i> Experiment duration (d): 30	100-1000	Monesin (21-37), salinomycin (22-42), narasin (22-47)	Hussain et al. (2012)
	Dimension (m): 30 x 120 x 0.13 HRT (h): 6 Type of plants: <i>Acorus</i> , <i>Typha</i> sp. Experiment duration (d): 30	0.01-0.90	SMX (15-80), atenolol (90-100), dilantin (5-65), CARB (NR-65), diazepam (NR-10), DCF (80-95), NAP (70-95), triclosan (NR-100), Tri(2-chloroethyl) phosphate (NR-22)	Park et al. (2009)

Table 4.3.1 Examples of occurrence and removal of EOCs in different studies conducted with distinct types of CWs at various scales (**continued**).

	Full	<p>Surface area (m²): 10000 HRT (d): 30 Type of plants: <i>Phragmites australis</i>, <i>Typha Latifolia</i> Experiment duration (d): 7</p>	0.04-2.86	<p>IBP (95-96), NAP (52-92), DCF (73-96), ketoprofen (97-99), CLF AC (32-36), CARB (30-47), flunixin (NR-64), galaxolide (85-88), tonalide (88-90), mecoprop (79-91), MCPA (79-93), terbutylazine (1-80)</p>	Matamoros et al. (2008b)
Hybrid systems					
SFCW+ HFCW	Pilot	<p>SFCW Surface area (m²): 75 HRT (h): 55 Type of plants: <i>Juncus effusus</i> HFCW Surface area (m²): 75 HRT (h): 55 Type of plants: <i>Juncus effusus</i> Experiment duration (d): 21</p>	0.14-55.00	<p>SFCW: IBP (2-35), NAP (28-40), ketoprofen (NR-40), CAFF (40-45), CARB (1-15), salicylic acid (40-45), triclosan (1-35), butylated hydroxyanisole (20-22), butylated hydroxytoluene (8-10), methyl dihydrojasmonate (42-45), tonalide (20-29), galaxolide (15-20)</p> <p>HFCW: IBP (1-5), NAP (1-15), ketoprofen (3), CAFF (10-20), CARB (2-3), salicylic acid (10-30), triclosan (5-10), butylated hydroxyanisole (13-18), butylated hydroxytoluene (1-10), methyl dihydrojasmonate (5-15), tonalide (1-19), galaxolide (1-16)</p>	Reyes-Contreras et al. (2011)

Table 4.3.1 Examples of occurrence and removal of EOCs in different studies conducted with distinct types of CWs at various scales (**continued**).

					<p>Overall WWTP performance: IBP (20-50), NAP (40-65), ketoprofen (30-70), CAFF (65-95), CARB (8-28), salicylic acid (90-92), triclosan (32-70), butylated hydroxyanisole (45-50), butylated hydroxytoluene (12-30), methyl dihydrojasmonate (50-75), tonalide (35-60), galaxolide (28-50)</p>	
<p>PP + SFCW</p>	Full	<p>PP area (m²): 20000 HRT (d): 4 Type of plants: n.a. SFCW Surface area (m²): 8000 x 3 SFCWs HRT (d): 8.5 Type of plants: <i>Phragmites australis</i>, <i>Typha latifolia</i> Experiment duration (d): 7</p>	Surface	0.03-2.16	<p>PP: Ketoprofen (70-98), DCF (30-80), CARB (30-40), NAP (15-75), IBP (5-70), CAFF (25-60), cashmeran (30-85), celestolide (15-55), galaxolide (20-70), tonalide (35-80), methyl dihydrojasmonate (45-85), triclosan (45-70), oxybenzone (20-80), tri(2-chloroethyl) phosphate (NR-40), tributyl phosphate (20-60), diazinon (NR-60), terbutrin (50-100), benzothiazole, 2-(methylthio)-(25-60)</p> <p>SFCW: Ketoprofen (70-100), DCF (78-97), CARB (5-50), NAP (70-96), IBP (85-97), CAFF (40-80), cashmeran (0-90), celestolide (0-55), galaxolide (85-95), tonalide (65-90), methyl dihydrojasmonate (5-70), triclosan (40-95), oxybenzone (55-80), tri(2-chloroethyl) phosphate (10-30), tributyl phosphate (NR-70), diazinon (NR-60), terbutrin (58-100), benzothiazole, 2-(methylthio)-(35-80)</p>	Matamoros and Salvadó (2012)

Table 4.3.1 Examples of occurrence and removal of EOCs in different studies conducted with distinct types of CWs at various scales (**continued**).

Aerated lagoons + SFCW	Full	Aerated lagoons Dimension (m): 61 x 183 x 3 HRT (d): 9 Type of plants: n.a. SFCW Surface area (m ²): n.a. HRT (d): n.a. Type of plants: <i>Hydrocottle spp.</i> , <i>Phragmites australis</i> Experiment duration (d): 27	0.03-25.57	Aerated lagoons: Cotinine (97), CAFF (100), CARB (NR), fluoxetine (ND), ACAPh (100), NAP (99), IBP (100), gemfibrozil (61), atenolol (80), nadolol (NR), PROPR (ND), metoprolol (88), sotalol (15), SPY (74), SMX (78) SFCW: Cotinine (99), CAFF (100), CARB (NR), fluoxetine (ND), ACAPh (100), NAP (98), IBP (99), gemfibrozil (NR), atenolol (93), nadolol (77), PROPR (ND), metoprolol (92), sotalol (33), SPY (76), SMX (92)	Conkle et al. (2008)
FP+ SFCW+ HFCW	Full	FP Surface area (m ²): 1073 HRT (d): 75.9 Type of plants: <i>Lemna minor</i> SFCW Surface area (m ²): 44 HRT (d): 1.2 Type of plants: <i>Typha latifolia</i> HFCW Surface area (m ²): 585 HRT (d): 5.7 Type of plants: <i>Salix atrocinerea</i> Experiment duration (d): 30	<0.58-60.00	FP: Ketoprofen (67), NAP (66), IBP (34), DCF (67), salicylic acid (88), CARB (NR), CAFF (64), methyl dihydrojasmonate (75) SFCW: Ketoprofen (79), NAP (71), IBP (29), DCF (68), salicylic acid (92), CARB (NR), CAFF (73), methyl dihydrojasmonate (77) HFCW: Ketoprofen (>81), NAP (82), IBP (42), DCF (78), salicylic acid (94), CARB (NR), CAFF (95), methyl dihydrojasmonate (90)	Hijosa-Valsero et al. (2010a)

Table 4.3.1 Examples of occurrence and removal of EOCs in different studies conducted with distinct types of CWs at various scales (**continued**).

FP+ SFCW+ HFCW	Full	FP Surface area (m ²): 230 HRT (d): 4.21 Type of plants: algae	<0.05-69.16	FP: Ketoprofen (>62), NAP (33), IBP (56), DCF (72), salicylic acid (97), CARB (NR), CAFF (18), methyl dihydrojasmonate (>92)	Hijosa-Valsero et al. (2010a)
		SFCW Surface area (m ²): 210 HRT (d): 3.53 Type of plants: <i>Typha latifolia</i>		SFCW: Ketoprofen (>62), NAP (63), IBP (89), DCF (>79), salicylic acid (97), CARB (NR), CAFF (89), methyl dihydrojasmonate (>95)	
		HFCW Surface area (m ²): 362.5 HRT (d): 3.16 Type of plants: <i>Salix atrocinerea</i>		HFCW: Ketoprofen (>62), NAP (74), IBP (89), DCF (>86), salicylic acid (93), CARB (NR), CAFF (>95), methyl dihydrojasmonate (>95)	
		Experiment duration (d): 30			

(Matamoros et al., 2005; Matamoros and Bayona, 2006; Matamoros et al., 2007; Conkle et al., 2008; Matamoros et al., 2008a; Matamoros et al., 2008b; Matamoros et al., 2009b; Park et al., 2009; Song et al., 2009; Ávila et al., 2010; Dordio et al., 2010; Hijosa-Valsero et al., 2010a; Hijosa-Valsero et al., 2010b; Reinhold et al., 2010; Dordio et al., 2011; Hijosa-Valsero et al., 2011; Ranieri et al., 2011; Reyes-Contreras et al., 2011; Zhang et al., 2011; Hussain et al., 2012; Matamoros et al., 2012b; Matamoros and Salvadó, 2012; Reyes-Contreras et al., 2012; Zhang et al., 2012a; Zhang et al., 2012b; Zhang et al., 2012c; Ávila et al., 2013; Zhang et al., 2013a; Zhang et al., 2013c; Zhang et al., 2013b).

n.a. Not available information; ND Not detected; NR Not removed.

Table 4.3.2. Examples of occurrence and removal of EOCs in different studies conducted with distinct types of ponds, daphnia and fungal reactors at various scales.

Ponds, Daphnia reactors, Fungal reactors					
Type	Scale	System characteristics	Influent concentration ($\mu\text{g L}^{-1}$)	Removal efficiency (%)	Reference
PP	Microcosm	Dimension (L): 2.5 Type of plants: <i>Spyrogira</i> sp./ <i>Zannichellia palustris</i> Experiment duration (d): 20	200	STZ (35-36), SPY (37-45), SMZ (8-15), SMX (14-20), TC (83-89), OTC (90-93)	Garcia-Rodríguez et al. (2013)
		Dimension: n.a. Type of plants: <i>Anabaena cylindrica</i> , <i>Chlorococcus</i> , <i>Spirulina platensis</i> , <i>Chlorella</i> , <i>Scenedesmus quadricauda</i> , <i>Anabaena</i> var / <i>Lemna</i> sp. Experiment duration (d): 6	1	17-ET (>95), estrone (>95), 17 β -estradiol (>95)	Shi et al. (2010)

Table 4.3.2. Examples of occurrence and removal of EOCs in different studies conducted with distinct types of ponds, daphnia and fungal reactors at various scales (**continued**).

WSPs (AP + FP + MP)	Full	AP Surface area (m ²): 335 x 2 HRT (d): 0.4 Type of plants: n.a. FP Surface area (m ²): 8481 HRT (d): 4.1 Type of plants: n.a. MP Surface area (m ²): 3169 HRT (d): 1 Type of plants: n.a. Experiment duration (d): 30	<0.36-77.80	AP: Ketoprofen (>54), NAP (76), IBP (70), DCF (>46), salicylic acid (94), CARB (>54), CAFF (50), methyl dihydrojasmonate (59) FP: Ketoprofen (NR), NAP (79), IBP (79), DCF (>46), salicylic acid (92), CARB (>54), CAFF (62), methyl dihydrojasmonate (>60) MP: Ketoprofen (>54), NAP (85), IBP (>99), DCF (>46), salicylic acid (93), CARB (>54), CAFF (83), methyl dihydrojasmonate (>76)	Hijosa-Valsero et al. (2010a)
Aerated lagoons	Full	Dimension: n.a. Type of plants: n.a. Experiment duration (d): 30	0.02-5.44	CAFF (>99), CARB (NR), NAP (97-99), IBP (79-99), gemfibrozil (NR), triclosan (95-99), triclocarban (74-87), trimethoprim (NR-93), SMX (92-97), diphenhydramine (67-88)	Li et al. (2013)
HRAP	Mesocosm	Dimension (m): 0.4 x 0.2 x 0.3 HRT (d): 7 Type of plants: n.a. Experiment duration (d): n.a.	790	TC (60-69)	de Godos et al. (2012)

Table 4.3.2. Examples of occurrence and removal of EOCs in different studies conducted with distinct types of ponds, daphnia and fungal reactors at various scales (**continued**).

Daphnia reactors	Pilot	Dimension (m): 0.8 x 1.3 x 0.5 HRT (d): 1-4 Experiment duration (d): 30	0.02-1.25	Cashmeran (49-75), IBP (58-94), benzothiazole, 2-(methylthio)- (27-66), tributyl phosphate (10-22), methyl dihydrojasmonate (1-97), tri(2-chloroethyl)phosphate (1-5), diazinone (3-14), CAFF (19-52), galaxolide (46-89), tonalide (50-90), terbutrin (20-94), CARB (5-13), NAP (14-72), oxybenzone (51-92), triclosan (57-87), ketoprofen (81-99), DCF (36-93)	Matamoros et al. (2012c)
Fungal reactors	Bioslurry reactor	Dimension (L): 6 Type of fungus: <i>Trametes versicolor</i> Experiment duration (d): 26	0.01-0.21	Ketoprofen (50), NAP (45), IBP (92), indomethacine (62), DCF (60), mefenamic acid (41), phenazone (45), bezafibrate (45), gemfibrozil (41), atorvastatin (50), diazepam (26), CARB (31), ranitidine (100), famotidine (100), cimetidine (100), SMZ (91), SPY (100), STZ (86), atenolol (100), sotalol (100), butalbital (100), hydrochlorothiazide (83), flurosemide (65), gibenclamide (100)	Rodríguez-Rodríguez et al. (2012)
		Dimension (L): 10 Type of fungus: <i>Trametes versicolor</i> Experiment duration (d): 8	0.04-84.71	NAP (NR-100), IBP (100), ACAPh (100), salicylic acid (NR), ketoprofen (35-100), codeine (NR-100), erythromycin (NR-100), metronidazole (NR-100), ciprofloxacin (NR-35), azithromycin (NR-100), cefalexine (NR), PROPR (NR-100), CARB (NR), acridone (NR-100), citalopram (100)	Cruz-Morató et al. (2013)

(Hijosa-Valsero et al., 2010a; Shi et al., 2010; de Godos et al., 2012; Matamoros et al., 2012c; Rodríguez-Rodríguez et al., 2012; Cruz-Morató et al., 2013; Garcia-Rodríguez et al., 2013; Li et al., 2013).

n.a. Not available information; ND Not detected; NR Not removed

4.3.1 The influence of light exposure, water quality and vegetation on the removal of sulfonamides and tetracyclines: A laboratory-scale study

Overview

TCs and SAs have a continual input into ecosystems and hence they are considered as “pseudopersistent” contaminants (Seifrtová et al., 2009). TCs behave amphoterically due to their three different pKa values. TCs are ionized at environmental pHs from 3 to 8. TC solutions are quite stable at neutral and weakly acidic pH values, but unstable at alkaline pH (Reemtsma and Jekel, 2006). SAs also show amphoteric behaviour as they have two different pKa values, indicating protonation and deprotonation, depending on the pH of the aqueous solution.

The removal of these antibiotics in WWTPs depends on their physicochemical properties and on the type of the treatment employed (Massey et al., 2010). Advanced reclamation systems such as ozonization, photo-fenton and reverse osmosis are effective for removing antibiotics from secondary-treated WWs (Deng, 2009; Maroga Mboula et al., 2012). However, advanced treatment processes consume a high level of energy, require expensive installations and cannot remove antibiotics completely (Maroga Mboula et al., 2012). Biologically-based WW reclamation systems such as PPs, which are typically located after secondary WW units, may be almost as effective whilst overcoming the disadvantages of advanced treatment systems and generating an ecologically diverse water (Matamoros and Salvadó, 2012). Although this treatment has the capacity to remove certain pharmaceuticals (Hijosa-Valsero et al., 2010a; Matamoros et al., 2012b), its ability for removing antibiotics has not yet been widely studied. Furthermore, recent laboratory-scale studies have found that some pharmaceuticals such as IBP and CAFF can be eliminated by aquatic plants (e.g. *Lemna sp.* and *Ceratophyllum*) (Reinhold et al., 2010; Matamoros et al., 2012c), but the influence of vegetation on the removal of antibiotics in real treated WW has not yet been addressed.

Our aim here is to assess the capacity of two species of vegetation (*Spyrogira sp.* and *Z. palustris*) commonly found in PPs, to remove TCs (OTC and TC) and SAs (STZ, SMZ, SMX, SPY). The kinetic parameters and intermediate degradation products are also evaluated.

Results and discussion

Depletion plots

Fig. 4.3.6 and Fig. 4.3.7 show the variations of the concentrations of the selected antibiotics in water throughout the experiments. The physicochemical properties of the TCs and SAs studied here reveal that these antibiotics were present as anions at pH 8 (Fig. 1.5). Given this, sorption processes due to hydrophobic interactions with organic matter are not expected to play a significant role and it is likely that either photodegradation or biodegradation is the main processes in the reactors.

- *The effect of light-radiation and water quality on the removal of antibiotics*

Fig. 4.3.6 shows the degradation profiles of the antibiotics when two different matrices were evaluated (secondary-treated WW and UW) with or without exposure to light. The concentration of TCs declined rapidly in all uncovered reactors, as a result of their high photolability (Dürckheimer, 1975). This effect was observed in both water matrices, with TC being almost completely eliminated after 20 d (>80%) as opposed to very little removal in the case of the covered control reactors (<15%). These results generally agree with those found by Chen et al. (2008) who observed that TC photodegradation was mainly attributable to direct photolysis. The OTC was nearly totally depleted in the uncovered reactors, due to both indirect and direct photodegradation processes, whereas the depletion of OTC in the covered reactor fed with treated WW cannot be explained by photodegradation. The differences in OTC removal between the treated WW covered reactors (88%) and UW covered reactor (48%) can only be attributable to processes of biodegradation or hydrolysis. However, as TCs have been shown to be recalcitrant to biodegradation (Prado et al., 2009), hydrolysis is likely to be the most plausible as about 20% of the OTC was hydrolysed after 14 d in darkness (Pouliquen et al., 2007). Furthermore, Xuan et al. (2009) reported that hydrolysis at neutral pH appeared to be much faster than in both acidic and alkaline solutions and that OTC half-life decreased from 120 to 0.15 d when the temperature was increased from 4 to 60 °C. In the conditions of our study, pH 8 and 22 °C, hydrolysis is likely to be the most important process in OTC elimination. Significantly different results were obtained for SAs. After

20 d of incubation, similarly low levels of degradation (<20%) were observed for SMZ and SMX for both covered

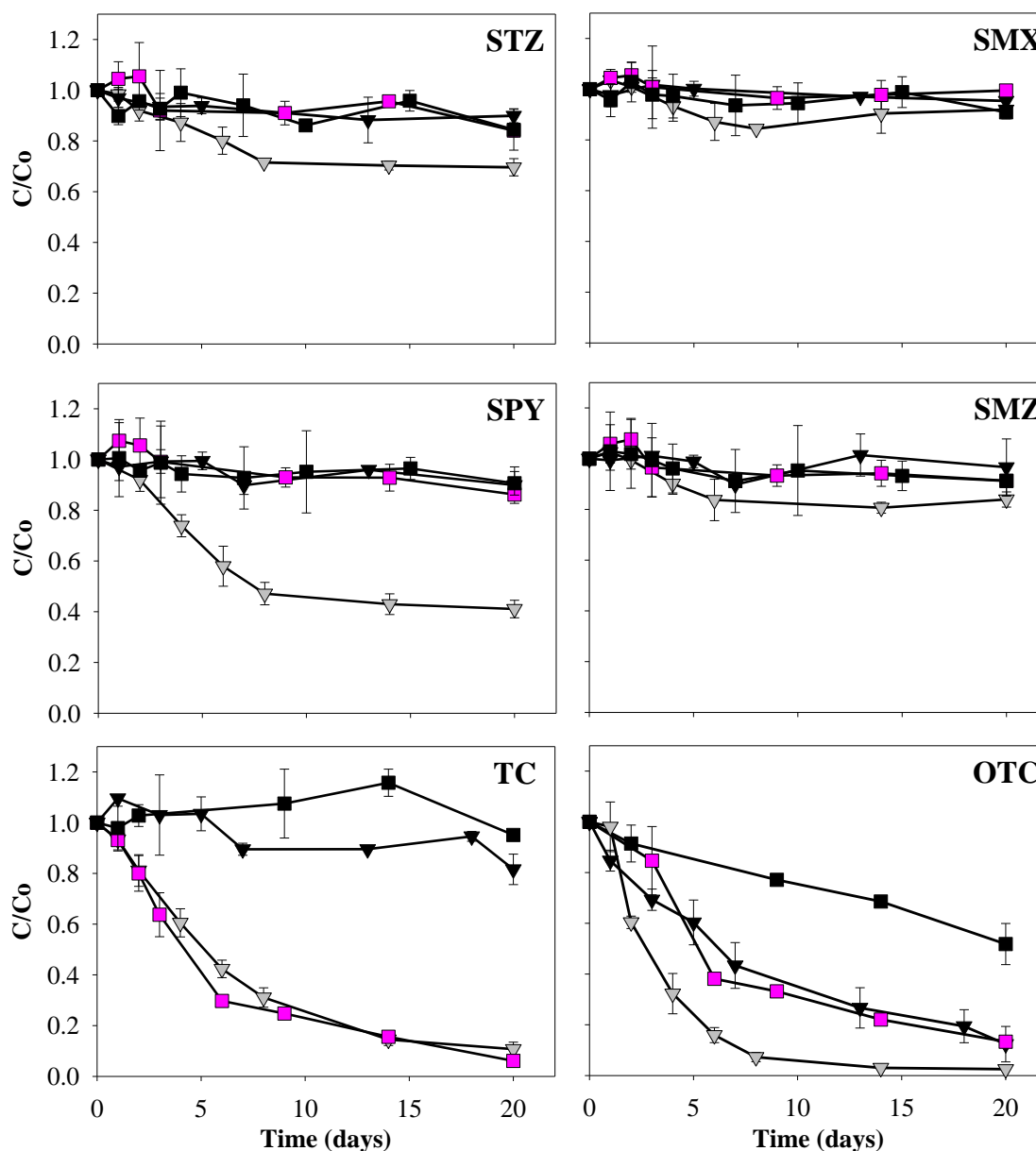


Figure 4.3.6. Decline in concentration of selected antibiotics in the covered reactors fed with secondary-treated WW (black triangles) or UW (black squares) and uncovered control reactors fed with secondary-treated WW (grey triangles) or UW (purple squares) (n=3).

and uncovered reactors. The best performance was in the case of SPY and STZ, where the elimination was more than 30% and 60% respectively in uncovered reactors fed with treated WW whereas no removal was observed in uncovered reactors fed with UW. These results suggest that biodegradation and photodegradation may have an effect in the case of these two SAs. Biodegradation due to the presence of organic matter and

bacteria in the reactors and indirect photolysis due to the adsorption of the light by other compounds present in the reclaimed water that produces reactive species and afterwards breakdown the compounds of interest. In line with the results of Baran et al. (2006), who concluded that STZ was not biodegradable but it can be photodegraded by advanced UV radiation, we hypothesize that SPY and STZ are removed by indirect photodegradation through the organic matter present in treated wastewater.

- *The effect of algae and aquatic plants on the removal of antibiotics*

Fig. 4.3.7 shows the depletion of antibiotics in reactors planted with *Z. palustris* or *Spyrogira* sp. and unplanted reactors fed with treated WW throughout the experiment. After 20 d of incubation, no significant differences in the elimination of most of the studied antibiotics were observed between planted and unplanted reactors. TCs were eliminated at between 80% and 97%, SPY and STZ at between 30% and 45%, and SMX and SMZ at <20%. Although the elimination rates were similar, they were slightly higher in unplanted reactors in the case of SPY and OTC, presumably due to the presence of aquatic vegetation attenuating the light radiation. Although the biomass weight increased from 2 g FW to up 8 g FW after 20 d, the effect of the plants (*Z. palustris* and *Spyrogira* sp.) on the elimination of antibiotics was minor. The fact that aquatic vegetation was found to play a slight role in the elimination of antibiotics may be due to the ionic form of TCs and SAs impeding their root uptake by electrical repulsions between the negative charge of the anions and the negative charged biomembrane (Trapp, 2009).

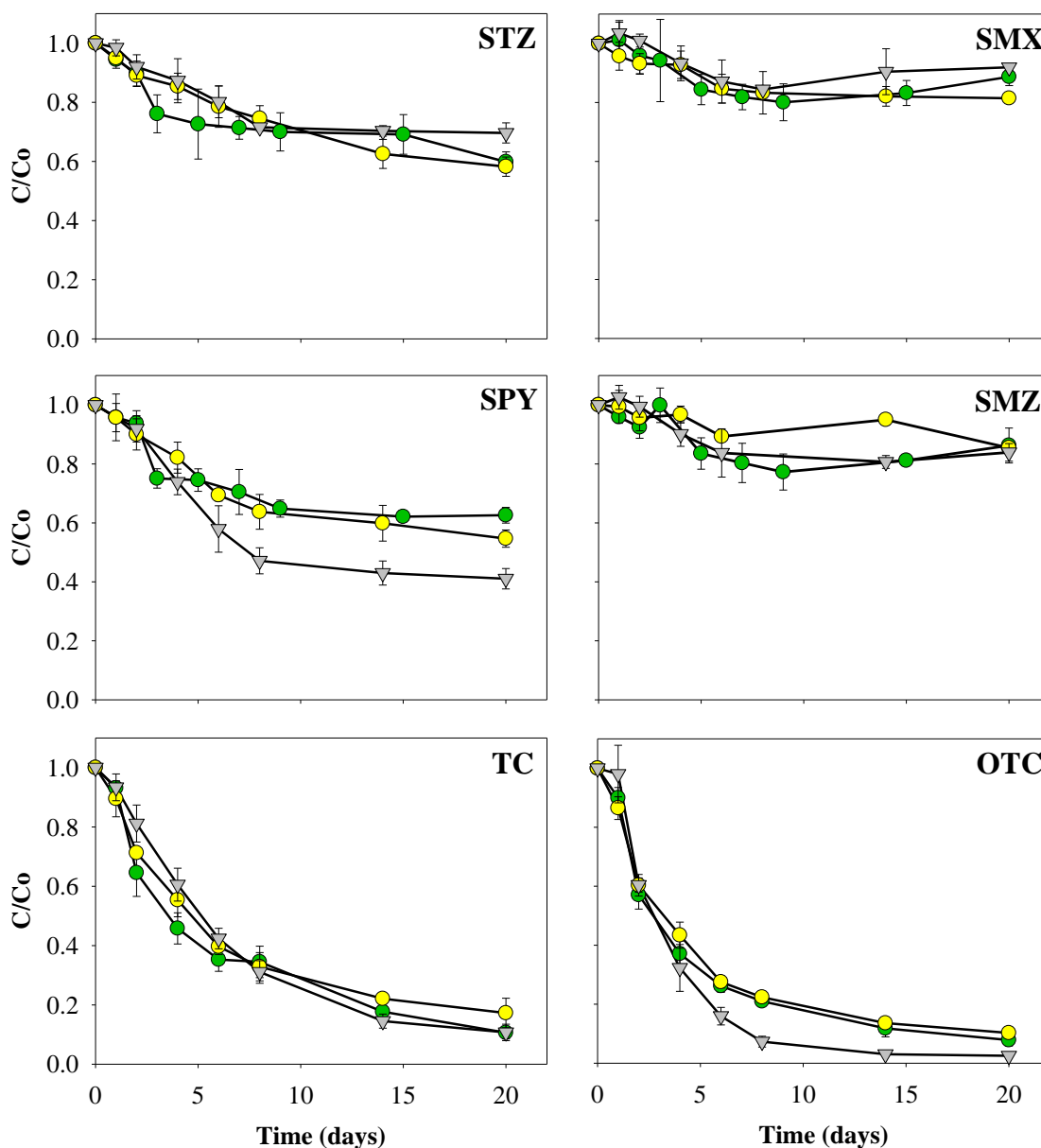


Figure 4.3.7. Decline in concentration of the selected antibiotics in the algae (green dots), *Z. Pallustris* (yellow dots) and uncovered control (grey triangles) reactors fed with secondary-treated WW (n=3).

Kinetic removal rates

The kinetic parameters were calculated from the variation of the concentration over time. The reduction in the concentration of antibiotics in water under the different experimental conditions fits well with a pseudo-first order kinetic (k) with Pearson correlation coefficients higher than 0.9 ($p < 0.05$; Table 4.3.3).

The simplified pseudo-first order equation (Eq. (4.3.1)) used in this study is as follows:

$$\ln [A] = -kt + \ln[A]_0 \quad (4.3.1)$$

where $[A]$ is the concentration of the compound at any moment of time, $[A]_0$ is the initial concentration of the compound, k is the removal rate coefficient (in units of L/time), and t is the time since the experiment started.

This good fit may be due to the significant effect of the photodegradation processes, which are generally described as pseudo-first order reactions (Matamoros et al., 2009a). Pseudo-first order removal rates were compound dependent and fluctuated between 0.003 and 0.007 for SMZ and TC in covered reactors and between 0.13 and 0.21 d⁻¹ for TC and OTC in the uncovered control reactors respectively, with half-lives ($t_{1/2}$) of between 3 and 350 d. The photodegradable compounds studied (TC and OTC) presented the lowest half-lives in all uncovered experiments ($t_{1/2} = 3\text{--}8$ d). These results are similar to those reported by Sanderson et al. (2005). Furthermore, TC removal kinetic rates were higher in uncovered reactors fed with treated wastewater with $k = 0.13$ d⁻¹ for TC and $k = 0.21$ d⁻¹ for OTC than in UW where $k = 0.10$ d⁻¹ and $k = 0.14$ d⁻¹ for TC and OTC, respectively. These findings are in agreement with the results found by López-Péñalver et al. (2010), who reported a higher photodegradation rate for OTC in real waters by indirect photolysis due to the presence of organic matter.

The half-lives of SAs ranged from 21 to 94 d in uncovered planted and unplanted reactors. Although these results differ from those found in earlier laboratory-scale studies (Lai and Hou, 2008; García-Galán et al., 2012), the fact that much higher concentrations (in the range of mg L⁻¹) and artificial sun-light devices were used makes comparison difficult.

Table 4.3.3. Pseudo-first order kinetic rate constants (k), correlation coefficients, $t_{1/2}$, and removal of selected antibiotics in the microcosm reactors.

Trade name	Reactor	k (d ⁻¹)	Pearson cor.coef.	p-value	$t_{1/2}$ (d)	Removal (%)
STZ	Covered control	0.008±0.001	0.906 ± 0.040	0.002*	88	10± 3
	Uncovered control	0.018±0.001	0.585 ± 0.378	0.160	39	30± 3
	<i>Spyrogira</i> sp.	0.021±0.004	0.808 ± 0.052	0.037*	33	36± 8
	<i>Z. palustris</i>	0.026±0.001	0.963 ± 0.009	0.001*	27	35± 12
SPY	Covered control	0.004±0.002	0.694 ± 0.395	0.355	165	9± 5
	Uncovered control	0.049±0.004	0.905 ± 0.007	0.011*	14	59± 3
	<i>Spyrogira</i> sp.	0.023±0.003	0.839 ± 0.053	0.007*	30	37± 3
	<i>Z. palustris</i>	0.032±0.002	0.924 ± 0.043	0.001*	21	45± 3
SMZ	Covered control	0.006±0.004	0.544 ± 0.182	0.243	112	10± 8
	Uncovered control	0.011±0.001	0.789 ± 0.117	0.033*	61	16± 3
	<i>Spyrogira</i> sp.	0.013±0.011	0.601 ± 0.326	0.068	53	15± 5
	<i>Z. palustris</i>	0.008±0.005	0.737 ± 0.140	0.019*	91	8± 6
SMX	Covered control	0.003±0.003	0.467 ± 0.049	0.393	350	10± 8
	Uncovered control	0.008±0.001	0.675 ± 0.258	0.210	91	3± 5
	<i>Spyrogira</i> sp.	0.007±0.001	0.540 ± 0.089	0.312	94	14± 5
	<i>Z. palustris</i>	0.016±0.011	0.823 ± 0.105	0.011*	44	20± 2
TC	Covered control	0.007±0.001	0.698 ± 0.005	0.124	94	15± 8
	Uncovered control	0.126±0.015	0.988 ± 0.006	0.000*	5	89± 3
	<i>Spyrogira</i> sp.	0.113±0.013	0.978 ± 0.010	0.000*	6	89± 2
	<i>Z. palustris</i>	0.095±0.011	0.968 ± 0.013	0.000*	7	83± 5
OTC	Covered control	0.090±0.021	0.990 ± 0.008	0.000*	8	88± 7
	Uncovered control	0.213±0.006	0.955 ± 0.010	0.000*	3	97± 0
	<i>Spyrogira</i> sp.	0.139±0.018	0.975 ± 0.009	0.000*	5	93± 2
	<i>Z. palustris</i>	0.121±0.005	0.960 ± 0.008	0.000*	6	90± 1

* Statistically significant correlation (p<0.05).

Identification of intermediate degradation products

Comparison of the chromatograms obtained by LC-MS/MS in full-scan mode from all collected water samples from the algae reactor fed with WW led to the identification of one intermediate degradation product whose peak overlapped that of OTC. Published mass spectra seem to identify this compound as a TC degradation product after a photocatalytic process or oxidation process in the presence of MnO_2 (Chen and Huang, 2011; Maroga Mboula et al., 2012), but the chemical structure of this intermediate, with a reported MW of 458, remains unknown. Fig. 4.3.8 shows the formation and degradation of the intermediate degradation product throughout the experiment in the algae reactor.

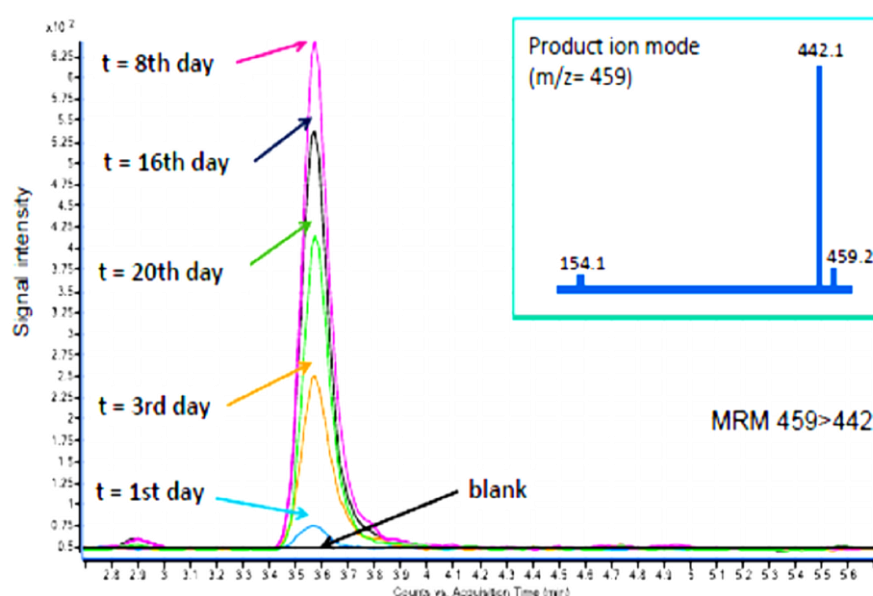


Figure 4.3.8. Chromatograms of a tetracycline intermediate degradation product tentatively identified in the algae reactors. Ion product spectrum of the m/z 459 at 135 V fragmentor is shown.

The TC degradation product peaked at day 8 and decreased drastically afterwards, presumably because of the high photodegradation rate of this compound. The presence of this intermediate product in algae reactors suggests that photodegradation was the most significant removal pathway for TC in PPs.

From our studies we saw that TC is mainly removed by photodegradation, OTC is removed by photodegradation, biodegradation or hydrolysis, SPY and STZ are removed by biodegradation or indirect photodegradation processes, and SMZ and SMX are not effectively removed. k for TCs and SAs ranged from 0.007 to 0.21 d⁻¹ and 0.003 to 0.049 d⁻¹ with $t_{1/2}$ from 3 to 94 d and from 14 to 350 d, respectively. Photodegradable compounds are eliminated at higher rates in uncovered reactors fed with treated WW due to indirect photodegradation. The elimination rates obtained in planted reactors are similar to those obtained in uncovered and unplanted reactors, which leads us to conclude that the presence of aquatic vegetation such as *Spyrogira* sp. and *Z. palustris* has no significant influence on the removal of TCs and SAs. A TC intermediate photodegradation product was tentatively identified in the uncovered reactor suggesting that photodegradation is the removal pathway for TC. In conclusion, this study highlighted that the use of PPs with large surface areas exposed to significant amounts of sun-light radiation may effectively remove TCs and SAs from secondary-treated WW.

4.3.2 The influence of *Lemna* sp. and *Spirogyra* sp. on the removal of pharmaceuticals and endocrine disruptors in treated wastewaters

Overview

Biologically-based WW reclamation systems have proven to be almost as effective as advanced treatment systems and to be effective for the removing certain pharmaceuticals (Hijosa-Valsero et al., 2010a; Matamoros et al., 2012c; Petrović et al., 2014). As described, a variety of physical, chemical and biological processes are associated with the removal of pharmaceuticals and EDCs in this type of WW treatment (White et al., 2006; Matamoros et al., 2008b; Imfeld et al., 2009).

These processes, which can occur concurrently, depend on the physicochemical properties of the compound to be eliminated and on the internal plant metabolism when organic pollutants are uptaken by plants. In the latter case, a serial chain of biochemical reactions can occur, including the transformation of parent pollutants, the conjugation of metabolites with macromolecules, and the incorporation of conjugated products into cell walls and vacuoles (Pilon-Smits, 2005; Reinhold et al., 2010). Recent laboratory-scale studies carried out using UW or tap water have found that aquatic plants (e.g. *Lemna* sp. and *Ceratophyllum* sp.) can enhance the removal of pharmaceuticals such as IBP and CAFF (Reinhold et al., 2010; Matamoros et al., 2012b), whereas in the case of DCF, SAs and TCs they are mainly removed by photodegradation (Andreozzi et al., 2003; Garcia-Rodríguez et al., 2013).

However, little attention has been paid neither to the effect of vegetation in the removal of other microcontaminants with different physicochemical properties nor to the combined role of vegetation, organic matter, microbial communities, and sunlight exposure in the removal of microcontaminants by PPs. In the case of plants, there is also a lack of information on the differences between using superior rooted aquatic plants, such as *Lemna* sp., and filamentous algae, such as *Spirogyra* sp.

The aim of this study is to assess the capacity of PPs to remove six pharmaceuticals (DCF, ACAPh, IBP, CARB, CLF AC and PROPR), two EDCs (17 α -17-ET and BPA) and one stimulant (CAFF). To this end, a laboratory-scale study was performed using different reactors, some containing aquatic vegetation (*Lemna* sp. and *Spirogyra* sp.).

As well as evaluating the removal capability of each reactor, the study identifies the different elimination processes taking place. The compounds were selected according to their ubiquity in WWTP effluents and different physicochemical properties (Table 1.5). The study was carried out at the research laboratory of the University of Girona (Spain) from September to December 2011.

Results and discussion

Behaviour of the selected microcontaminants

The variation in the concentration of the selected pharmaceuticals and EDCs in water throughout the experiments described in chapter 3 (page 73) can be seen in Figs. 4.3.9, 4.3.10, and 4.3.11. Compounds were grouped according to their removal efficiencies in uncovered planted or unplanted reactors fed with secondary-treated WW. They were classified as follows, highly degradable compounds (CAFF and ACAPh) when removal efficiencies were around 100 % in <10 d, moderately degradable compounds (BPA, 17-ET, IBP, and PROPR) when removal efficiencies ranged from 88 to 100 % after 20 incubation d, and poorly degradable compounds (DCF, CLF AC, and CARB) when removal efficiencies ranged from 20 to 41 % in 20 d. Afterwards, the results obtained for each of these groups were evaluated taking into account different parameters, such as the physicochemical properties of the compounds (e.g. $\log K_{ow}$, and pK_a (Table 1.5)), water composition (UW and secondary-treated WW), light effect (differences between the degradation profiles obtained from the covered and uncovered experiments fed with treated WW or UW) and plant effect (degradation profiles obtained from planted and unplanted reactors fed with secondary-treated WW).

Finally, the main processes affecting the removal of microcontaminants — biodegradation, photodegradation (direct or indirect), and plant uptake— are identified for each group, although it must be remembered that different removal processes can occur simultaneously in biologically-based wastewater treatment systems (Macek et al. 2000; Zhang et al. 2010).

- Highly degradable compounds

Fig. 4.3.9 (a) and (b) show that after 10 d of incubation, almost complete elimination was obtained for both ACAPh and CAFF in all reactors filled with secondary-treated WW. Moreover, the reactors containing *Lemna* sp. and *Spirogyra* sp. presented

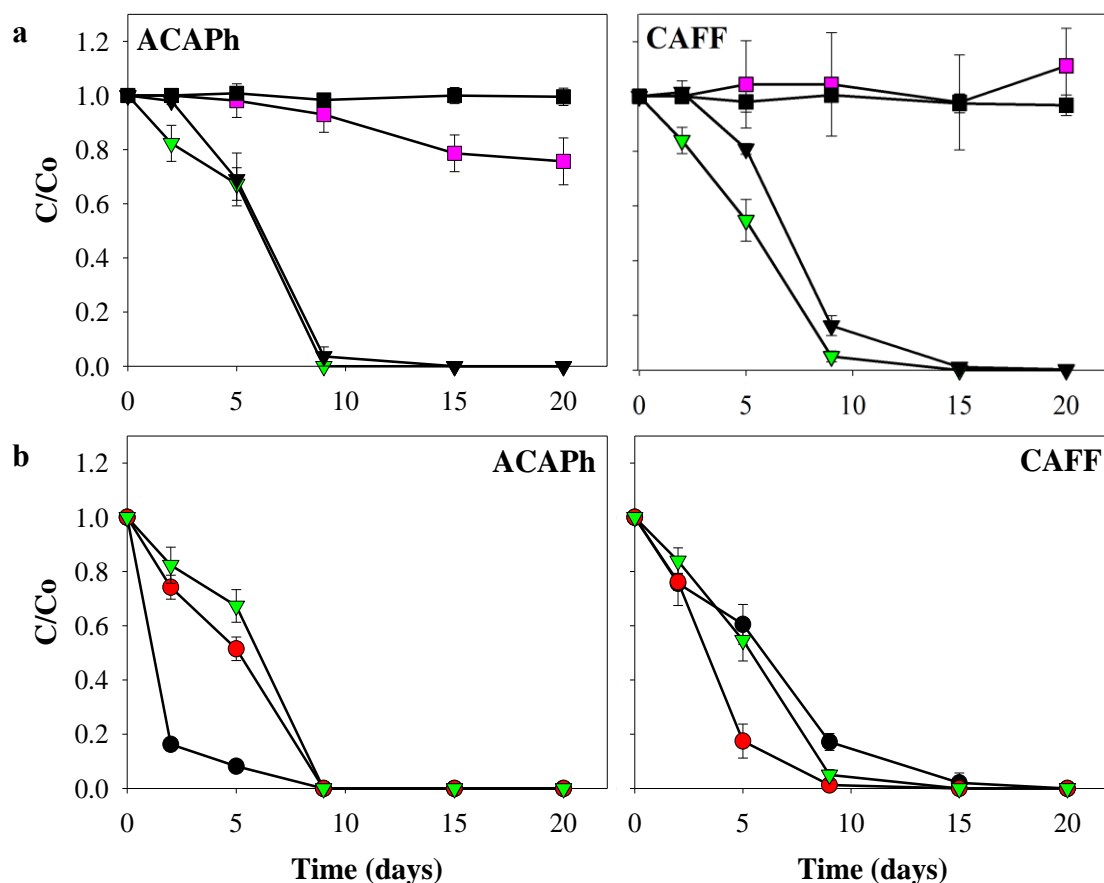


Figure 4.3.9. Decline concentration of highly degradable compounds in the (a) covered control reactors fed with secondary-treated WW (inverted filled black triangles) or UW (filled black squares) and uncovered control reactors fed with secondary-treated WW (inverted filled green triangles) or UW (filled purple squares). (b) *Lemna* sp. (filled red circles), *Spirogyra* sp. (filled red circles), and uncovered control reactors fed with secondary-treated WW (inverted filled green triangles).

the fastest elimination rates resulting in efficiencies of 84 ± 1 % in the removal of ACAPh after 2 d of incubation and of 83 ± 8 % in the removal of CAFF after 5 d of incubation. However, in reactors filled with UW, elimination was much lower (<25 % after 20 d). Therefore, biodegradation and removal due to the presence of plants can be considered as the main processes involved in the elimination of ACAPh and CAFF. The

presence of bacteria and organic matter in secondary-treated WW can enhance biodegradation of compounds such as ACAPh and CAFF, whose high biodegradation rates in surface waters and WW have been reported in different studies (Conkle et al., 2008; Zhang et al., 2012a). Moreover, the presence of plants, producing exudates and containing microorganisms on their surfaces, aids the removal of these compounds through both biodegradation and plant uptake (Dordio et al., 2010; Reinhold et al., 2010). These results agree with those found by Matamoros et al. (2012c) for CAFF in laboratory assays using hydroponic cultures as well as those obtained by Zhang et al. (2012b, 2013c) in a mesocosm study planted with *Scirpus* sp., in which removals of >85 % were achieved. The high polarity and lack of charge at environmental pH of both ACAPh and CAFF explain their uptake by aquatic plants (Trapp, 2009). Zhang et al. (2013c) also observed that non-charged compounds, such as CAFF, were easily incorporated by *Scirpus validus*, an aquatic plant. Nevertheless, in comparison with previous laboratory studies with synthetic river water, the effect of vegetation on the removal of CAFF was much lower (Matamoros et al., 2012b). This can be explained by the differences in the water composition used in the different studies, as the content of organic matter and bacteria is, in general, higher in secondary-treated WW than surface water.

- Moderately degradable compounds

Fig. 4.3.10 (a) and b show significant concentration declines for BPA, 17-ET, IBP, and PROPR in the uncovered reactors fed with secondary-treated WW. BPA, 17-ET and PROPR were removed more efficiently in planted than unplanted reactors during the first 10 d, with removal efficiencies ranging from 68 to 95 %. However, in the covered control reactors filled with the same type of water, the percentages of elimination were lower (<34 %). No elimination was observed in either covered or uncovered control reactors filled with UW. Based on these findings, it can be deduced that the presence of microorganisms, organic matter, nitrates, and other matrix components in the secondary-treated WW favours the removal of these compounds by indirect photodegradation or biodegradation, as has previously been reported by White et al. (2006) in CWs. Moreover, as stated by Ávila et al. (2013) and Kumar et al. (2011), in the presence of adequate photosensitizers, such as bacteria or plant exudates, BPA, 17-

ET, IBP, and PROPR can be biodegraded or spontaneously photodegraded in natural waters, corroborating the high removal rates obtained in our study.

The differences observed between planted and unplanted reactors may be explained by indirect effects such as the presence of plant exudates and microbial activity associated with the biofilm development on the plants' surface or by the direct uptake of these compounds by the plants (Matamoros et al., 2012b; Matamoros et al., 2012c). Moreover, as *Spirogyra* sp. grew in the uncovered reactors filled with secondary-treated WW 10–15 d after starting the experiment, this plant may contribute to increasing the removal of contaminants, resulting in similar levels of elimination in both planted and unplanted reactors. In a laboratory-scale study, Shi et al. (2010) obtained removal efficiencies of >80 % for 17-ET when duckweed or algae was present in the water. Kumar et al. (2011) also obtained removal efficiencies of >90 % in a microcosm of a surface flow constructed wetland containing different type of plants. Ávila et al. (2010), when evaluating the capacity of horizontal flow constructed wetlands for the removal of emerging organic contaminants, achieved removals ranging from 85 to 99 % for BPA and IBP, similar to the results obtained by Li et al. (2013) in a lagoon system where removals of 79–99 % of IBP were obtained. These results suggest that non-charged compounds with a log K_{ow} of between 3 and 4 (BPA, 17-ET, and PROP) are consistently uptaken by plants (Deegan et al., 2011), as it has been previously postulated by different authors for other compounds with similar log K_{ow} (Tsao, 2003; Pilon-Smits, 2005). Moreover, sorption into solid particles must also be taken into account. The concentration decline observed in the different type of reactors for IBP, an ionic compound at environmental pH conditions, cannot be mainly associated to plant uptake as the electrical repulsion between the negative charge of anions and the negative charge of the biomembrane does not permit the plant uptake of ionized compounds (Trapp, 2009). Therefore, indirect photodegradation and biodegradation are considered to be the main removal processes for IBP, whereas plant uptake and sorption on the surface of the vegetation and into solid particles are for non-ionized compounds such as BPA, 17-ET, and PROP (log K_{ow} = 3–4).

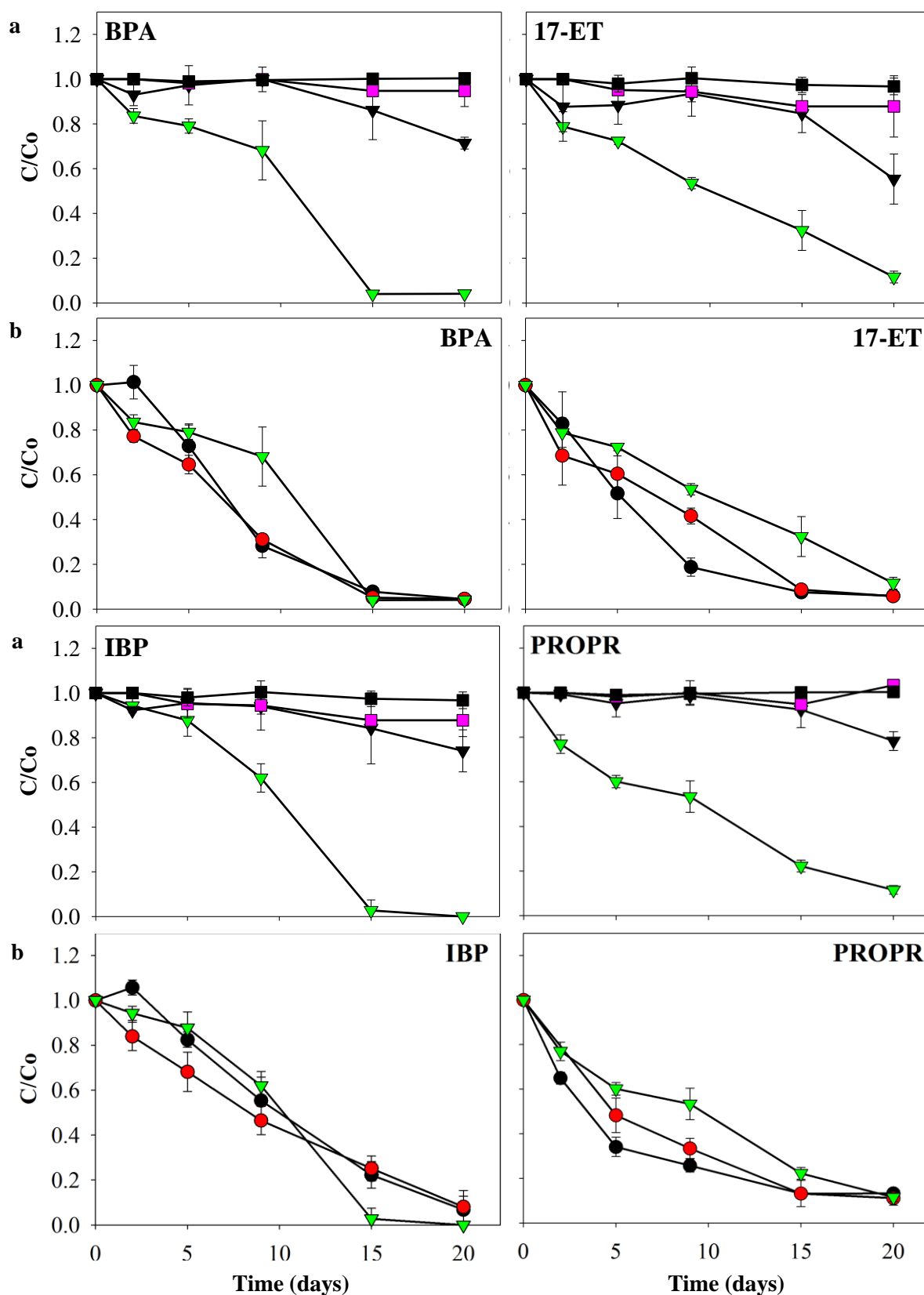


Figure 4.3.10. Decline concentration of moderately degradable compounds in the (a) covered control reactors fed with secondary-treated WW (inverted filled black triangles) or UW (filled black squares) and uncovered control reactors fed with secondary-treated WW (inverted filled green triangles) or UW (filled purple squares). (b) *Lemna* sp. (filled black circles), *Spirogyra* sp. (filled red circles), and uncovered control reactors fed with secondary-treated WW (inverted filled green triangles).

- Poorly degradable compounds

DCF, CLF AC, and CARB were the compounds found to have been least removed after the 20 experimental d with final removal rates of <45 %. No significant differences were observed between the reactors compared with any of the studied compounds (Fig. 4.3.11(a,b)) except for DCF and CARB in uncovered and planted reactors, respectively, where the removal efficiencies were greater. The removal efficiencies obtained agree with the reported recalcitrance of these compounds (Matamoros and Salvadó, 2012; Zhang et al., 2012b). However, as stated by Andreozzi et al. (2003), when some microcontaminants, such as DCF, are exposed to sunlight, they are photodegraded either by direct or indirect mechanisms, which is consistent with the higher removal efficiency observed for DCF in uncovered, light-exposed reactors. Moreover, some studies have reported the influence of sunlight radiation on the removal of DCF in natural lakes and biologically-based reclamation plants (Matamoros et al., 2012c).

The results obtained for CARB and CLF AC agree with their already stated high recalcitrance to biodegradation and photodegradation in surface waters, conventional activated sludge WWTPs and PPs (Heberer, 2002; Tixier et al., 2003; Moldovan et al., 2009; Hijosa-Valsero et al., 2010a; Matamoros and Salvadó, 2012). Finally, the greater elimination of CARB (71 ± 4 % in 2 d) in reactors planted with *Lemna* sp. can be explained by the fact that this plant has roots that may facilitate its uptake ($\log K_{ow} = 2.45$). Conversely, plant uptake does not seem to be relevant for CLF AC, an ionic compound in environmental pH conditions (pK_a 3.2). Dordio et al. (2010, 2011a) also obtained high removals of CARB (88–97 %) in the presence of rooted plants (*Typha* sp.) in a microcosm study and Zhang et al. (2013b) reported that CARB was easily incorporated to *S. validus*, an aquatic plant.

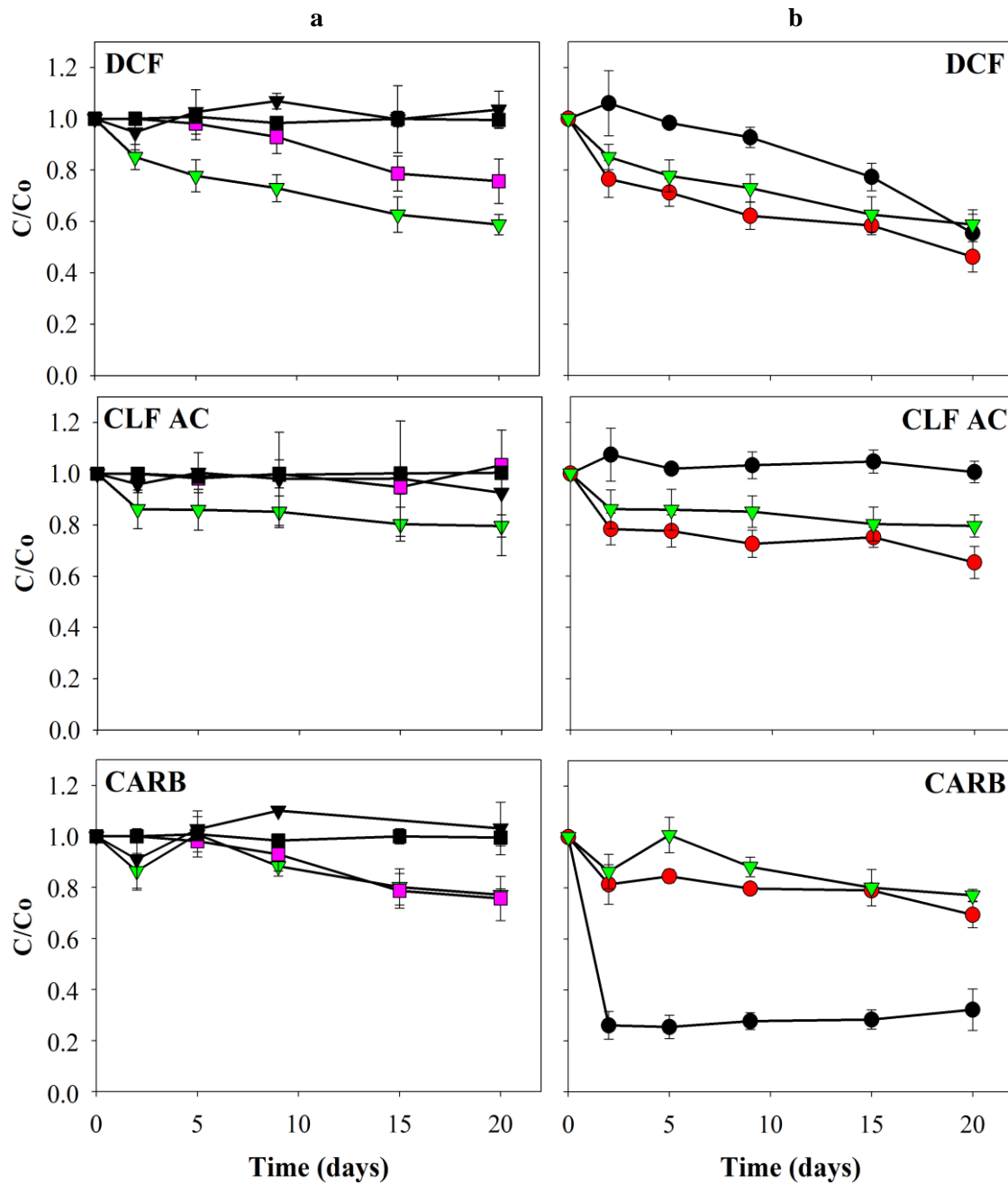


Figure 4.3.11. Decline concentration of poorly degradable compounds in the (a) covered control reactors fed with secondary-treated WW (inverted filled black triangles) or WW (filled black squares) and uncovered control reactors fed with secondary-treated WW (inverted filled green triangles) or UW (filled purple squares). (b) *Lemna* sp. (filled black circles), *Spirogyra* sp. (filled red circles), and uncovered control reactors fed with secondary-treated WW (inverted filled green triangles).

Kinetics removal rates

Table 4.3.4 shows the kinetic parameters obtained for the different experiments. The decay of the concentration of studied compounds in water under the different experimental conditions fits well with a pseudo-first order kinetic, which should be related to the effect of biodegradation and photodegradation processes, generally described as first order reactions (Matamoros et al., 2009a).

k were compound dependent and ranged from <0.001 to 0.503 day^{-1} in reactors filled with secondary-treated WW and from <0.001 to 0.017 day^{-1} in reactors filled with UW. These latest reactors were those which presented higher values of $t_{1/2}$ for all the studied compounds (45 to >800 d). The lowest values were obtained for reactors fed with secondary-treated WW, whereas the greatest were for the control covered reactors, indicating that photodegradation had a large influence on compounds removal.

The uncovered reactors fed with secondary-treated WW showed the highest removal for CAFF, IBP and ACAPh ($>99\%$ after 20 incubation d), presenting k values from 0.049 to 0.503 d^{-1} and $t_{1/2}$ between 1 and 9 d. The values for CAFF and IBP calculated in this study were higher than those previously found in a laboratory-scale study with *Lemna* sp. incubated under synthetic surface water (0.18 and 0.02 d^{-1} , respectively) (Matamoros et al., 2012b). In the present study, the composition of the secondary-treated WW substantially enhanced the IBP k values. $t_{1/2}$ for BPA, 17-ET, IBP, and PROPR ranged from 4 to 14 d. In general, highest removal rates were obtained in uncovered reactors, ranging from 0.050 to 0.181 d^{-1} for both IBP and BPA in unplanted uncovered reactors. The removal of these compounds is explained by biodegradation and indirect photodegradation processes taking place in the reactor. The k obtained are similar to those reported in CWs for the removal of these compounds (Ávila et al., 2010; Hijosa-Valsero et al., 2010a; Kiran Kumar et al., 2011).

Recalcitrant compounds DCF, CLF AC and CARB showed the lowest removal efficiencies, as photodegradation, biodegradation, and plant uptake do not seem to be significant processes in their elimination (Conkle et al., 2008; Matamoros and Salvadó, 2012), and CLF AC was the most recalcitrant compound according to the $t_{1/2}$ (50 to >800 d). Nevertheless, it is worth mentioning that the k values of CLF AC seem to be affected by the presence of plants. In the case of CARB, the lowest $t_{1/2}$ was achieved in

reactors containing the rooted superior plant (*Lemna* sp., $t_{1/2} = 20$ d) as has been discussed above (Dordio et al., 2011).

Table 4.3.4. Kinetic parameters (k and $t_{1/2}$), correlation coefficients and percentage of removal of selected microcontaminants in the microcosms reactors.

Trade name	Type of water	Reactor	k (d ⁻¹)	Pearson cor.coef.	p-value	$t_{1/2}$ (d)	Removal (%)
CARB	Secondary-treated WW	Covered control	0.006±0.001	0.674	0.109	118	19±12
		Uncovered control	0.011±0.002	0.880	0.008	62	23±7
		<i>Spirogyra</i> sp	0.013±0.004	0.799	0.041	58	31±5
		<i>Lemna</i> sp	0.035±0.001	0.534	0.272	20	72±1
	UW	Covered control	< 0,001	-	-	>800	nr
		Uncovered control	< 0,001	-	-	>800	nr
CAFF	Secondary-treated WW	Covered control	0.300±0.010	0.405	0.008	2	99±1
		Uncovered control	0.337±0.042	0.931	0.013	2	99±1
		<i>Spirogyra</i> sp	0.503±0.006	0.979	0.047	1	99±1
		<i>Lemna</i> sp	0.185±0.016	0.950	0.003	4	99±1
	UW	Covered control	< 0.001	-	-	>800	nr
		Uncovered control	0.010±0.000	0.925	0.005	67	16±1
ACAPh	Secondary-treated WW	Covered control	0.076±0.025	0.938	0.014	10	99±1
		Uncovered control	0.079±0.017	0.991	0.017	9	99±1
		<i>Spirogyra</i> sp	0.132±0.017	0.996	0.019	5	99±1
		<i>Lemna</i> sp	0.479±0.026	0.931	0.642	1	99±1
	UW	Covered control	< 0.001	-	-	>800	nr
		Uncovered control	0.008±0.002	0.639	0.049	86	24±7
PROPR	Secondary-treated WW	Covered control	0.011±0.002	0.806	0.074	67	22±4
		Uncovered control	0.093±0.010	0.984	0.001	7	88±2
		<i>Spirogyra</i> sp	0.120±0.022	0.937	0.049	7	89±3
		<i>Lemna</i> sp	0.130±0.032	0.946	0.032	8	87±2
	UW	Covered control	< 0.001	-	-	>800	nr
		Uncovered control	0.003±0.002	0,636	0.151	300	7±2
IBP	Secondary-treated WW	Covered control	0.009±0,002	0.570	0.036	78	38±26
		Uncovered control	0.049±0.008	0.926	0,002	14	99±1
		<i>Spirogyra</i> sp	0.099±0.008	0.982	<0.001	7	92±7
		<i>Lemna</i> sp	0.109±0.008	0.972	<0.001	6	93±6
	UW	Covered control	< 0.001	-	-	>800	nr
		Uncovered control	< 0.001	-	-	>800	nr

Table 4.3.4. Kinetic parameters (k and $t_{1/2}$), correlation coefficients and percentage of removal of selected microcontaminants in the microcosms reactors (**continued**).

DCF	Secondary-treated WW	Covered control	< 0.001	-	-	>800	nr
		Uncovered control	0.024±0.003	0.962	0.004	29	41±4
		<i>Spirogyra</i> sp	0.032±0.004	0.940	0.013	22	54±6
		<i>Lemna</i> sp	0.029±0.004	0.948	0.001	24	48±9
	UW	Covered control	< 0.001	-	-	>800	nr
		Uncovered control	0.017±0.006	0.919	0.002	45	24±9
CLF AC	Secondary-treated WW	Covered control	< 0.001	-	-	>800	nr
		Uncovered control	0.009±0.001	0.818	0.056	80	20±4
		<i>Spirogyra</i> sp	0.015±0.002	0.806	0.063	48	35±6
		<i>Lemna</i> sp	< 0.001	-	-	>800	nr
	UW	Covered control	< 0.001	-	-	>800	nr
		Uncovered control	< 0.001	-	-	>800	nr
BPA	Secondary-treated WW	Covered control	0.014±0.002	0.795	0.101	50	29±3
		Uncovered control	0.181±0.012	0.960	0.001	4	96±1
		<i>Spirogyra</i> sp	0.174±0.002	0.973	0.002	4	95±1
		<i>Lemna</i> sp	0.167±0.003	0.988	0.002	4	96±1
	UW	Covered control	< 0.001	-	-	>800	nr
		Uncovered control	< 0.001	-	-	>800	nr
17-ET	Secondary-treated WW	Covered control	0.046±0.023	0.722	0.008	18	45±11
		Uncovered control	0.099±0.016	0.949	<0.001	7	88±3
		<i>Spirogyra</i> sp	0.151±0.003	0.984	0.003	5	94±1
		<i>Lemna</i> sp	0.155±0.006	0.980	0.009	4	94±1
	UW	Covered control	< 0.001	-	-	>800	nr
		Uncovered control	< 0.001	-	-	>800	nr

nr - no removal

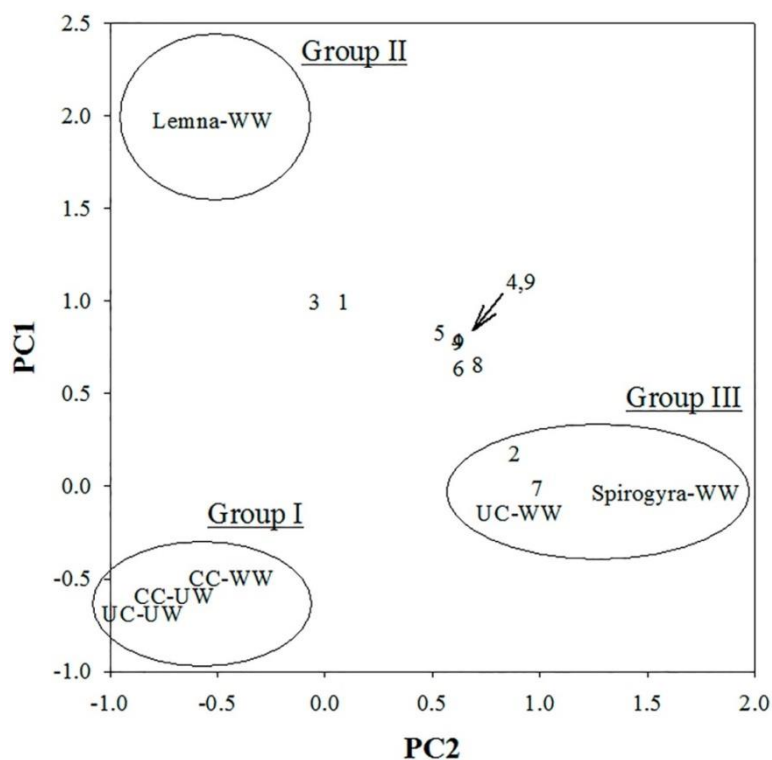
PCA

A PCA was performed using the whole data set in order to deepen our understanding of the main processes involved in the removal of selected contaminants in aquatic systems. The PCA reduced the nine measured variables to two principal components with eigenvalues >1, which explain 93 % of the variability of the system. The first principal component (PC1) had positive loadings (>0.9) for CARB (1) and ACAPh (3), which presented the highest removal rates in *Lemna* sp. reactors fed with secondary-treated wastewater. The second component (PC2) had high positive values for CAFF (2) and CLF AC (7), which had the highest removal rates in *Spirogyra* sp. reactors fed with

secondary-treated wastewater. The positive values of both PC1 and PC2 are associated with the greater removal efficiency that is achieved when aquatic plants are present in the reactors. The other compounds, 4, 5, 6, 8, and 9, had similarly positive values for both PC1 and PC2, indicating that the two plant species had similar effects on their removal rates.

Fig. 4.3.12 is the scores plot for PC1 versus PC2. The six different experimental conditions were grouped into three clusters depending on the influence of the vegetation. Group I, which presented low values for both PC1 and PC2, consisted of covered control reactors fed with secondary-treated wastewater and the covered and uncovered control reactors fed with ultrapure water. No vegetation effect was observed in these reactors. Group II corresponds to the *Lemna* sp. reactors and finally Group III, which presented high positive values for PC2, was made up of *Spirogyra* sp. reactors and uncovered control reactors fed with secondary-treated wastewater. As has been mentioned above, the growth of *Spirogyra* sp. in the unplanted reactors explains their proximity to the group formed by reactors planted with algae.

Figure 4.3.12. Score plot of principal component 1 versus component 2 separating the reactor assays in three groups, namely the no vegetation effect (group I), the *Lemna* sp. Effect (group II), and the *Spirogyra* sp. Effect (group III). Loadings are also presented (1-CARB, 2-CAFF, 3-ACAPh, 4-PROPR, 5-IBP, 6-DCF, 7-CLF AC, 8-BPA, 9-17-ET). CC control covered, UC uncovered control, WW secondary-treated wastewater, UW ultrapure water.



In this research, we have grouped the studied compounds according to their removal efficiencies in uncovered reactors filled with secondary-treated WW after 20 incubation d: highly degradable compounds (CAFF and ACAPh) when removal efficiencies were near to 100 % in <10 d, moderately degradable compounds (BPA, 17-ET, IBP, and PROPR) when removal efficiencies ranged from 88 to 100 % after 20 d, and poorly degradable compounds (DCF, CLF AC, and CARB) when removal efficiencies ranged from 20 to 41 % after 20 d. Pseudo-first order removal rates ranged from 0.001 to 0.503 d⁻¹, with half-lives between 2 and >800 d. Finally, a PCA was successfully employed for the determination of the specific positive effect of *Lemna* sp. on the removal rate of CARB and ACAPh, as well as the effectiveness of algae on CAFF and CLF AC removal. Furthermore, this study has demonstrated that non-charged compounds with a log Kow between 2 and 4 (BPA, 17-ET, CARB and PROP) were affected by the presence of vegetation, probably due to their plant uptake, whereas negatively charged compounds (IBP, DCF, and CLF AC) were not. Hence, we can conclude that the presence of aquatic plants can play an important role in the removal efficiency of pharmaceuticals and EDCs from PPs and that the selection of the most appropriate plant species should be made in function of the compounds that are to be eliminated.

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CHAPTER 5

General discussion and future perspectives

Nowadays, because of the water cycle, large amounts of highly consumed compounds (e.g. pharmaceuticals and EDCs) reach WWTPs, where they can be distributed, depending on their physicochemical properties, between the aqueous phase (treated wastewater) and the solid phase (sewage sludge/biosolid). Usually, the techniques applied in WWTPs are not able to completely remove them, contributing to the release of EOCs into natural water bodies. As stated in previous chapters of this thesis, the presence of these contaminants in the environment is of concern and it is necessary to develop strategies for their analysis and monitoring, and to study their most likely attenuation routes during wastewater treatment and in the environment. In this thesis, diverse studies have been carried out to endeavor improvement, novelty, and knowledge in the preceding topics.

5.1 Determination of pharmaceutical compounds in sewage sludge using a standard addition approach

Sample preparation is a relevant step in the appropriate determination of EOCs, as it directly affects the quality of the obtained data on the sample analysis. The development of robust, simple, and low cost methods will help ease sample analysis when large numbers of samples need to be handled.

In this work, a standard addition approach has been used for the determination of pharmaceuticals in sewage sludge/biosolid samples from different WWTPs. As stated in Chapter 4, the sample matrix is a relevant parameter to consider when choosing a sample preparation method, as it is usually quite complex and may contain interferences that negatively influence the determination of the desired compounds. We have developed a simple and robust method for the determination of three NSAIDs, one lipid regulator and four antibiotics by applying the standard addition approach and using USE followed by LC-MS/MS. Our analytical method has avoided the laborious clean-up step, the use of multiple and expensive ILCs, and the complex task of finding an uncontaminated similar sample matrix. Recoveries greater than 80% were obtained. The method avoided typical electrospray ionization problems in mass spectrometry by reducing the amount of sample to be extracted. In this case we used 50 mg of dry

weight sewage sludge/biosolid, whereas typical methods based on ILCs use 1-5g (Nieto Cebrián, 2010). By reducing the amount of sample, we reduce the matrix extracted and interferences in MS. Although we used an MS detector, the standard addition approach allows the use of low cost detectors such as UV or DAD. This may considerably decrease the total cost of the analytical method.

5.2 Innovative analytical method based on PIMs for the determination of antibiotics

Antibiotics are present in the environment at very low concentrations and commonly sample treatment and pre-concentration techniques are applied to facilitate their determination. Moreover, to better study the impact of these compounds in the environment, special devices must be developed to be used as passive samplers. In our studies, we have explored the possibility of using polymer inclusion membranes for these purposes.

The target compounds were six antibiotics (four SAs and two TCs), and based on their physico-chemical properties, it was decided to use the commercial ionic liquid Aliquat 336 (an ammonium quaternary salt) as a carrier. The basis of the extraction is an anion exchange between the chloride of the Aliquat 336 and the antibiotics, present as anions at basic pH. To ensure maximum extraction efficiency, different PIMs were prepared by varying the polymer (CTA or PVC) and the plasticizer (NPOE, DBS, or BBPA).

PIM performance could be thought to only depend on carrier selection based on compound characteristics, but we observed that PIM characteristics also rely on the nature and concentration of all its components. Both polymers tested in this work provided membranes with high mechanical strength. After a 24h experiment using PIMs with similar Aliquat 336 content (25 wt% in CTA-based PIM and 31 wt% in PVC-based PIM), without plasticizers, only some of the compounds initially present at 5 mg L⁻¹ at pH 9 in the feed phase were slightly transported to the stripping phase (consisting of 1 mol L⁻¹ NaCl solution). The addition of NPOE in the PIM composition improved membrane fluidity and elasticity, which in turns increased its transport efficiency. It was discovered out that a PIM consisting of 30 wt% CTA, 26 wt% Aliquat 336, and 44 wt%

NPOE allowed the quantitative transport of TC, OTC, and SMX, and good transport values for STZ, SPY and SMZ.

The effect on pH of the feed phase was also investigated due to the strong pH-dependence of both SAs and TCs, which affects their interaction with the anion exchanger carrier. As expected, in general, higher transport efficiencies were observed when working with feed solutions at pH 9 rather than at pH 7, except for SMX, for which similar results were obtained at both tested pH levels. This behaviour can be explained because SMX is the most acidic compound among all those studied, and, pH 7 is enough to ensure the formation of anionic species. Based on these promising results, and taking into account that SMX is commonly detected in streams and groundwater, it was decided to explore the possibility of designing a passive sampler device incorporating a PIM for the monitoring of SMX in environmental matrices, which will be discussed later.

The fact that for the first time a PIM system was developed for the transport of different antibiotics shows the relevance of our studies. Moreover, it is important to highlight that both the complexity on the interactions between all PIM components, and the affinity of the target compounds for the PIM, makes the design of an appropriate PIM a tough task that must be studied systematically.

Due to the low concentration at which antibiotics are found in the aqueous media, we thought about the importance of pre-concentration to facilitate compounds determination. Thus, for the first time we incorporated the designed PIM in a special device that allowed using larger S phase volumes and small R phase volumes, extracting and pre-concentrating antibiotics at the same time. In these experiments the concentration of each antibiotic in the feed solution (120 mL at pH 9) was decreased to $100\ \mu\text{g L}^{-1}$, and 4 mL of a $1\ \text{mol L}^{-1}$ NaCl solution were used as a stripping phase.

After a 24h experiment the six studied antibiotics were successfully pre-concentrated, in particular SMX. To better investigate the parameters affecting the system, we evaluated different receiving solutions (NaCl and acetic acid solutions). Those consisting of 0.5 and $1\ \text{mol L}^{-1}$ NaCl were the most successful, as they contained enough Cl^- to avoid the reverse transport of antibiotics to the source solution.

Afterwards, the effect of the water matrix was investigated using two different environmental water samples (spiked at a known concentration) that were in contact

with the PIM-based device for 48h. From our results, comparable pre-concentration factors were obtained for each compound, independently of the water composition. These results were of great interest, since they highlighted the great affinity of the organic compounds for the PIM despite the non-selectivity of the carrier used, since their transport was not affected by the presence of other anions contained in the water.

Based on these encouraging results, we thought about the applicability of the PIM-based device not only as a laboratory pre-concentration method for antibiotics, but also as a passive sampler in natural waters. Taking into account that the pH of natural waters is around 7 and that the transport of SMX was not affected by pH, as previously seen, this sulfonamide was chosen as the model compound of the presence of sulfonamide antibiotics in waters. SMX is one of the most frequently reported sulfonamide antibiotics in WWTP effluents and environmental waters.

Using the PIM-based device several parameters were investigated, with special emphasis on the influence of the passive sampler design and the flow pattern of the source solution.

To ensure maximum passive sampler efficiency a PIM with the same composition as that used for the transport of antibiotics in our previous studies was finally fixed (30 wt% CTA, 26 wt% Aliquat 336, and 44 wt% NPOE). As a stripping phase, a 2 mol L⁻¹ NaCl solution was used to ensure a high Cl⁻ concentration that would guarantee the needed chloride concentration gradient.

Diverse water samples with different chemical compositions were spiked at a known concentration of SMX. After a 24h contact of the PIM-based device, no significant differences in the SMX concentrations were observed in the final receiving solutions, despite the divergent water compositions. This is a promising result considering our aim of using the proposed device for SMX monitoring in the environment. Moreover, the effect of SMX content on the transport efficiency was tested using two different water samples spiked at various SMX concentrations. After 24h of device exposure, a linear relationship was observed between the original SMX concentration in water and the antibiotic concentration in the receiving solution, with only slight differences between the two tested waters. Since a passive sampler calibration is usually conducted under controlled laboratory conditions prior to field application, and based on the obtained results, we believe that it would be possible to apply a constructed calibration curve for

similar types of natural waters. However, further studies should be conducted with an extensive range of water types to confirm this promising finding.

Passive sampling design is also an important factor of study. We evaluated two different passive sampling calibration approaches: “dip-in” and “flow-through”. Our results showed that the source solution flow rate and mixing were important parameters to be considered in the design of the passive sampler, as they significantly influenced its performance. As environmental waters are subjected to flow and mixing variability, an alternative design was proposed to minimize the above-mentioned effects, ensuring a SMX transport mechanism in the bulk solution only based on diffusion. Our results demonstrated the promising performance of the new device configuration, as after conducting different “dip-in” experiments to study the new configuration and the mixing effect, even though the new device configuration prompted to less SMX accumulation at the receiving solution, it was less sensitive to the flow and mixing effect.

Apart from the evaluated parameters, others such as the source solution temperature and the increase of the PIM area in the device would be interesting to study, as they are supposed to have a relevant effect on the compounds’ transport rates through the PIM. Moreover, it would also be interesting to consider the applicability of the proposed device to monitor other antibiotics.

PIMs are simple to prepare, require few reagents, and can virtually extract anything with the right carrier and chemical conditions. As stated, we believe that PIMs can play an important role in the future as a new passive sampling technique. This will reduce the number of analytical steps involved when compared with the most common passive sampling techniques available nowadays for the monitoring of EOCs, such as POCIS, DGTs, or Quechers. They are based on solid absorbents in which the compounds of interest interact, but an elution step is always needed prior to compound determination in the laboratory. Thus, the use of PIM-based devices can facilitate the sample treatment process, as they allow a direct analysis of the receiving phase, granting at the same time the acquisition of time-weighted average (TWA) concentrations of the studied compounds.

In addition, PIMs are much cheaper than the solid sorbents used in the most common passive samplers. Furthermore, the proposed devices are small, reusable and cheap, and

by simply changing the PIM composition, the proposed passive sampler can be applied to monitor the EOCs, making it a versatile tool for passive sampling monitoring.

On the other hand, it is very important to optimize PIM composition and to control the loss of the membrane extractant/carrier (usually occurring during the first exposure hours in the field).

Thus, it is important to control and fix the parameters involved in PIM synthesis (e.g. compound solubility and mixing, environmental temperature and humidity) to ensure good reproducibility and repeatability of the passive sampling device.

In the future, it would be interesting to compare the effectiveness of some of the commonly used passive samplers for EOCs (e.g. POCIS and DGTs) with the PIM-passive sampling based devices (i.e. extraction efficiency, time involved on the analysis, reusability of the passive sampling and costs involved).

5.3 The ability of phytotechnologies to remove EOCs from wastewater

There are several studies based on biologically-based WW treatment systems (e.g. CWs or PPs). Most of them are focused on the effectiveness of EOC removal, but few evaluate how the different processes occurring in this type of treatment (e.g. plant uptake, photodegradation, biodegradation) contribute to compound removal. Given the importance of this knowledge in designing an effective biologically-based WW treatment system, we carried out two studies to assess how different processes likely to occur in PPs (i.e. photodegradation, biodegradation, and plant uptake) contribute on the removal of a varied group of EOCs in WWs. By designing diverse experiments, it was possible to determine the relevance of different processes taking place in this type of natural treatment.

TCs, both TC and OTC, were mainly degraded by a photodegradation processes, although the contribution of biodegradation should also be considered for OTC. Observing compound concentration decline over time, the highest concentration decrease was observed in directly light exposed reactors filled with the two types of water studied (i.e. UW and secondary-treated WW) in comparison to the non-exposed

ones. The presence of plants was found not to be influential. This was confirmed by the tentative identification of a TC phototransformation product.

The results of our study demonstrate that biodegradation is the main removal process for the attenuation of CAFF and ACAPh. No removal was observed in those reactors without microbiological activity and no differences were observed between light exposed and non-exposed light reactors.

17-ET, BPA, IBP, PROPR, SPY and STZ were considered as photodegradable and biodegradable. Only light exposed reactors containing water with microbiological activity and wastewater constituents allowed us to see a clear removal tendency over days, indicating biodegradation and also an indirect photodegradation effect. The presence of vegetation enhanced the removal rate of most of the EOCs studied. This effect was studied by using the following equation:

Vegetation effect = $(k_{\text{vegetated reactor}} - k_{\text{uncovered control reactor}}) / k_{\text{uncovered control reactor}}$ where k is the kinetic removal rate calculated in the Chapter 6 for each of the compounds studied.

Therefore, the vegetation effect on the kinetic removal rates was observed for the following compounds: ACAPh (*Lemna* sp. 500%, *Spirogyra* sp. 67%) and CAFF (*Lemna* sp. -47%, *Spirogyra* sp. 49%), 17-ET (*Lemna* sp. 57%, *Spirogyra* sp. 52%), PROPR (*Lemna* sp. 40%, *Spirogyra* sp. 29%), and STZ (*Spirogyra* sp. 17%, *Z. pallustris* 44%).

CLF AC, DCF, CARB, SMZ and SMX were classified as recalcitrant, as practically no removal was observed in any of the designed experiments (maximum removals lower than 40% after 20 incubation days). Plant effect was considered not to be an important factor in most compounds' removal.

Our results show that water quality is a relevant factor for removing EOCs. Therefore, the larger the concentration of nutrients and bacteria is, the greater is the efficiency of EOCs removal. This means that biodegradation due to the presence of bacteria and therefore co-metabolism, seems to play an important role in the removal of EOCs, as well as the effect of indirect photodegradation.

Even though the main removal driving forces identified in the studies were biodegradation and photodegradation, it also would have been very interesting to

determine the concentration of EOCs in plant tissues, at least for those compounds in which a plant effect was viewed to have contributed to EOCs removal. This effect can be attributed to direct plant uptake or to the release of plant exudates that would enhance compound biodegradation. Therefore, because of the lack of plant tissue analysis, any of these hypotheses could be disregarded.

Furthermore, we observed that removal rates are compound dependant. Thus, considering the total experimental time of twenty days, compounds can be divided into three groups:

Highly removed compounds (i.e. CAFF, ACAPh, TC, OTC, IBP, PROPR, 17-ET, and BPA), presented values of near 100% removal after the total experimental time and half time life ($t_{1/2}$) between 1 and 14 d in light exposed reactors containing secondary-treated WW and aquatic plants. Some of them (i.e. CAFF, 17-ET, IBP) affected by the presence of plants, while others (i.e. TC and BPA) were not.

In the same experimental conditions, moderately removed compounds (i.e. SPY, DCF, and STZ), with removal rates between 60% and 30% after twenty days, presented $t_{1/2}$ ranging from 14 to 39. Finally, poorly removed compounds (i.e. CLF AC, CARB, SMZ, and SMX) showed $t_{1/2}$ from 20 to 80 and removal rates lower than 35%.

$t_{1/2}$ can provide useful information for the design of biologically-based WW technologies by increasing their HRT up to the desired effectiveness. This is normally done to achieve a final concentration at the water effluent that is not harmful to the ecosystem.

Our results demonstrate that this type of WW treatment is an emerging cost-effective technology for wastewater remediation to be used in WWTPs. Our studies allow us to better understand how a variety of EOCs can be removed through different processes in biologically-based WW treatment systems. The fate of EOCs in these systems is highly dependent on their physicochemical properties, such as their hydrophobicity ($\log K_{ow}$) and ionization state (pK_a), even though this information is not relevant enough to predict which process (e.g. photodegradation, biodegradation, plant uptake) will remove EOCs, as there is some controversy about it. It is not obvious which physicochemical parameter dominates the compounds' removal behaviour.

Our work has been carried out at laboratory scale under controlled conditions. It would be very interesting to develop the same type of study, but with a wider variety of secondary-treated WWs, at a greater scale, and reproducing diverse climate conditions, as all the removal effects mentioned above are highly influenced by a variety of parameters such as water composition, sunlight radiation and temperature.

Biologically-based WW treatment systems are a promising alternative to other existing technologies such as advanced treatment systems (oxidation and membrane based technologies). Although advanced systems are usually highly efficient at removing recalcitrant compounds such as CARB, they consume a great amount of energy, require expensive installations (to build and maintain), and cannot completely remove all the EOCs. Natural treatment systems allow obtaining ecologically richer water and can be almost as effective as advanced treatment systems while avoiding their disadvantages. The idea behind the use of these systems is to provide a water effluent with the same ecological characteristics as the surface water into which the treated water will finally be discharged. Nevertheless, biologically-based treatment technologies still need to address some drawbacks: large surface areas are required, certain compounds have low removal rates and, depending on climate conditions, the rates of EOC removal effectiveness vary.

A combination of different biologically-based WW treatment systems based on a variety of configurations of multistep CWs and/or ponds (i.e. hybrid systems) would remove nutrients efficiently and would facilitate the removal of EOCs (Reyes-Contreras et al., 2011). Moreover, these treatment systems can also be combined with other technologies such as conventional activated sludge WWTPs or advanced oxidation technologies in order to increase the overall removal efficiency of EOCs (Badawy et al., 2009; Verlicchi et al., 2013). In addition, there are other interesting compact biologically-based technologies, such as microalgae or fungal reactors, may have very promising futures and will need further evaluation. Furthermore, the ability of willow systems, a wastewater treatment technology that has a zero discharge of effluent, to remove EOCs and their possible plant uptake has not yet been explored.

Finally, only a few (<30) of the hundreds of EOCs described in the literature have been assessed in biologically-based WW treatment systems. Also, information about the behaviour of EOCs transformation products in these systems is lacking, which is relevant since recent studies have reported that some of them can be more toxic than

parental compounds. Future studies in biologically-based treatment systems will therefore need to address the assessment of new EOCs and their transformation products as well as their toxicity at different trophic levels.

5.4 Future perspectives

Future applications of the studies carried out in this thesis include, for example, the use of the developed analytical method (Chapter 4) for the determination of EOCs in biosolids intended for crop production. This, in combination with plant tissue analysis, would allow the plant uptake rate of different EOCs to be determined. However, because crops need to be irrigated, it would also be interesting to monitor the presence of EOCs in the reclaimed water used for this purpose. Therefore, after an appropriate laboratory calibration, the developed PIM-based passive samplers could be settled in the last step of the wastewater treatment process, where reclaimed water is intended for reuse. Moreover, due to the small size of the PIM-based passive samplers, they could be settled in different reactors containing different water matrix compositions, different aquatic plants and exposed to a variety of environmental conditions, to determine the removal of EOCs over a fixed period of time. Therefore, they may be used to monitor EOCs in real biologically-based WW treatment systems, which could be used as a system to attenuate the occurrence of EOCs in reclaimed water before its use in agricultural irrigation.

To go further into the results found as a result of the laboratory experiments mentioned in Chapter 6 a comparative study regarding the identification and quantification of TPs of EOCs between biologically-based WW treatments and advanced wastewater treatments may help to clarify which kind of treatments produce less toxic TPs.

Even though the occurrence of EOCs in environmental samples remains an unresolved issue, we must be more critical about the necessity of the need to completely remove these compounds. Although current analytical methods detect concentrations below one part per trillion in water, the question remains: What is the relevance of the presence of these compounds at such a low level in the ecosystem? While there is evidence that compounds such as 17-ET cause feminization of male fish at such levels, most of the

EOCs do not. All of us may therefore need to reflect about what we should do as researchers and what society needs. In some cases it might be better to provide a water effluent with high ecological biodiversity than to remove EOCs. In others, depending on the final use of the reclaimed water, we may need to completely remove EOCs. In these cases advanced physicochemical technologies such as oxidation or membrane based treatments will be needed. Therefore, decision makers may need to assess the water quality needed and choose from among the best available water reclamation technologies (biologically-based or advanced physicochemical-based technology) or in some special cases a combination of both.

CHAPTER 6

Conclusions

The general main conclusions extracted from the research conducted in this thesis are summarised as follows:

- A reliable, simple-to-use and robust analytical methodology based on ultrasound-assisted extraction and LC-MS/MS with standard addition calibration has been successfully developed for the determination of eight pharmaceuticals in biosolid and sludge samples from WWTPs.
- A membrane-based separation device, incorporating a polymer inclusion membrane (PIM) consisting of 30% CTA, 26% Aliquat 336, and 44% NPOE, has allowed the transport and simultaneous preconcentration of six organic compounds belonging to two widely used families of antibiotics, SAs and TCs from waters at basic pH to a 0.1M NaCl solution.
- A new passive sampler incorporating the developed PIM has been prepared and tested for the monitoring of SMX. A proper design of the device has been achieved to avoid the effect of flow pattern of the source solution on the performance of the passive sampler, ensuring that SMX mass transfer towards the PIM was based on diffusion, and, thus, removing the major uncertainty in passive sampling caused by flow pattern of the source solution (aquatic system).
- Biologically-base wastewater treatment systems tested at laboratory-scale are capable of effectively removing tetracycline compounds ($t_{1/2}$ =5-7 day), but not SA compounds ($t_{1/2}$ =20-350 day).
- Direct and indirect photodegradation are the main driven process for the removal of TCs and SAs antibiotics in PPs, whereas the presence of aquatic vegetation (*Spyrogira* sp. and *Z. pallustris*) has no significant influence.
- Biologically-based WW treatment systems tested at laboratory-scale area capable of nearly completely removing CAFF and ACAPh (99% removal after 10 incubation days, $t_{1/2}$ =1 day) due to biodegradation processes. BPA, 17-ET, IBP, and PROPR were removed from 88 to 99 % after 20 days with a $t_{1/2}$ = 4-7days whereas CLF AC, DCF, CARB, SMZ and SMX were poorly removed ($t_{1/2}$ >20 day).

- Non-charged compounds with a log K_{ow} between 0 and 4 (ACAPh, CAFF, 17-ET, CARB and PROPR) are affected by the presence of vegetation (*Lemna* sp, *Spyrogira* sp.), probably due to their plant uptake or biodegradation enhancement due to the release of plant exudates, whereas negatively charged compounds (IBP, DCF, and CLF AC) are not.

CHAPTER 7

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