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2019

Environment International 127 (2019) 558–572

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Stereoisomeric profiling of chiral pharmaceutically active compounds in wastewaters and the receiving environment – A catchment-scale and a laboratory study

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ARTICLE INFO

Handling Editor: Olga-Ioanna Kalantzi

Keywords:
Enantioselectivity
Chiral
Microcosm
Activated sludge
River water

ABSTRACT

Chiral pharmaceutically active compounds (cPACs) are not currently governed by environmental regulation yet are expected to be in the future. As cPACs can exert stereospecific toxicity in the aquatic environment, it is essential to better understand their stereoselective behaviour here. Therefore, this study aims to provide a new perspective towards comprehensive evaluation of cPACs at a river catchment level, including their stereochemistry as a chemical phenomenon driving fate of chiral molecules in the environment. A large spatial and temporal monitoring program was performed in Southwest England. It included 5 sewage treatment works and the receiving waters of the largest river catchment in Southwest England. Simultaneously, lab-scale microcosm studies in simulated activated sludge bioreactors and river water microcosm were performed to evaluate stereoselective degradation of cPACs. A multi-residue enantioselective method allowed the analysis of a total of 18 pairs of enantiomers and 3 single enantiomers in wastewater and river water samples.

Our monitoring program revealed: (1) spatial and temporal variations of cPACs in influent wastewaters resulting from different patterns of usage as well as an (2) enantiomeric enrichment of cPACs, likely due to human metabolism, despite their commercialization as racemic mixtures. A similar chiral signature was observed in effluent and receiving waters. Stereoselective degradation was observed in trickling filters (TF) for naproxen, ketoprofen, cetirizine and 10,11-dihydroxy-10-hydroxycarbamazepine, in sequencing batch reactors (SBR) for ifosfamide and in activated sludge (AS) for cetirizine. The extent of enantiomer-specific fate was wastewater treatment dependent in the case of naproxen (TF showed higher stereoselectivity than AS and SBR) and cetirizine (TF and AS showed higher stereoselectivity than SBR) due to differing microbial population. Furthermore, stereoselective degradation of naproxen was highly variable among STWs using similar treatments (TF) and operating in the same region. Microbial stereoselective degradation was also confirmed by both activated and river water simulated microcosm for chloramphenicol, ketoprofen, indoprofen, naproxen and 10,11-dihydroxy-10-hydroxycarbamazepine. Results from our large scale river catchment monitoring study and lab simulated microcosm show wide-ranging implications of enantiomerism of cPACs on environmental risk assessment (ERA). As two enantiomers of the same compound show different biological effects (e.g. toxicity), their non-racemic presence in the environment might lead to inaccurate ERA. This is because current ERA approaches do not require analysis at enantiomeric level.

1. Introduction

Substantial attention has been given to the presence of pharmaceutically active compounds (PACs) in environmental matrices because of their ubiquity and potential ecological risks (Cizmas et al., 2015; Ebele et al., 2017). However, there is a limited understanding of the environmental fate and effects of chiral PACs (cPACs) at the enantiomeric level mainly due to the absence of adequate enantioselective

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https://doi.org/10.1016/j.envint.2019.03.050
Received 6 January 2019; Received in revised form 3 March 2019; Accepted 21 March 2019

analytical methods (Camacho-Muñoz et al., 2016a; Kasprzyk-Hordern, 2010; Sanganyado et al., 2017; Zhou et al., 2018). Enantiomers of cPACs often exhibit different pharmacokinetics and pharmacodynamics that can result in enantiomer dependent toxicity, due to the inherent stereospecificity of biological processes. For example, the therapeutic effect of non-steroidal anti-inflammatory drugs resides almost exclusively in the S-enantiomer (S-(+)-ibuprofen is 110 times more active than R-(−)-ibuprofen (Adams et al., 1976; Villaneuva et al., 1993)) whereas the R-enantiomer are partly active, inactive or toxic (naproxen is prescribed as S-(+)-naproxen because the R-enantiomer is suspected to be a liver toxin (Harrington and Lodewijk, 1997)); they can also be enantiomer-specific towards aquatic organisms (R-(−)-fluoxetine is 30 times more toxic to the protozoan *T. thermophila* and more harmful to the algae *P. subcapitata* than S-(+) fluoxetine whereas S-(+)-fluoxetine is 10 times more toxic to the fish *P. promelas* than its antipode (Andrés-Costa et al., 2017; De Andrés et al., 2009). In spite of this well-established enantiomer-specific toxicity, many cPACs are commercialized as an equal mixture of both enantiomers known as a racemic mixture and indicated by an enantiomeric fraction (EF) of 0.5.

Little is known about the enantiomeric compositions and fate of cPACs in the aquatic environment. cPACs are continuously excreted or disposed into the sewer systems as the unaltered parent compound or as metabolites in racemic or non-racemic mixtures. Sewage treatment works (STWs) are designed to reduce loads of nutrients and organic matter but not to remove cPACs, making them a major point-source of pollution in many aquatic systems (Archer et al., 2017; Camacho-Muñoz et al., 2016a; Camacho-Muñoz et al., 2016b; Duan et al., 2018; Evans et al., 2017; Sanganyado et al., 2017). Moreover, removal efficiencies depend on a high number of variables such as the wastewater treatment process, operational variables of the STW, physicochemical properties of the cPAC and/or meteorological conditions.

Growing evidence has demonstrated the stereoselective behaviours of cPACs in wastewater, sludge and river water systems (Camacho-Muñoz and Kasprzyk-Hordern, 2015; Camacho-Muñoz et al., 2016b; Evans et al., 2017; Kasprzyk-Hordern and Baker, 2012). During wastewater treatment and in the receiving aquatic environment, cPACs undergo a series of physical (e.g., dilution, volatilization, and sorption), chemical (e.g., hydrolysis, photolysis, sorption, and oxidation) and biological (e.g., biodegradation and biotransformation) attenuation processes. Whereas abiotic processes such as volatilization or photodegradation are non-enantioselective, biotic processes such as metabolism and microbial degradation may alter the EF due to selective transformations (Baker and Kasprzyk-Hordern, 2013; Kasprzyk-Hordern, 2010; Kasprzyk-Hordern and Baker, 2012; MacLeod et al., 2007; MacLeod and Wong, 2010; Nikolai et al., 2006; Ribeiro et al., 2012). These changes in the EF can provide insights of the compound's history, as well as pointing to the nature and sources of environmental pollution (Camacho-Muñoz et al., 2016a; Petrie et al., 2015). For example, since biodegradation can be enantioselective, EF has been proposed as an innovative tool for in situ assessment of biodegradation processes in the environment or to distinguish between direct or indirect disposal (Bagnall et al., 2013; Camacho-Muñoz et al., 2016a; Escuder-Gilabert et al., 2018; Hashim et al., 2011; Maia et al., 2017; Petrie et al., 2015). Furthermore, the development of appropriate environmental legislation is governed by having robust data sets on the occurrence and fate of cPACs in the environment. As toxicity of cPACs in the environment can be stereospecific, it is essential to better understand the enantiomeric composition and behaviour of cPACs in the environment. This will enable more accurate environmental risk assessment to be undertaken as current guidelines do not consider stereochemistry (Directive 2013/39/EU).

This study aims to provide a new perspective towards comprehensive evaluation of cPACs at a river catchment level, including their stereochemistry as a chemical phenomenon driving the fate of chiral molecules in the environment. Therefore, we designed and conducted a field study which involved spatial and temporal sampling campaigns along a river catchment that covers an area of approximately 2000 km^2 in Southwest England. Spatial and temporal composite sampling campaigns in 5 STWs, with different wastewater treatments, that account for 75% of contributing population (≈1.5 million) in the catchment. The objectives of this study were: (i) to investigate the fate and behaviour of cPACs during wastewater treatment; and (ii) to assess the impact of effluent discharges to river quality. In addition, evidence suggests that stereoselectivity is biological in nature; therefore research in this area is crucial to get a better understanding of environmental impacts resulting from the stereoselective disposition of cPACs in any biodegradation pathway that could lead to a significant under or overestimation of the risks posed by these compounds. Consequently, we performed controlled lab-scale microcosm studies: (iii) to evaluate the stereoselective degradation of a wide group of cPACs in river water and activated sludge simulating microcosm experiments under different experimental conditions (biotic, abiotic, light and dark conditions); (iv) to investigate if EF can be used to distinguish between different natural attenuation processes; and (v) to provide insights into biodegradation pathways and the capacity of receiving waters to deal with those cPACs.

2. Experimental

2.1. Chemicals and reagents

Selection of cPACs was based on their high usage, occurrence and ubiquity in the environment and possibility of chiral inversion during biotic processes. HPLC-grade acetonitrile (≥99.9%), methanol (≥99.9%), ammonium acetate (≥99.0%), formic acid (≥96.0%) and sodium azide were supplied by Sigma-Aldrich. Ultrapure water was obtained from a water purification system (MilliQ system, UK). R/S(±)-aminorex, caffeine, R/S(±)-carboxyibuprofen (mixture of diastereomers), 6R,7R,2R-cephalexin, S-(+)-*O*-desmethylnaproxen, 1R,2S-(−)-florfenicol, R/S(±)-2-hydroxyibuprofen, R/S(±)-ibuprofen, R/S(±)-ifosfamide, R/S(±)-indoprofen, R/S(±)-ketoprofen, S-(+)-ketoprofen, R/S(±)-mandelic acid, R/S(±)-2-phenylpropionic acid, R/S(±)-praziquantel, R/S(±)-tetramisole hydrochloride, S-(−)-tetramisole hydrochloride (known as levamisole) and 1R,2R-(−)-chloramphenicol were purchased from Sigma-Aldrich (Gillingham, UK). 6R,7R,2S-cephalexin, R/S(±)-3-N-dechloroethylifosfamide, R/S(±)-dihydroketoprofen (mixture of diastereomers), R/S(±)-naproxen and S-(+)-naproxen were obtained from Toronto Research Chemicals Inc. (Ontario, Canada). R/S(±)-10,11-dihydro-10-hydroxycarbamazepine, R/S(±)-fexofenadine hydrochloride, R/S(±)-cetirizine dihydrochloride and 1S,2S-(+)-chloramphenicol were supplied by LGC Standards (Teddington, UK). 1R,2R-(−)-chloramphenicol base and S-(+)-ibuprofen were purchased from Fisher. Chemical structures, and physicochemical properties of the studied compounds can be found in Camacho-Muñoz and Kasprzyk-Hordern (2015).

Surrogate/internal standards (IS): (±)-chloramphenicol-d_5, R/S(±)-ibuprofen-d_3, R/S(±)-ifosfamide-d_4, R/S(±)-ketoprofen-d_3, R/S(±)-naproxen-d_3, R/S(±)-praziquantel-(cyclohexyl-d_{11}) and R/S(±)-tetramisole-d_5 hydrochloride were purchased from LGC Standards (Teddington, UK) and Sigma-Aldrich (Gillingham, UK).

All standards were of high purity grade. Stock solutions of each compound (1 mg mL^{-1}) were prepared in methanol and stored at −18 °C. Working solutions were prepared by diluting stock solution in methanol and stored at −18 °C. All glassware was deactivated with dimethylchlorosilane (5% DMDCS in toluene, Sigma-Aldrich). Oasis HLB (3 cm^3, 60 mg) and MAX (3 cm^3, 60 mg) cartridges were purchased from Waters (Milford, MA, USA).

2.2. The catchment sampling campaign

The study area is located in the Southwest of the UK. The river catchment covers an area of approximately 2000 km^2 (Fig. S1). The population within the catchment is ~1.5 million. The sampling campaign was conducted in 2015 from June to October. Care was taken to

avoid days in which heavy rainfall occurred to avoid losses due to overflow. At each site, sampling was conducted for 7 consecutive days running from Wednesday to Tuesday. Wastewater was collected as volume proportional 24 h composites (80 mL every 15 min, 4 °C) and pooled after 24 h from five major STWs (named STWs A–E) receiving wastewater from 75% of population. STW A uses conventional activated sludge (AS), STW B–D uses tricking filter beds (TF) and STW E uses sequencing batch reactors (SBR). Influent wastewater was collected between screening and primary sedimentation. Grab activated sludge samples were collected from STW E. River waters (8 L) were collected as grab samples upstream and downstream of each effluent discharge point at varying distances depending on accessibility. Downstream samples were collected at a minimum of 0.5 km below the effluent discharge point (Table S1), except in the case of STW A which was taken close to the effluent discharge point due to no suitable access point. STW A is the site studied furthest up the river catchment and is in a rural location. River water was not collected for site E as the STW discharges directly to the estuary. All samples were then returned to the laboratory on ice to maintain a temperature of approximately 4 °C and processed immediately upon arrival to the laboratory. ISs were added before samples were filtered through 0.7-μm glass fiber filters (Whatman, UK).

2.3. Microcosm bioreactors

2.3.1. Sample collection

Samples were collected as discussed in Section 2.2 in February 2015. Microcosm bioreactors were set up within 2 h after sample collection.

2.3.2. Study of mixed compounds bioreactors in activated sludge microcosm: Influence of biotic processes

Activated sludge microcosms were conducted in the dark with or without sodium azide (as an inhibitor to biotic processes) in duplicate (Fig. S2B). Six conical flasks (made of borosilicate 3.3 glass) were autoclaved prior to use. Four empty flasks were spiked with a mixture of the compounds (R/S(±)-naproxen, R/S(±)-ketoprofen, R/S(±)-indoprofen, R/S(±)-tetramisole, R/S(±)-praziquantel, R/S(±)-ifosfamide, R/S(±)-10,11-dihydro-10hydroxycarbamazepine and R/S (±)-chloramphenicol) prepared in methanol and the methanol allowed to evaporate before adding the sample. Subsequently, 2 L of unfiltered activated sludge were added to each flask and they were placed onto a magnetic stirrer. Three bioreactors were spiked with sodium azide (1 g L^{-1}). Over a 24 h-sampling period, samples (100 mL) were collected at 0 h (before and after spiking), 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 5 h, 8 h, 12 h and 24 h. After collection samples were spiked with a mixed solution of deuterated ISs (1 μg L^{-1}). Dissolved oxygen, pH and temperature were measured each sampling time to verify bioreactor performance (Fig. S3).

2.3.3. Study of mixed compounds bioreactors in river water microcosm: influence of biotic (microbial degradation) and abiotic processes (photochemical processes)

River water microcosms were conducted as previously described by Bagnall et al. (2013). In summary, degradation experiments were conducted in the light and dark (to study photochemical processes), with or without sodium azide (as an inhibitor to biotic processes) in duplicate (Fig. S2A). Eight conical flasks (made of borosilicate 3.3 glass with no visible light absorption and UV light cut-off at < 275 nm) were autoclaved prior to use. Each empty flask was spiked with a mixture of the compounds (R/S(±)-naproxen, R/S(±)-ketoprofen, R/S(±)-indoprofen, R/S(±)-tetramisole, R/S(±)-praziquantel, R/S(±)-ifosfamide, R/S(±)-10,11-dihydro-10hydroxycarbamazepine and R/S (±)-chloramphenicol) prepared in methanol and the methanol allowed to evaporate before adding the sample. Subsequently, 2 L of unfiltered river water were added to each flask and they were placed onto a magnetic stirrer. Four bioreactors were spiked with sodium azide (1 g L^{-1}) to inhibit biotic processes while the four remaining were not spiked to allow biotic processes take place. Four bioreactors were incubated in the dark (two replicate microcosms with and two without sodium azide) and four bioreactors were incubated in the light (two replicate microcosms with and two without sodium azide). Daylight conditions were simulated using an Osram 400 W HQI BT daylight lamp for 8 h each day of the experiment. Over a month sampling period, samples (200 mL) were collected on day 1 (before and after spiking), 2, 3, 5, 7, 9, 12, 15, 22 and 30. After collection, samples were spiked with a mixed solution of deuterated ISs (1 μg L^{-1}). Dissolved oxygen, pH, temperature and photon flux measurements were measured each sampling day during the sampling period to verify bioreactor performance (Fig. S3). Average photon flux measured at the level of the bottle base was 388 μmol/m^2/s.

2.4. SPE and analysis

The analytical methodology used and validation parameters are discussed previously in Camacho-Muñoz and Kasprzyk-Hordern (2015). Briefly, Oasis HLB-MAX cartridges were set up in tandem and conditioned with methanol and deionized water at pH 7.5. Samples were spiked with a mixture of ISs, filtered and loaded onto the cartridges. SPE cartridges were washed individually and then connected in series afterwards. Analytes were eluted with 4 × 1 mL methanol and 2 × 1 mL methanol (2% formic acid), eluates evaporated to dryness and reconstituted with 0.5 mL of mobile phase.

The samples were then analysed by chiral LC-MS/MS using an ACQUITY UPLC™ system (Waters, UK) and a triple quadrupole mass spectrometer (TQD, Waters, UK) equipped with an electrospray ionisation source. Separation was carried out with a Chiral-AGP (100 × 2 mm, i.d. 5 μm) column (Chromtech, UK) and isocratic elution with mobile phase of 10 mM ammonium acetate with 1% acetonitrile (pH 6.7) at a flow rate of 0.08 mL min^{-1}. Analysis was performed in positive and negative mode simultaneously. Further details about chromatographic conditions, MS settings and data analysis are reported elsewhere (Camacho-Muñoz and Kasprzyk-Hordern, 2015). A quality assurance and quality control were followed during environmental sample analysis. It included a series of procedural blanks to monitor for background contamination, spiked samples and standards prepared in mobile phase to assess the variability of the analysis. They were injected in triplicate and randomly dispersed throughout sample batches.

2.5. Calculations

Removal efficiency from the aqueous phase during wastewater treatment was calculated by comparison of the concentration in μg L^{-1} of each cPAC found in influent ($C_{influent}$) and in effluent ($C_{effluent}$) wastewater. In this context, removal efficiencies reported are a combination of degradation and/or formation of transformation products. Negative removal rates can occur due to desorption of the cPAC from the solid phase and/or conversion of conjugated metabolites back to the parent compound (Burns et al., 2018; Verlicchi et al., 2012).

$$\%RE = \frac{C_{influent} - C_{effluent}}{C_{influent}} \times 100$$

The mass load of the PACs in the aqueous phase (mg day^{-1}) was determined using the following equation:

$$Mass\ load\ (mg\ day^{-1}) = \frac{C_{aqueous} * F}{1000}$$

where $C_{aqueous}$ refers to the concentration of each cPAC expressed as mg L^{-1} and F refers to the flow rate expressed as m^3 day^{-1}.

The enantiomeric fraction (EF) was calculated with relative (normalised with IS) peak areas as follows:

$$EF = \frac{E1}{E1 + E2} \text{ or } \frac{(+)}{(+) + (-)}$$

where E1 and E2 are concentrations of the first and the second eluting enantiomer, respectively and (+) and (−) enantiomers if the elution order is known. EF equals 1 or 0 in the case of single enantiomer form and 0.5 in the case of racemate.

3. Results and discussion

3.1. Fate of cPACs in the river catchment

3.1.1. cPACs in full scale wastewater treatment plants

3.1.1.1. Occurrence of cPACs in wastewater. A total of 18 pairs of enantiomers and 3 single enantiomers were analysed in wastewater and river water samples. Out of the 21 target compounds, 14 were detected in wastewater samples (R/S(±)-tetramisole, 6R,7R,2R-cephalexin, 6R,7R,2S-cephalexin, R/S(±)-ifosfamide, R/S(±)-10,11-dihydro-10-hydroxycarbamazepine, R/S(±)-naproxen, R/S(±)-ketoprofen, R/S(±)-ibuprofen, R/S(±)-2-phenylpropionic acid, R/S(±)-mandelic acid, R/S(±)-carboxyibuprofen, R/S(±)-hydroxyibuprofen, R/S(±)-fexofenadine, R/S(±)-cetirizine and S-(+)-*O*-desmethylnaproxen) (Fig. 1). In influent wastewater, the most dominant cPACs (frequency of detection and mean concentration in brackets) were S(+)-naproxen (100%, 24.5 μg L^{-1}), R/S(±)-2-hydroxyibuprofen (100%, 17.8 μg L^{-1}), R/S(±)-fexofenadine (83%, 2.19 μg L^{-1}), R/S(±)-cetirizine (83%, 2.21 μg L^{-1}) and R/S(±)-tetramisole (74%, 0.09 μg L^{-1}) and in effluents were R/S(±)-fexofenadine (100%, 2.02 μg L^{-1}), R/S(±)-cetirizine (100%, 5.32 μg L^{-1}), R/S(±)-tetramisole (100%, 0.13 μg L^{-1}) and S-(+)-naproxen (94%, 2.61 μg L^{-1}).

The highest mass load of cPACs in influent wastewater were found in STW E (5.64 mg day^{-1}) followed by STW C (1.81 mg day^{-1}), STW B (0.52 mg day^{-1}), STW A (0.47 mg day^{-1}) and STW D (0.23 mg day^{-1}). While the highest mass load in effluent were observed in samples taken at STW E (0.62 mg day^{-1}), followed by STW C (0.59 mg day^{-1}), STW B (0.10 mg day^{-1}), STW D (0.88 mg day^{-1}) and STW A (0.53 mg day^{-1}). S-(+)-Naproxen and R/S(±)-2-hydroxyibuprofen were found to be the compounds at the highest concentrations in influent wastewater at all STWs, up to 54.3 μg L^{-1} and 29.7 μg L^{-1}, respectively, whereas in effluent the profile was different (Fig. S4).

3.1.1.2. Enantioselective transformation of cPACs in wastewater. The enantiomer composition of the commercialized cPACs and their propensity to stereoselectivity during metabolism are the main factors which influence the EF values of cPACs observed in influent wastewater. In effluent samples the microbial activity during wastewater treatment can lead to further changes in the EF values observed. Despite being marketed mostly as racemic mixtures, most of the studied cPACs were found enriched with one enantiomer form in influent wastewater (Fig. 2). As a note, with the present analytical method the pair of enantiomers of cephalexin, phenylpropionic acid, mandelic acid, carboxyibuprofen and hydroxyibuprofen were not enantioseparated so EF values could not be provided.

3.1.1.2.1. NSAIDs. R/S(±)-Ibuprofen (Fig. 3) was found in effluent wastewaters from STW B, C and D at mean concentrations of 0.24, 1.00 and 1.21 μg L^{-1}, respectively. Effluent wastewater showed an excess of S-(+)-ibuprofen (mean EF of 0.71 ± 0.18), likely due to stereoselective human metabolism and/or microbial metabolic transformation during wastewater treatment. Although in this study R/S(±)-ibuprofen was not found in influent wastewater due to analysis issues (Rs was lost due to high concentration of R/S(±)-ibuprofen in influent wastewater), published reports confirm that the S-enantiomer is more prevalent in both influent and effluent wastewater (Buser et al., 1999; Matamoros et al., 2009; Wang et al., 2013). Despite S-(+)-ibuprofen being around 110 times more potent than the R-enantiomer, ibuprofen is marketed as a racemic mixture. During metabolism, R-(−)-ibuprofen undergoes chiral inversion increasing the proportion of S-(+)-ibuprofen in urine, an effect that has been observed in influent wastewater (Caballo et al., 2015; Hashim et al., 2011; Matamoros et al., 2009). Removal efficiencies of R/S(±)-ibuprofen have been reported above 90% regardless of the wastewater treatment used (Archer et al., 2017; Camacho-Muñoz et al., 2012; Camacho-Muñoz et al., 2011). Both metabolites of ibuprofen, R/S(±)-2-hydroxyibuprofen and R/S(±)-carboxyibuprofen were detected in influents (mean concentration: 17.8 and 2.10 μg L^{-1}, respectively) and effluents (mean concentration: 0.79 and 0.48 μg L^{-1}, respectively) (Fig. 1). R/S(±)-2-hydroxyibuprofen was found in the influents of all the STWs but only in the effluents of STW C (Fig. S4). Mean removal efficiency of this cPAC was 94.1%. On the other hand R/S(±)-carboxyibuprofen was only detected in the influents of STW A and C and in the effluents of STW C and D (Fig. S4) and the removal efficiencies varied from −40 to 100% between STWs (Fig. 4). The analytical methodology used in this study did not allow to separate the pair of enantiomers but in previous studies an enrichment of the first eluted enantiomer of R/S(±)-2-hydroxyibuprofen and R/S(±)-carboxyibuprofen were reported (Camacho-Muñoz and Kasprzyk-Hordern, 2017). This demonstrates the preferential metabolism of R-(−)-ibuprofen. R/S(±)-2-phenylpropionic acid was detected exclusively in influent samples of STW C and E at mean concentrations of 0.68 and 1.41 μg L^{-1}, respectively, showing removal efficiencies of 100% in both STWs.

Naproxen is commercialized as the S-enantiomer due to the known hepatotoxicity of the R-enantiomer. High concentrations of S-(+)-naproxen were found in all influent wastewater samples (mean concentration: 32.8, 26.7, 17.6, 28.5, 17.0 μg L^{-1} in STW A, B, C, D and E, respectively) (Fig. 3). R-(−)-naproxen was also found in the influents of STW D at a mean concentration of 0.93 μg L^{-1}. Regardless wastewater treatment, mean removal efficiency of S-(+)-naproxen resulted to be higher than 87% and chiral inversion took place in two STWs. R-(−)-naproxen was found after wastewater treatment in effluents from STW C and D at mean concentrations of 0.77 and 0.40 μg L^{-1}, respectively (Fig. 4). Mean removal efficiency observed for the R-enantiomer in STW D was 23%, lower than the one observed for the S-enantiomer (88%) due to the chiral inversion of the S-enantiomer or to different deconjugation rates of the conjugate. After wastewater treatment the mean EF values decreased from 1 to 0.89 ± 0.02 in STW C and from 0.97 ± 0.02 to 0.89 ± 0.03 in STW D (Fig. 3). These changes in the EF indicate that stereoselective processes took place during wastewater treatment. EF values reported were consistent with values previously published elsewhere (Caballo et al., 2015; Hashim et al., 2013; Khan et al., 2014). The metabolite S-(+)-*O*-desmethylnaproxen was detected exclusively in influent wastewater of STW A at mean concentrations of 3.63 μg L^{-1} and in effluents of STW B (1.24 μg L^{-1}) and C (1.04 μg L^{-1}) (Fig. S4). The presence of the single S-enantiomer of *O*-desmethylnaproxen in effluents and surface water has been reported elsewhere (Selke et al., 2010). These values were similar to those reported in effluent wastewater from Sweden (2.51 μg L^{-1}), Pakistan (1.36 μg L^{-1}), Germany (0.23 μg L^{-1}) and UK (0.22 μg L^{-1}) (Camacho-Muñoz and Kasprzyk-Hordern, 2015; Larsson et al., 2014; Selke et al., 2010).

Ketoprofen is sold as a racemic mixture despite only S(+)-ketoprofen exerting the therapeutic effects (Barbanoj et al., 2001). Both enantiomers of R/S(±)-ketoprofen (Fig. 3) were detected in influent wastewaters from STW A (0.03 μg L^{-1}) and B (0.22 μg L^{-1}) and in the treated wastewaters of STW B (0.02 μg L^{-1}) and E (0.31 μg L^{-1}) (Fig. S4). The values of EF in influents were quite close to the racemic mixture, 0.56 ± 0.06 whereas higher EF of 0.64 ± 0.05 was observed in effluents (Fig. 2). EF values were similar to those reported by Hashim et al. (2011) in tertiary treated wastewater. Degradation of R/S(±) during wastewater treatments accounted for > 59% of S-(+)-ketoprofen and > 62% of R-(−)-ketoprofen and was found to be stereoselective leading to the enrichment of ketoprofen with the S-(+)-enantiomer in effluent wastewaters. The metabolite R/S(±)-dihydroketoprofen was not detected in any sample.

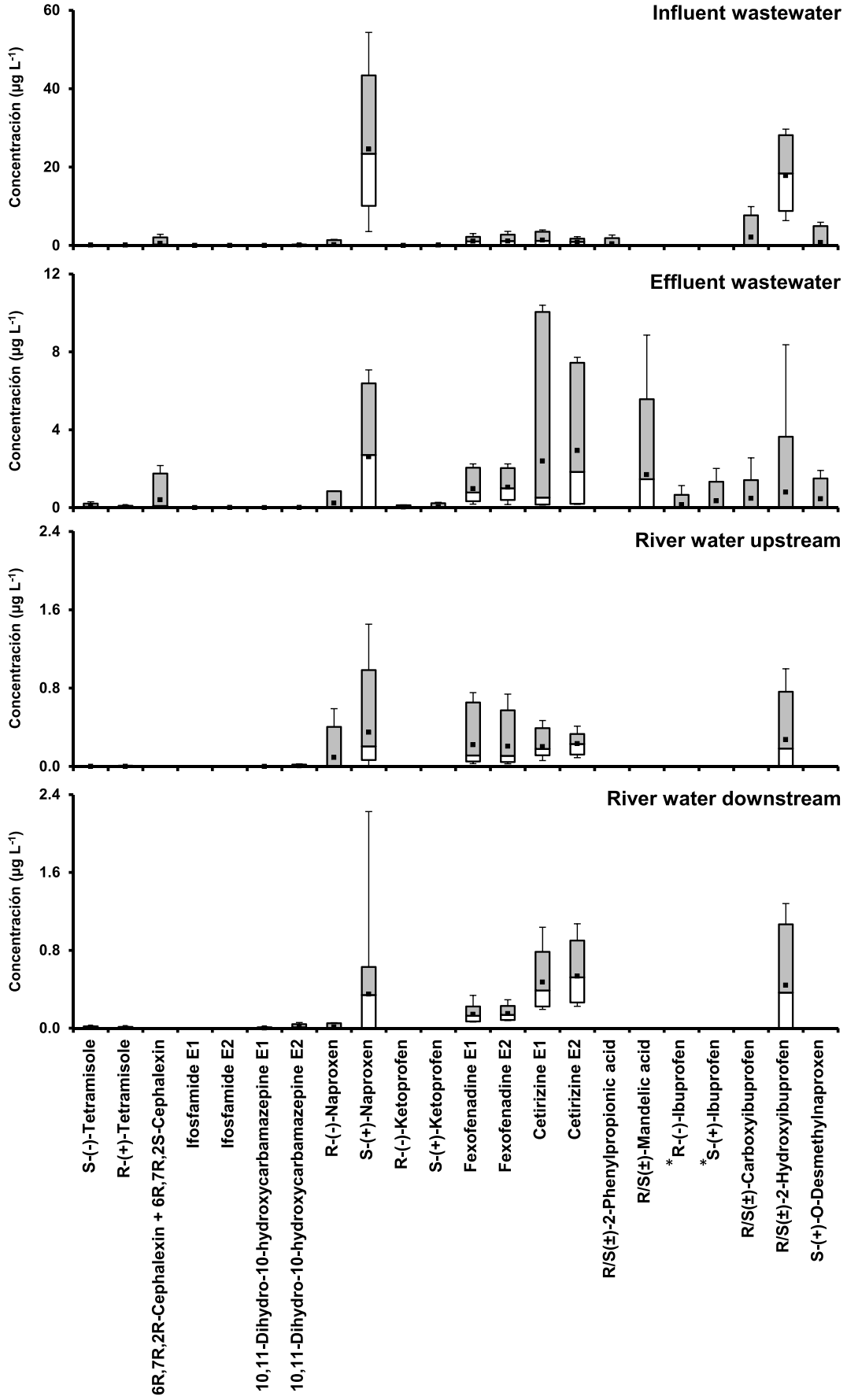

Fig. 1. Concentration ($\mu g\,L^{-1}$) of cPACs in influent and effluent wastewater and upstream and downstream river water. Lines in each box show the lower (5%), median (50%) and upper (95%) percentile. Lines from each box show maximum and minimum concentration values. The point inside each box shows the average concentration. *Data not available for R/S(±)-ibuprofen in influent wastewater.

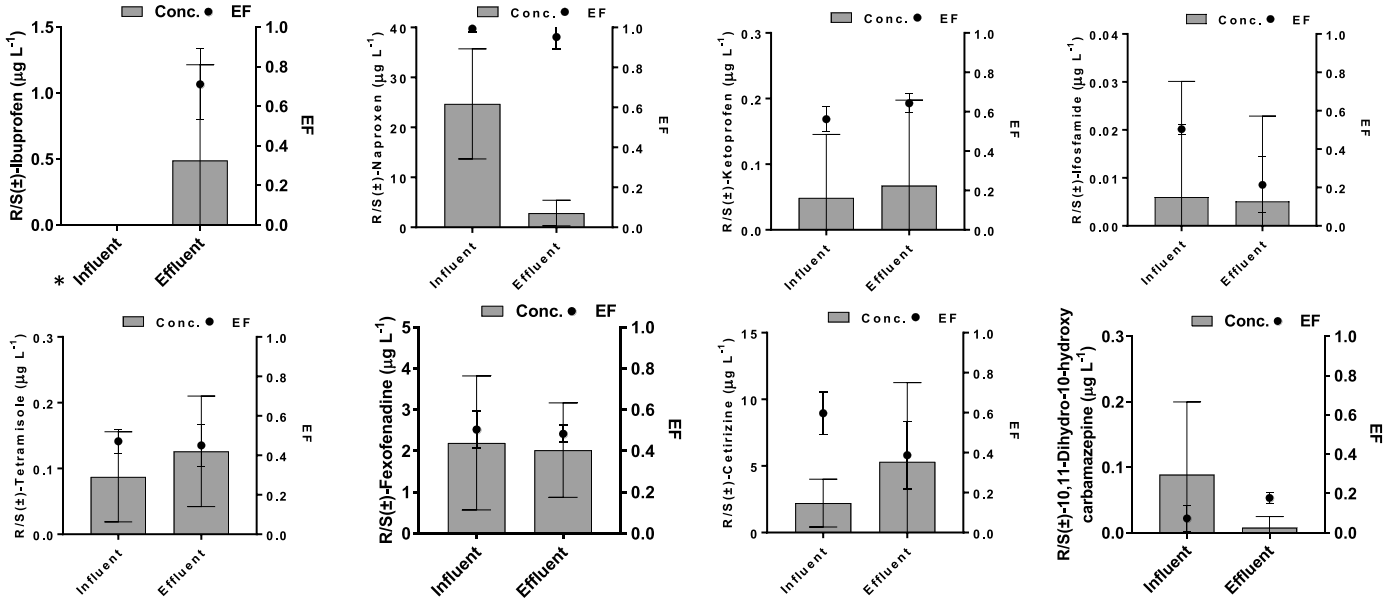

Fig. 2. Concentration (represented by bars) and enantiomeric fraction (represented by symbols) of R/S(±)-ibuprofen, R/S(±)-naproxen, R/S(±)-ketoprofen, R/S (±)-ifosfamide, R/S(±)-tetramisole, R/S(±)-fexofenadine, R/S(±)-cetirizine and R/S(±)-10,11-dihydro,10-hydroxycarbamazepine in influent and effluent wastewater. *Data not available for R/S(±)-ibuprofen in influent wastewater.

3.1.1.2.2. Anthelmintic drugs. Both enantiomers of tetramisole were found at similar mean concentrations in influent (0.05 and 0.04 µg L^{-1} of S-(−)-tetramisole and R-(+)-tetramisole, respectively) and effluent (0.07 and 0.05 µg L^{-1} of S-(−)-tetramisole and R-(+)-tetramisole) wastewater.

Wastewater treatment processes were found not to be effective in the removal of tetramisole enantiomers from wastewater (mean removal efficiency: < 12% S-(−)-tetramisole and < 6% R-(+)-tetramisole) and the mean EF value remained unchanged (EF: 0.47 ± 0.06 and 0.45 ± 0.11 in influent and effluent, respectively) (Fig. 2). However, differences were found between wastewater treatment plants (Fig. 4). SBR led to no reduction of R/S(±)-tetramisole, which resulted in both enantiomers detected in the SBR effluents with an enrichment of the S-enantiomer (EF: 0.31 ± 0.07). TF and AS revealed a similar removal efficiency of both enantiomers with no stereoselective preference among them (TF removal: 17% and 8%; AS removal: 8% and 4% of S and R-tetramisole, respectively) (Fig. 4). Concentrations of S-(−)-levamisole in wastewater up to 0.23 (influent) and 0.18 µg L^{-1} (effluent) and mean removal efficiencies of 30% were reported by Čizmić et al. (2017a).

The anthelmintic activity is associated with the S-enantiomer of tetramisole. As a result, tetramisole is marketed as enantiomerically pure S-enantiomer (Thienpont et al., 1969). However, the racemic and the S-tetramisole have also been used to adulterate illicit drugs (Bertucci et al., 2014; Casale et al., 2012) which could explain the presence of the R-enantiomer in wastewater samples. Chiral inversion or enantiomers deconjugated at different rates could be another explanations for its presence but the information regarding this drug is scarce. Its metabolite, R/S(±)-aminorex was not detected in any sample. Further work is therefore required to understand metabolism and transformation of this compound in the environment.

3.1.1.2.3. Anticancer drugs. The pair of enantiomers of ifosfamide (Fig. 3) was detected solely in STW E (≈900,000 inhabitants; serving an urban area with many hospitals) at mean concentrations of 0.006 µg L^{-1} (E1 and E2) in influent wastewater and at 0.003 µg L^{-1} of E1 and 0.008 µg L^{-1} of E2 in effluent wastewater. Removal efficiencies of ifosfamide enantiomers varied from 0 to 76% (Fig. 4). Ifosfamide has been reported quite resistant to biodegradation and UV light, making the use of advanced oxidation processes necessary for its complete elimination (Franquet-Griell et al., 2017). In this study, stereoselective degradation of ifosfamide was observed for the first time during wastewater treatment. A racemic mixture was found in influent (EF: 0.50 ± 0.03) but the effluent was enriched in the second eluted enantiomer (EF: 0.21 ± 0.12) (Fig. 3). Concentrations and removal efficiencies of racemic mixtures are in agreement with those previously reported by other authors for ifosfamide (Buerge et al., 2006; Martin et al., 2014; Negreira et al., 2014). The ifosfamide metabolite was not detected in any analysed sample.

3.1.1.2.4. Antihistamine drugs. Mean concentration of R/S (±)-cetirizine was lower in influent wastewater (2.21 µg L^{-1}) compared to treated wastewater (5.32 µg L^{-1}). Moreover, the EF changed from 0.60 ± 0.11 to 0.39 ± 0.17 after wastewater treatment, indicating an enrichment of cetirizine with E1-enantiomer in wastewater influent with subsequent interconversion leading to an enrichment of cetirizine with E2-enantiomer in wastewater effluent (likely due to microbial metabolic processes, Fig. 2). In previous studies, the R-enantiomer has been reported to be stable for racemization in the living body (Benedetti et al., 2009) and this over-the counter drug is commercialized both as a single enantiomer (S-form) and as a racemic mixture what could explain the excess of one of the enantiomers in the influent and not by stereoselective transformation in the body. Although cetirizine showed poor removal, E1 was removed to a greater extent compared to E2, especially after AS (removal efficiency: 44% E1 and − 241% E2) and TF (removal efficiency: −57% E1 and − 178% E2) treatment (Fig. 4). It is likely that stereoselective degradation took place. This phenomenon was not observed after SBR (removal efficiency: 12% E1 and 8% E2) (Fig. 4). This observation indicates that stereoselective transformation of cetirizine is wastewater treatment process dependent. Cetirizine enantiomers have not been investigated before in environmental samples, and most of the research has been focused on biological samples. To our knowledge this is the first study reporting a chiral switch and stereoselective degradation of cetirizine. The R-enantiomer is the active enantiomer with approximately 30-fold higher affinity for human histamine H_1-receptors than the S-enantiomer. In addition it shows higher bioavailability than the racemic mixture and due to its slower metabolism it has a long half-life in the body (Tillement et al., 2003).

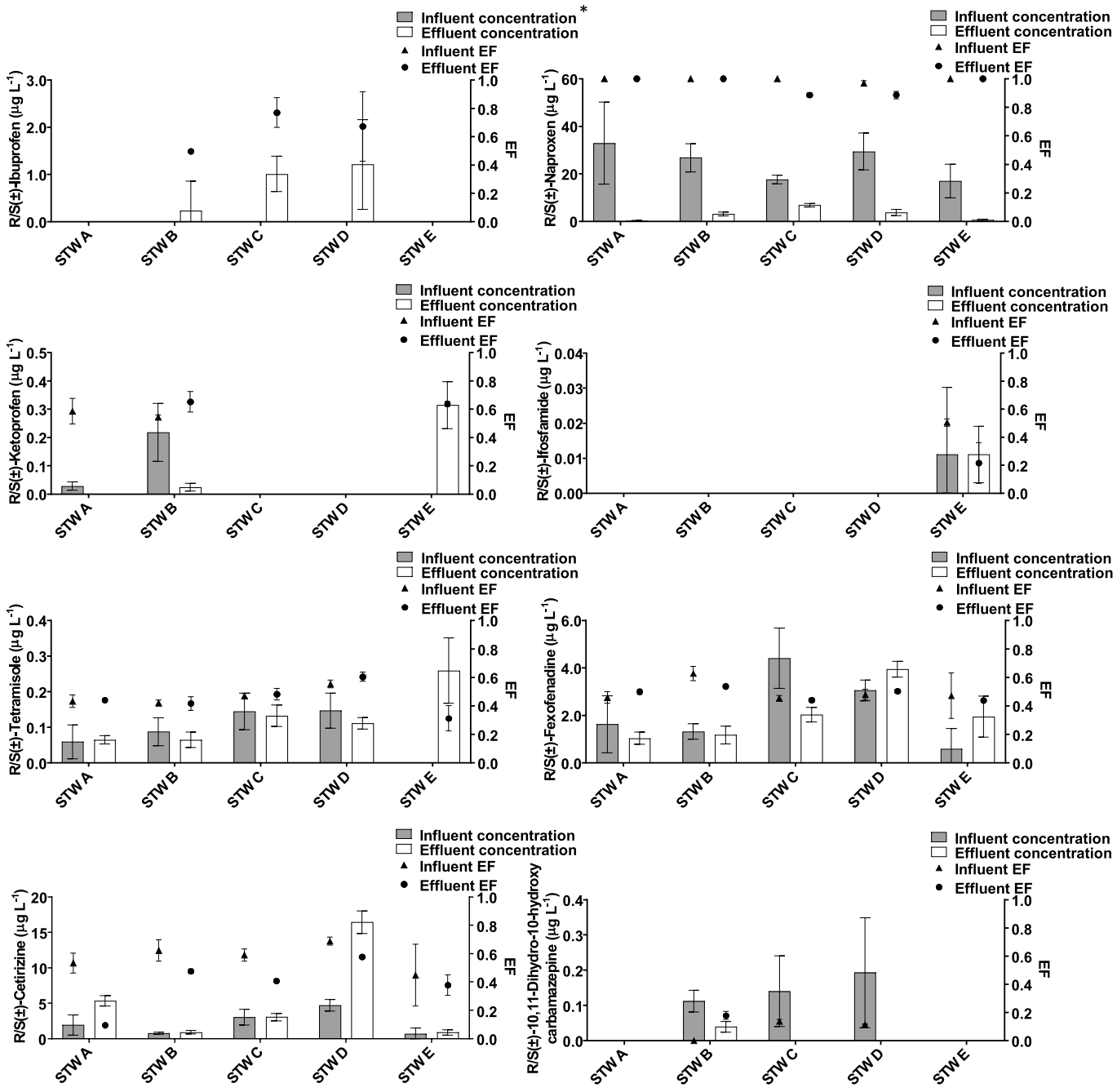

Fig. 3. Concentration (represented by bars) and enantiomeric fraction (represented by symbols) of R/S(±)-tetramisole, R/S(±)-10,11-dihydro-10-hydroxycarbamazepine, R/S(±)-fexofenadine, R/S(±)-cetirizine, R/S(±)-naproxen, R/S(±)-ketoprofen, R/S(±)-ibuprofen and R/S(±)-ifosfamide in influent and effluent wastewater of STW A-E. *Data not available for R/S(±)-ibuprofen in influent wastewater.

Fexofenadine is administered as a racemic mixture because both enantiomers are biologically active. In this study, mean concentration of R/S(±)-fexofenadine remained unchanged after wastewater treatment, 2.19 µg L^{-1} and 2.02 µg L^{-1} in influent and effluent, respectively (Fig. 2). Mean removal efficiencies were low: < 36% after AS, < 6% after TF and < −48% after SBR. No differences on removal efficiencies were observed between enantiomers (Fig. 4). Unlike cetirizine, EF values were similar in both influent and effluent wastewater, 0.50 ± 0.09 and 0.48 ± 0.04, respectively. No stereoselective degradation was observed for fexofenadine. Similar or lower concentrations of racemic mixtures were reported elsewhere (Burns et al., 2018; Loos et al., 2013).

3.1.1.2.5. Carbamazepine metabolite. 10,11-Dihydro-10-hydroxycarbamazepine E1 was present at lower concentration than its antipode E2 both in influent (0.01 and 0.08 µg L^{-1} of E1 and E2, respectively) and effluent (0.001 and 0.006 µg L^{-1} of E1 and E2, respectively) samples (Fig. 2) resulting in an EF of 0.07 ± 0.06 and 0.18 ± 0.03, respectively.

10,11-Dihydro-10-hydroxycarbamazepine enantiomers were present only in STW B, C and D (Fig. 3). Whereas both enantiomers were below limit of detection (LOD) in effluents from STW C and D, they behaved differently in STW B. Removal efficiency of 10,11-dihydro-10-hydroxycarbamazepine E2 in STW B ranged from 49 to 83%, whereas E1 was only found in effluent samples but not influent samples accounting for a null removal (Fig. 4). Enantioselective behaviour was observed in STW B, whereas in influent samples the mean EF value was 0 because only the E2 enantiomer was detected. The mean EF value in effluent samples was 0.18 ± 0.03. This change in the EF value could be

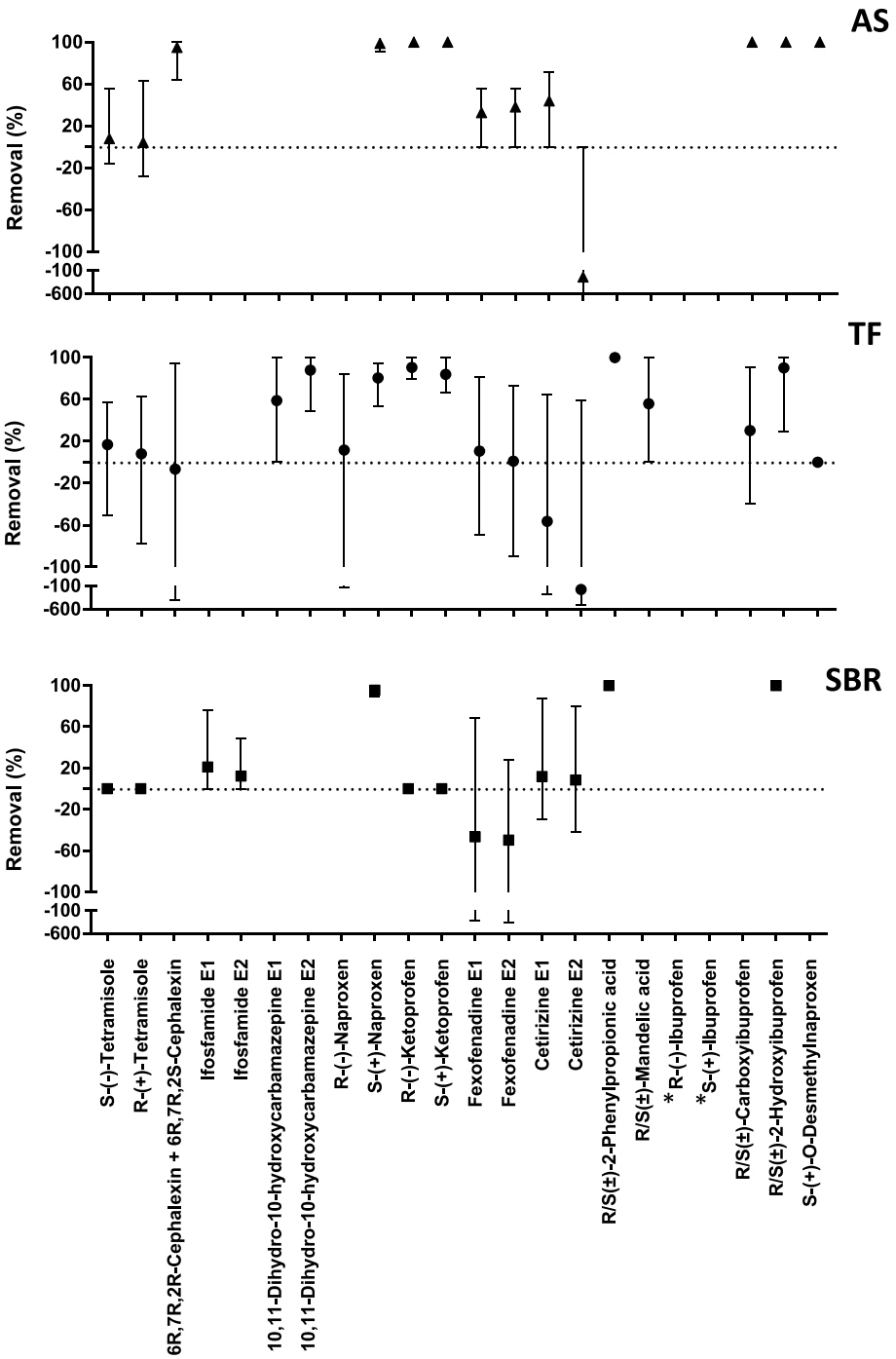

Fig. 4. Removal efficiencies of cPACs during activated sludge (AS), trickling filter beds (TF) and sequencing batch reactors (SBR). Symbols show the mean removal efficiency and lines show maximum and minimum removal efficiencies. *Data not available for R/S(±)-ibuprofen.

due to chiral inversion or more likely to the transformation of the parent compound into its metabolites. Further work is needed to fully understand stereochemistry of carbamazepine's transformation.

Concentrations of R/S(±)-10,11-dihydro-10-hydroxycarbamazepine were lower than those reported (at non-stereospecific level) in Germany (0.5 μg L^{-1}), Portugal (0.3 μg L^{-1}) (Bahlmann et al., 2014) but were similar to those reported in Canada (0.03 μg L^{-1}) (Miao et al., 2005).

3.1.1.2.6. Antibiotic. Mean concentration of cephalexin remained nearly constant during wastewater treatment (< LOD to 2.88 μg L^{-1} in influent wastewater and < LOD to 2.17 μg L^{-1} in effluent wastewater) (Fig. S4). Removal efficiency of cephalexin varied depending on the wastewater treatment used. AS treatment reduced the concentration of racemic cephalexin by up to 95%, whereas TF treatment achieved removal efficiencies from −400 to 95% (Fig. 4). It has been previously reported that TF treatment is superior to AS and anaerobic reactors in removing microbial loads (Lamba and Ahammad, 2017) which could lead to a lesser degradation of cephalexin due to less specialized bacterial communities. The presence of antibiotics in wastewater has turned STW into hot spots for antibiotic-resistant bacteria and antibiotic resistant genes because they can alter the bacterial community structure present in the biological reactors (Novo et al., 2013; Yang et al., 2014). Due to its similar structure to penicillin and other β-lactams, cephalexin can be enzymatically hydrolysed by the β-lactamase enzyme similar to acid hydrolysis. Previous studies have

shown that conventional wastewater treatment processes are fairly effective at removing antibiotics but not antibiotic-resistant bacteria and antibiotic resistant genes (Mao et al., 2015; Narciso-da-Rocha et al., 2018).

Concentrations reported in this study are higher than those reported in Iran (up to 0.46 μg L^{-1} in influents and 0.03 μg L^{-1} in effluents), Australia (up to 0.08 μg L^{-1} in effluents) and China (up to 1.8 μg L^{-1} in effluents) (Costanzo et al., 2005; Gulkowska et al., 2008; Mirzaei et al., 2018).

3.1.1.3. Impact of wastewater treatment technology on cPACs removal. Considering, the results presented in Section 3.1.1.2, significant differences were observed in the EF of some cPACs depending on the wastewater treatment technology used (Figs. 3 and S6).

Significant changes on the EF value of R/S(±)-naproxen ($p < 0.01$), R/S(±)-ketoprofen ($p < 0.05$), R/S(±)-cetirizine ($p < 0.0001$) and R/S(±)-10,11-Dihydro-10-hydroxycarbamazepine ($p < 0.01$) were observed after TF wastewater treatment (Fig. S6). Moreover, stereoselective degradation of naproxen was highly variable among STWs using TF and operating in the same region (Fig. 4). TF is an aerobic wastewater treatment that also allows anaerobic organisms to develop due to its configuration. The significant changes on the EF values is most likely associated with the presence of a wide microbial community that comprises aerobic, anaerobic and facultative bacteria, fungi, algae, and protozoa. In comparison with TF, biological treatment of AS resulted to be significantly stereoselective only in the case of R/S (±)-cetirizine ($p < 0.0001$); and SBR only for R/S(±)-ifosfamide ($p < 0.05$). Both AS and SBR are aerobic biological treatments and although they both have a dense aerobic microbial population it is not as diverse as the one present in TF. The extent of enantiomer-specific fate was also wastewater treatment dependent in the case of naproxen (TF showed higher stereoselectivity than AS and SBR) and cetirizine (TF and AS showed higher stereoselectivity than SBR) due to differing microbial populations. Stereoselective fate of cPACs depending on wastewater treatment (AS showed higher stereoselectivity than TF in the case of atenolol) was previously reported (Kasprzyk-Hordern and Baker, 2012). This necessitates further research to fully understand phenomena driving stereoselective fate of cPACs during wastewater treatment.

3.1.2. Occurrence of cPACs in the upstream (control) and downstream (impact) sites

The frequency of detection, concentration levels and EF of cPACs were determined to provide information about their distribution and behaviour and to assess the impact of effluent discharge to river water quality. R/S (±)-Tetramisole, R/S(±)-10,11-dihydro-10-hydroxycarbamazepine, R/S(±)-naproxen, R/S(±)-fexofenadine, R/S(±)-cetirizine and R/S (±)-2-hydroxyibuprofen were detected in upstream (control) and downstream (impact) river water.

The control sites that showed the highest cPACs mass loads were the ones further downstream, the ones that have received the highest number of effluent discharges: site D (1.19 mg day^{-1}) > site B (0.26 mg day^{-1}) > site C (0.20 mg day^{-1}) > site A (0.07 mg day^{-1}). In the control sites there were no significant daily differences on the total concentration of cPACs, which ranged from 5.47 μg L^{-1} to 8.27 μg L^{-1} (Fig. S5). Overall S-(+)-naproxen (0.35 μg L^{-1}), R/S(±)-2-hydroxyibuprofen (0.28 μg L^{-1}), R/S(±)-fexofenadine (0.22 μg L^{-1} E1 and 0.21 μg L^{-1} E2), and cetirizine (0.20 μg L^{-1} E1 and 0.23 μg L^{-1} E2), were found at the highest mean concentration levels in control sites (Fig. 1).

In descending order, the highest mass loads discharges were found in effluents from STW C (0.59 mg day^{-1}) > STW B (0.10 mg day^{-1}) > STW D (0.09 mg day^{-1}) > STW A (0.05 mg day^{-1}). Taking into account the river flow effluent wastewater should be diluted by a factor of 14 in Site A, 15 in Sites B and C and 113 in Site D. It must be noted that there was no suitable access points to the river downstream of STW A, so sample was taken close to the effluent discharge point. No river water was collected for Site E because the STW discharges directly to the estuary. The impact of effluent discharges was clearly observed in the mass loads of cPACs found in the impact sites: site C (1.18 mg day^{-1}) > site D (0.90 mg day^{-1}) > site B (0.27 mg day^{-1}) > site A (0.17 mg day^{-1}). Total amount of cPACs in impact sites increased about 67, 63, 50 and 90% in Site, A, B, C and D, respectively, compared to the ones reported in effluent wastewaters.

In terms of mean concentration levels (Fig. 1), concentrations found in downstream river water were higher than the ones reported in upstream river water (except in the case of fexofenadine), but lower than the ones reported in effluent wastewater, except in the case of R/S (±)-10,11-dihydro-10-hydroxycarbamazepine which was similar. No significant daily differences on the total concentration of cPACs were observed in the impact sites (7.48–9.35 μg L^{-1}) (Fig. S5). Similar to control sites, the highest mean concentration levels in impact sites were found for R/S(±)-cetirizine (0.47 μg L^{-1} E1 and 0.54 μg L^{-1} E2), R/S (±)-hydroxyibuprofen (0.44 μg L^{-1}), S-(+)-naproxen (0.35 μg L^{-1}) and R/S(±)-fexofenadine (0.14 μg L^{-1} E1 and 0.15 μg L^{-1} E2 (Fig. 1).

The EF profile observed in downstream river revealed that it was enriched in one of the enantiomers (Fig. 5), corresponding, in most cases, with the chiral profile observed in effluent wastewater.

3.2. Microcosm bioreactors

In order to evaluate processes involved in stereoselective degradation of a wide group of cPACs, river water and activated sludge simulating microcosm experiments were undertaken under different experimental conditions (biotic, abiotic, light and dark conditions).

3.2.1. Activated sludge simulating microcosm bioreactors

No stereoselectivity for ifosfamide (Fig. 6F), praziquantel (Fig. 6E), tetramisole (Fig. 6D) and ketoprofen (Fig. 6B) was observed for the activated sludge bioreactors, concentration levels and EF remained unchanged over the 24 h under dark biotic and abiotic conditions. In a study about the elimination of anticancer drugs during conventional activated sludge wastewater using a fungal treatment it was observed that ifosfamide and cyclophosphamide were quite inalterable, in contrast with the other anticancer drugs, and it was suggested that more specific biodegradation systems (bacteria, another fungi, etc.) might be needed (Ferrando-Climent et al., 2015). In the case of praziquantel advanced oxidation technologies with UV-C/TiO_2 with or without scavengers (H_2O_2) are necessary to degrade it (Čizmić et al., 2017b; Havliková et al., 2016). A slight decrease in tetramisole concentration (19%; Fig. S7D) was observed under biotic conditions, but neither of its enantiomers showed to be most preferably degraded. Concentration levels of ketoprofen enantiomers remained constant through the whole 24 h experiment (Fig. S7B). Non-enantioselective transformations and low removal have been previously reported for both ketoprofen enantiomers in a membrane bioreactor (Hashim et al., 2011), an activated sludge bioreactor (Caballo et al., 2015; Escuder-Gilabert et al., 2018) or in an enzymatic membrane bioreactor (Nguyen et al., 2017) pointing to the presence of the carboxylic group as responsible for being resistant to enzymatic degradation.

Stereoselective degradation was observed for naproxen (Fig. 6A), indoprofen (Fig. 6C), chloramphenicol (Fig. 6H) and carbamazepine metabolite (Fig. 6G).

The initial excess of S-(+)-naproxen would normally help to observe changes in the EF during microcosm experiments. After 3 h in the biotic reactor the concentration of the enantiomers of naproxen were < LOD. Although R/S(±)-naproxen was used to feed the experiment, S-(+)-naproxen was degraded faster than its antipode (Fig. S7A) or chiral inversion took place. In the abiotic experiment, the removal of naproxen enantiomers was slower, it reached 50% after 24 h (Fig. S7A). The most surprising observation to emerge during the experiment was the increase in absolute concentration of R-(−)-naproxen compared to the initial point which caused a change in the EF from 0.79

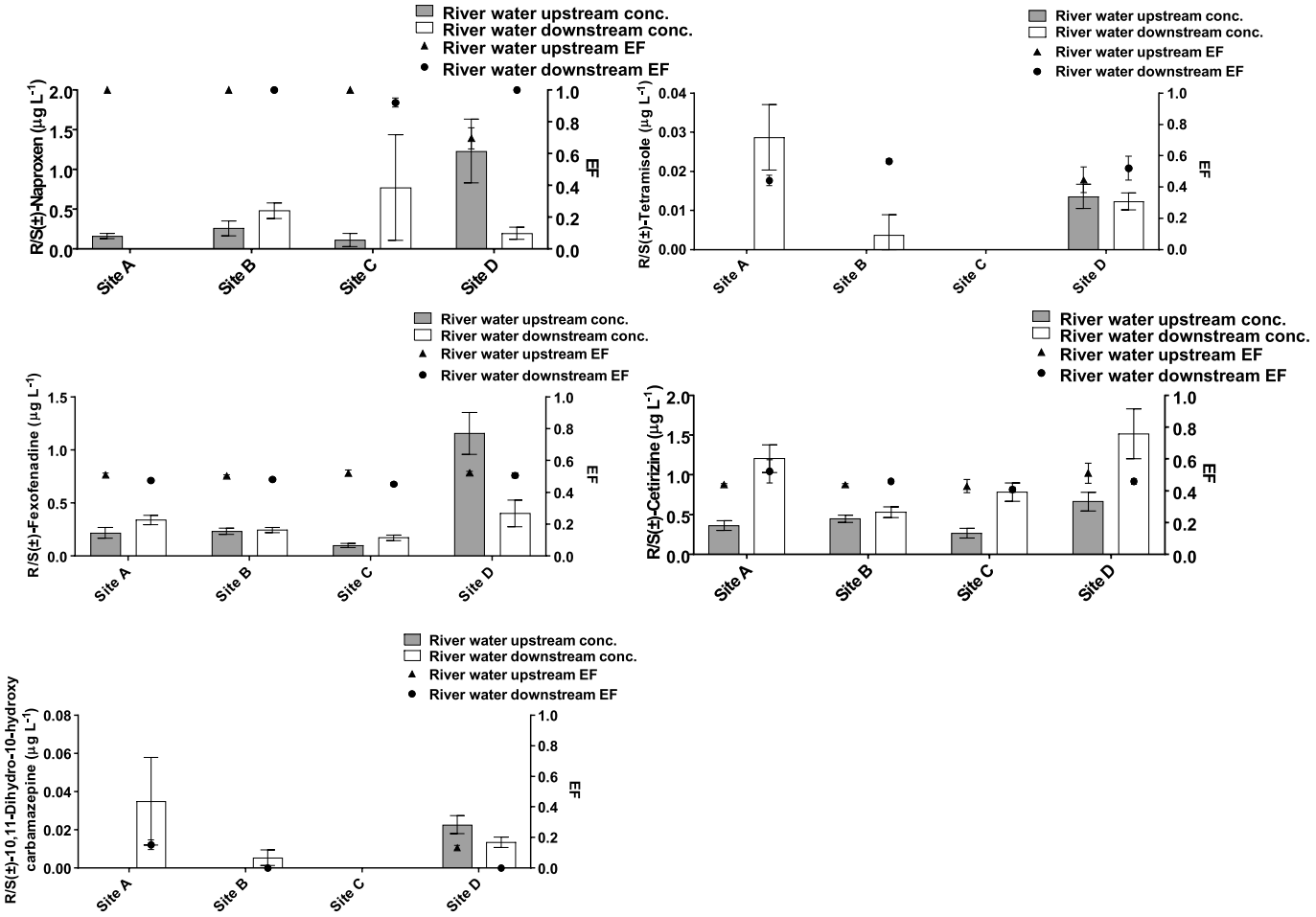

Fig. 5. Concentration (represented by bars) and enantiomeric fraction (represented by symbols) of R/S(±)-tetramisole, R/S(±)-10,11-dihydro-10-hydroxycarbamazepine, R/S(±)-naproxen, R/S(±)-fexofenadine and R/S(±)-cetirizine in upstream and downstream river water of Sites A–D.

to 0.54 ($p < 0.01$) after 24 h. Although unidirectional chiral inversion of profens have mostly been reported (Hashim et al., 2011; Kasprzyk-Hordern, 2010) our results could indicate bidirectional inversion of naproxen enantiomers during microcosm experiments. This compound-specific bidirectional inversion has been reported for naproxen (14% of R-(−)-naproxen was inverted to the S-enantiomer whereas only 4% of S-(+)-naproxen was inverted to R-(−)-naproxen) (Nguyen et al., 2017)). The opposite behaviour of the EF under biotic and abiotic conditions could be explained due to a combination of chiral inversion and enantioselective degradation of S-(+)-naproxen in the biotic experiment. Complete degradation of indoprofen was achieved after 5 h of incubation (Fig. S7C) in the biotic activated sludge microcosm. The EF value of indoprofen was also altered. It changed from an initial EF of 0.52 ± 0.01 to an EF of 0.38 ± 0.01 in 5 h ($p < 0.05$). To the best of our knowledge, this is the first time degradation of chiral indoprofen has been evaluated. It is well established that profens undergo chiral inversion during metabolism (Khan et al., 2014; Sanganyado et al., 2017), therefore changes in the EF values during biodegradation processes have been mainly explained by faster degradation of one enantiomer over the other or chiral inversion (Rossetti et al., 1992).

Enantioselective degradation was observed for chloramphenicol under biotic (EF 0.45 ± 0.01 to 0.37 ± 0.04) and most surprisingly under abiotic (EF 0.52 ± 0.03 to 0.40 ± 0.01) conditions (Fig. 6H). It led to the enrichment of the 1R,2R-(−)-enantiomer in the system which is the only one of the possible eight different isomers of chloramphenicol with reported antimicrobial activity (Shabad et al., 1977). 1R,2R-(−)-chloramphenicol was removed from the aqueous phase by 49% and 35% under biotic and abiotic conditions respectively in 24 h; concentration of 1S,2S-(+)-chloramphenicol was attenuated by 65% and 60% under biotic and abiotic conditions, respectively (Fig. S7H). Enantioselective degradation is often observed by the result of microbial activity (Hashim et al., 2011). The degree and direction of chiral inversion in different organisms depend on multiple enzymes (Kasprzyk-Hordern, 2010; Khan et al., 2014; Sanganyado et al., 2017), but so far chiral inversion of chloramphenicol has not been reported. Changes in the EF value in the abiotic activated sludge were unexpected and they will need further research to explain them. It has been reported that the bacteriostatic effects of sodium azide appear to be limited to Gram-negative Bacteria, whereas Gram-positive Bacteria are mostly resistant to sodium azide (Lichstein, 1944; Stannard and Horecker, 1948). Therefore, the presence of sodium azide-resistant bacteria could have preferentially degraded one enantiomer over the other. Another explanation could be stereoselective sorption. Sanganyado et al. (2017) pointed out that chiral minerals and organic matter may serve as a chiral environment resulting in one enantiomer being less available for transport, microbial degradation or uptake by non-target organisms than the other.

Under biotic conditions, the absolute concentration of 10,11-dihydro,10-hydroxycarbamazepine enantiomers remained constant throughout the 24 h experiment (Fig. 6G). However, after 8 h the EF value (0.47 ± 0.02) started to increase until it reached 0.63 ± 0.07 in 24 h. In the abiotic reactor the concentration of both enantiomers were < LOD after 8 h with no stereoselectivity observed.

3.2.2. River water simulating microcosms

The results of the mixed-compound river water microcosms

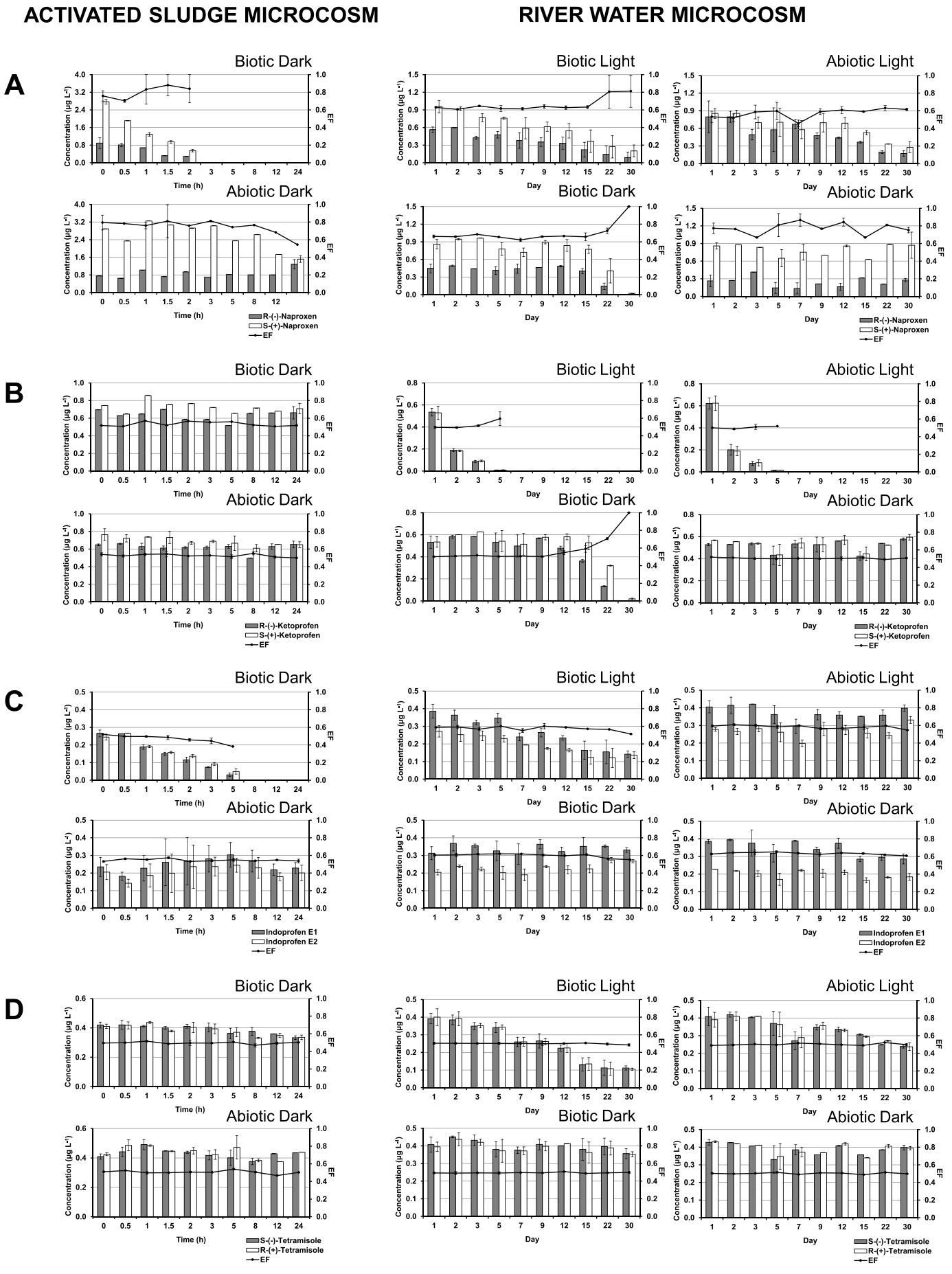

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Fig. 6. Concentration (represented by bars) and enantiomeric fraction (represented by symbols) of naproxen (A), ketoprofen (B), indoprofen (C), tetramisole (D), praziquantel (E), ifosfamide (F), 10,11-dihydro-10-hydroxycarbamazepine (G) and chloramphenicol (H) enantiomers in mixed compound bioreactors in activate sludge and river water.

indicated that R/S(±)-ifosfamide was not degraded over the course of the 30 days experiment in biotic and/or abiotic conditions both in the presence and absence of light in river water bioreactors (Figs. 6F, S7F). Ifosfamide has been shown to exhibit poor biodegradability and negligible light absorption in the solar wavelength range (Buerge et al., 2006; Kümmerer et al., 1997), also the presence of halogenated functional groups in its molecular structure may contribute to hinder aerobic biodegradation (Tadkaew et al., 2011).

R/S(±)-Praziquantel (Figs. 6E, S7E) and R/S(±)-10,11-dihydroxy,10-hydroxycarbamazepine (Figs. 6G, S7G) were also considered persistent but in contrast to ifosfamide concentration levels of both enantiomers of praziquantel and carbamazepine metabolite decreased under biotic light conditions although not stereoselectively. Few reports have shown that praziquantel is photodegraded when directly exposed to sunlight in aqueous media but over a period of few months (Suleiman et al., 2004). A combination of microbial and photochemical degradation could speed up the degradation of praziquantel and account for the changes observed in the biotic light experiment over a period of 30 days. Regarding carbamazepine metabolites they have been reported to be as persistent as the parent compound during wastewater treatment (Leclercq et al., 2009). Enantioselective degradation was observed in the biotic activated sludge microcosm but not in the river water microcosm what would indicate high biomass density or specialized microbial communities are needed to enantioselectively degrade this compound.

The combination of photochemical and biological degradation was key to dissipate the concentration of R/S(±)-ketoprofen (Figs. 6B, S7B) and R/S(±)-naproxen (Figs. 6A, S7A). Photodegradation had an important impact on concentration whereas biodegradation had it also on stereoselectivity. By day 5 ketoprofen enantiomers were removed by 98% of their initial concentration in biotic and abiotic light conditions. Photodegradation of naproxen was slower, by day 30, S-(+)-naproxen was removed by 84% and 79% and R-(−)-naproxen by 79% and 68% in the biotic and abiotic light microcosms, respectively. When light was not present stereoselective biodegradation was clearly observed. The R-enantiomers of naproxen and ketoprofen were preferentially degraded over the S-enantiomers. At the beginning of the experiment the EF of ketoprofen was 0.50 ± 0.01 and the EF of naproxen was 0.66 ± 0.02 (due to the presence of endogenous S-(+)-naproxen), half way the EF of both cPACs increased (EF = 0.71 ± 0.01 ketoprofen, $p < 0.001$, day 12; EF = 0.73 ± 0.04 naproxen, $p < 0.05$, day 22) and they reached EF = 1 ($p < 0.001$) at the end of the experiment.

Among the studied profens, R/S(±)-indoprofen (Figs. 6C, S7C) turned to be more stable to photochemical and biological degradation. The dissipation of indoprofen enantiomers happened only under biotic light conditions and at the end of the experiment (30 days) suggesting more time is needed to achieve complete removal. An excess of indoprofen E1 was found at the beginning of the experiment (EF = 0.59 ± 0.01), but at the end of the experiment the concentration of E1 and E2 were similar probably as a result of E1 being degraded faster than E2 or due to chiral inversion (EF = 0.51 ± 0.01). In vitro metabolic inversion of R/S(±)-indoprofen towards an excess of the S-enantiomer was previously reported after liver microsomes where incubated with the racemic mixture and the individual enantiomers (Rossetti et al., 1992).

Photodegradation was an important process to dissipate the concentration of R/S(±)-chloramphenicol (Figs. 6H, S7H) and R/S(±)-tetramisole (Figs. 6D, S7D) in the microcosm experiments. R/S(±)-chloramphenicol was not present after 12 and 15 days in the biotic light and abiotic light reactors, respectively. Biotic processes also contributed to the degradation of R/S(±)-chloramphenicol although in a minor extent than photolysis. When biotic processes were involved 1R,2R-(−)-chloramphenicol was degraded faster than the 1S,2S-(+)-enantiomer (EF = 0.77 ± 0.20). This behaviour was opposite to the one observed in the activated sludge microcosm in which the EF value decreased. Different microbial communities and the presence of microalgae could explain these differences. As an example, different environmental studies have reported varied results showing S-(−)-metoprolol enrichment (Evans et al., 2017) and R-(+)-metoprolol enrichment (Ribeiro et al., 2013) in activated sludge microcosms or the preferential degradation of S-(−)-metoprolol by microalgae (Lv et al., 2018). Changes in the biotic light reactor could not be observed because photolysis was such an important dissipation effect that probably overshadowed the preferential degradation of one over the other. Photodegradation has been reported as an important process for the degradation of chloramphenicol in natural waters (Trovo et al., 2014). Regarding R/S(±)-tetramisole, 73% and 40% of both enantiomers were removed under biotic and abiotic light conditions, respectively, whereas under dark conditions removal efficiency was under 10% after 30 days. As it happened, neither of the tetramisole enantiomers was shown to be more preferentially degraded than the other in the activated sludge microcosms.

4. Conclusions

In this study, we performed a catchment-scale study (macrocosm) to investigate the occurrence, removal efficiencies and enantiomeric distribution of a comprehensive set of cPACs in 5 major STWs and in receiving waters of the largest river catchment in Southwest England. At the same time we performed controlled lab-scale microcosm studies in simulated activated sludge bioreactors and river water microcosm to evaluate stereoselective degradation of cPACs and to investigate the role of EF as a marker of in situ biodegradation. cPAC concentrations in influent wastewater were: (i) found to vary significantly spatially and temporally and (ii) are enriched in one of the enantiomers as a signature of human and microbial metabolism despite being marketed as racemic mixtures. Receiving waters revealed a chiral signature that corresponded to the one observed in effluent wastewaters. Stereoselective degradation during wastewater treatment was observed in the case of naproxen, ketoprofen, cetirizine, ifosfamide and 10,11-dihydroxy-10-hydroxycarbamazepine. It is hypothesized that differences in density and diversity of microbial population resulted in wastewater treatment dependent stereoselectivity and also in the extent of enantiomer-specific fate. Higher stereoselectivity was observed for naproxen in TF and for cetirizine in TF and AS.

Both activated and river water simulated microcosm confirmed that enantioselective degradation was biological in nature. It led to an excess of S-(+)-naproxen, 1R,2R-(−)-chloramphenicol and one of the enantiomers of indoprofen and 10,11-dihydroxy-10-hydroxycarbamazepine in activated sludge microcosm and to an excess of S-(+)-naproxen, S-(+)-ketoprofen, 1S,2S-(+)-chloramphenicol and one of the enantiomers of indoprofen. Differences in the preferential degradation of one enantiomer in both microcosms could be attributed to biomass density, nature of microbial community and time of exposition. Further work is required to study this phenomenon.

Results from our large scale river catchment monitoring study and lab simulated microcosms show the impact of stereoisomerism of cPACs on their fate. It is hypothesized that cPACs stereoselective fate will have effects on biota. As two enantiomers of the same compound show different biological effects (e.g. toxicity), their non-racemic presence in the environment might lead to inaccurate environmental risk assessment (ERA). This is because current ERA approaches do not require analysis at enantiomeric level.

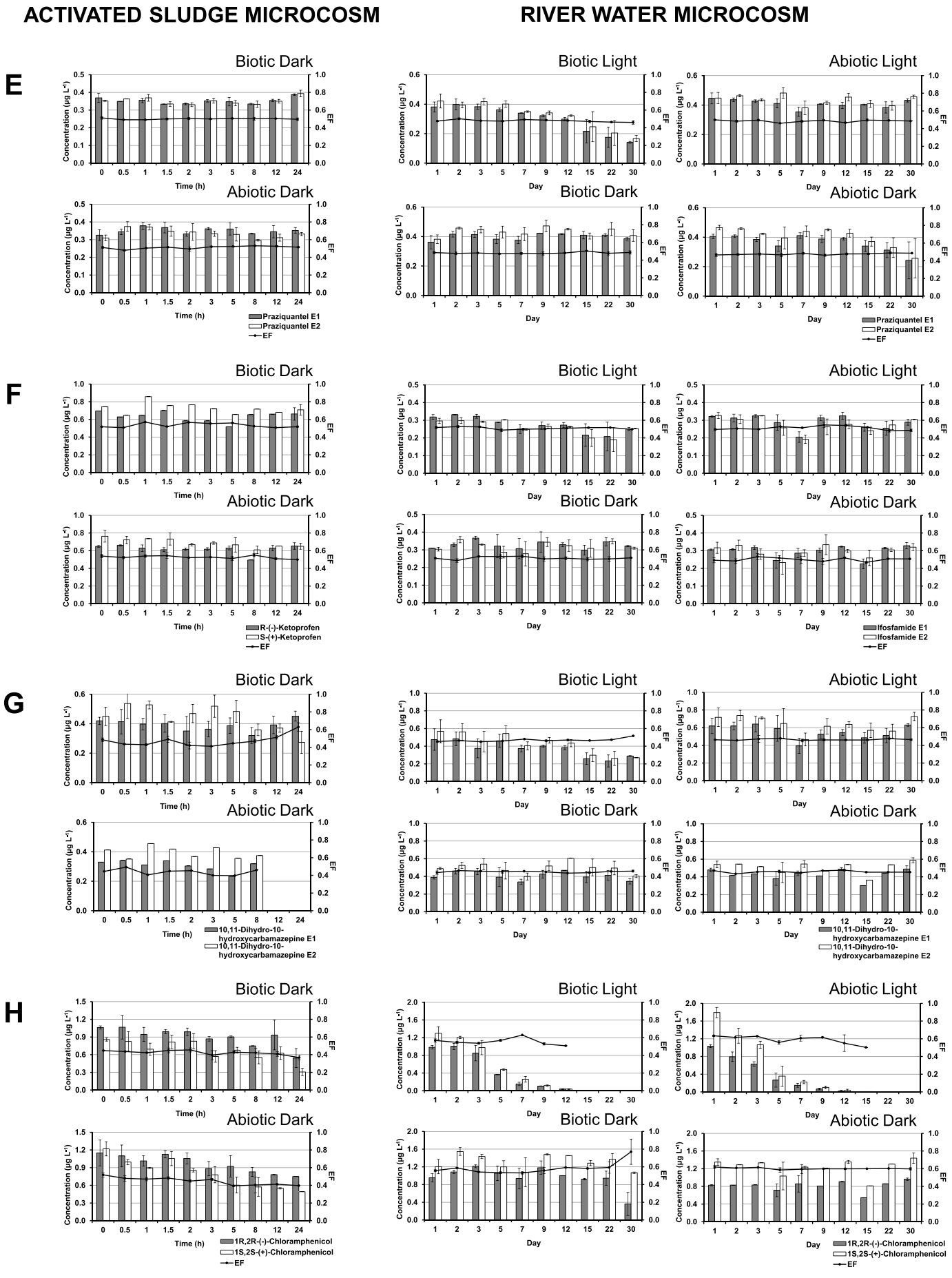

Fig. 6. (*continued*)

Acknowledgments

This work was supported by the European Union's Seventh Framework Programme for research, technological development and demonstration [grant agreement 629015, the MC IEF project 'Chiral veterinary medicines in the environment'], Wessex Water, the University of Bath's EPSRC Impact Acceleration Account (Project number: EP/K503897/1 and ZR-Z0248) and Natural Environment Research Council (NE/L009579/1). The authors would like to thank all members of Environmental Chemistry and Public Health Research Group at Bath for their support during the project.

Conflict of interest

The authors declare no conflict of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.03.050.

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Supplementary material

Table S1. Site information of studied STWs and corresponding river locations

Site	Sewer residence time[a] (h)	STW secondary process	SRT (d)	HRT[a] (h)	Population served	Mean flow (m^3 d^{-1})	Effluent dilution factor	River sampling, distance to discharge point (km)	
								Upstream	Downstream
A	<0.5-4	AS	19	46.2	37,000	8,242±3,085	14	0.5	n/a
B	<0.5-4	TF	n/a	24.5	67,870	11,202±3,202	15	0.5	0.5
C	<0.5-9	TF	n/a	13.9	105,847	24,875±2,167	15	2	2
D	<0.5-2	TF	n/a	17.6	17,638	2,924±199	113	1	1
E	<1-24	90% SBR 10% AS	4 8	10.9 25.8	909,617	153,061±12,245	n/a[b]	-	-

Key: STW, wastewater treatment process; SRT, solids retention time; HRT, hydraulic retention time; AS, activated sludge; TF, trickling filter; SBR, sequencing batch reactor

[a]Under summer (dry weather) flow

[b]Effluent discharged into estuary

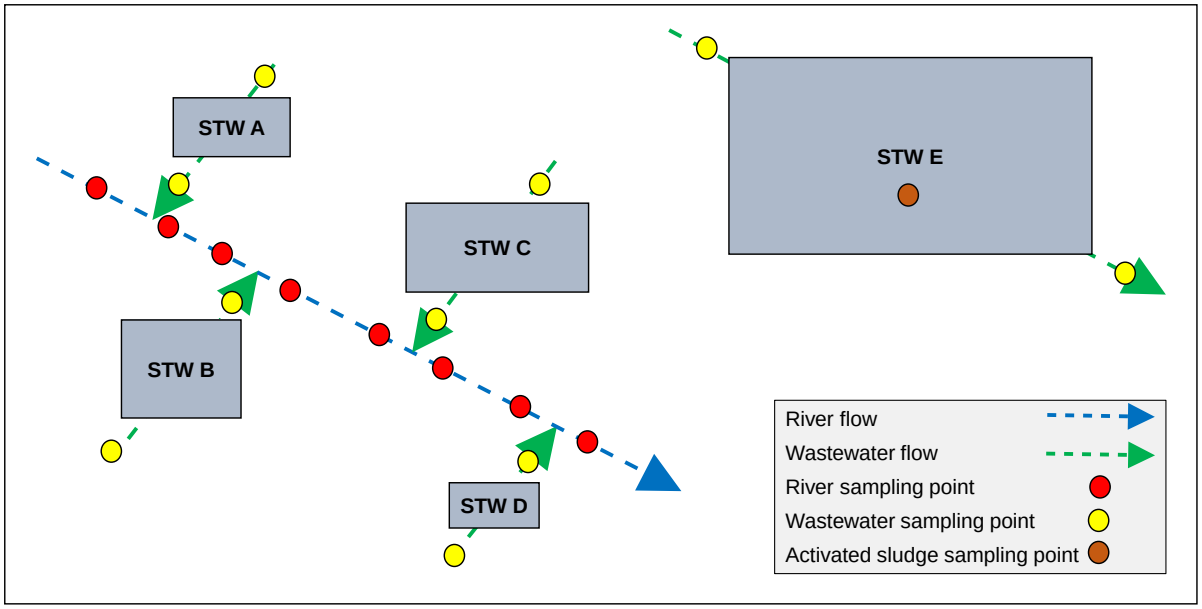


Figure S1. Catchment area schematic illustrating sampling points

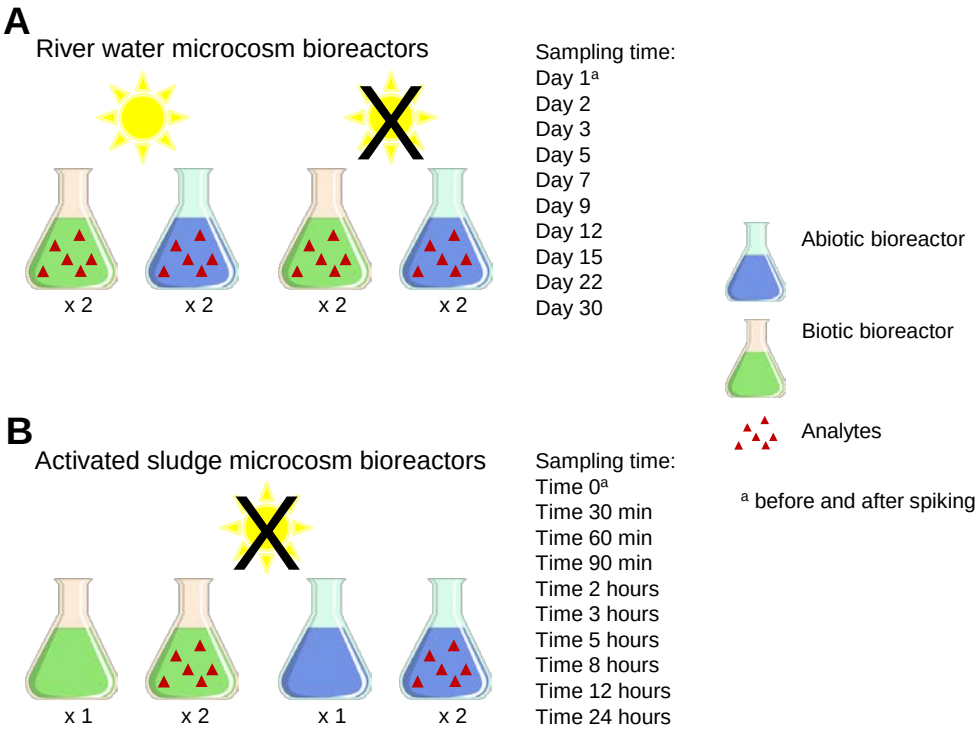


Figure S2. Schematic for the river water (A) and activated sludge (B) microcosm bioreactors.

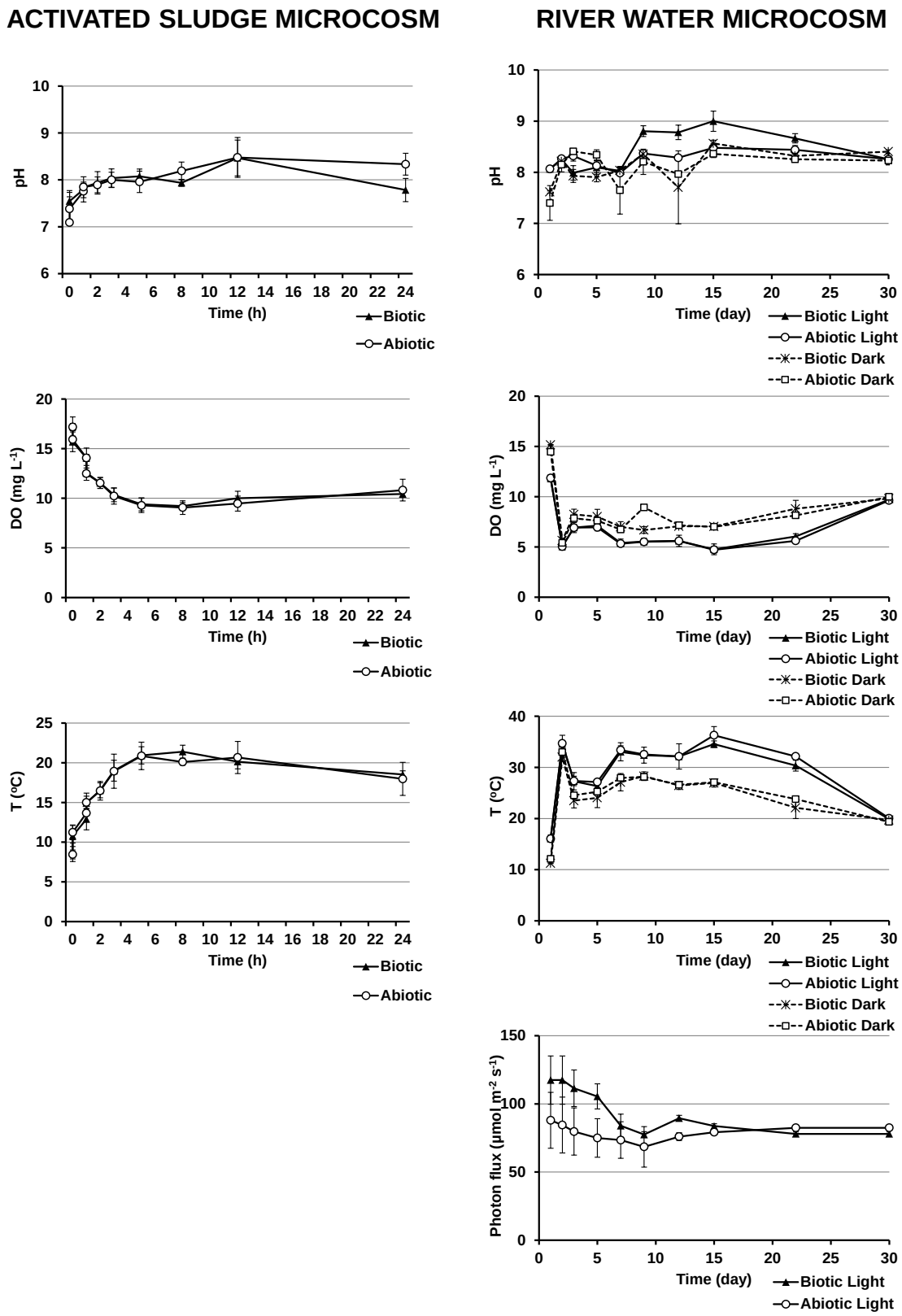
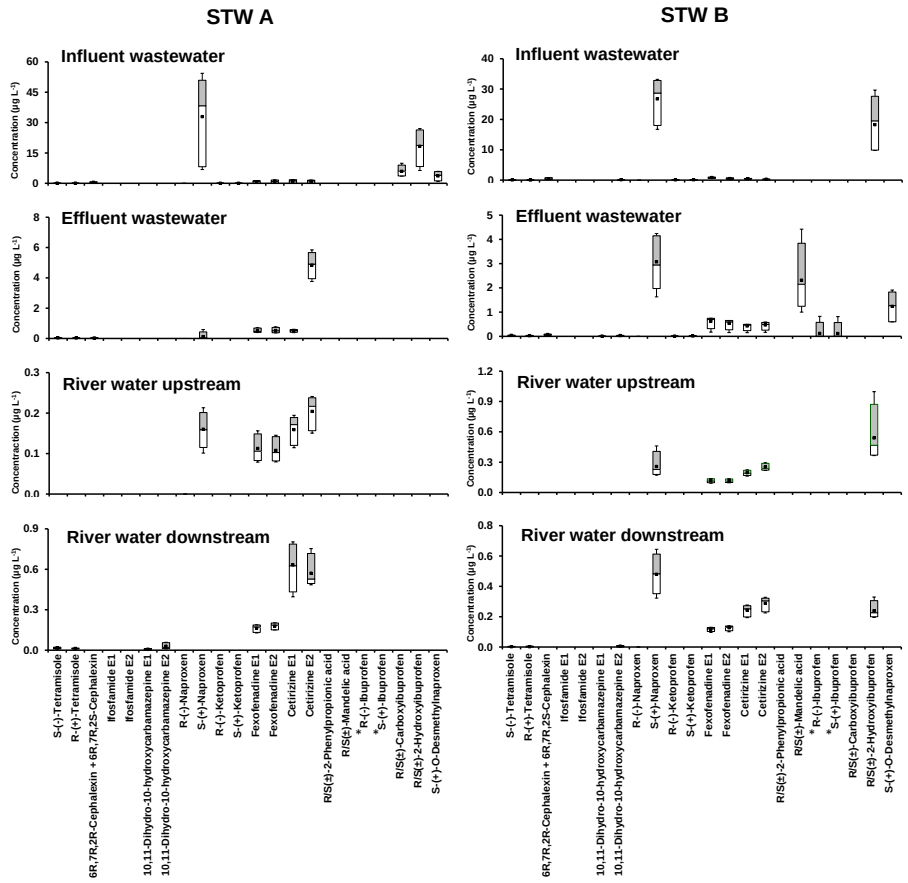
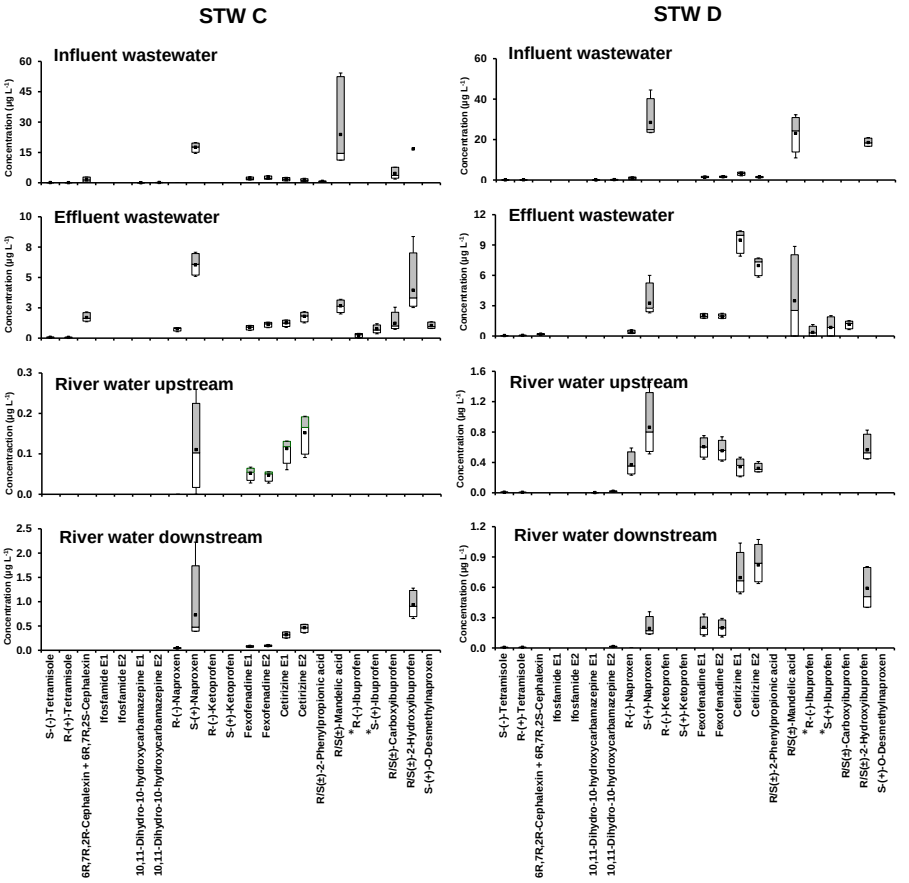


Figure S3. pH, dissolved oxygen (DO) and temperature (T) recorded during 24 h in the activated sludge microcosm bioreactors and during 30 days in the river water microcosm bioreactors.


STW A
STW B
Influent wastewater
Effluent wastewater
River water upstream
River water downstream
Concentration (µg L-1)
S-(-)-Tetramisole
R-(+)-Tetramisole
6R,7R,2R-Cephalexin + 6R,7R,2S-Cephalexin
Ifosfamide E1
Ifosfamide E2
10,11-Dihydro-10-hydroxycarbamazepine E1
10,11-Dihydro-10-hydroxycarbamazepine E2
R-(-)-Naproxen
S-(+)-Naproxen
R-(-)-Ketoprofen
S-(+)-Ketoprofen
Fexofenadine E1
Fexofenadine E2
Cetirizine E1
Cetirizine E2
R/S(±)-2-Phenylpropionic acid
R/S(±)-Mandelic acid
*R-(-)-Ibuprofen
*S-(+)-Ibuprofen
R/S(±)-Carboxyibuprofen
R/S(±)-2-Hydroxyibuprofen
S-(+)-O-Desmethylnaproxen


STW C
STW D
Influent wastewater
Effluent wastewater
River water upstream
River water downstream
Concentration (µg L-1)
S-(-)-Tetramisole
R-(+)-Tetramisole
6R,7R,2R-Cephalexin + 6R,7R,2S-Cephalexin
Ifosfamide E1
Ifosfamide E2
10,11-Dihydro-10-hydroxycarbamazepine E1
10,11-Dihydro-10-hydroxycarbamazepine E2
R-(-)-Naproxen
S-(+)-Naproxen
R-(-)-Ketoprofen
S-(+)-Ketoprofen
Fexofenadine E1
Fexofenadine E2
Cetirizine E1
Cetirizine E2
R/S(±)-2-Phenylpropionic acid
R/S(±)-Mandelic acid
*R-(-)-Ibuprofen
*S-(+)-Ibuprofen
R/S(±)-Carboxyibuprofen
R/S(±)-2-Hydroxyibuprofen
S-(+)-O-Desmethylnaproxen

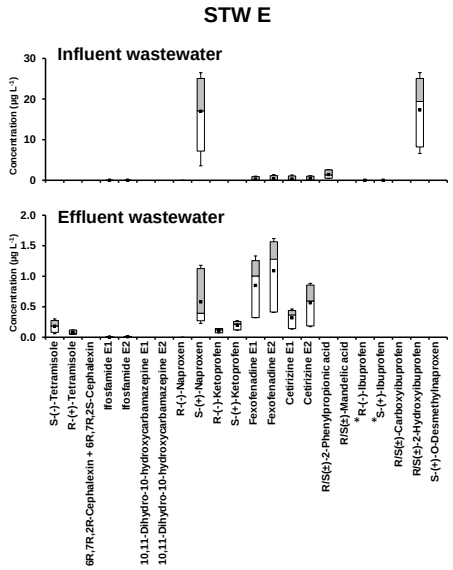


Figure S4. Concentration (µg L^{-1}) of cPACs in influent and effluent wastewater of STW A-E and upstream and downstream river water of each site (STW A-D). Lines in each box show the lower (5%), median (50%) and upper (95%) percentile. Lines from each box show maximum and minimum concentration values. The point inside each box shows the average concentration. *Data not available for R/S(±)-ibuprofen in influent wastewater.

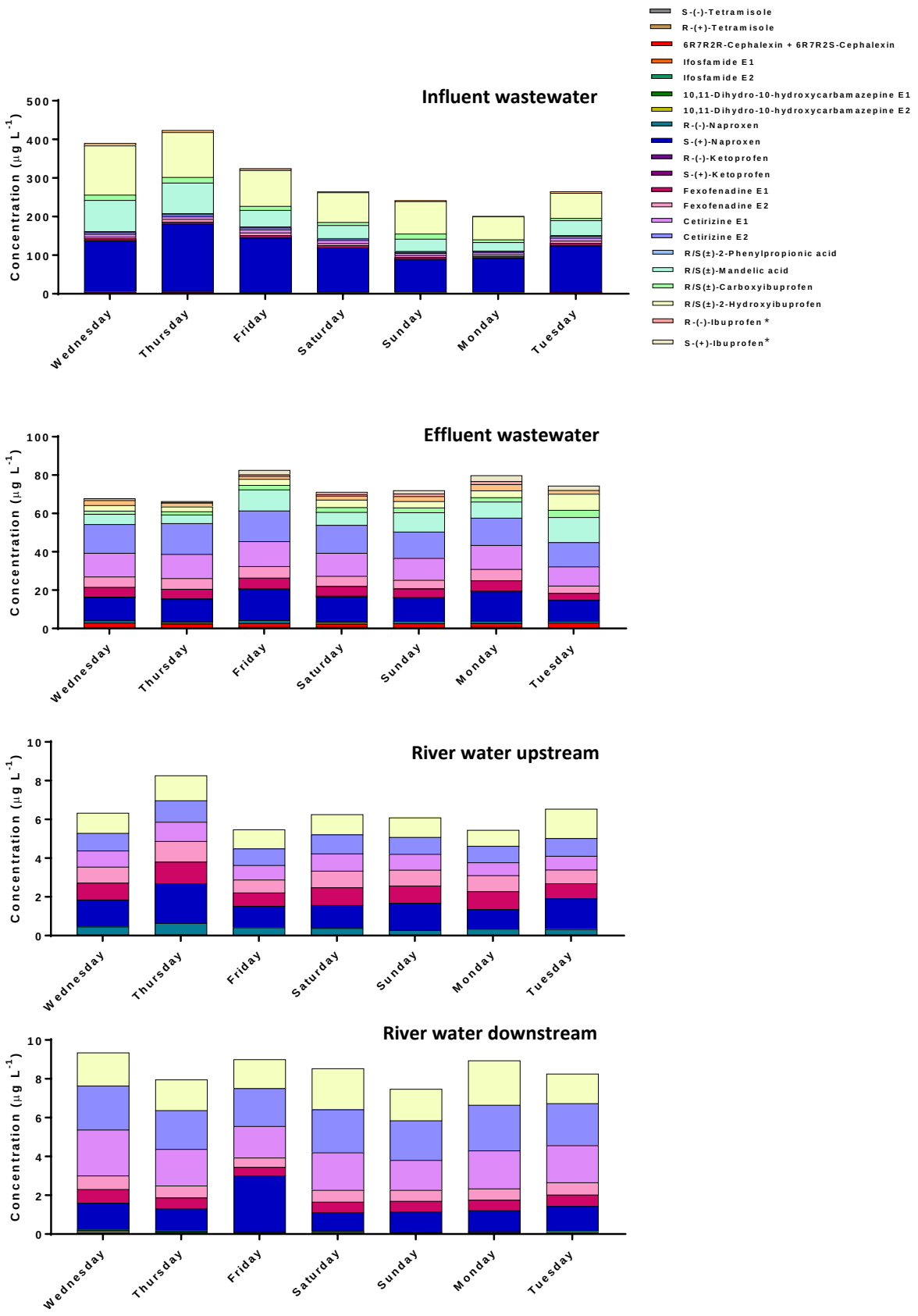


Figure S5. Concentration (µg L^{-1}) of cPACs in influent (A) and effluent (B) wastewater and upstream (C) and downstream (D) river water during a week. *Data not available for R/S(±)-ibuprofen in influent wastewater.

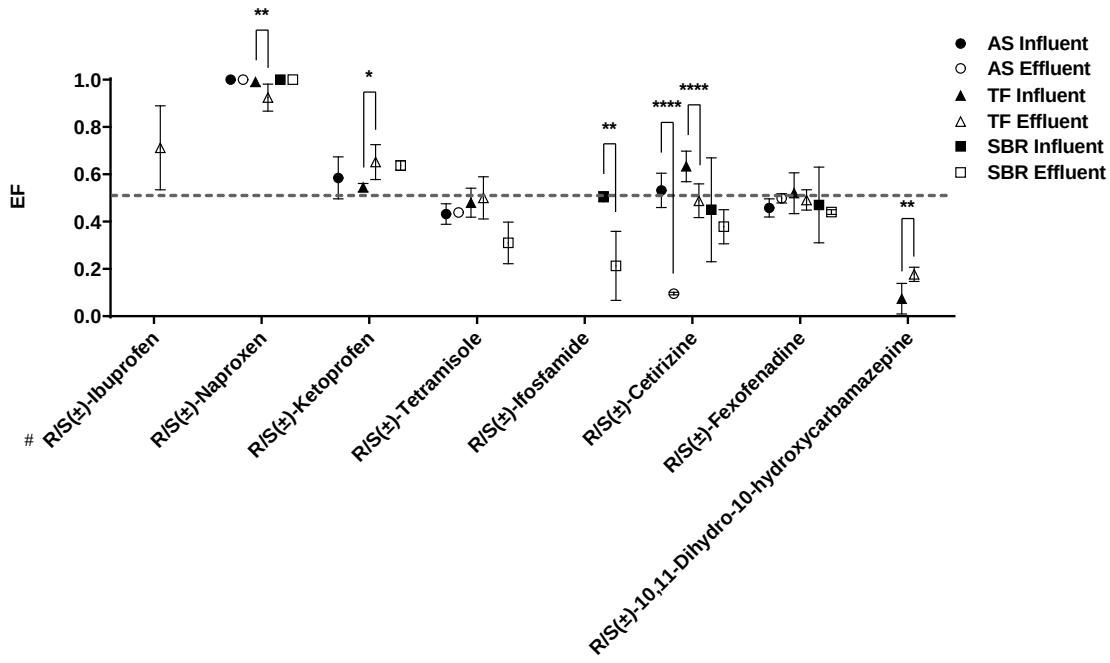


Figure S6. Enantiomeric fraction (EF) (mean ± SD) of cPACs in influent and effluent wastewater of activated sludge (AS), trickling filter beds (TF) and sequencing batch reactors (SBR). Significance was assessed by multiple ANOVA using Sidak's multiple comparisons test, *p<0.05; **p<0.01, ****p<0.0001. # Data not available for R/S(±)-ibuprofen in influent wastewater.

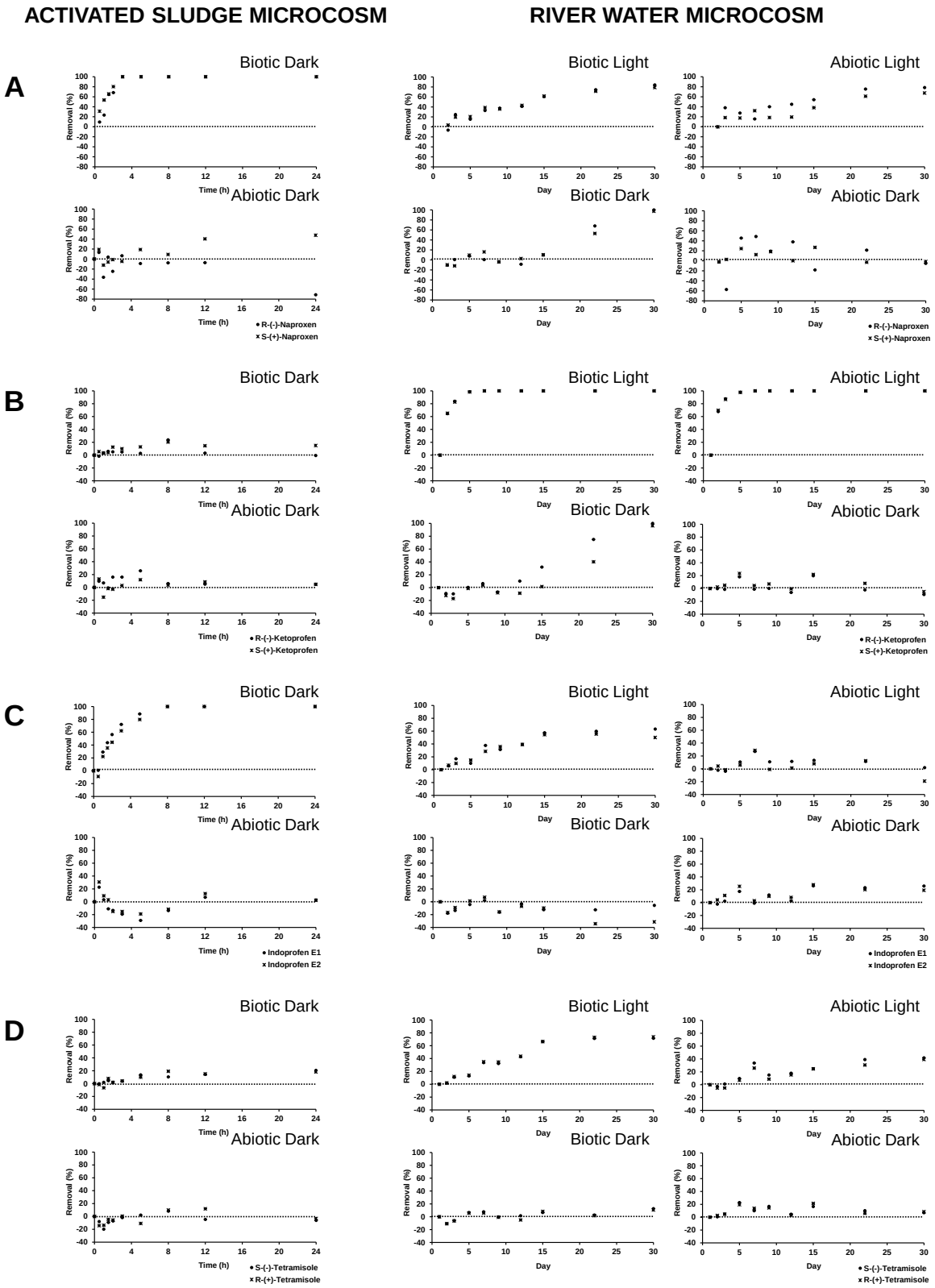


Figure S7. Removal of naproxen (A), ketoprofen (B), indoprofen (C), tetramisole (D), praziquantel (E), ifosfamide (F), 10,11-dihydro-10-hydroxycarbamazepin (G) and chloramphenicol (H) enantiomers in mixed compound bioreactors in activate sludge and river water.

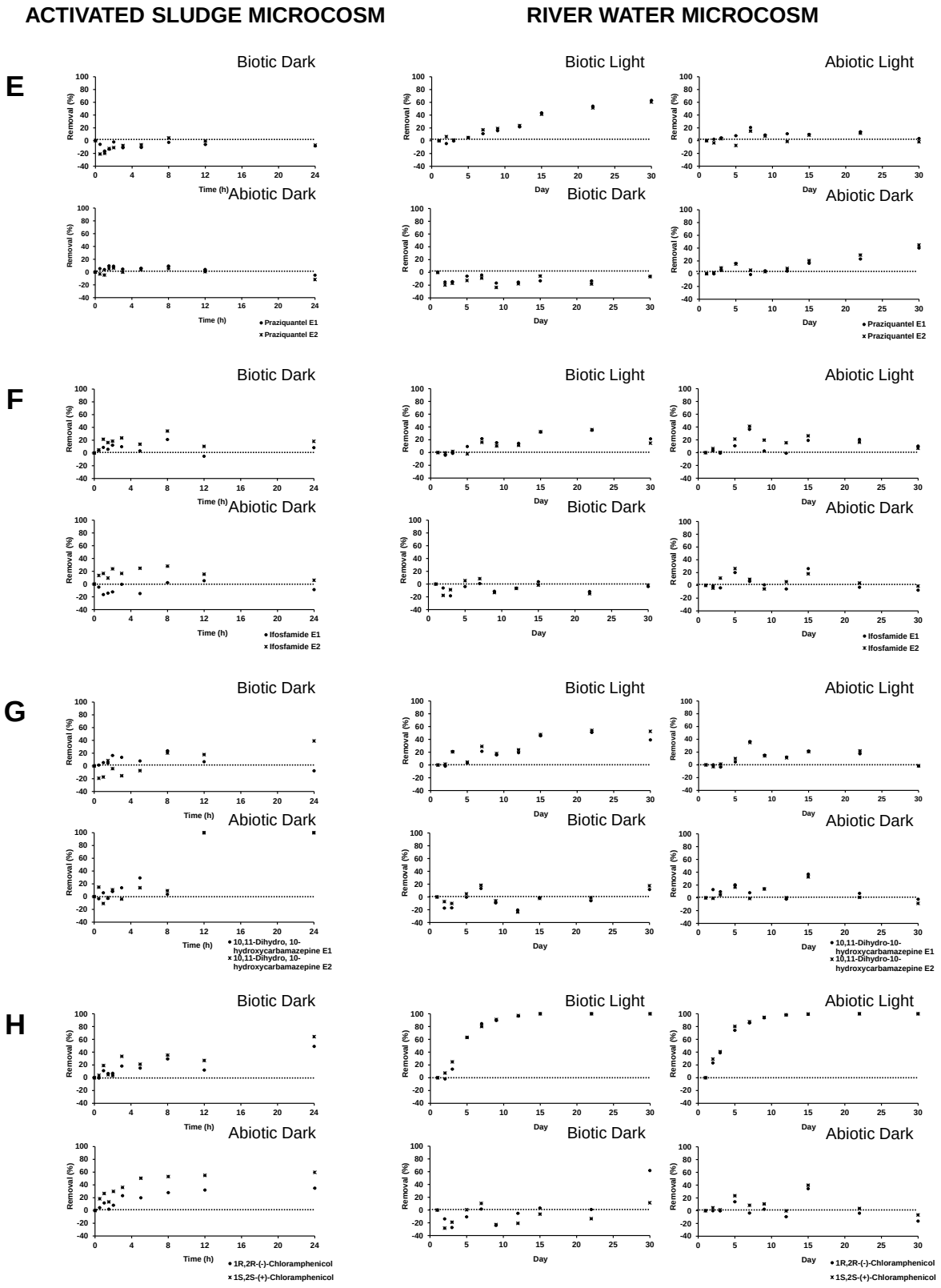


Figure S7. (Continuation).