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Determination of a broad spectrum of pharmaceuticals and endocrine disruptors in biofilm from a waste water treatment plant-impacted river

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HIGHLIGHTS

- Method for analysis of pharmaceuticals & endocrine disruptors in river biofilm
- Bioaccumulation in biofilm of a WWTP-impacted river evaluated
- Seven PhACs and five EDCs detected in biofilm downstream of the WWTP

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ABSTRACT

Wastewater treatment plants (WWTPs) are one of the main sources of pharmaceuticals and endocrine disrupting compounds in freshwater ecosystems, and several studies have reported bioaccumulation of these compounds in different organisms in those ecosystems. River biofilms are exceptional indicators of pollution, but very few studies have focused on the accumulation of these emerging contaminants. The objectives of this study were first to develop an efficient analytical methodology for the simultaneous analysis of 44 pharmaceuticals and 13 endocrine disrupting compounds in biofilm, and second, to assess persistence, distribution, and bioaccumulation of these contaminants in natural biofilms inhabiting a WWTP-impacted river. The method is based on pressurized liquid extraction, purification by solid-phase extraction, and analysis by ultra performance liquid chromatography coupled to a mass spectrometer (UPLC–MS/MS) in tandem. Recoveries for pharmaceuticals were 31–137%, and for endocrine disruptors 32–93%. Method detection limits for endocrine disruptors were in the range of 0.2–2.4 ng g^{-1}, and for pharmaceuticals, 0.07–6.7 ng g^{-1}. A total of five endocrine disruptors and seven pharmaceuticals were detected in field samples at concentrations up to 100 ng g^{-1}.

1. Introduction

Hundreds of pharmaceuticals (PhACs) are ubiquitously detected in freshwater ecosystems at concentrations ranging between ng L^{-1} to μg L^{-1} (Daughton and Ternes, 1999). Despite these relatively low concentrations, PhACs may pose a risk to aquatic organisms because they are designed to modify biochemical pathways in the human body at low doses. Pharmaceuticals are developed to remain in the human body for an adequate period of time to reach their therapeutic effect, which means that a great majority of them are excreted mostly unchanged and may persist in the environment (Boxall et al., 2004). Another group of emerging contaminants widely detected in freshwater ecosystems are endocrine disrupting compounds (EDCs). These compounds belong to different chemical families, and are able to interfere with the hormonal system of exposed organisms by mimicking or counteracting natural hormones (Céspedes et al., 2005; Pojana et al., 2007). The presence of these compounds in freshwater ecosystems is of special concern considering that organisms are chronically exposed to a mixture of PhACs and EDCs. Well-known examples of harmful effects due to exposure to emerging contaminants are the feminisation of male fish (Kidd et al., 2007; Sumpter, 1998), inhibition of molting in crustaceans (Rodriguez et al., 2007), and altered fish behavior (Margiotta-Casaluci et al., 2014; Valenti et al., 2012). Wastewater treatment plants (WWTP) have been identified as one of the main sources of PhACs and EDCs for freshwater ecosystems (Daughton and Ternes, 1999; Fent et al., 2006; Petrovic et al., 2002). The threat posed by the release of those contaminants through WWTP effluents is particularly worrisome in streams or small rivers, where the dilution capacity of the receiving freshwater ecosystem is small (Brooks et al., 2005).

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Studies reporting toxic effects of PhACs and EDCs have led to some attempts of regulation for some of these compounds in the European Union (Collado et al., 2014), such as the anti-inflammatory diclofenac or the synthetic hormones EE2, which have been included in the so called 'watch list' of priority substances under the Water Framework Directive for the "specific purpose of facilitating the determination of appropriate measures to address the risk posed by these substances" (European Commision, 2013). In the US, the Drinking Water Contaminant Candidate List also contains several PhACs and EDCs, including antibiotics, and hormones (Environmental Protection Agency U.S., 2012). Other PhACs, such as carbamazepine, sulfamethoxazole, diclofenac, ibuprofen, naproxen, bezafibrate, atenolol, erythromycin and gemfibrozil have been classified as high priority pharmaceuticals to the water cycle by the GWRC, Global Water Research Coalition (2008).

A comprehensive knowledge of the fate of these pollutants in all the environmental compartments involved may be crucial to assessing the potential risk associated with the discharge of WWTP effluents. Previous studies have reported bioaccumulation of PhACs and EDCs in different environmental compartments. For instance, some studies have shown that sediments may be a sink of PhACs, due to the links with microbial degradation, in particular for those compounds not affected by hydrolysis or photodegradation (Kunkel and Radke, 2008). Other studies have reported bioaccumulation of PhACs and EDCs in invertebrates (Berlioz-Barbier et al., 2014; Huerta et al., 2015) and fish (Brooks et al., 2005; Chu and Metcalfe, 2007; Du et al., 2012; Huerta et al., 2013; Jakimska et al., 2013; Pojana et al., 2007; Ramirez et al., 2009). The question that remains is whether river biofilms could be a significant compartment for accumulation and transformation of these emerging contaminants.

River biofilms are communities composed mainly of bacteria, algae, and fungi embedded in an organic polymer matrix. This matrix of extracellular polymeric substances (EPS) is particularly relevant in the sorption of compounds from the water phase, acting as a molecular sieve, sequestering cations, anions, apolar compounds and particles (Flemming and Wingender, 2010). Biofilms are fundamental constituents of river ecosystems, as they are involved in vital functions such nutrient retention (Bechtold et al., 2012). Their relatively rapid development, widespread distribution and large biomass, together with their capacity to absorb contaminants, suggest that biofilms are exceptional indicators of pollution (Sabater et al., 2007). Several studies have already shown that the presence of contaminants such as PhACs and EDCs can affect the biofilm negatively, altering its structure and metabolism (Corcoll et al., 2014, 2015; Ricart et al., 2010; Rosi-Marshall et al., 2013). Biofilms have an important role in water purification capacity (Chenier et al., 2003; Tien and Chen, 2013). In fact, transport and fate of contaminants in aquatic environments may be affected significantly by their sorption and remobilization interaction with biofilms (Headley et al., 1998), as they follow a transient development and collapse, and in their detachment may move even kilometers downstream (Sabater et al., 2015), transporting contaminants within them. Thus, biofilms influence the transport and fate of emerging contaminants such as PhACs and EDCs through biotic (bioaccumulation and biotransformation by algae and bacteria) (Chenier et al., 2003; Tien and Chen, 2013) and abiotic (physical sorption to EPS) means (Headley et al., 1998) . In this study, bioaccumulation refers to the concentration of target compounds found within the biofilm, both inside the cells and in the matrix surrounding them, which may be led by active biological uptake or passive physical sorption. Bioaccumulation in biofilms has been reported for a wide variety of contaminants, such as metals (Arini et al., 2012; Morin et al., 2008; Serra and Guasch, 2009; Tien et al., 2013), pesticides (Headley et al., 1998), hormones, surfactants and a psychiatric drug (Correa-Reyes et al., 2007; Writer et al., 2011a, 2011b, 2013). Because of their acknowledged capacity to bioaccumulate different contaminants, they could also play a critical role in transferring PhACs and other EDCs to higher trophic levels of riverine food webs within freshwater ecosystems.

However, information about bioaccumulation of PhACs and other EDCs in river biofilms is still non-existent for the great majority of these compounds. To fill the gaps in knowledge regarding the fate of emerging contaminants in freshwater ecosystems, such as PhACs and EDCs, it is essential to develop and validate appropriate analytical methods. Therefore, the objectives of this study were first to develop an efficient analytical methodology for the simultaneous analysis of PhACs and EDCs in river biofilm, and second, to assess persistence, distribution, and bioaccumulation of these trace contaminants in river biofilms affected by WWTP effluents.

2. Materials and methods

2.1. Standards and solutions

A total of 44 PhACs and 13 EDCs were analyzed. A list of the target analytes, molecular structures, and chemical properties are listed in the supplementary material (Table S1). Individual stock standards and labeled internal standards were prepared in methanol at a concentration of approximately 1000 mg L^{-1}. Stock solutions and 20 mg L^{-1} mixtures in methanol were stored at −20 °C and diluted to 1 mg L^{-1} before each analytical run.

2.2. Sample collection and pre-treatment

The study was conducted in a section of the River Segre (Spain) affected by the discharge of a WWTP effluent. Water and biofilm samples were collected at five sites: one site upstream (500 m from the WWTP) and four downstream of the local WWTP (from 500 to 4500 m). Water samples (100 mL) were filtered through 0.45 μm nylon membrane filters and kept at −20 °C until analysis. Biofilm was collected from surfaces of rocks that were removed from near-shore areas of the stream. Biofilm of at least one river cobble was gently scraped (volume = 40 mL) and used for each replicate. The biofilm was placed directly into Falcon® tubes and transported to the laboratory in a dark cool box. Samples were lyophilised and kept frozen (−20 °C) until analysis.

2.3. Water extraction

Water was extracted according to the method developed by Gros et al. (2012) for the analysis of PhACs, and also applied for the analysis of EDCs. Briefly, 3 mL of EDTA 1 M (4%, v/v) were added to the samples. SPE cartridges (Oasis HLB, 60 mg) were conditioned with 5 mL of methanol followed by 5 mL of ultra-pure water at a flow rate of 2 mL min^{-1}. Samples were loaded onto the cartridge at a flow rate of 1 mL min^{-1}. Cartridges were rinsed with 6 mL of HPLC grade water, and were dried in air for 30 min. Finally, analytes were eluted with 6 mL of methanol and evaporated to dryness under a nitrogen stream and reconstituted in 1 mL of methanol/water (10:90) for the analysis of PhACs and methanol/water (50:50) for the analysis of EDCs. Finally, 5 μL of a 1 mg L^{-1} standard mixture containing labeled compounds were added in the water extracts before analysis.

2.4. Biofilm extraction and clean-up

Initially, sonication was pre-selected as the possible extraction method, together with pressurized liquid extraction (PLE). Four solvents were tested, including methanol, methanol/water (1:1), methanol with 0.1% EDTA, and citric buffer (pH4)/acetonitrile (1:1, v/v), all based on the authors' previous experience with pharmaceutical and EDC extraction. To reduce the number of experiments and solvent consumption, the results of one of these solvents were compared in both extraction methods to find which one had the best extraction recoveries. For sonication extraction, 200 mg of freeze-dried biofilm was placed in a 14-mL Falcon tube with 10 mL of the corresponding solvent. Extraction entailed 3 cycles of 10 min, and the supernatant was collected in a

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