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Effects of pesticides and pharmaceuticals on biofilms in a highly impacted river

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ABSTRACT

We investigated the effects of pharmaceuticals and pesticides detected in a Mediterranean river, on fluvial biofilms by translocation experiments performed under controlled conditions. Water was sampled from three sites along a pollution gradient. Biofilms grown in mesocosms containing relatively clean water were translocated to heavily polluted water. Several biofilm descriptors were measured before and after translocations. Fifty-seven pharmaceuticals and sixteen pesticides compounds were detected in river waters. The translocation from less to more polluted site was the most effective. Autotrophic biomass and peptidase increased while phosphatase and photosynthetic efficiency decreased. Multivariate analysis revealed that analgesics and anti-inflammatories significantly affected biofilm responses. Ibuprofen and paracetamol were associated with negative effects on photosynthesis, and with the decrease of the green algae/cyanobacteria ratio, while diclofenac was associated with phosphatase activity. The effects of these emerging compounds on biofilms structure and function may cause important alterations in river ecosystem functioning.

1. Introduction

A large number of organic compounds reach freshwaters and pose a risk to the structure and functioning of ecosystems. Although pesticides and pharmaceutical drugs are the most commonly detected compounds (Azevedo et al., 2000; Daughton and Ternes, 1999), they differ in the ways they enter water bodies. Pesticides mainly originate from agricultural activities and enter aquatic environments through diffuse sources via runoff. Their concentration normally peaks after rainfalls following spraying on agricultural fields (Rabiet el al., 2010). Although various pesticides are currently included in the list of priority substances in the European Union regulations (Decision 2455/2001/EC), many others are still unregulated. Pharmaceuticals mainly enter aquatic environments via wastewater, and their concentrations in rivers are normally lower than those of pesticides (Petrović et al., 2005). However, their relevance is related to the chronic character of their input, and their concentrations may increase as a consequence of lower dilution situations characteristically occurring under water scarcity (Kuster et al., 2008). Pharmaceuticals are intrinsically bioactive compounds and little is known about their effects on the aquatic ecosystems resulting from long-term, low-dose exposure (Ginebreda et al., 2010). Although pharmaceuticals are considered as new emerging pollutants by the EC, only a few have been included in regulatory policies within the Water Framework Directive (European Commission, 2000). Environmental risk assessment (ERA) procedures for both pesticides and pharmaceuticals are based on short-term, single-species laboratory tests that only partially reflect real ecosystem situations (Ginebreda et al., 2010).

To date, a few studies have investigated the effects of priority and non-priority pollutants on real ecosystems (Hernando et al., 2006; Crane et al., 2006; Sanderson et al., 2004; Nunes et al., 2005; Pascoe et al., 2003), but less attention has been paid to the effects on biofilms (Pesce et al., 2006; Ricart et al., 2010a). River biofilms are complex microbial benthic communities composed of autotrophic and heterotrophic organisms (Romaní, 2010), which act as an interface between the water and the riverbed by interacting and responding rapidly to changes in environmental conditions (Sabater et al., 2007). Biofilms play a fundamental role in the trophic web and in the biogeochemical cycles within aquatic

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ecosystems (Battin et al., 2003; Lock et al., 1993). The short life cycle of biofilm microorganisms and the trophic interactions between the microbiota (algae, bacteria, fungi, protozoa) allow for the detection of both short and long-term, and of direct and indirect effects on the biofilm consortia (Proia et al., 2012a). River biofilms can therefore be useful in determining the effects of pollutants on freshwater ecosystems (Sabater et al., 2007).

This study aims to investigate the effects of pharmaceuticals and pesticides detected in the waters of the Llobregat River on the structure and function of its biofilms. The Llobregat is the most important source of drinking water for the city of Barcelona (Catalonia, NE Spain). However, this area is densely populated and affected by intense industrial and agricultural activities. As a consequence of these anthropogenic pressures, high concentrations of priority and emerging compounds occur in both water and sediments (Casas et al., 2003; Guerra et al., 2009). The environmental risk assessment of the pharmaceuticals detected in the Llobregat waters (Ginebreda et al., 2010), as well as the relationships between the occurrence of pharmaceuticals (Muñoz et al., 2009) and pesticides (Ricart et al., 2010a) and the structural composition of benthic communities (macroinvertebrates and diatoms) has been already carried out in this river. In search for causal evidence of the effects of these pollutants on biofilms, determining the effect of polluted water on reference communities can be of help. This approach was applied in the present study in mesocosms experiments involving the translocation of biofilms from less polluted to more polluted waters, aiming to reveal the links between the biofilm communities' responses to pollutant concentrations and to co-occurring environmental factors. Three sampling sites were selected to define a pollution gradient, translocations from less to progressively more polluted waters were performed, and the effects on biofilms were determined. We hypothesized that bioactive compounds affect the responses of biofilms, and the magnitude of the responses should be related to the incoming higher concentration of organic pollutants. To test our hypotheses we related the observed biofilm responses in each translocation experiment to the environmental factors and chemical water quality of the sites.

2. Methods

2.1. Study sites

The Llobregat is a Mediterranean river located in NE Spain, which flows into the Mediterranean Sea, south of Barcelona. The Llobregat is 165 km long and drains a catchment of 4948 km^2 (Marcé et al., 2012). Its flow is characterized by a high variability including periodic floods and droughts related to seasonal heavy rainfall and drought respectively (Ricart et al., 2010a). The mean annual discharge is 693 Mm^3 (Ginebreda et al., 2010), nearly 30% of which is used for drinking water purposes. As such, the Llobregat River is a paradigm of an overexploited river (Muñoz et al., 2009). Its watershed supports over 3 million people and receives significant inputs of industrial and urban wastewater ($\sim$137 Mm^3/year, Ginebreda et al., 2010) as well as surface runoff from agricultural areas (Kuster et al., 2008). Moreover, salt inputs from the salt mines of the tributary Cardener have caused an increase in water salinity, worsening the already poor conditions of the lower reaches of the river. In this study, three sampling sites were selected in the middle-lower part of the Llobregat following a pollution gradient: Castellbell (Reference, R) and Mina de Terrassa (Polluted, P), with low and moderate pollution respectively, and Sant Joan Despí (Highly Polluted, HP) being a pollution hotspot.

2.2. Experimental design

The biofilm responses to increasing pollutant concentrations were investigated by means of translocation experiments performed under controlled conditions. The river water was collected three times a week between the 16th of October and the 19th of November 2009 from the three sites, and then used as inoculum to grow biofilms in 18 independent mesocosms installed in the laboratory. Biofilms were colonized on glass slides (1 cm^2 each) placed at the bottom of each mesocosm (35–40 slides per mesocosm). The mesocosms consisted of sterile glass jars (19 cm in diameter, 9 cm high), filled with 1.5 L of water recirculated by a submersible pump (Hydor, Pico 300, 230 V 50 Hz, 4.5 W). All mesocosms were kept in an incubator (SCLAB) with controlled temperature (18 °C) and light irradiance (150–180 μmol photons m^{-2} s^{-1}; dark/light cycle of 12 h/12 h). After 25 days of colonization, the biofilms were translocated to more polluted waters. Three translocation experiments were carried out (three replicate jars per translocation): glass jars with biofilms previously incubated with R water were then filled with P water (R→P), and HP water (R→HP). Glass jars previously incubated with P water were then incubated with HP water (P→HP). Finally, in nine replicate glass jars (three per site) previously incubated with R, P and HP water, the original conditions were maintained and used as controls. The biofilms were sampled four times: one before the translocations (3 November, day 0) and three after; day 2 (5 November), day 9 (12 November) and day 16 (19 November). During the experiments, the water was replaced three times a week with river water from the respective sites. At each sampling date one glass slide for each biofilm metric was collected from each replicate mesocosm.

2.3. Environmental conditions

2.3.1. Physical and chemical parameters

Conductivity, temperature, pH and dissolved oxygen were measured with the sensor probes (HACH LANGE GMBH, Germany) both in the field and in the mesocoms, before and after each water renewal ($n = 15$). Water samples were collected from the glass jars and filtered (Nylon Membrane Filters 0.2 μm, WHATMAN, UK) before and after water renewals, prior to analysis. Soluble reactive phosphorus was measured following Murphy and Riley (1962). Samples for anion and cation were preserved frozen until the analysis ($n = 14$) by ion chromatography (761 Compact IC, METROHM, Switzerland).

2.3.2. Pharmaceuticals and pesticides

The concentrations of 66 pharmaceuticals were analysed in surface waters using the multiresidue analytical method based on LC-MS/MS after solid-phase extraction described by Osorio et al. (2012). The concentrations of 16 pesticides were analysed following the method based on online SPE–LC–MS/MS described by Köck-Schulmeyer et al. (2012). The analysis of both pharmaceuticals and pesticides was performed on triplicate at each sampling date ($n = 18$).

2.4. Biofilm metrics

2.4.1. Chlorophyll in vivo fluorescence measurements

The chlorophyll fluorescence emission of the biofilms was measured with a Phyto-PAM (Pulse Amplitude Modulated) chlorophyll fluorometer (Heinz Walz GmbH), which uses a set of light-emitting diodes that excite chlorophyll using four different wavelengths (470, 520, 645 and 665 nm). For each glass slide sampled from each glass jar, three measurements were performed to represent the small-scale heterogeneity of biofilms. All measurements were based on the procedure described by Serra et al. (2009). The photosynthetic efficiency (Yeff) and capacity (Ymax) of PSII were measured based on the fluorescence signal recorded at 665 nm and given as relative units of fluorescence. The minimum fluorescence level of the dark-adapted samples was used as an estimate of autotrophic biomass. This estimate was based on the fluorescence recorded at four different excitation wavelengths (F1 at 470 nm, F2 at 520 nm, F3 at 645 nm, and F4 at 665 nm). F1 is linked to green algae, whereas F2 is mostly related to diatoms. The F3 signal is related to cyanobacteria and the F4 signal is related to the whole algal community (Ricart et al., 2010a). The ratio between F1 and F3 was calculated for each replicate as an indicator of changes in the autotrophic community structure.

2.4.2. Chlorophyll-a density

On each sampling day, one glass slide from each glass jar was collected and the chlorophyll-*a* was extracted using 90% acetone for 12 h. Sonication during two minutes (40 W power, 40 kHz frequency, SELECTA, Spain) improved the pigment extraction. The chlorophyll-*a* concentration was determined using spectrophotometric measurements (UV, 1800 Shimadzu) following the method described in Jeffrey and Humphrey (1975).

2.4.3. Extracellular enzyme activities

The activities of the extracellular enzymes leucine-aminopeptidase (EC 3.4.11.1), alkaline phosphatase (EC 3.1.3.1–2) and β-D-1,4-glucosidase (EC 3.2.1.21) in the biofilms were measured spectrofluorometrically as described in Proia et al. (2013).

Leucine-aminopeptidase and β-D-1,4-glucosidase are mainly bacterial activities while alkaline phosphatase may be produced by both algae and bacteria.

2.4.4. Phosphorus uptake capacity

Phosphorus (P) uptake capacity was estimated by measuring the decrease in SRP after a calculated spike. In each experiment, background samples were analysed in advance in order to reach 4–8 times increase of the basal phosphorus concentration with the spike. The phosphorus uptake rate (U, μg P cm^{-2} h^{-1}) was calculated as the mass of P per unit area per unit time (Proia et al., 2011). As U could depend on biomass and the colonized area was different in each day, the values were normalized by expressing the mass of P removed from water per chlorophyll-*a*