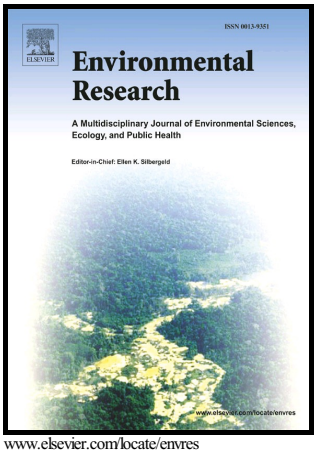

Impact of fullerenes in the bioaccumulation and biotransformation of venlafaxine, diuron and triclosan in river biofilms

Lúcia H.M.L.M. Santos, Anna Freixa, Sara Insa, Vicenç Acuña, Josep Sanchís, Marinella Farré, Sergi Sabater, Damià Barceló, Sara Rodríguez-Mozaz

www.elsevier.com/locate/envres

PII: S0013-9351(18)30618-2
DOI: https://doi.org/10.1016/j.envres.2018.11.036
Reference: YENRS8169

To appear in: *Environmental Research*

Received date: 5 October 2018
Revised date: 20 November 2018
Accepted date: 22 November 2018

Cite this article as: Lúcia H.M.L.M. Santos, Anna Freixa, Sara Insa, Vicenç Acuña, Josep Sanchís, Marinella Farré, Sergi Sabater, Damià Barceló and Sara Rodríguez-Mozaz, Impact of fullerenes in the bioaccumulation and biotransformation of venlafaxine, diuron and triclosan in river biofilms, *Environmental Research,* https://doi.org/10.1016/j.envres.2018.11.036

Impact of fullerenes in the bioaccumulation and biotransformation of venlafaxine, diuron and triclosan in river biofilms

Lúcia H.M.L.M. Santos[a,*], Anna Freixa[a], Sara Insa[a], Vicenç Acuña[a], Josep Sanchís[b], Marinella Farré[b], Sergi Sabater[a,c], Damià Barceló[a,b], Sara Rodríguez-Mozaz[a]

[a]Catalan Institute for Water Research (ICRA), Carrer Emili Grahit 101, 17003 Girona, Spain

[b]Department of Environmental Chemistry, IDAEA, CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

[c]Institute of Aquatic Ecology, University of Girona, Campus de Montivili, 17071 Girona, Spain

[*]Corresponding author: Lúcia H.M.L.M. Santos, Catalan Institute for Water Research (ICRA), Carrer Emili Grahit 101, 17003 Girona, Spain; Telephone: +34 972183380; Fax number: +34 972183248, lhsantos@icra.cat

Abstract:

A huge variety of organic microcontaminants are presently detected in freshwater ecosystems, but there is still a lack of knowledge about their interactions, either with living organisms or with other contaminants. Actually, carbon nanomaterials like fullerenes (C_{60}) can act as carriers of organic microcontaminants, but their relevance in processes like bioaccumulation and biotransformation of organic microcontaminants by organisms is unknown. In this study, mesocosm experiments were used to assess the bioaccumulation and biotransformation of three organic microcontaminants (venlafaxine, diuron and triclosan) in river biofilms, and to understand how much the concomitant presence of C_{60} at environmental relevant concentrations could impact these processes. Results indicated that venlafaxine exhibited the highest bioaccumulation (13% of the initial concentration of venlafaxine in water), while biotransformation was more evident for triclosan (5% of the initial concentration of triclosan in water). Furthermore, biotransformation products such as

methyl-triclosan were also present in the biofilm, with levels up to 42% of the concentration of accumulated triclosan. The presence of C_{60} did not involve relevant changes in the bioaccumulation and biotransformation of microcontaminants in biofilms, which showed similar patterns. Nevertheless, the study shows that a detailed evaluation of the partition of the organic microcontaminants and their transformation products in freshwater systems are important to better understand the impact of the co-existence of others microcontaminants, like carbon nanomaterials, in their possible routes of bioaccumulation and biotransformation.

Keywords:

Emerging microcontaminants, nanoparticles, transformation products, river biofilms, co-exposure

1. Introduction

An increasingly incidence of organic microcontaminants in freshwater ecosystems has been pointed out, embracing a wide variety of compounds such as pharmaceuticals and personal care products (PPCPs), endocrine disrupting compounds, pesticides, nanomaterials, among others (Farré et al., 2010; Thomaidi et al., 2015; Meador et al., 2016; Sousa et al., 2018). Although they are detected in the environment at low concentrations (ng L^{-1}, µg L^{-1}), many organic microcontaminants are considered pseudo-persistent contaminants, since their input surpass their removal/degradation (Ahmed et al., 2017). In this way, these pollutants may exert potential toxic effects on aquatic organisms (Santos et al., 2010) and may affect the biological communities (Corcoll et al., 2014). Some organic microcontaminants also show the potential to bioaccumulate (Huerta et al., 2012; Du et al., 2014; Rodríguez-Mozaz et al., 2016) and propagate through the food web (Du et al., 2014; Ruhí et al., 2016).

The concern about the presence and impact of nanomaterials in the environment has increased in the last years. However some reviews have recently reported that carbon nanomaterials do not show relevant bioaccumulation and do not pose a direct threat on aquatic organisms when present at environmental concentrations (Bjorkland et al., 2017; Freixa et al., 2018a). Nevertheless, due to their sorption properties, it is expected that nanomaterials can interact with co-existing microcontaminants,

influencing their original distribution, toxicity, bioaccumulation and risk (Deng et al., 2017; Freixa et al., 2018a). For instance, carbon nanomaterials have been reported to affect the toxicity of different organic microcontaminants by promoting antagonistic or synergistic responses in species of different trophic levels (Knauer et al., 2007; Baun et al., 2008; Sanchís et al., 2016). Particularly, fullerenes, a type of carbon nanomaterial composed by 60 atoms of carbon, showed to change the bioaccumulation of organic microcontaminants in fish by impacting their bioavailability (Hu et al., 2010; Park et al., 2010).

Given that organic microcontaminants occur in the environment as mixtures of pollutants, it is of relevance to evaluate how their simultaneous presence may affect the toxicity, biotransformation and bioaccumulation processes in freshwater organisms. In this context, river biofilm appears as a good model to assess the impact of organic microcontaminants as well as their interaction at a community level. River biofilms are complex communities of microorganisms mainly formed by bacteria, algae, fungi and protozoa, which are embedded in a mucopolysaccharide matrix (Sabater et al., 2007). Due to its close contact with the water column and streams, biofilm uptakes organic and inorganic nutrients from the water, but also microcontaminants and other toxicants, either by uptake or physical adsorption, mainly by their retention in the mucopolysaccharide matrix (Sabater et al., 2007). In this way, biofilm showed the ability to accumulate organic microcontaminants (Huerta et al., 2016; Ruhí et al., 2016; Wilkinson et al., 2018) at concentrations up to few hundred ng g^{-1}, as well as to degrade them (Sabater et al., 2007; Writer et al., 2011). In fact, river biofilms have been used in ecotoxicological studies to evaluate the impact of multiple stressors (e.g. physical, chemical or biological) in freshwater ecosystems (Sabater et al., 2016; Romero et al., 2018; Serra-Compte et al., 2018). Effects perform at the community level and provide an integrated response of the community to the environmental changes (Sabater et al., 2007).

Therefore, this study aimed to evaluate i) the bioaccumulation and biotransformation of three selected organic microcontaminants (venlafaxine, diuron and triclosan) by river biofilms; and ii) the influence of the presence of fullerenes (C_{60}), on the bioaccumulation and biotransformation of selected organic microcontaminants by river biofilms. Bioaccumulation accounts for the organic microcontaminants retained in the biofilm, either by the uptake in microbial and algal cells or because of their adsorption to the cells walls or to the extracellular mucopolysaccharide matrix. With

this purpose, individual mesocosm experiments were launched with the three organic microcontaminants (venlafaxine, diuron and triclosan) and fullerenes (C_{60}). The presence of the three target microcontaminants as well as some selected transformation products (TPs) was monitored in water along the 72h of the experiments and in the river biofilms at the end of the exposure. The hypothesis tested was that i) organic microcontaminants with distinct physical-chemical properties will present different patterns of bioaccumulation in river biofilms; ii) the presence of C_{60} will interfere with the bioaccumulation and the biotransformation of the selected organic microcontaminants by river biofilms.

2. Materials and Methods

2.1. Reagents and Chemicals

Venlafaxine, diuron, triclosan, methyl-triclosan and 2,4-dichlorophenol were purchased from Sigma-Aldrich. N-desmethylvenlafaxine, O-desmethylvenlafaxine, N,N-didesmethylvenlafaxine, N,O-didesmethylvenlafaxine, N,N-didesmethyl-O-desmethylvenlafaxine, 1-(3,4-dichlorophenyl)urea (DCPU) and 1-(3,4-dichlorophenyl)-3-methylurea (DCPMU) were purchased from Toronto Research Chemicals (Ontario, Canada). Isotopically labeled compounds used as internal standards were venlafaxine-d6 (VLF-d6) purchased from CDN Isotopes (Quebec, Canada), diuron-d6 (DIU-d6) purchased from Sigma-Aldrich, and triclosan-d3 (TCS-d3) and triclosan-methyl-d3-ether (MTCS-d3) purchased from Toronto Research Chemicals (Ontario, Canada). All the standards were of high grade purity (>95%). Fullerene C_{60} with 99.5% of purity was also purchased from Sigma-Aldrich. Methanol, acetonitrile and water LC-MS grade were provided by Merck (Darmstadt, Germany). All solvents were of MS grade. Sodium chloride and di-sodium hydrogen phosphate dehydrate for solid phase microextraction (SPME) analyses were purchased from Scharlau (Sentmenat, Spain).

Individual stock solutions of all compounds were prepared on a weight basis in methanol (final concentration of 1000 mg L^{-1}). An intermediate mixture containing the selected organic microcontaminants and their TPs of 10 mg L^{-1} was also prepared in methanol by appropriate dilution of the individual stock solutions. Stock solutions and the 10 mg L^{-1} intermediate mixture solution were stored at -20 ºC. Before each analytical run, working standard solutions were prepared in a mixture of methanol:water (50:50, *v/v*) for LC-MS/MS analysis or in water for GC-MS/MS analysis.

An aqueous fullerene stock suspension of 100 mg L^{-1} was prepared by adding C_{60} fullerene powder to an amber glass bottle containing rainwater (previously filtered with 0.2 µm pore-size nylon-fiber filters). Aggregates were dispersed by 2 months stirring with a PTFE-coated magnetic nucleus at room temperature. The bottle was loosely caped with pre-slitted aluminum fold in order to allow free exchange of ambient air while preventing dust deposition. No surfactant, organic solvent or ultrasounds were employed in order to artificially enhance the stability of the aggregates. The resulting aggregates were round-shaped and highly polydispersed, with some micron-sized clusters co-occurring with a fraction of nanosized aggregates (mainly from 100 to 200 nm). More detailed information on the characterization of the C_{60} aggregates is described elsewhere (Freixa et al., 2018b).

2.2. Experimental design and set-up

Experiments were performed according to Freixa et al. (2018b), using glass mesocosms (diameter = 25 cm, height = 15 cm), with a central glass cylinder and a glass blade incorporated to a rotor (12 V, 2.2 W, 60 rpm, Philips), in order to constantly move the water, allowing a homogenous flow circulation. Each mesocosm was filled with 4.5 L of rainwater with the following composition: 2.2 µg L^{-1} $P\text{-}PO_3^{4-}$, 0.75 mg L^{-1} $N\text{-}NO_3$ and 2.8 mg L^{-1} dissolved organic carbon. Water level was kept constant by adding water at a constant daily rate of 4.5 mL day^{-1} using a peristaltic pump (Ismatec, MCP, 150W). During the exposure time, mesocosms were maintained at a constant temperature of 20 ºC and day-night cycles (12-hour light /12-hours dark), applied using LED lamps (Lumina Led 62, 48W).

Three organic microcontaminants were selected based on their physical-chemical properties (Table S1), toxicity, environmental levels, and potential to accumulate in aquatic organisms:

- Venlafaxine is an antidepressant, widely detected in surface waters at concentrations up to 387 ng L^{-1} (González Alonso et al., 2010). This pharmaceutical has shown potential to bioaccumulate in biofilms (Huerta et al., 2016). Nevertheless, there is a gap of knowledge on its toxicity, and just a few studies are available in literature (Fong et al., 2017; Di Poi et al., 2018), mainly focusing on fish (Best et al., 2014; Bisesi Jr et al., 2014). Nevertheless, an EC_{50} of 6900 µg L^{-1} was reported for algae (Minguez et al., 2018);

- Diuron is a herbicide and antifouling biocide, currently included in the list of priority substances of Directive 2013/39/EU (European Comission, 2013). It is highly persistent in the environment (Giacomazzi and Cochet, 2004), being found at levels up to 3.05 µg L^{-1} (Okamura et al., 2003). It shows toxicity to algae (EC_{50} = 15 µg L^{-1}) (Proia et al., 2011) and can bioaccumulate in biofilms (Chaumet et al., 2019); and

- Triclosan is an antibacterial that can be ubiquitously found in the environment, reaching concentrations up to 310 ng L^{-1} (Lyndall et al., 2017). A wide variety of organisms shows toxicity to triclosan (Bedoux et al., 2012), including bacteria (EC_{50} = 43.9 µg L^{-1}) (Ricart et al., 2010) and algae (EC_{50} = 0.7-4.46 µg L^{-1}) (Orvos et al., 2002). It can bioaccumulate in aquatic organisms as well (Coogan et al., 2007; Rüdel et al., 2013; Huerta et al., 2016).

2.2.1. Preliminary experiments

In order to evaluate the interaction of the organic microcontaminants with the C_{60} as well as with the inner glass structure of the mesocosm, and their possible abiotic transformation under the experimental conditions, a previous experiment was carried out in abiotic conditions, i.e. without biofilm. In this way, 12 mesocosms were filled with rainwater and spiked with each individual organic microcontaminant at the tested nominal concentrations (10 µg L^{-1} for diuron and triclosan, and 50 µg L^{-1} for venlafaxine). Nominal concentrations of the organic microcontaminants were selected based on differences in their toxicity, which shows EC_{50}s for algae varying from 0.7-4.46 µg L^{-1} (triclosan) (Orvos et al., 2002), 15 µg L^{-1} (diuron) (Proia et al., 2011) to 6900 µg L^{-1} (venlafaxine) (Minguez et al., 2018). An EC_{50} for bacteria of 43.9 µg L^{-1} has been described for triclosan (Ricart et al., 2010). Six of these mesocosms were also spiked with C_{60} at a nominal concentration of 1 µg L^{-1}. Each condition was tested in duplicate. Water samples were collected every 24h during the 72h of experiment. During the experiment, the concentration of organic microcontaminant freely dissolved in the aqueous phase and the fraction of microcontaminant adsorbed to suspended C_{60} aggregates were measured separately. For that, samples were centrifuged (7500 rpm, 10 min, 4 ºC) to separate both fractions. Direct analysis was performed for water fraction (see section 2.4.1), whereas 1 mL of methanol was added for the extraction of the organic microcontaminants adsorbed to C_{60} with ultrasonic assisted extraction for 20 min. Then, the methanolic extract was centrifuged (15,000 rpm, 15 min) and the supernatant was transferred to a glass tube. This procedure was repeated twice and all

the supernatants were combined and evaporated under a gentle stream of nitrogen. Finally, dried extracts were reconstituted in 1 mL of methanol:water (10:90, *v/v*) and were analysed by LC-MS/MS (diuron and venlafaxine) or GC-MS/MS (triclosan) (see LC-MS/MS and GC-MS/MS methods description in Supplementary Material).

2.2.2. Exposure experiments

Three sets of experiments were performed for each of the three organic microcontaminants considered (i.e. venlafaxine, diuron and triclosan). A scheme of the experimental set-up is shown in Figure 1. Each set included 4 different exposure conditions.

- C_{60} exposure: biofilm was exposed to a nominal concentration of C_{60} of 1 µg L^{-1};
- Organic microcontaminant exposure: biofilm was individually exposed to each of the 3 selected organic microcontaminants. A nominal concentration of 10 µg L^{-1} was chosen for diuron and triclosan, and 50 µg L^{-1} for venlafaxine;
- Organic microcontaminant + C_{60} exposure: biofilm was simultaneously exposed to the organic microcontaminant and C_{60}, using the same nominal concentrations selected for the single exposure experiments;
- Control: biofilm was kept under normal experimental conditions of light and temperature and neither organic microcontaminants nor nanoparticles were added.

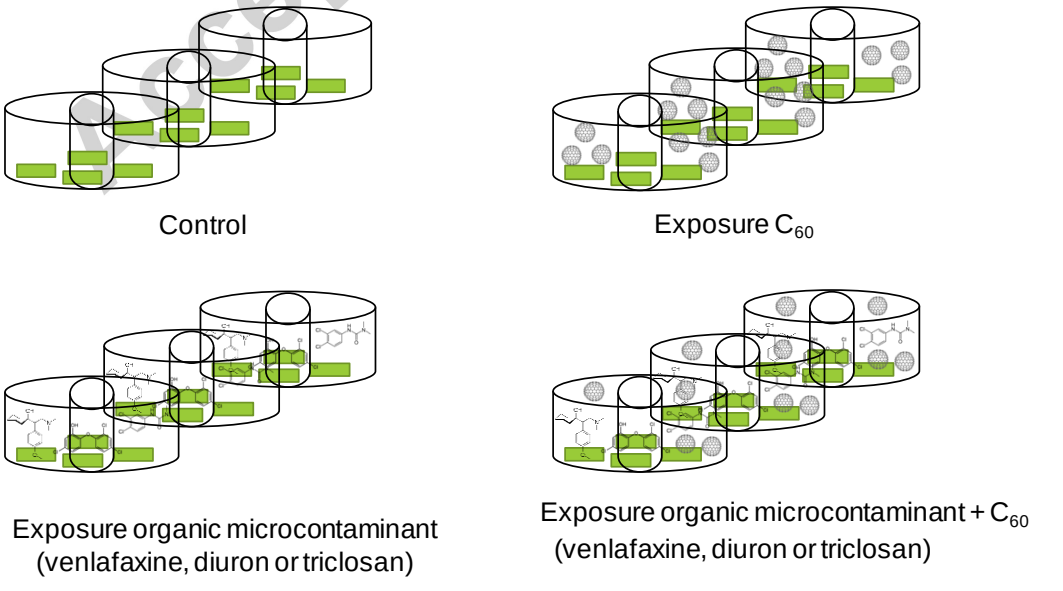

Figure 1 - Experimental setup. Each experimental set consisted in 4 test conditions (n = 3), namely: Control, Exposure to C_{60}, Exposure to organic microcontaminant, and Exposure to organic microcontaminant + C_{60}. An individual experimental set was performed for each organic microcontaminant (venlafaxine, diuron or triclosan), using a nominal concentration of 50 µg L^{-1} (venlafaxine) or 10 µg L^{-1} (diuron or triclosan). The nominal concentration of C_{60} was 1 µg L^{-1} in all the tested conditions.

All experiments were performed in triplicate using 5 week-old epilithic biofilm previously grown in artificial channels (200 cm long, 10 cm wide, 7.5 cm deep). Colonizing biofilm was obtained from a non-impacted oligotrophic stream (Llémena River, NE, Spain) (GPS coordinates: 42°04'16.3"N, 2°36'13.7" E), and allowed to grow on square glass tiles (1.5 × 1.5 cm). After 5 weeks, colonised biofilm was transferred to glass mesocosms and acclimated for a period of 12h before starting the exposure experiments, with exposure time of 72h.

For the exposure experiment, stock solutions of each organic microcontaminant were previously prepared on methanol (concentration = 1000 mg L^{-1}). Afterwards they were diluted with rainwater to get the final concentration of 50 µg L^{-1} (venlafaxine) and 10 µg L^{-1} (diuron and triclosan) on the mescosms. This represents a maximum final concentration of methanol in the mesocosm of 50 µL L^{-1} (equivalent to 0.005%, *v/v*), which is below the reported toxicity concentration for aquatic organisms, especially algae, as well as the maximum threshold recommended by OECD (100 µL L^{-1}) (Hutchinson et al., 2006). In the case of C_{60}, an aqueous stock suspension of 100 mg L^{-1} prepared using filtered rainwater (see section 2.1) was used for spiking purposes.

Besides the three organic microcontaminants, some of their target TPs (Table S1) were chosen for analysis based on biodegradation and/or phototransformation pathways of the parent compounds described in literature, as well as the availability of analytical standards. Five TPs of venlafaxine, which are mainly correlated to its human metabolization (Magalhães et al., 2014), were selected, with some of them being detected in the environment (Kern et al., 2010; Writer et al., 2013; Aymerich et al., 2016; Boix et al., 2016). Two TPs of diuron, which are formed by N-demethylation by bacteria and/or fungi, were selected, namely: 1-(3,4-dichlorophenyl)urea (DCPU) and 1-(3,4-dichlorophenyl)-3-methylurea (DCPMU); these TPs have indeed been detected in the aquatic environment (Thomas et al., 2002; Gonzalez-Rey et al., 2015). Methyl-triclosan and 2,4-dichlorophenol have been described in literature as two characteristic

TPs of triclosan biodegradation (Dann and Hontela, 2011; Kim et al., 2011); the latest has also been described as a photodegradation product (Latch et al., 2005). These TPs have been detected in the aquatic environment (Chen et al., 2015; Wluka et al., 2016; Tohidi and Cai, 2017), including biota (Coogan et al., 2007; Kookana et al., 2013), and were thus chosen to be monitored in this study.

2.3. Water and biofilm sampling

Water samples from each mesocosm were collected every 24h for the quantification of organic microcontaminants and their TPs. At the beginning and end of the exposure experiment, additional water samples were collected for the quantification of C_{60}. Samples at time 0h were collected after 1h of the initial spiking (in the case of experiments with organic microcontaminants and/or C_{60}) in order to allow a homogeneous dispersion of the microcontaminant in the water of the mesocosms. Water samples were collected in amber glass bottles and kept at -20 ºC until analysis of organic microcontaminants. For the analysis of C_{60}, water samples (150 mL) were consecutively filtered through glass microfiber filters (0.7 µm) (Whatmann) and nylon membrane filters (0.45 µm) (Millipore) and the filters were kept at -20 ºC until analysis.

Biofilm from all treatments and replicates was randomly sampled at the end of the exposure experiment (72h) for the quantification of the organic microcontaminants and their TPs. Biofilm samples were scraped from the glass tiles, freeze-dried and kept at -20 ºC until further analysis.

2.4. Analysis of organic microcontaminants and their transformation products

2.4.1. Water

Water samples (1 mL) collected in the mesocosms of venlafaxine and diuron experiments were analyzed by liquid chromatography coupled with a hybrid mass spectrometry detector (UPLC-QqLIT) from Waters-AB Sciex using a method adapted from Gros et al. (2012). In the case of triclosan and its TPs (methyl-triclosan and 2,4-dichlorophenol), they were analyzed using an on-line HS-SPME-GC-MS/MS methodology adapted from Regueiro et al. (2009). Detailed information on sample preparation, the optimized mass spectrometer parameters for the target compounds and GC-MS/MS analysis is given in Supplementary Material.

2.4.2. Biofilm

Freeze-dried biofilm samples (100 mg) were extracted with 1 mL of citric buffer pH 4:ACN (1:1, *v/v*) using a mini bead beater (Vortex-Genie 2, MoBio Laboratorios, Inc, USA) with glass beads, followed by a clean-up step using solid phase extraction (SPE). Venlafaxine, diuron and their TPs were analyzed by LC-MS/MS, whilst triclosan and its TPs were analyzed by on-line HS-SPME-GC-MS/MS. More detailed information on biofilm sample preparation as well as the quality parameters of the analytical methodologies used for all the selected compounds are described in Supplementary Material.

2.5. Assessment of bioaccumulation

The bioaccumulation of the target organic microcontaminants (venlafaxine, triclosan and diuron) and their TPs in biofilm was assessed by evaluating their concentration in biofilm, expressed in ng g^{-1} dry weight; as well as determining their percentage relatively to the initial mass of the parent compounds. Additionally, bioconcentration factors (BCF) of the three target microcontaminants were also calculated to compare their accumulation potential.

2.5.1. Mass balance calculation

The mass distribution of the selected organic microcontaminants among water, biofilm and C_{60} fraction at the end of the exposure experiment was calculated using the concentrations obtained for each microcontaminant after the 72h of exposure as well as the water volume (4.5 L) and biofilm mass in each mesocosm. The following equations were used (Eq. 1-4):

$$\text{Contaminant remaining in water (\%)} = \frac{Cw\,(72h) \times V}{Cw\,(0h) \times V} \times 100 \quad \text{(Eq. 1)}$$

where Cw (µg L^{-1}) is the concentration of the corresponding microcontaminant in water at time 0h or 72h (begin and end of the exposure experiment, respectively), and V (L) corresponds to the volume of water used in the exposure experiment (4.5 L).

- Bioaccumulation:

$$\text{Bioaccumulation (\%)} = \frac{Cbiofilm\,(72h) \times m\,biofilm}{Cw\,(0h) \times V} \times 100 \quad \text{(Eq. 2)}$$

where $C_{biofilm}$ (µg g^{-1}) is the concentration of the corresponding microcontaminant in biofilm at time 72h (end of the exposure experiment), $m_{biofilm}$ (g) corresponds to the mass of biofilm, in dry weight, Cw (µg L^{-1}) is the concentration of the corresponding microcontaminant in water at time 0h, and V (L) corresponds to the volume of water used in the exposure experiment (4.5 L).

- Biotransformation:

Biotransformation was evaluated taking into account the sum of all the TPs formed in each mesocosm and considering their presence in water and biofilm. Herein, it is considered all kinds of transformation (e.g. biotransformation, phototransformation, etc.) that may occur in the mesocosm during the exposure experiments. The following equation was considered:

$$\text{Biotransformation (\%)} = \frac{\Sigma\, Cw\, TP\, (72h) \times V + \Sigma\, Cbiofilm\, TP\, (72h) \times m\, biofilm}{Cw\, (0h) \times V} \times 100 \quad \text{(Eq. 3)}$$

where Σ Cw TP (µg L^{-1}) is the sum of concentration of all TPs in water at 72h, V (L) corresponds to the volume of water used in the exposure experiment (4.5 L), $\Sigma\, C_{biofilm\ TP}$ (µg g^{-1}) is the sum of concentration of all TPs in biofilm at 72h, $m_{biofilm}$ (g) corresponds to the mass of biofilm, in dry weight, Cw (µg L^{-1}) is the concentration of the parent compound in water at time 0h.

- Adsorption to C_{60}:

The fraction of organic microcontaminant absorbed to C_{60}-aggregates was analyzed according to the procedure described in section 2.2.1. The percentage of adsorption to C_{60} of each organic microcontaminants is described in Table S5.

- Others:

Others (%) = 100-(%Contaminant remaining in water + %Bioaccumulation + %Biotransformation+ %Adsorption C_{60}) (Eq. 4)

In "Others" it was considered other possible fate of the organic microcontaminants, namely: the formation of other TPs that were not considered in the present study; the adsorption of the organic microcontaminants to the inner glass structure of the mesocosm; and the mineralization of the microcontaminant.

2.5.2. Calculation of bioconcentration factors (BCF)

Since two different nominal exposure concentrations were considered (50 µg L^{-1} for venlafaxine and 10 µg L^{-1} for diuron and triclosan), bioconcentration factors (BCF) were calculated in order to do a more adequate comparison of the accumulative potential of the different organic microcontaminants. BCFs, expressed in L kg^{-1} dry weight (dw), were calculated for the organic microcontaminants taking into account their measured concentration in water and in biofilm at the end of the exposure experiment (72h) (Gobas et al., 2009), following the equation (5):

$$\text{BCF (L kg}^{-1}\text{ dw)} = \text{conc. biofilm 72h (µg kg}^{-1}\text{ dw)/conc. water 72h (µg L}^{-1}\text{)} \quad \text{(Eq. 5)}$$

2.6. Statistical analysis

Normal distribution and normality of the data was tested using Shapiro-Wilks test and Levene's test for homogeneity of variance, respectively, after a log(x+1) transformation. Since the data was normal distributed, a *t*-test for independent samples was performed to compare the concentrations of the different compounds in biofilm and water at 72h without and in the presence of C_{60}. Statistically significant values were set at $p \leq 0.05$. All statistical analysis was performed using SPSS software, version 21 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.).

3. Results and Discussion

3.1. Preliminary experiments

Preliminary experiments in the absence of biofilm were performed to evaluate the potential loss of the organic microcontaminants by abiotic transformation and/or sorption processes with the C_{60} aggregates and at the inner glass surface of the mesocosms. Figure 2 shows the variation of the concentration of organic microcontaminants in water in the presence and absence of C_{60} aggregates during 72h.

Concentration of venlafaxine and diuron remained constant along the experiment irrespective of the presence of C_{60} (Figures 2A and 2B), whereas triclosan showed a decrease in its concentration along the time, which could be attributed to both abiotic degradation and sorption processes. Triclosan depletion in the absence of C_{60} (48%) was higher than in the in the presence of C_{60} (24%) (Figure 2C). This might be justified by a high affinity of C_{60} to the inner glass surface of mesocosms, reducing the surface area available for adsorption of triclosan. Besides sorption, abiotic degradation processes, like phototransformation (Latch et al., 2005) should also be taken into account as proved by the detection of 2,4-dichlorophenol (a triclosan transformation product) in all preliminary experiments (TP formed corresponded to 0.7% against 0.3% of the initial concentration of triclosan in the absence and in the presence of C_{60}, respectively).

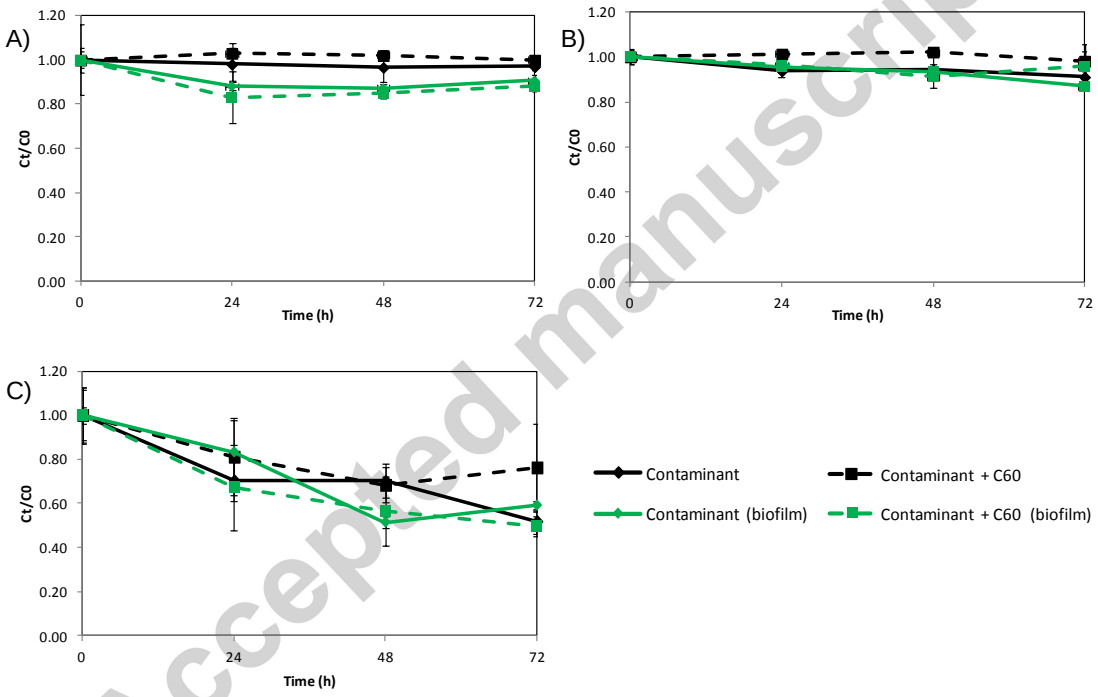

Figure 2 – Relative concentration of the organic microcontaminants in water over 72h in the preliminary experiments (black lines) and in the exposure experiments (green lines) with and without C_{60}. A) Venlafaxine; B) Diuron; C) Triclosan. C_0 is the initial concentration of the corresponding organic microcontaminant and C_t is the concentration of the organic microcontaminant at the time considered.

Data about the adsorption of the organic microcontaminants in the C_{60} under the exposure conditions is shown in Table S5. Up to 20% and 3% of initial concentration of

triclosan and venlafaxine was, respectively adsorbed in C_{60} after 72h. No sorption was measured for diuron. The adsorption of triclosan to carbon nanomaterials has been previously described in the literature (Zhou et al., 2013).

3.2. Bioaccumulation of organic microcontaminants

The bioaccumulation of three selected organic microcontaminants (venlafaxine, diuron and triclosan) in river biofilm was assessed in the exposure experiments with or without C_{60}. The concentration of the organic microcontaminants in water and biofilm (Tables 1 and S6-S8) showed that none of the microcontaminants were detected in water or biofilm of control and C_{60} exposure mesocosms. However, triclosan was detected in biofilm of the C_{60} exposure mesocosm at residual levels (29 ± 7 ng g^{-1} dw) (Table S8).

Table 1 - Concentration of the organic microcontaminants in water (µg L^{-1}) at 0 and 72h and in biofilm (µg g^{-1} dry weight) at 72h of exposure without and in the presence of C_{60}. Asterisk marks statistically significant differences in the presence of C_{60} at the end of the exposure experiment (72h) for a $p < 0.05$. Bioconcentration factors (BCF), expressed in L kg^{-1}, dry weight, of biofilm for the selected organic microcontaminants in the experiments without C_{60} are also indicated.

			Organic microcontaminant		
			Venlafaxine	**Diuron**	**Triclosan**
Without C_{60}					
Water	**Conc. (µg L^{-1}) ± SD**	**0h**	56.3 ± 2.1	10.3 ± 0.3	8.24 ± 1.15
		72h	51.0 ± 1.3	8.95 ± 0.15	4.87 ± 0.52
Biofilm	**Conc. (µg g^{-1} dw) ± SD**	**72h**	48.9 ± 3.1	4.29 ± 0.35	2.87 ± 0.19
	BCF (L kg^{-1} dw) ± SD	**72h**	959 ± 49	480 ± 32	607 ± 13
In the presence of C_{60}					
Water	**Conc. (µg L^{-1}) ± SD**	**0h**	49.9 ± 2.83	9.72 ± 0.33	6.80 ± 0.87
		72h	43.9 ± 1.01*	9.31 ± 0.96	3.39 ± 0.30*
Biofilm	**Conc. (µg g^{-1} dw) ± SD**	**72h**	48.1 ± 7.1	3.59 ± 0.59	2.53 ± 0.06*
	BCF (L kg^{-1}	**72h**	—	—	—

dw) ± SD

After 72 h, a decrease in the water concentration of the organic microcontaminants was observed, this being 9 - 41% (without C_{60}) and 4 - 50% (with C_{60}) (Figure 2). The decrease in the presence of C_{60} was statistically significant for venlafaxine ($p = 0.002$) and triclosan ($p = 0.012$) (Table 1). Triclosan showed the highest reduction in water, as it was already observed in the experiment without biofilm (section 3.1.). Diuron decreased more pronouncedly in the absence of C_{60} (13% against 4%), showing that abiotic or biotic degradation processes could have a more pronounced effect in the removal of diuron than the sorption to C_{60}; or that diuron and C_{60} need a longer period to reach the sorption equilibrium as it is described for other carbon nanomaterials (up to 14 days) (Sobek et al., 2009).

The concentration of C_{60} in water (Table 2) at the end of the exposure experiment (72h) remained steady at 1.0 µg L^{-1} in the experiments of venlafaxine and diuron, but decreased in the experiment of triclosan (0.3 µg L^{-1}). In general, in the presence of the organic microcontaminants a small decrease in the concentration of C_{60} in water was observed, that in the case of venlafaxine was statistically significant ($p = 0.008 < 0.05$).

Table 2 - Concentration of fullerenes (C60) in water (µg L^{-1}) at 0 and 72h in the experiments performed with the selected organic microcontaminants. Asterisk marks statistically significant differences in the presence of organic microcontaminant at the end of the exposure experiment (72h) for a $p < 0.05$.

Experiment	**Concentration C_{60} (µg L^{-1}) ± SD**	
	0h	**72h**
Only C_{60}		
Venlafaxine	3.08 ± 0.25	1.30 ± 0.07
Diuron	2.50 ± 0.25	1.20 ± 0.39
Triclosan	1.35 ± 0.03	0.30 ± 0.07
C_{60} + Organic microcontaminant		
Venlafaxine	3.02 ± 0.12	1.07 ± 0.04*
Diuron	2.57 ± 0.04	1.04 ± 0.14
Triclosan	1.25 ± 0.04	0.37 ± 0.04

All the selected organic microcontaminants bioaccumulated in biofilms independently of the presence of C_{60} (Table 1). As mentioned above, BCFs were calculated in order to do a more adequate comparison of the accumulative potential of the different organic microcontaminants (Table 1), since two different nominal exposure concentrations were considered (50 µg L^{-1} for venlafaxine and 10 µg L^{-1} for diuron and triclosan). Concentrations of the organic microcontaminants in biofilm per dry weight allowed comparing the bioaccumulation potential of the selected organic microcontaminants in the presence of C_{60}. As highlighted, herein it is considered as bioaccumulation the organic microcontaminants retained in the biofilm, either by uptake of the microbial and algal cells or just adsorbed at the cells walls, together with those entrapped in the extracellular mucopolysaccharide matrix. The interaction of organic microcontaminants with biofilm is compound-dependent and can be affected by factors like the hydrophobicity of the microcontaminant (log D), its pKa and/or the electrostatic interactions between the compound and the surface of biofilm, which is negatively charged (Headley et al., 1998; Torresi et al., 2017). It was indeed expected that organic microcontaminants with a high log D should have a high bioaccumulation in biofilm, even though this was not observed in the present study. Despite its lowest log D (1.78) (Table S1), venlafaxine showed the highest potential to accumulate (BCF = 959 ± 49), followed by triclosan (BCF = 607 ± 13) and diuron (BCF = 480 ± 32) (Table 1). The observed accumulation trend could be related to the pKa of the organic microcontaminants. At the experimental pH (8.1 ± 0.2), venlafaxine is positively charged (Table S1), and a higher sorption to biofilm than in neutral (diuron) or negatively charged (triclosan) compounds may occur, due to the establishment of electrostatic interactions between venlafaxine and the negatively charged surface of biofilm (Torresi et al., 2017).

The assessment of the impact of the presence of C_{60} in the bioaccumulation of the selected organic microcontaminants was performed in terms of concentration of the organic microcontaminants in the river biofilms. BCFs could not be calculated in this case, given that they might not accurately reflect the bioaccumulation of the organic microcontaminants in river biofilms in the compounds that had the capacity to adsorb to C_{60}, as is the case of venlafaxine and triclosan (Table S5). As a matter of fact, the decrease in the concentration of the organic microcontaminant in water after 72h could be due either to the bioaccumulation in biofilm or to its adsorption to C_{60}. The venlafaxine levels in biofilm were almost not affected due to the presence of C_{60} (48.1

vs 48.9 μg g^{-1} dw with or without C_{60}, respectively) (Table 1; $p = 0.812 > 0.05$). Since venlafaxine was at a much higher concentration than C_{60} (50 against 1 μg L^{-1}), the full sorptive capacity of C_{60}-aggregates was rapidly reached in these experiments without significantly affecting the concentration of free venlafaxine in water and its corresponding bioaccumulation.

The presence of C_{60} decreased the diuron concentration from 4.29 μg g^{-1} dw to 3.59 μg g^{-1} dw (Table 1), but the difference was not statistically significant ($p = 0.162 > 0.05$). The adsorption of C_{60}-aggregates on biofilm could reduce the biofilm surface area available for diuron sorption, but also the competition of the microcontaminant with the C_{60}-aggregates for the membrane receptor binding sites could reduce, or even block, the uptake of microcontaminants and its bioaccumulation (Deng et al., 2017). These findings might confirm the capability of carbon nanomaterials like C_{60}-aggregates to adsorb to algal cells, facilitating a close contact between nanoparticles and organisms (Baun et al., 2008; Schwab et al., 2013; Schwab et al., 2014). In addition, an antagonistic response in toxicological endpoints such as basal fluorescence or photosynthetic activity has also been reported (Freixa et al., 2018b) and confirms that C_{60} might have a protective role for biofilm against the toxicity of diuron.

Bioaccumulation of triclosan showed a statistically significant decrease in the presence of C_{60} (from 2.87 μg g^{-1} dw to 2.53 μg g^{-1} dw; $p = 0.039 < 0.05$). This decrease could be related to the high sorption of triclosan to the C_{60}-aggregates (around 20%; Table S5) which could reduce its free concentration in water and, consequently, its availability to be accumulated (Hu et al., 2010; Hu et al., 2015). Moreover, the lower thickness of the external mucopolysaccharide (EPS) in biofilms exposed to triclosan was more pronounced in the presence of C_{60} (Freixa et al., 2018b). Overall, this would contribute to a decrease in the retention of triclosan in the EPS: thinner biofilms provide less surface area to interact with organic microcontaminants (Torresi et al., 2017). Besides, the co-exposure to triclosan and C_{60} also showed a synergistic effect over biofilm respiration (Freixa et al., 2018b). The present observations are in agreement with others that described changes in toxicity due to the interaction of organic microcontaminants with carbon nanomaterials (Knauer et al., 2007; Baun et al., 2008; Park et al., 2010; Schwab et al., 2013).

3.3. Biotransformation of organic microcontaminants

Among the five selected TPs of venlafaxine, just the demethylated ones (O-desmethylvenlafaxine and N-desmethylvenlafaxine) were detected after 72h of exposure to venlafaxine (Figure 3), proving that they are formed by biological degradation. Also Rúa-Gómez and Püttmann (2013) reported a very low photodegradation of venlafaxine, while demethylation has been indicated as an important transformation route of venlafaxine (Boix et al., 2016; Lambropoulou et al., 2017). The O-desmethylvenlafaxine was the only TP detected in water samples, though at a much lower concentration than the parent compound (around 200-fold less) (Figure 3) and reaching similar levels at the end of the exposure experiments independently of the presence of C_{60} (Figure S1). In the presence of C_{60}, the concentration of O-desmethylvenlafaxine in biofilms showed a slight decrease, while N-desmethylvenlafaxine recorded a small increase (Figure 3). Nevertheless, no statistically significant differences were observed for the concentration of the venlafaxine TPs in water or biofilm ($p > 0.05$).

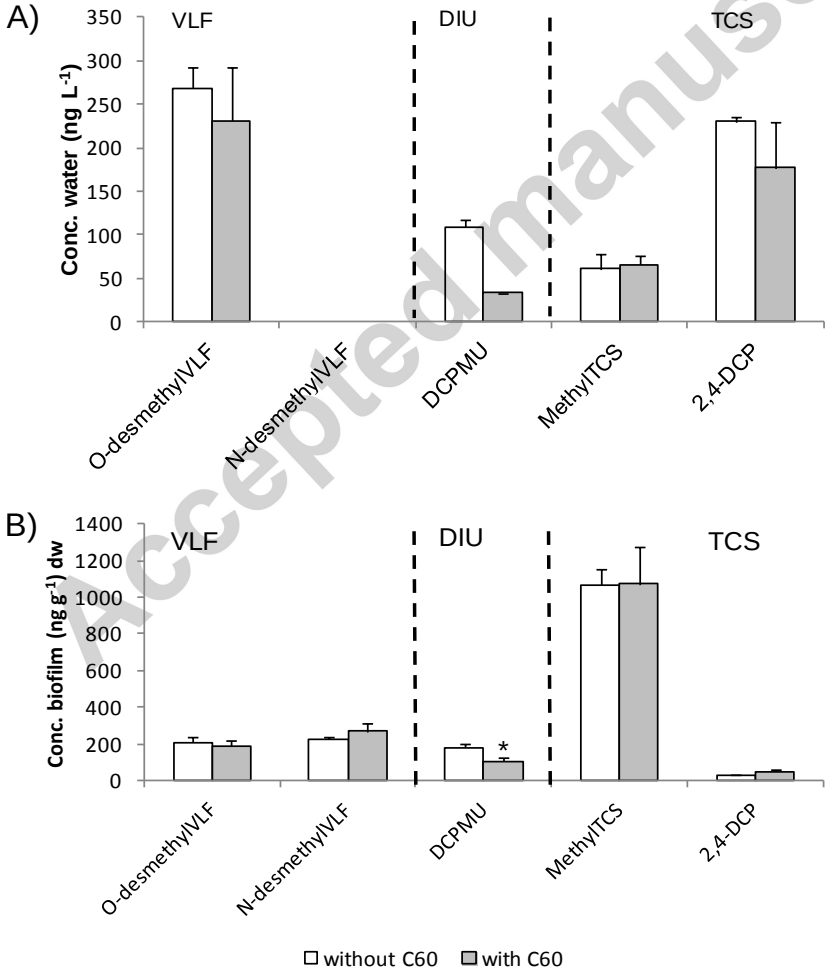

Figure 3 – Concentration of transformation products in water (expressed in ng L^{-1}) (A) and biofilm (expressed in ng g^{-1}, dry weight) (B) after 72h of exposure. VLF = venlafaxine; DIU = diuron; TCS = triclosan. Asterisk marks statistically significant differences in the presence of C_{60}.

Since diuron is resistant to photodegradation (Giacomazzi and Cochet, 2004; Vercraene-Eairmal et al., 2010), none of the selected diuron TPs were detected in the preliminary experiments (without biofilm). Biotransformation is the most important degradation mechanism of this compound (Giacomazzi and Cochet, 2004). In the treatments with diuron, DCPMU was the only TP detected in water and biofilm, though at much lower concentrations than the parent compound (1.2% of diuron in water), while in the presence of C_{60} its concentration was below the limit of quantification (<0.033 µg L^{-1}) (Figure S1 and Table S7). The concentration of DCPMU (relative to diuron concentration) in biofilms showed a statistically significant decrease (from 4.2% to 2.3%) in the presence of C_{60} ($p = 0.029$; Figure 3). This could be related to a reduction in the bacterial biofilm biomass when biofilms were co-exposed to diuron and C_{60} (Freixa et al., 2018b), and/or by a possible internalization of the C_{60}-aggregates by the cells that could impair the biodegradation pathway of diuron, preventing the formation of DCPMU (Deng et al., 2017).

Among the two selected triclosan TPs, only 2,4-dichlorophenol was detected in the preliminary experiments (without biofilm), showing a slightly higher concentration in the absence of C_{60} (0.7% against 0.3% of the initial triclosan concentration), which is in agreement with a higher decrease on the concentration of triclosan without C_{60} (c.a. 50%) (Figure 2). Photodegradation of triclosan is pH-dependent, being its anionic form more photo-reactive than the neutral one (Huang et al., 2016). Since the anionic form is the dominant one at the experimental pH (pH = 8.1), triclosan was more susceptible to be photodegraded. Besides that, C_{60}-aggregates could compete with triclosan for irradiation and 2,4-dichlorophenol is photolabile, showing a photodegradation faster than its formation by photodegradation of triclosan, all leading to lower concentrations of 2,4-dichlorophenol in water (Latch et al., 2005). On the other hand, both TPs were detected in water and biofilm in the exposure experiments with triclosan (Figures 3 and S1), though no statistically significant differences were observed ($p > 0.05$) in the presence of C_{60}. 2,4-dichlorophenol was present in water at higher concentration than methyl-triclosan, but its relative concentration comparatively to triclosan did not exceed

5%, regardless of the presence of C_{60} (Figure S1). The concentration of this TP was also 2.75 times higher in the experiments with biofilm than in the abiotic experiments, showing that besides phototransformation, it can also be formed by biological degradation (Kim et al., 2011). Oppositely, methyl-triclosan was the most abundant TP in biofilm, reaching levels up to 42% of the concentration of triclosan accumulated in biofilm (Figure 3 and Table S8), which is in agreement with its high lipophilicity and resistance to degradation (Dann and Hontela, 2011).

3.4. Overall distribution of the organic microcontaminants and their transformation products between water and biofilm

In general, after 72h of exposure, most of the selected organic microcontaminants remained in water (between 51 to 98% of the initial mass; Figure 4). Independently of the presence of C_{60}, venlafaxine showed a similar distribution profile in the exposure experiments, differing just in the 3% adsorbed to C_{60} in the experiments of co-exposure to C_{60} and venlafaxine. Venlafaxine was also the microcontaminant that showed higher potential to accumulate in biofilm (13-15%) while biotransformation was very low (0.6%).

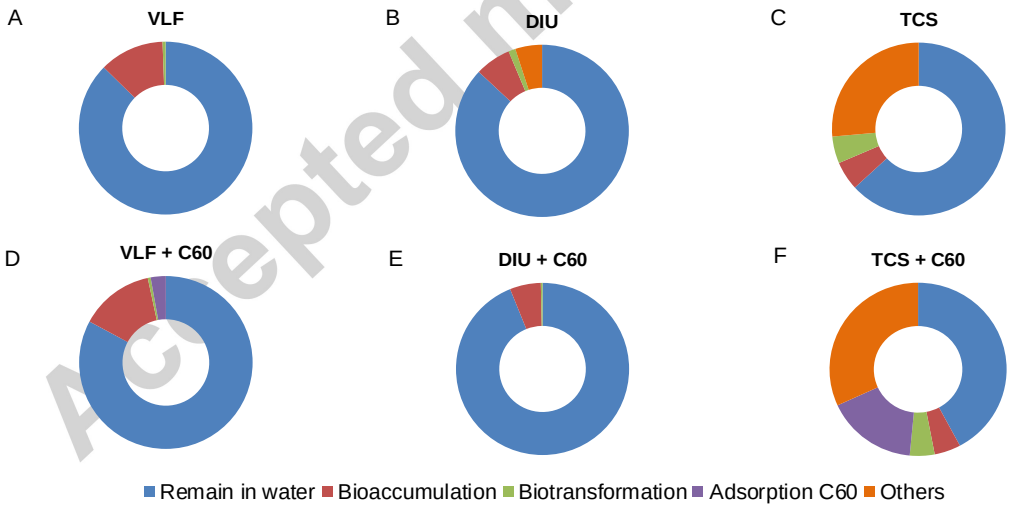

Figure 4 - Mass balance analysis of the selected organic contaminants after 72h of exposure of biofilm without (A-C) and in the presence of C_{60} (D-F), in terms of percentage of initial mass of organic contaminant in water. A and D – venlafaxine (VLF); B and E – diuron (DIU); C and F – triclosan (TCS). Note: "Others" group includes the formation of any TP not detected in the present study, the adsorption of the

organic microcontaminant to the mesocosm inner glass, and the mineralization of the microcontaminant.

In the case of diuron, 6.7% was bioaccumulated in biofilm and 1.3% biotransformed at the end of the exposure experiment. However, there was 5% of the initial mass of diuron that was removed from water and could not be found in neither biofilm nor as TPs. This could be justified by the possible formation of other TPs that were not monitored in the present study. On the other hand, in the presence of C_{60}, the mass of diuron that remained in water showed an increment of 10% and there was less biotransformation of the microcontaminant (0.3%). This might be due to a competition of C_{60} to the membrane receptor binding sites present in the microbial/algal cells, preventing the uptake of diuron and its biotransformation.

In the case of triclosan, 5.4% was bioaccumulated and 5.0% biotransformed at the end of the exposure experiment. Although significant differences were observed in the concentration of triclosan in river biofilms in the presence of C_{60}, when these differences were represented in terms of percentage relatively to the initial mass of triclosan, a similar pattern of bioaccumulation and biotransformation of the organic microcontaminant was seen regardless the presence of C_{60}. However, the percentage of triclosan that remained in water in the presence of C_{60} was much lower, mostly due to its sorption to C_{60} (20%). Nevertheless, a considerable percentage of triclosan that was removed from water could not be found in biofilm (bioaccumulation) nor as TPs (biotransformation and/or phototransformation): 26% and 38% in the absence and in the presence of C_{60}, respectively. These findings could be attributed to different factors, namely: i) possible adsorption of triclosan to the inner glass structure of the mesocosms; ii) formation of other TPs that were not evaluated in this study, for instance, in literature it is described the potential formation of phototransformation products of triclosan by reaction with other molecules of triclosan or with dissolved organic matter (Latch et al., 2005); iii) underestimation of the formation of 2,4-dichlorophenol, because its photodegradation surpass its formation by photodegradation of triclosan (Latch et al., 2005); iv) possibility of total mineralization of triclosan.

4. Conclusions

Venlafaxine showed the highest bioaccumulation (up to 15% of the initial mass removed from water phase was retained in biofilm), followed by triclosan and diuron.

On the other hand, just a small percentage of the organic microcontaminants were degraded, with triclosan showing the highest degradation (5.0%). In the presence of C_{60}, there were not large differences in the bioaccumulation and biotransformation of the organic microcontaminants. A slight increment in the bioaccumulation of venlafaxine and triclosan in biofilm occurred in the presence of C_{60} (15% and 6% against 13% and 5%, respectively), while for diuron only a very small decrease was observed (6.0% against 6.7%). Relatively to biotransformation, only diuron and triclosan showed slight changes, which never exceeded a variation of 1%. Although not significant, the slight differences observed in bioaccumulation and biotransformation can be due to the interaction organic microcontaminant-C_{60}, organic microcontaminant-biofilm or C_{60}-biofilm.

A detailed evaluation of the partition of the organic microcontaminants and their TPs in test systems are crucial to better understand their possible routes of bioaccumulation and biodegradation and the impact of the co-existence of other microcontaminants like carbon nanomaterials. Besides that, river biofilms represent a good model to evaluate the impact of single and co-exposure of organic microcontaminants, since they allow to investigate the effect at community level.

Funding sources

This work has been funded by the Spanish Ministry of Economy and Competitiveness (project NANOTRANSFER; ERA SIINN PCIN-2015-182-C02-02 and project PLAS-MED; CTM2017-89701-C3-2-R). Authors also acknowledge the support from the Economy and Knowledge Department of the Catalan Government through Consolidated Research Group (ICRA-ENV 2017 SGR 1124 & 2017 SGR 1404). Lúcia H.M.L.M. Santos acknowledges the Juan de la Cierva program (FJCI-2014-22377) and Sara Rodríguez-Mozaz acknowledges the Ramon y Cajal program (RYC-2014-16707).

Acknowledgments

This work has been funded by the Spanish Ministry of Economy and Competitiveness (project NANOTRANSFER; ERA SIINN PCIN-2015-182-C02-02

and project PLAS-MED; CTM2017-89701-C3-2-R). Authors also acknowledge the support from the Economy and Knowledge Department of the Catalan Government through Consolidated Research Group (ICRA-ENV 2017 SGR 1124 & 2017 SGR 1404). Lúcia H.M.L.M. Santos acknowledges the Juan de la Cierva program (FJCI-2014-22377) and Sara Rodríguez-Mozaz acknowledges the Ramon y Cajal program (RYC-2014-16707).

References:

Ahmed, M.B., Zhou, J.L., Ngo, H.H., Guo, W., Thomaidis, N.S., Xu, J., 2017. Progress in the biological and chemical treatment technologies for emerging contaminant removal from wastewater: A critical review. J. Hazard. Mat. 323, Part A, 274-298, https://doi.org/10.1016/j.jhazmat.2016.04.045.

Aymerich, I., Acuña, V., Barceló, D., García, M.J., Petrovic, M., Poch, M., Rodriguez-Mozaz, S., Rodríguez-Roda, I., Sabater, S., von Schiller, D., Corominas, L., 2016. Attenuation of pharmaceuticals and their transformation products in a wastewater treatment plant and its receiving river ecosystem. Water Res. 100, 126-136, https://doi.org/10.1016/j.watres.2016.04.022.

Baun, A., Sørensen, S.N., Rasmussen, R.F., Hartmann, N.B., Koch, C.B., 2008. Toxicity and bioaccumulation of xenobiotic organic compounds in the presence of aqueous suspensions of aggregates of nano-C60. Aquat. Toxicol. 86, 379-387, https://doi.org/10.1016/j.aquatox.2007.11.019.

Bedoux, G., Roig, B., Thomas, O., Dupont, V., Le Bot, B., 2012. Occurrence and toxicity of antimicrobial triclosan and by-products in the environment. Environ. Sci. Pollut. Res. 19, 1044-1065, 10.1007/s11356-011-0632-z.

Best, C., Melnyk-Lamont, N., Gesto, M., Vijayan, M.M., 2014. Environmental levels of the antidepressant venlafaxine impact the metabolic capacity of rainbow trout. Aquat. Toxicol. 155, 190-198, http://dx.doi.org/10.1016/j.aquatox.2014.06.014.

Bisesi Jr, J.H., Bridges, W., Klaine, S.J., 2014. Effects of the antidepressant venlafaxine on fish brain serotonin and predation behavior. Aquat. Toxicol. 148, 130-138, http://dx.doi.org/10.1016/j.aquatox.2013.12.033.

Bjorkland, R., Tobias, D.A., Petersen, E.J., 2017. Increasing evidence indicates low bioaccumulation of carbon nanotubes. Environmental Science: Nano 4, 747-766, 10.1039/c6en00389c.

Boix, C., Ibáñez, M., Sancho, J.V., Parsons, J.R., Voogt, P.d., Hernández, F., 2016. Biotransformation of pharmaceuticals in surface water and during waste water treatment: Identification and occurrence of transformation products. J. Hazard. Mat. 302, 175-187, http://dx.doi.org/10.1016/j.jhazmat.2015.09.053.

Chaumet, B., Morin, S., Boutry, S., Mazzella, N., 2019. Diuron sorption isotherms in freshwater biofilms. Sci. Total Environ. 651, 1219-1225, https://doi.org/10.1016/j.scitotenv.2018.09.286.

Chen, X., Casas, M.E., Nielsen, J.L., Wimmer, R., Bester, K., 2015. Identification of Triclosan-O-Sulfate and other transformation products of Triclosan formed by activated sludge. Sci. Total Environ. 505, 39-46, https://doi.org/10.1016/j.scitotenv.2014.09.077.

Coogan, M.A., Edziyie, R.E., La Point, T.W., Venables, B.J., 2007. Algal bioaccumulation of triclocarban, triclosan, and methyl-triclosan in a North Texas wastewater treatment plant receiving stream. Chemosphere 67, 1911-1918, https://doi.org/10.1016/j.chemosphere.2006.12.027.

Corcoll, N., Acuña, V., Barceló, D., Casellas, M., Guasch, H., Huerta, B., Petrovic, M., Ponsatí, L., Rodríguez-Mozaz, S., Sabater, S., 2014. Pollution-induced community tolerance to non-steroidal anti-inflammatory drugs (NSAIDs) in fluvial biofilm communities affected by WWTP effluents. Chemosphere 112, 185-193, http://dx.doi.org/10.1016/j.chemosphere.2014.03.128.

Dann, A.B., Hontela, A., 2011. Triclosan: environmental exposure, toxicity and mechanisms of action. Journal of Applied Toxicology 31, 285-311, 10.1002/jat.1660.

Deng, R., Lin, D., Zhu, L., Majumdar, S., White, J.C., Gardea-Torresdey, J.L., Xing, B., 2017. Nanoparticle interactions with co-existing contaminants: joint toxicity, bioaccumulation and risk. Nanotoxicology 11, 591-612, 10.1080/17435390.2017.1343404.

Di Poi, C., Costil, K., Bouchart, V., Halm-Lemeille, M.-P., 2018. Toxicity assessment of five emerging pollutants, alone and in binary or ternary mixtures, towards three aquatic organisms. Environ. Sci. Pollut. Res. 25, 6122-6134, 10.1007/s11356-017-9306-9.

Du, B., Haddad, S.P., Luek, A., Scott, W.C., Saari, G.N., Kristofco, L.A., Connors, K.A., Rash, C., Rasmussen, J.B., Chambliss, C.K., Brooks, B.W., 2014.

Bioaccumulation and trophic dilution of human pharmaceuticals across trophic positions of an effluent-dependent wadeable stream. Phil. Trans. R. Soc. B 369, 20140058, 10.1098/rstb.2014.0058.

European Comission, 2013. Directive 2013/39/EU of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Directive 2013/39/EU, Off. J. Europ. Union, pp. 1-17.

Farré, M., Perez, S., Gajda-Schrantz, K., Osorio, V., Kantiani, L., Ginebreda, A., Barcelo, D., 2010. First determination of C-60 andC(70) fullerenes and N-methylfulleropyrrolidine C-60 on the suspended material of wastewater effluents by liquid chromatography hybrid quadrupole linear ion trap tandem mass spectrometry. J. Hydrol. 383, 44-51, 10.1016/j.jhydrol.2009.08.016.

Fong, P.P., Bury, T.B.S., Donovan, E.E., Lambert, O.J., Palmucci, J.R., Adamczak, S.K., 2017. Exposure to SSRI-type antidepressants increases righting time in the marine snail *Ilyanassa obsoleta*. Environ. Sci. Pollut. Res. 24, 725-731, 10.1007/s11356-016-7855-y.

Freixa, A., Acuña, V., Sanchís, J., Farré, M., Barceló, D., Sabater, S., 2018a. Ecotoxicological effects of carbon based nanomaterials in aquatic organisms. Sci. Total Environ. 619-620, 328-337, https://doi.org/10.1016/j.scitotenv.2017.11.095.

Freixa, A., Acuña, V., Gutierrez, M., Sanchís, J., Santos, L.H.M.L.M., Rodriguez-Mozaz, S., Farré, M., Barceló, D., Sabater, S., 2018b. Fullerenes Influence the Toxicity of Organic Micro-Contaminants to River Biofilms. Frontiers in Microbiology 9, 10.3389/fmicb.2018.01426.

Giacomazzi, S., Cochet, N., 2004. Environmental impact of diuron transformation: a review. Chemosphere 56, 1021-1032, https://doi.org/10.1016/j.chemosphere.2004.04.061.

Gobas, F.A.P.C., de Wolf, W., Burkhard, L.P., Verbruggen, E., Plotzke, K., 2009. Revisiting Bioaccumulation Criteria for POPs and PBT Assessments. Integrated environmental assessment and management 5, 624-637, 10.1897/ieam_2008-089.1.

Gonzalez-Rey, M., Tapie, N., Le Menach, K., Dévier, M.-H., Budzinski, H., Bebianno, M.J., 2015. Occurrence of pharmaceutical compounds and pesticides in aquatic systems. Mar. Pollut. Bull. 96, 384-400, http://dx.doi.org/10.1016/j.marpolbul.2015.04.029.

González Alonso, S., Catalá, M., Romo Maroto, R., Rodríguez Gil, J.L., Gil de Miguel, Á., Valcárcel, Y., 2010. Pollution by psychoactive pharmaceuticals in the Rivers of

Madrid metropolitan area (Spain). Environ. Int. 36, 195-201, 10.1016/j.envint.2009.11.004.

Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2012. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. J. Chromatogr. A 1248, 104-121, 10.1016/j.chroma.2012.05.084.

Headley, J.V., Gandrass, J., Kuballa, J., Peru, K.M., Gong, Y., 1998. Rates of Sorption and Partitioning of Contaminants in River Biofilm. Environ. Sci. Technol. 32, 3968-3973, 10.1021/es980499l.

Hu, X., Li, J., Shen, M., Yin, D., 2015. Fullerene-associated phenanthrene contributes to bioaccumulation but is not toxic to fish. Environ. Toxicol. Chem. 34, 1023-1030, 10.1002/etc.2876.

Hu, X., Liu, J., Zhou, Q., Lu, S., Liu, R., Cui, L., Yin, D., Mayer, P., Jiang, G., 2010. Bioavailability of organochlorine compounds in aqueous suspensions of fullerene: Evaluated with medaka (*Oryzias latipes*) and negligible depletion solid-phase microextraction. Chemosphere 80, 693-700, https://doi.org/10.1016/j.chemosphere.2010.05.042.

Huang, X., Tu, Y., Song, C., Li, T., Lin, J., Wu, Y., Liu, J., Wu, C., 2016. Interactions between the antimicrobial agent triclosan and the bloom-forming cyanobacteria *Microcystis aeruginosa*. Aquat. Toxicol. 172, 103-110, https://doi.org/10.1016/j.aquatox.2016.01.002.

Huerta, B., Rodriguez-Mozaz, S., Barcelo, D., 2012. Pharmaceuticals in biota in the aquatic environment: analytical methods and environmental implications. Anal. Bioanal. Chem. 404, 2611-2624, 10.1007/s00216-012-6144-y.

Huerta, B., Rodriguez-Mozaz, S., Nannou, C., Nakis, L., Ruhí, A., Acuña, V., Sabater, S., Barcelo, D., 2016. Determination of a broad spectrum of pharmaceuticals and endocrine disruptors in biofilm from a waste water treatment plant-impacted river. Sci. Total Environ. 540, 241-249, http://dx.doi.org/10.1016/j.scitotenv.2015.05.049.

Hutchinson, T.H., Shillabeer, N., Winter, M.J., Pickford, D.B., 2006. Acute and chronic effects of carrier solvents in aquatic organisms: A critical review. Aquat. Toxicol. 76, 69-92, https://doi.org/10.1016/j.aquatox.2005.09.008.

Kern, S., Baumgartner, R., Helbling, D.E., Hollender, J., Singer, H., Loos, M.J., Schwarzenbach, R.P., Fenner, K., 2010. A tiered procedure for assessing the formation

of biotransformation products of pharmaceuticals and biocides during activated sludge treatment. J. Environ. Monit. 12, 2100-2111, 10.1039/c0em00238k.

Kim, Y.-M., Murugesan, K., Schmidt, S., Bokare, V., Jeon, J.-R., Kim, E.-J., Chang, Y.-S., 2011. Triclosan susceptibility and co-metabolism – A comparison for three aerobic pollutant-degrading bacteria. Bioresource Technol. 102, 2206-2212, https://doi.org/10.1016/j.biortech.2010.10.009.

Knauer, K., Sobek, A., Bucheli, T.D., 2007. Reduced toxicity of diuron to the freshwater green alga *Pseudokirchneriella subcapitata* in the presence of black carbon. Aquat. Toxicol. 83, 143-148, https://doi.org/10.1016/j.aquatox.2007.03.021.

Kookana, R.S., Shareef, A., Fernandes, M.B., Hoare, S., Gaylard, S., Kumar, A., 2013. Bioconcentration of triclosan and methyl-triclosan in marine mussels (*Mytilus galloprovincialis*) under laboratory conditions and in metropolitan waters of Gulf St Vincent, South Australia. Mar. Pollut. Bull. 74, 66-72, https://doi.org/10.1016/j.marpolbul.2013.07.030.

Lambropoulou, D., Evgenidou, E., Saliverou, V., Kosma, C., Konstantinou, I., 2017. Degradation of venlafaxine using TiO2/UV process: Kinetic studies, RSM optimization, identification of transformation products and toxicity evaluation. J. Hazard. Mat. 323, Part A, 513-526, https://doi.org/10.1016/j.jhazmat.2016.04.074.

Latch, D.E., Packer, J.L., Stender, B.L., VanOverbeke, J., Arnold, W.A., McNeill, K., 2005. Aqueous photochemistry of triclosan: Formation of 2,4-dichlorophenol, 2,8-dichlorodibenzo-p-dioxin, and oligomerization products. Environ. Toxicol. Chem. 24, 517-525, 10.1897/04-243r.1.

Lyndall, J., Barber, T., Mahaney, W., Bock, M., Capdevielle, M., 2017. Evaluation of triclosan in Minnesota lakes and rivers: Part I – ecological risk assessment. Ecotoxicol. Environ. Saf. 142, 578-587, https://doi.org/10.1016/j.ecoenv.2017.04.049.

Magalhães, P., Alves, G., Llerena, A., Falcão, A., 2014. Venlafaxine pharmacokinetics focused on drug metabolism and potential biomarkers. Drug Metabolism and Drug Interactions 29, 129-141, DOI 10.1515/dmdi-2013-0053.

Meador, J.P., Yeh, A., Young, G., Gallagher, E.P., 2016. Contaminants of emerging concern in a large temperate estuary. Environ. Pollut. 213, 254-267, https://doi.org/10.1016/j.envpol.2016.01.088.

Minguez, L., Bureau, R., Halm-Lemeille, M.-P., 2018. Joint effects of nine antidepressants on *Raphidocelis subcapitata* and *Skeletonema marinoi*: A matter of

amine functional groups. Aquat. Toxicol. 196, 117-123, https://doi.org/10.1016/j.aquatox.2018.01.015.

Okamura, H., Aoyama, I., Ono, Y., Nishida, T., 2003. Antifouling herbicides in the coastal waters of western Japan. Mar. Pollut. Bull. 47, 59-67, https://doi.org/10.1016/S0025-326X(02)00418-6.

Orvos, D.R., Versteeg, D.J., Inauen, J., Capdevielle, M., Rothenstein, A., Cunningham, V., 2002. Aquatic toxicity of triclosan. Environ. Toxicol. Chem. 21, 1338-1349, 10.1002/etc.5620210703.

Park, J.-W., Henry, T.B., Menn, F.-M., Compton, R.N., Sayler, G., 2010. No bioavailability of 17α-ethinylestradiol when associated with nC60 aggregates during dietary exposure in adult male zebrafish (*Danio rerio*). Chemosphere 81, 1227-1232, https://doi.org/10.1016/j.chemosphere.2010.09.036.

Proia, L., Morin, S., Peipoch, M., Romaní, A.M., Sabater, S., 2011. Resistance and recovery of river biofilms receiving short pulses of Triclosan and Diuron. Sci. Total Environ. 409, 3129-3137, https://doi.org/10.1016/j.scitotenv.2011.05.013.

Regueiro, J., Becerril, E., Garcia-Jares, C., Llompart, M., 2009. Trace analysis of parabens, triclosan and related chlorophenols in water by headspace solid-phase microextraction with in situ derivatization and gas chromatography–tandem mass spectrometry. J. Chromatogr. A 1216, 4693-4702, https://doi.org/10.1016/j.chroma.2009.04.025.

Ricart, M., Guasch, H., Alberch, M., Barceló, D., Bonnineau, C., Geiszinger, A., Farré, M.l., Ferrer, J., Ricciardi, F., Romaní, A.M., Morin, S., Proia, L., Sala, L., Sureda, D., Sabater, S., 2010. Triclosan persistence through wastewater treatment plants and its potential toxic effects on river biofilms. Aquat. Toxicol. 100, 346-353, https://doi.org/10.1016/j.aquatox.2010.08.010.

Rodríguez-Mozaz, S., Huerta, B., Barceló, D., 2016. Bioaccumulation of Emerging Contaminants in Aquatic Biota: Patterns of Pharmaceuticals in Mediterranean River Networks. in: Petrovic, M., Sabater, S., Elósegui, A. (Eds.). Emerging Contaminants in River Ecosystems. Springer International Publishing, Switzerland, pp. 121-142.

Romero, F., Sabater, S., Timoner, X., Acuña, V., 2018. Multistressor effects on river biofilms under global change conditions. Sci. Total Environ. 627, 1-10, https://doi.org/10.1016/j.scitotenv.2018.01.161.

Rúa-Gómez, P.C., Püttmann, W., 2013. Degradation of lidocaine, tramadol, venlafaxine and the metabolites O-desmethyltramadol and O-desmethylvenlafaxine in surface

waters. Chemosphere 90, 1952-1959, https://doi.org/10.1016/j.chemosphere.2012.10.039.

Rüdel, H., Böhmer, W., Müller, M., Fliedner, A., Ricking, M., Teubner, D., Schröter-Kermani, C., 2013. Retrospective study of triclosan and methyl-triclosan residues in fish and suspended particulate matter: Results from the German Environmental Specimen Bank. Chemosphere 91, 1517-1524, https://doi.org/10.1016/j.chemosphere.2012.12.030.

Ruhí, A., Acuña, V., Barceló, D., Huerta, B., Mor, J.-R., Rodríguez-Mozaz, S., Sabater, S., 2016. Bioaccumulation and trophic magnification of pharmaceuticals and endocrine disruptors in a Mediterranean river food web. Sci. Total Environ. 540, 250-259, http://dx.doi.org/10.1016/j.scitotenv.2015.06.009.

Sabater, S., Barceló, D., De Castro-Català, N., Ginebreda, A., Kuzmanovic, M., Petrovic, M., Picó, Y., Ponsatí, L., Tornés, E., Muñoz, I., 2016. Shared effects of organic microcontaminants and environmental stressors on biofilms and invertebrates in impaired rivers. Environ. Pollut. 210, 303-314, http://dx.doi.org/10.1016/j.envpol.2016.01.037.

Sabater, S., Guasch, H., Ricart, M., Romaní, A., Vidal, G., Klünder, C., Schmitt-Jansen, M., 2007. Monitoring the effect of chemicals on biological communities. The biofilm as an interface. Anal. Bioanal. Chem. 387, 1425-1434, 10.1007/s00216-006-1051-8.

Sanchís, J., Olmos, M., Vincent, P., Farré, M., Barceló, D., 2016. New Insights on the Influence of Organic Co-Contaminants on the Aquatic Toxicology of Carbon Nanomaterials. Environ. Sci. Technol. 50, 961-969, 10.1021/acs.est.5b03966.

Santos, L.H.M.L.M., Araujo, A.N., Fachini, A., Pena, A., Delerue-Matos, C., Montenegro, M.C.B.S.M., 2010. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J. Hazard. Mat. 175, 45-95, 10.1016/j.jhazmat.2009.10.100.

Schwab, F., Bucheli, T.D., Camenzuli, L., Magrez, A., Knauer, K., Sigg, L., Nowack, B., 2013. Diuron Sorbed to Carbon Nanotubes Exhibits Enhanced Toxicity to *Chlorella vulgaris*. Environ. Sci. Technol. 47, 7012-7019, 10.1021/es304016u.

Schwab, F., Camenzuli, L., Knauer, K., Nowack, B., Magrez, A., Sigg, L., Bucheli, T.D., 2014. Sorption kinetics and equilibrium of the herbicide diuron to carbon nanotubes or soot in absence and presence of algae. Environ. Pollut. 192, 147-153, https://doi.org/10.1016/j.envpol.2014.05.018.

Serra-Compte, A., Corcoll, N., Huerta, B., Rodríguez-Mozaz, S., Sabater, S., Barceló, D., Álvarez-Muñoz, D., 2018. Fluvial biofilms exposed to desiccation and pharmaceutical pollution: New insights using metabolomics. Sci. Total Environ. 618, 1382-1388, https://doi.org/10.1016/j.scitotenv.2017.09.258.

Sobek, A., Stamm, N., Bucheli, T.D., 2009. Sorption of Phenyl Urea Herbicides to Black Carbon. Environ. Sci. Technol. 43, 8147-8152, 10.1021/es901737f.

Sousa, J.C.G., Ribeiro, A.R., Barbosa, M.O., Pereira, M.F.R., Silva, A.M.T., 2018. A review on environmental monitoring of water organic pollutants identified by EU guidelines. J. Hazard. Mat. 344, 146-162, https://doi.org/10.1016/j.jhazmat.2017.09.058.

Thomaidi, V.S., Stasinakis, A.S., Borova, V.L., Thomaidis, N.S., 2015. Is there a risk for the aquatic environment due to the existence of emerging organic contaminants in treated domestic wastewater? Greece as a case-study. J. Hazard. Mat. 283, 740-747, http://dx.doi.org/10.1016/j.jhazmat.2014.10.023.

Thomas, K.V., McHugh, M., Waldock, M., 2002. Antifouling paint booster biocides in UK coastal waters: inputs, occurrence and environmental fate. Sci. Total Environ. 293, 117-127, https://doi.org/10.1016/S0048-9697(01)01153-6.

Tohidi, F., Cai, Z., 2017. Fate and mass balance of triclosan and its degradation products: Comparison of three different types of wastewater treatments and aerobic/anaerobic sludge digestion. J. Hazard. Mat. 323, Part A, 329-340, https://doi.org/10.1016/j.jhazmat.2016.04.034.

Torresi, E., Polesel, F., Bester, K., Christensson, M., Smets, B.F., Trapp, S., Andersen, H.R., Plósz, B.G., 2017. Diffusion and sorption of organic micropollutants in biofilms with varying thicknesses. Water Res. 123, 388-400, https://doi.org/10.1016/j.watres.2017.06.027.

Vercraene-Eairmal, M., Lauga, B., Saint Laurent, S., Mazzella, N., Boutry, S., Simon, M., Karama, S., Delmas, F., Duran, R., 2010. Diuron biotransformation and its effects on biofilm bacterial community structure. Chemosphere 81, 837-843, https://doi.org/10.1016/j.chemosphere.2010.08.014.

Wilkinson, J.L., Hooda, P.S., Swinden, J., Barker, J., Barton, S., 2018. Spatial (bio)accumulation of pharmaceuticals, illicit drugs, plasticisers, perfluorinated compounds and metabolites in river sediment, aquatic plants and benthic organisms. Environ. Pollut. 234, 864-875, https://doi.org/10.1016/j.envpol.2017.11.090.

Wluka, A.-K., Rüdel, H., Pohl, K., Schwarzbauer, J., 2016. Analytical method development for the determination of eight biocides in various environmental compartments and application for monitoring purposes. Environ. Sci. Pollut. Res. 23, 21894-21907, 10.1007/s11356-016-7296-7.

Writer, J.H., Antweiler, R.C., Ferrer, I., Ryan, J.N., Thurman, E.M., 2013. In-Stream Attenuation of Neuro-Active Pharmaceuticals and Their Metabolites. Environ. Sci. Technol. 47, 9781-9790, 10.1021/es402158t.

Writer, J.H., Barber, L.B., Ryan, J.N., Bradley, P.M., 2011. Biodegradation and Attenuation of Steroidal Hormones and Alkylphenols by Stream Biofilms and Sediments. Environ. Sci. Technol. 45, 4370-4376, 10.1021/es2000134.

Zhou, S., Shao, Y., Gao, N., Deng, J., Tan, C., 2013. Equilibrium, Kinetic, and Thermodynamic Studies on the Adsorption of Triclosan onto Multi-Walled Carbon Nanotubes. CLEAN – Soil, Air, Water 41, 539-547, 10.1002/clen.201200082.

Highlights:

- River biofilms are a good model to assess the effect of pollutants at a community level
- Impact of C_{60} in bioaccumulation and biotransformation of VLF, DIU and TCS was assessed
- Venlafaxine showed the highest potential to accumulate in river biofilms
- Biotransformation was more evident for triclosan
- Presence of C_{60} did not alter bioaccumulation and biotransformation of OMC