Environmental Pollution 233 (2018) 1131–1137

Contents lists available at ScienceDirect

Environmental Pollution

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

Effects of chronic pollution and water flow intermittency on stream biofilms biodegradation capacity☆

Marko Rožman [a, *], Vicenç Acuña [a], Mira Petrović [a, b]

[a] Catalan Institute for Water Research (ICRA), Emili Grahit 101, 17003 Girona, Spain
[b] Catalan Institution for Research and Advanced Studies (ICREA), Passeig Lluís Companys 23, 08010 Barcelona, Spain

ARTICLE INFO

Article history:
Received 19 May 2017
Received in revised form
5 October 2017
Accepted 5 October 2017
Available online 26 October 2017

Keywords:
Pharmaceutically active compounds
Drought
Biofilm
Stressor combination
Biodegradation

ABSTRACT

A mesocosm case study was conducted to gain understanding and practical knowledge on biofilm emerging contaminants biodegradation capacity under stressor and multiple stressor conditions. Two real life scenarios: I) biodegradation in a pristine intermittent stream experiencing acute pollution and II) biodegradation in a chronically polluted intermittent stream, were examined via a multifactorial experiment using an artificial stream facility. Stream biofilms were exposed to different water flow conditions i.e. permanent and intermittent water flow. Venlafaxine, a readily biodegradable pharmaceutical was used as a measure of biodegradation capacity while pollution was simulated by a mixture of four emerging contaminants (erythromycin, sulfisoxazole, diclofenac and imidacloprid in addition to venlafaxine) in environmentally relevant concentrations. Biodegradation kinetics monitored via LC-MS/MS was established, statistically evaluated, and used to link biodegradation with stress events. The results suggest that the effects of intermittent flow do not hinder and may even stimulate pristine biofilm biodegradation capacity. Chronic pollution completely reduced biodegradation in permanent water flow experimental treatments while no change in intermittent streams was observed. A combined effect of water flow conditions and emerging contaminants exposure on biodegradation was found. The decrease in biodegradation due to exposure to emerging contaminants is significantly greater in streams with permanent water flow suggesting that the short and medium term biodegradation capacity in intermittent systems may be preserved or even greater than in perennial streams.

1. Introduction

Multiple stressors resulting from natural and anthropogenic disturbances are constantly impacting freshwater environment. “Traditional stressors”, such as eutrophication, organic and inorganic pollution, acidification and hydro-morphological pressures now coexist with newly discovered risks, such as climate change, alien species, emerging contaminants (ECs) etc. Although in some cases stressors may act independently of each other, they are more often expected to exert complex interactive behaviour, thus leading to synergistic/antagonistic effects, either directly (affecting the same target) or indirectly (affecting different targets) (Schuhmacher et al., 2016). On the other hand, most of the current knowledge is concentrated on the effects of single stressor on the chemical and ecological status of water bodies. The understanding of multiple stressors interactions remains limited in both theoretical concepts and empirical knowledge (Segner et al., 2014).

One of the key natural stressors is drought (Barceló and Sabater, 2010; Ludwig et al., 2011). Water abstractions in areas of intensive agriculture and around major cities can further amplify water intermittency. These same areas contribute to the releasing of emerging contaminants (pharmaceuticals, pesticides etc.) into the water systems (Murray et al., 2010; Petrie et al., 2014).

Water scarcity-water pollution is multiple stressors setting of high likelihood as well as natural stressor-chemical stressor scenario of particular interest for supplementing ecotoxicological and toxicological testing which could be considered in future risk assessment procedures (Holmstrup et al., 2010). Although knowledge on the combined effects of drought and chemical contaminants on aquatic communities is still limited, recent studies assessed the impact of multiple stressors on some traits of biological receptors in aquatic communities (Corcoll et al., 2015; Pesce

☆ This paper has been recommended for acceptance by Klaus Kummerer.

* Corresponding author. Present address: Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia.
E-mail address: marko@irb.hr (M. Rožman).

https://doi.org/10.1016/j.envpol.2017.10.019
0269-7491/

et al., 2016; Proia et al., 2013; Stampfli et al., 2013). A study on multiple stressors (Stampfli et al., 2013) investigated the effects of fluctuations in water level and exposure to a pesticide on zooplankton. Synergism between stressors was not detected due to the strong individual effect of the hydrological disturbance. Similarly, in an assessment of the combined effects of drought and the model fungicide tebuconazole on leaf litter decomposition process no combined effect was observed (Pesce et al., 2016). Again, most of the observed effects in leaf-microbial communities were related to the drought stress rather than the fungicide stress. Proia et al. investigated how an episode of drought might influence the response of river biofilms to pulses of highly concentrated triclosan (90 ppb) (Proia et al., 2013). Out of several biofilm descriptors only the viability of the diatom community showed statistically significant interaction suggesting a combined effect of two stressors. On the other hand, exposure of biofilm communities to a mixture of pharmaceutical compounds at environmental concentrations coupled with flow intermittency significantly affected green algae and cyanobacteria and, accordingly, the photosynthetic efficiency of autotrophs (Corcoll et al., 2015). The same study evaluated pharmaceutical accumulation in the stream biofilm and found no statistical difference in bioaccumulation between the control and the intermittent stream.

However, bioaccumulation is just one aspect of the natural attenuation process of organic chemicals. In order to completely assess the biofilm attenuation process, biodegradation needs to be considered. In this context, an important question is how biodegradation is affected by flow intermittency.

In this paper we present the results of a mesocosm experiment aimed at improving our understanding of the biofilm biodegradation process under simulated flow intermittency (altered water flow) and the combined effects of flow intermittency and exposure to a mixture of ECs. Case study was designed to examine two different real life scenarios: I) biodegradation in a pristine intermittent stream experiencing acute pollution and II) biodegradation in a chronically polluted intermittent stream. Using collected data, we attempt to elucidate how biodegradation efficiency of the biofilm changes in respect to those scenarios.

2. Materials and methods

2.1. Materials

Compounds for ECs mixture and corresponding internal standards: erythromycin (erithromycin-NN-dimethyl-^{13}C), diclofenac (diclofenac-d_4), imidacloprid (imidacloprid-d_4), venlafaxine (venlafaxine-d_6) and sulfisoxazole (sulfamethoxazole-d_4) were obtained from Sigma-Aldrich (Spain) and used without further purification. HPLC grade solvents and modifiers were purchased from Fisher Scientific (Spain). Source water for the artificial streams was rainwater, filtered through activated carbon filters. The sand used in artificial streams was extracted from an unpolluted segment of the Segre River near the city of Puigcerdà (NE Iberian Peninsula) (d_{50} = 0.38 mm) and sterilized with a Presoclave-II 30 L autoclave (120 °C for 20 min) (JP Selecta S.A., Barcelona, Spain).

2.2. Experimental streams facility conditions and biofilm colonisation

The experiment was performed in the indoor Experimental Streams Facility (ESF) of the Catalan Institute for Water Research (Girona, Spain) using artificial streams. Each stream consisted of an independent methacrylate channel (l × w × d = 200 cm × 10 cm × 10 cm), and a 70 l water tank from which water can be recirculated. The channels received a constant flow of 50 ml s^{-1}. The water exchange rate was 4.28% per hour, meaning that the water of each artificial stream was completely renewed once a day. Mean water velocity was 0.88 ± 0.03 cm s^{-1}, and water depth over the plane bed ranged between 2.2 and 2.5 cm. Each artificial stream was filled with 5 L of sand to create a plane bed that facilitated the growth of biofilm. At complete water saturation, the porosity of the sand yielded a water content of 20% of the wet weight. Daily cycles of photosynthetic active radiation (PAR) were defined as 9 h daylight and 15 h darkness, and were simulated by LED lights (Lightech, Girona, Spain). PAR was held constant at 173.99 ± 33 mEm^{-2} s^{-1} during the daytime, and was recorded every 10 min using 4 quantum sensors located across the entire array of streams (sensor LI-192SA, LiCOR Inc, Lincoln, USA). Water temperature was kept constant at 20 °C by means of a cryo-compact circulator (Julabo CF-31, Seelbach, Germany), and recorded every 10 min using VEMCO Minilog (TR model, AMIRIX Systems Inc, Halifax, NS, Canada) temperature data loggers. Overall, physico-chemical conditions in the artificial streams (water velocity, temperature, and light cycles) emulated those of the Segre River during late spring and under low flow conditions.

The biofilm was inoculated twice per week during the colonization period (Fig. 1.) using combined inocula from epilithic (growing on surface of rocks) and epipsammic (growing on the surface of sand) biofilms from the same unpolluted segment of the Segre River. Specifically, biofilm inocula were obtained after scraping 10-12 cobbles and washing around 10 L of fine sediments. When biofilms were five weeks old (end of colonisation period, Fig. 1.) homogeneity of biofilms in different streams was checked by assessing biofilm descriptors (ash-free dry mass (AFDM), Chl-a concentration, basal fluorescence (F_0) and photosynthetic efficiency of authotrphs (Y_{eff})). Multivariate Pillai's trace statistics did not find a significant difference between artificial streams, $V = 0.897$, $F(4, 1) = 2.17$, $P > 0.05$.

2.3. Experimental design

Overall experimental timeline is depicted at Fig. 1. After the colonisation period, the water flow was interrupted for 5 days to simulate a dry period in the treatments of flow intermittency. Accordingly two treatment levels were created: permanent flow and intermittent flow. Once flow was re-established artificial streams were involved in two different experimental phases:

- Experimental phase 1: biodegradation in pristine streams of different flow histories experiencing acute pollution. Selected compounds at the environmentally relevant concentration of 10^{-6} g/l were applied in pristine streams with permanent and intermittent flows. The streams were closed and the water flow was recirculated for 4 days.
- Experimental phase 2: biodegradation in chronically polluted streams of different flow history. After phase 1, a mixture of ECs at a concentration of 10^{-7} g/l was maintained over five days to simulate chronic pollution. During those 5 days the streams were operated under open-flow management and the constant concentration of pollutants was achieved using a peristaltic pump (IPC pump, Ismatec, Switzerland). Following the chronic pollution phase the experimental phase 2 started. A mixture of ECs at a concentration of 10^{-6} g/l was applied in the now "chronically polluted stream", the streams were closed and the water flow was recirculated for 4 days.

In addition, control streams of neither sediment nor light, streams with only light and streams with only sediment were assigned. Streams with only light were used as additional control since only photosynthetic active radiation has been used and no

	Colonisation	Flow manipulation	Experimental phase 1	Chronic pollution	Experimental phase 2
Permanent flow treatment	Open flow	Open flow	Spike Recirculated flow	ECs exposure Open flow	Spike Recirculated flow
Intermittent flow treatment	Open flow	Flow stop	Spike Recirculated flow	ECs exposure Open flow	Spike Recirculated flow
Time / day	1-36	37-41	42-46	47-51	52-55

Fig. 1. Mesocosm experimental design with two experimental phases.

photolysis was expected. Streams with only sediment were used to measure potential sorption component of ECs attenuation process Altogether 15 artificial streams were assigned following a randomized complete block design with 3 replicates per treatment. Water sampling frequency was from 3 to 12 h; on a logarithmic scale; more frequent at the beginning of experiment.

2.4. Liquid chromatography separation and tandem mass spectrometry

Online LC-MS/MS analysis of ECs mixture was performed on the TSQ Vantage triple quadrupole mass spectrometer (Thermo Fisher Scientific, USA) coupled with Aria TLX-1 HPLC system (Thermo Fisher Scientific, USA). The sample injection, enrichment, separation, and MS acquisition was carried out automatically. Solvents used for HPLC separation were methanol (solvent A) and 20 mM ammonium formate (solvent B) at pH 4. The water samples were loaded (2 ml injection volume) onto the trapping column (Hypersil GOLD aQ 20 mm × 2.1 mm i.d., 12 μm particle size, Thermo Fisher Scientific, USA) with a solvent mixture of 5% An at the flow rate of 3 ml/min. For ECs separation an analytical column (KINETEX C18 50 mm × 2.1 mm i.d., 1.7 μmm particle size, Phenomenex Inc., USA) was used. ECs were separated using the following 6.2 min gradient: initial conditions 10% A; 0.0–1.3 min, 10% A; 1.3–2.3 min, 25% A; 2.3–4.0 min, 65% A; 4.0–4.5 min, 100% A; 4.5–6.0 min, 100% A; 6.0–6.1 min, 10% A; 6.1–6.2 min, 10% A. The mass spectrometer operated under unit resolution in the selected reaction monitoring (SRM) mode using a fast polarization switching mode. The electrospray capillary voltage was set as -3500 V (positive) and 3000 V (negative), while the temperature of the drying gas was set at 350 °C. The target masses were monitored at two different transitions for each compound by SRM (Selected Reaction Monitoring) (SRM1: quantifier transition and SRM2: qualifier transition), details can be found in supporting information Table 1.

2.5. Data analysis

Target compounds were quantified using an internal standard method by a newly developed script (Bquant) for batch quantification of liquid chromatography mass spectrometry data using the procedure described in detail in (Rožman and Petrović, 2016). The internal standard calibration curves, limits of detection (LODs) and quantification (LOQs) are depicted in Fig. 1 Supporting information. All final concentrations were corrected for water evaporation during the experiment. The rate of decline of the target compound is assumed to follow first-order kinetics. The first-order rate constants were determined by using non-linear regression to fit data and using linear regression to fit ln transformed data.

One way ANOVA and pairwise comparison ANCOVA were conducted to compare rate constants of different treatments with their respective controls. Rate constants were reported if treatment was significantly different from control. Sorption rate constants were corrected for control rate constants. Biofilm rate constants were reported once corrected for control and sorption values. Reported values are represented as mean ± standard error.

One way ANOVA was used for comparing rate constants in the first experimental phase while a repeated measures ANOVA was used for testing differences between rate constants obtained in first and second phase of the experiment.

In order to comply with normality and homogeneity of variance outliers were removed and negative rate constants were allowed (i.e. when rate constants should be zero). ANOVA *post hoc* analysis was done by applying Bonferroni method. Statistical significance was set up at $P < 0.05$. ANCOVA pairwise comparisons were performed with a Bonferroni style adjustment of significance levels to correct for multiple testing. Each comparison was tested at P/n where P is the nominated significance level (i.e. 0.05) and n is the number of comparisons.

Scripts for data extraction, determination of rate constants and statistical evaluation were written in Mathematica 10.0 (Wolfram Research, Oxfordshire, UK).

3. Results and discussion

Biofilm as a biological receptor is characterised by biodegradation (Flemming and Wingender, 2010; Lawrence et al., 2001; Writer et al., 2013). This special trait is one of the main natural detoxification processes for organic chemicals in the aquatic environment. The latter is of uppermost relevance for the entire community as it prevents the ecological degradation of the system. However, biodegradation is inherently confounded with contaminants. Therefore, we can only test how prolonged exposure to sustained concentration of ECs (chronic pollution) affects biodegradation. The first scenario of our case study tests how a drought episode influences biodegradation and the second scenario test how a drought subsequently followed by exposure to sustained concentration of ECs affects biodegradation capacity.

A mixture of ECs is designed using erythromycin, sulfisoxazole (the representatives of macrolide and sulphonamide antibiotics), a wieldy used analgesic diclofenac and the neonicotinoid pesticide imidacloprid. The compounds included in the mixture were selected because of their widespread occurrence in polluted aquatic environments, high ecotoxicological relevance and inclusion on the watch list of substances for EU-wide monitoring. A mixture was chosen over a single compound experiment in order to better mimic environmentally relevant conditions and not to omit the possible interaction (not focus of this study) of contaminants in the mixture. All these compounds exhibit no (or very low) biodegradability (Corcoll et al., 2015; García-Galán et al., 2008; Pomiés et al., 2015). On the other hand venlafaxine is readily biodegraded (Li et al., 2013) and it was used as a reference for biodegradation capacity. As has been previously reported, photolysis is the known degradation pathway of erythromycin, sulfisoxazole, diclofenac and imidacloprid (Batchu et al., 2014; Packer et al., 2003; Wamhoff and Schneider, 1999). The absence of photolysis in our experiments (Figs. 2–11, supporting information) is a good indicator that only photosynthetic active radiation has been used in ESF. The constant concentration of diclofenac and imidaclopird during all

experiments (see supporting information) indicates no (or very low) attenuation by sorption and biodegradation processes. Erythromycin exhibited sorption on the sediment in both flow conditions and both experimental setups (Figs. 2 and 3 Supporting information), in accordance with previous observations (Kim et al., 2004).

In the first scenario pristine streams of different flow history were spiked with ECs mixture at concentration of 10^{-6} g/l. Fig. 2 a) illustrates the observed degradation kinetics of venlafaxine during the first phase of the experiment. The degradation kinetics were established by monitoring the concentration of the parent compound as a function of time. The k_{bio} and $t_{1/2}$ values obtained from

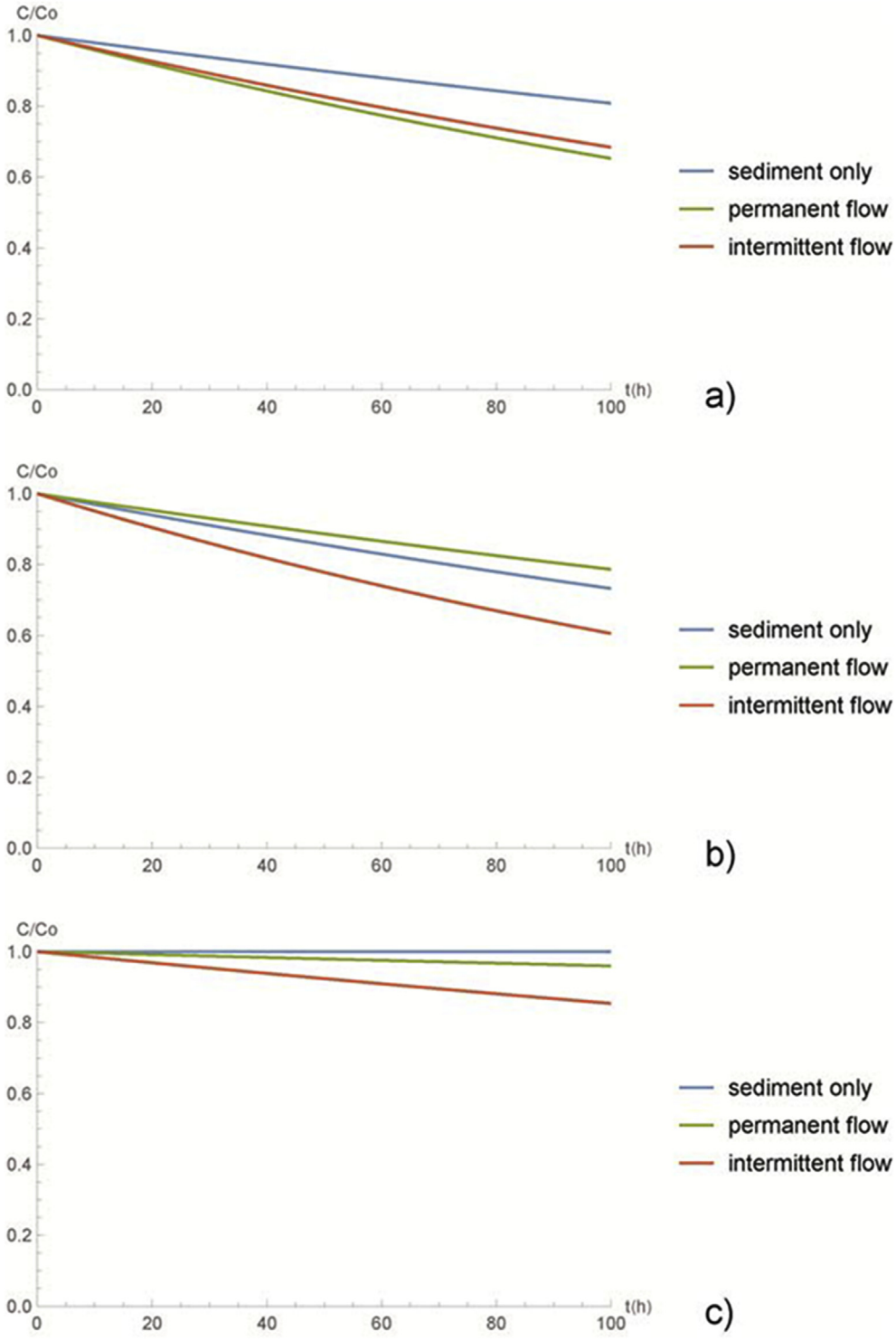

Fig. 2. Time decay of a) venlafaxine in experimental phase 1, b) venlafaxine in experimental phase 2 and c) sulfisoxazole in experimental phase 1. First order rate constants used to construct the curves are the average of three experimental replicates corrected for control. Rate constants are obtained by using non-linear regression. Different treatments, only sediment, permanent flow and intermittent flow, are indicated in legend. Experimental concentrations and corresponding theoretical fit for every replicate can be found in supporting information.

the experiments are summarized in Table 1. In both experimental treatments (permanent and intermittent flow) 19% of initial concentration of venlafaxine was adsorbed on sediment while additional an 18% was biodegraded, Fig. 2 a)., suggesting an equal contribution of sorption and biodegradation to venlafaxine attenuation during the time frame of the experiment. ANOVA analysis yielded significant variation among experimental treatments, $F(4, 10) = 56.9$, $P < 0.05$. Both, ANOVA *post hoc* Bonferroni test and ANCOVA pairwise comparison suggested a statistically significant difference between sediment only and permanent or intermittent flow channels indicating that sorption and biodegradation are two separate processes, (Table 3 supporting information). Observations are in accordance with previously reported behaviour of venlafaxine (Boix et al., 2016; Gasser et al., 2012; Li et al., 2013) confirming both sorption and biodegradation as significant environmental processes. The calculated half-life time of 15.2 ± 3.2 d is within the range (from 4.6 to 114 d) of reported laboratory biotransformation half-life times of venlafaxine (Li et al., 2013; Rúa-Gómez and Püttmann, 2013), albeit closer to value of 4.6 d reported by (Li et al., 2013).

Out of several venlafaxine degradation products reported (Boix et al., 2016; Gasser et al., 2012) only the *O*-desmethylation transformation product (TP) could be just tentatively identified (mass error of 5.3 ppm, RT 5.1 min). Further identification of *O*-desmethylvenlafaxine via a tandem mass spectrometry experiment was not possible due to low concentration levels of TP. The low abundance of TP is attributed to the low concentration of parent molecule as well as to the possibility that part of the TPs ensemble was adsorbed to the sediment. No other TPs were identified most likely because *O*-desmethyl product is the dominant metabolite of venalfaxine (Gasser et al., 2012) and suggested restrictions likely impair identification of other less abundant TPs.

No statistical difference between channels with and without flow intermittency was observed, suggesting no immediate impact of intermittency on biodegradation process in previously pristine streams. Similar trend, the absence of the effect of intermittency on bioaccumulation of pharmaceuticals had been reported previously by (Corcoll et al., 2015).

Biodegradation differed in experimental phase two. Fig. 2 b) illustrates attenuation kinetics of venlafaxine in the second phase of the experiment where all experimental channels were exposed to sustained concentration of ECs and consequent measurement of parent compounds decay. Nearly 24% of the venlafaxine decay was attributed to sorption on sediment. There was no statistical difference between sorption and biodegradation under permanent flow conditions (Table 4, supporting information). On the other hand, treatments with biofilm community of intermittent flow in addition to sorption displayed further 20% decrease of the initial venlafaxine concentration. Repeated measures ANOVA determined a significant main effect of the chronic pollution on rates of biodegradation, $F(1, 2) = 27.32$, $P < 0.05$. Rates were significantly lower after biofilm exposure to the mixture of ECs. On the other hand, the results show that biodegradation rate constants were not significantly affected by water flow conditions (the second main effect), $F(1, 2) = 8.81$, $P > 0.05$.

A statistically significant interaction effect between water flow history and exposure to ECs, $F(1, 2) = 30.35$, $P < 0.05$ was detected. This indicates that exposure to ECs had different effects on venlafaxine biodegradation rate constants depending on water flow conditions. Looking at the interaction graph (Fig. 3), venlafaxine rate constants after chronic pollution were significantly more lowered in permanent flow conditions than in intermittent flow

Table 1
Biodegradation rate constant (k_{bio}) and half-life time ($t_{1/2}$) of the target compounds obtained in the first and the second experimental phase. Values are represented as mean ± standard error. Rate constants are corrected for blank controls and (if detected) adsorption to the sediment.

Compound	Flow	Experiment 1		Experiment 2	
		k_{bio} (h^{-1})	$t_{1/2}$ (d)	k_{bio} (h^{-1})	$t_{1/2}$ (d)
Venlafaxine	Permanent	0.0021 ± 0.0004	15.2 ± 3.2	–	–
	Intermittent	0.0017 ± 0.0002	17.7 ± 1.5	0.0019 ± 0.0001	15.3 ± 0.8
Sulfisoxazole	Permanent	–	–	–	–
	Intermittent	0.0016 ± 0.0002	18.8 ± 1.7	–	–

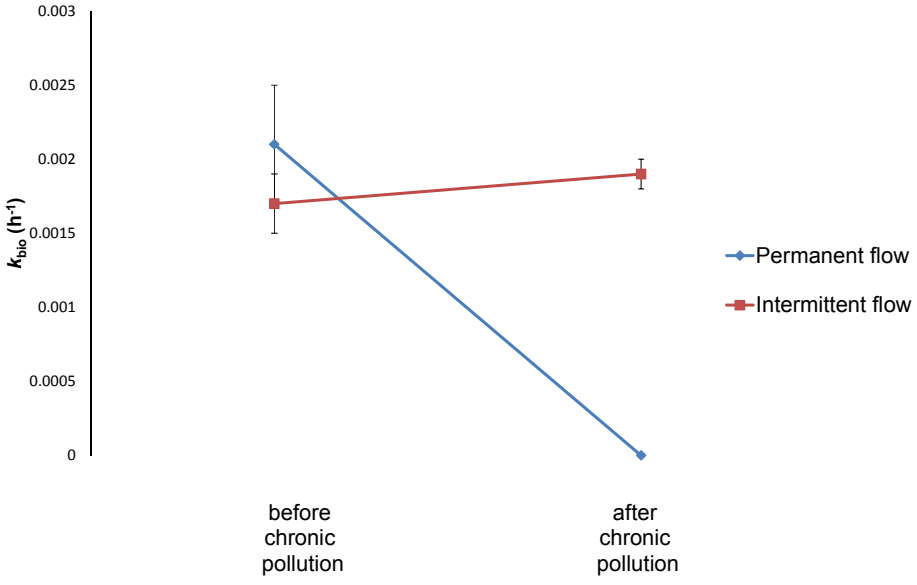

Fig. 3. The interaction plot: effect of permanent (SLB) v.s. intermittent (SLBD) flow before and after chronic pollution on venlafaxine biodegradation rate constant.

conditions. It seems that intermittent flow maintained biofilm degradation ability in polluted conditions. Since biodegradation is mainly related to bacterial communities this finding may indicate higher toxicity resilience of bacterial communities when introduced to flow intermittency. Biofilm community is shown to increase the heterotroph compartment after drought episode (Corcoll et al., 2015) possibly favouring (in the short term) more toxicity resistant bacteria. Observations on sulfisoxazole could further support our assumption on increased resilience of drought impacted bacterial communities. Sulfisoxazole, along with the entire sulphonamide antibiotic group, is known to exhibit very low or no biodegradation (García-Galán et al., 2008). However, in experimental phase 1, the drought strongly impacted biodegradation of sulfisoxazole (Fig. 2 c and Table 2 supporting information). Sulfisoxazole was gradually reduced by approximately 15% after 100 h with a calculated half-life of 18.8 ± 1.7 d, Table 1. No decrease of the initial concentration was observed in other treatment channels (Fig. 10 supporting information). In experimental phase 2 no change in sulfisoxazole concentration was detected in all treatments (Fig. 11 supporting information).

Overall, our findings establish a new testable hypothesis: water systems under multiple stressors conditions that combine the occurrence of ECs with hydrological discontinuities may preserve biofilm biodegradation ability. In addition to ecological level, molecular level (meta) assessment of biofilm function under multiple stressor interactions is needed.

4. Conclusions

The mesocosm experimental case study presented here provides insight into the understanding of the biofilm biodegradation capacity under flow intermittency and continuous exposure to a mixture of ECs. Even though mesocosm experiments may not always adequately represent natural systems, they are crucial in providing valuable data about effects noticeable at ecosystem level. Therefore, important findings of the current study can be summarised as follows.

Pristine biofilm biodegradation capacity is not affected by a water flow conditions suggesting that the flow history of pristine rivers may not alter the immediate response of biofilms to acute pollutant exposure. On a contrary, it seems that in some cases intermittent flow can stimulate short term biodegradation of compounds known to have very low biodegradation properties e.g. sulfisoxazole.

Consequent prolonged exposure to a mixture of ECs completely reduces biodegradation in permanent flow experimental treatments. On the other hand, treatments with intermittent flow preserved biofilm degradation capacity in polluted conditions highlighting that stream flow regime may modulate the biodegradation capacity of the biofilm community.

Significant interaction between the water flow history and the chronic pollution was observed indicating that decrease in biodegradation due to chronic pollution is greater in streams with permanent water flow. This finding has implications for aquatic ecosystems, as it suggests that the short and medium term biodegradation capacity in intermittent systems may be comparable to or even greater than in perennial streams.

Acknowledgment

This work has been supported by the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No. 657425 - TRANSFORMER project. We thank Maria Casellas (ICRA, Spain) for the help with the Experimental Streams Facility. Helpful discussions with S. Sabater (ICRA, Spain) are gratefully acknowledged. Proofreading by Marwan Braco Zeitoun (NHS Bournemouth, UK) is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2017.10.019.

References

Barceló, D., Sabater, S., 2010. Water quality and assessment under scarcity: prospects and challenges in Mediterranean watersheds. J. Hydrol. 383, 1–4. https://doi.org/10.1016/j.jhydrol.2010.01.010.

Batchu, S.R., Panditi, V.R., O'Shea, K.E., Gardinali, P.R., 2014. Photodegradation of antibiotics under simulated solar radiation: implications for their environmental fate. Sci. Total Environ. 299–310. https://doi.org/10.1016/j.scitotenv.2013.09.057, 470–471.

Boix, C., Ibáñez, M., Sancho, J.V., Parsons, J.R., Voogt, P. de, Hernández, F., 2016. Biotransformation of pharmaceuticals in surface water and during waste water treatment: identification and occurrence of transformation products. J. Hazard. Mater 302, 175–187. https://doi.org/10.1016/j.jhazmat.2015.09.053.

Corcoll, N., Casellas, M., Huerta, B., Guasch, H., Acuña, V., Rodríguez-Mozaz, S., Serra-Compte, A., Barceló, D., Sabater, S., 2015. Effects of flow intermittency and pharmaceutical exposure on the structure and metabolism of stream biofilms. Sci. Total Environ. 503–504, 159–170. https://doi.org/10.1016/j.scitotenv.2014.06.093.

Flemming, H., Wingender, J., 2010. The biofilm matrix. Nat. Rev. Microbiol. 8, 623–633. https://doi.org/10.1038/nrmicro2415.

García-Galán, M.J., Silvia Díaz-Cruz, M., Barceló, D., 2008. Identification and determination of metabolites and degradation products of sulfonamide antibiotics. Trac. - Trends Anal. Chem. 27, 1008–1022. https://doi.org/10.1016/j.trac.2008.10.001.

Gasser, G., Pankratov, I., Elhanany, S., Werner, P., Gun, J., Gelman, F., Lev, O., 2012. Field and laboratory studies of the fate and enantiomeric enrichment of venlafaxine and O-desmethylvenlafaxine under aerobic and anaerobic conditions. Chemosphere 88, 98–105. https://doi.org/10.1016/j.chemosphere.2012.02.074.

Holmstrup, M., Bindesbøl, A.M., Oostingh, G.J., Duschl, A., Scheil, V., Köhler, H.R., Loureiro, S., Soares, A.M.V.M., Ferreira, A.L.G., Kienle, C., Gerhardt, A., Laskowski, R., Kramarz, P.E., Bayley, M., Svendsen, C., Spurgeon, D.J., 2010. Interactions between effects of environmental chemicals and natural stressors: a review. Sci. Total Environ. 408, 3746–3762. https://doi.org/10.1016/j.scitotenv.2009.10.067.

Kim, Y.H., Pak, K., Pothuluri, J.V., Cerniglia, C.E., 2004. Mineralization of erythromycin A in aquaculture sediments. FEMS Microbiol. Lett. 234, 169–175. https://doi.org/10.1016/j.femsle.2004.03.027.

Lawrence, J.R., Kopf, G., Headley, J.V., Neu, T.R., 2001. Sorption and metabolism of selected herbicides in river biofilm communities. Can. J. Microbiol. 47, 634–641. https://doi.org/10.1139/cjm-47-7-634.

Li, Z., Gomez, E., Fenet, H., Chiron, S., 2013. Chiral signature of venlafaxine as a marker of biological attenuation processes. Chemosphere 90, 1933–1938. https://doi.org/10.1016/j.chemosphere.2012.10.033.

Ludwig, R., Roson, R., Zografos, C., Kallis, G., 2011. Towards an inter-disciplinary research agenda on climate change, water and security in Southern Europe and neighboring countries. Environ. Sci. Policy 14, 794–803. https://doi.org/10.1016/j.envsci.2011.04.003.

Murray, K.E., Thomas, S.M., Bodour, A.A., 2010. Prioritizing research for trace pollutants and emerging contaminants in the freshwater environment. Environ. Pollut. 158, 3462–3471. https://doi.org/10.1016/j.envpol.2010.08.009.

Packer, J.L., Werner, J.J., Latch, D.E., McNeill, K., Arnold, W.A., 2003. Photochemical fate of pharmaceuticals in the environment: naproxen, diclofenac, clofibric acid, and ibuprofen. Aquat. Sci. 65, 342–351. https://doi.org/10.1007/s00027-003-0671-8.

Pesce, S., Zoghlami, O., Margoum, C., Artigas, J., Chaumot, A., Foulquier, A., 2016. Combined effects of drought and the fungicide tebuconazole on aquatic leaf litter decomposition. Aquat. Toxicol. 173, 120–131. https://doi.org/10.1016/j.aquatox.2016.01.012.

Petrie, B., Barden, R., Kasprzyk-Hordern, B., 2014. A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. Water Res. 72, 3–27. https://doi.org/10.1016/j.watres.2014.08.053.

Pomiés, M., Choubert, J.M., Wisniewski, C., Miége, C., Budzinski, H., Coquery, M., 2015. Lab-scale experimental strategy for determining micropollutant partition coefficient and biodegradation constants in activated sludge. Environ. Sci. Pollut. Res. 22, 4383–4395. https://doi.org/10.1007/s11356-014-3646-5.

Proia, L., Vilches, C., Boninneau, C., Kantiani, L., Farré, M., Romaní, A.M., Sabater, S., Guasch, H., 2013. Drought episode modulates the response of river biofilms to triclosan. Aquat. Toxicol. 127, 36–45. https://doi.org/10.1016/j.aquatox.2012.01.006.

Rožman, M., Petrović, M., 2016. Bquant – novel script for batch quantification of LCMS data. MethodsX 3, 520–524. https://doi.org/10.1016/j.mex.2016.09.001.

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.

Schuhmacher, M., Navarro-Ortega, A., Sabater, L., Barceló, D., 2016. River conservation under multiple stressors: integration of ecological status, pollution and hydrological variability. Sci. Total Environ. 540, 1–2. https://doi.org/10.1016/j.scitotenv.2015.08.100.

Segner, H., Schmitt-Jansen, M., Sabater, S., 2014. Assessing the impact of multiple stressors on aquatic biota: the Receptor's side matters. Environ. Sci. Technol. 48, 7690–7696. https://doi.org/10.1021/es405082t.

Stampfli, N.C., Knillmann, S., Liess, M., Noskov, Y.A., Schäfer, R.B., Beketov, M.A., 2013. Two stressors and a community - effects of hydrological disturbance and a toxicant on freshwater zooplankton. Aquat. Toxicol. 127, 9–20. https://doi.org/10.1016/j.aquatox.2012.09.003.

Wamhoff, H., Schneider, V., 1999. Photodegradation of imidacloprid. J. Agric. Food Chem. 47, 1730–1734. https://doi.org/10.1021/jf980820j.

Writer, H., Antweiler, R.C., Ferrer, I., Ryan, J.N., Thurman, E.M., 2013. In-stream Attenuation of Neuro-active Pharmaceuticals and Their Metabolites.