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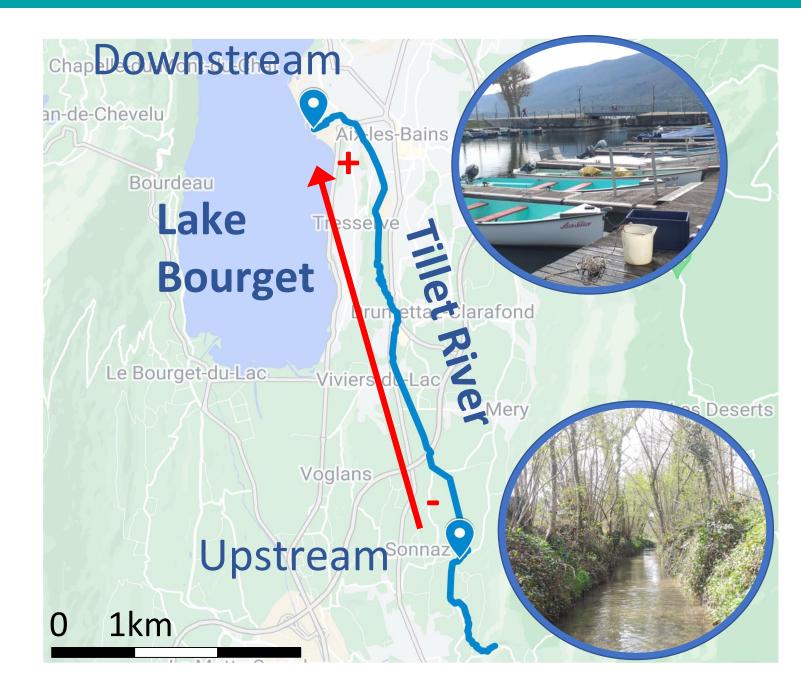
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In situ translocation experiment to assess the adaptation and resilience of periphytic communities to pharmaceutical substances using a PICT approach

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Introduction



Aquatic environments are contaminated by pharmaceutical substances worldwide [1]. This pollution poses ecotoxicological risks to exposed organisms, including microbial communities, such as periphyton. Given the ecological role of periphytic communities (e.g. biomass production, nutrient recycling, etc.), preserving their integrity is essential for well-functioning aquatic ecosystems. However, knowledge about effects of pharmaceuticals on freshwater periphyton is still very limited.

In this context, we performed an *in situ* study by implementing a Pollution Induced Community Tolerance (PICT) approach, a relevant diagnostic tool to establish *in situ* direct causal relationships between toxicants and their effects [2].

Two main hypotheses were tested:

Chronic exposure to pharmaceuticals increases periphytic community tolerance to these substances
 Tolerance dynamics (i.e. gain or loss of tolerance) reflect changes in exposure levels.

To this aim, we carried out a 10-week translocation study in the Tillet River (Savoie, France), considering an upstream reference station and a downstream station located in an urban area, and previously shown to be contaminated-by different classes of pharmaceuticals. PICT measurements were combined with chemical analyses of pharmaceuticals in surface water using polar organic chemical integrative samplers (POCIS).

Gradient of pharmaceutical concentrations
Map of the study site: the Tillet River

Materials and methods

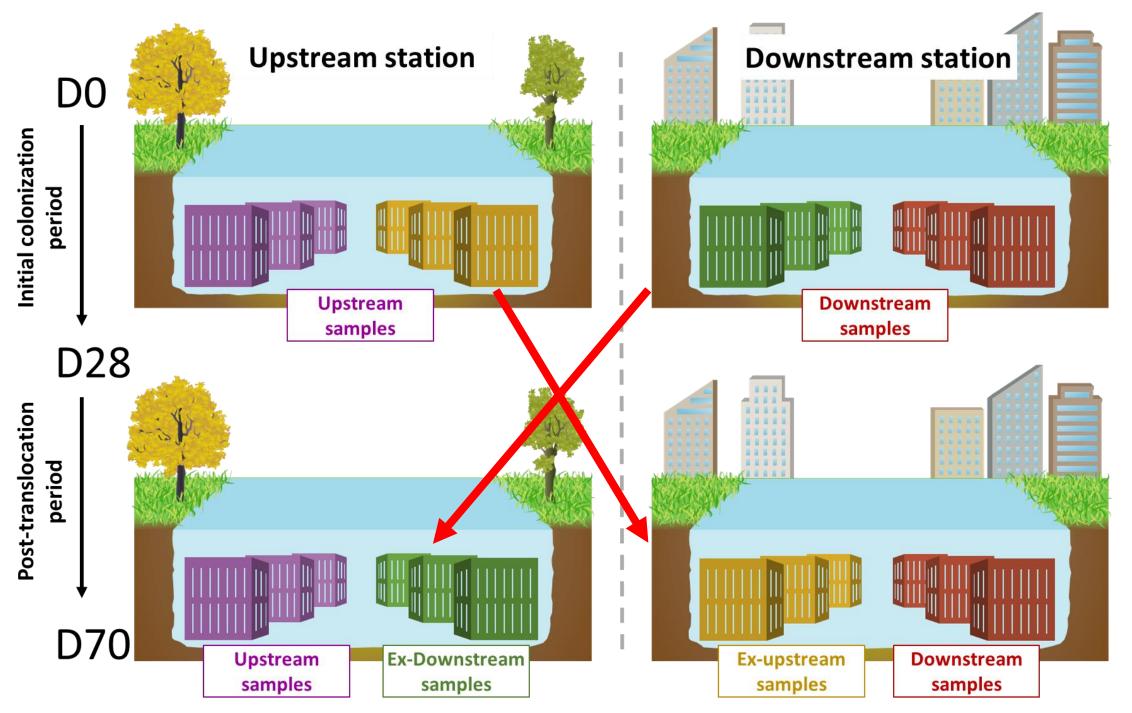


Illustration of the in situ experimental strategy

In Situ experimental strategy:

 After a 28-day initial colonization period, some periphytic biofilms were translocated (from up- to downstream and from down- to upstream).

⇒ Samplings were performed at days 28, 42, 56, and 70.

• Time averaged concentrations of selected pharmaceuticals (n = 42) in surface water were estimated using POCIS deployed during successive 2-week periods at the two sampling stations throughout the study.

PICT measurements and tolerance comparisons:

- Acute toxicity tests on bioflms were performed with 7 pharmaceuticals: *atenolol, diclofenac, erythromycin, paracetamol, ofloxacin, Ex sulfamethazine, and sulfamethoxazole 4*
- One of the three following activities was used as toxicity endpoint: β-glucosidase (4 hours), photosynthesis (4 h) or growth (72 h)

• Tolerance comparisons were based on estimated median effective concentrations (EC50).

P	resr	on	SPO

	upstream	ex-downstream	ex-upstream	downstream
າດໃ				



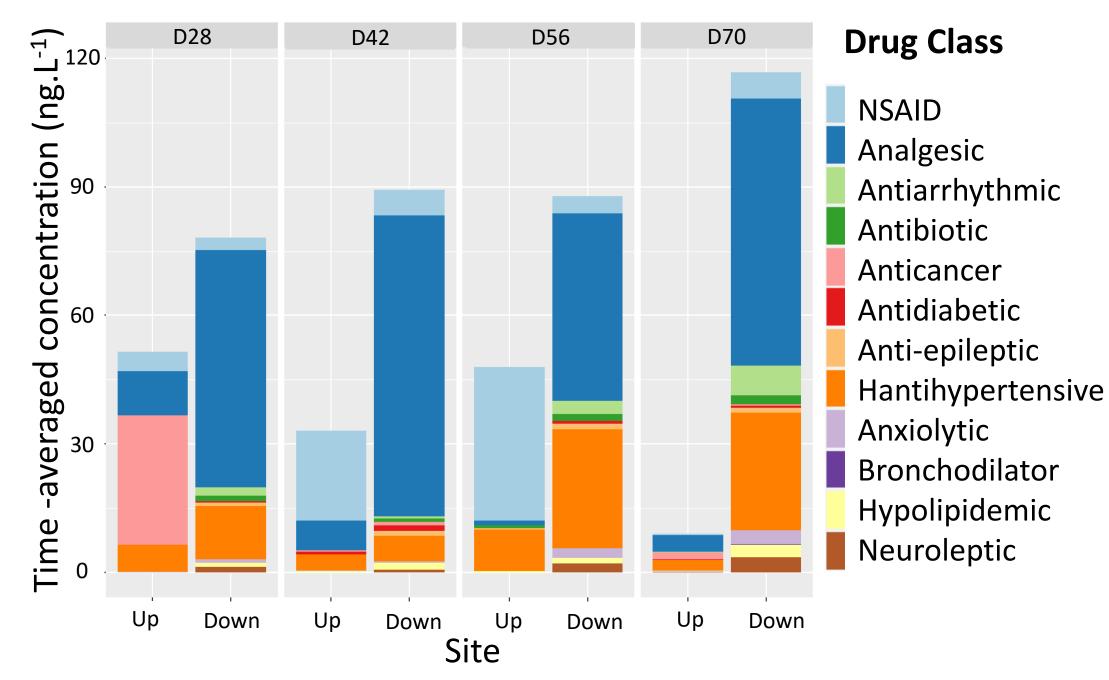
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24-0.387 25-0.37626-0.48227-0.48528-0.51129-0.5130-0.255 31-0.26132-0.3953-0.395
36-0.260 37-0.28138-0.40639-0.41140-0.41441-0.40642-0.20143-0.194 44-0.30145-0.31 46-0.40647-0.396
48-0.189 49-0.18550-0.35751-0.36752-0.35553-0.36554-0.13155-0.12556-0.19557-0.1658-0.33159-0.325
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84-0.09:85-0.12:86-0.26:87-0.275 88-0.09:85-0.12:86-0.26:87-0.275 88-0.277:89-0.258 88-0.277 88-0.277 89-0.258 88-0.277 88-0.277 88-0.277 88-0.277 88-0.277 88-0.277 88-0.277 88-0.277 88-0.277 88-0.277 88-0.277 89-0.258 88-0.277 89-0.258 88-0.277 89-0.258 88-0.277 89-0.258 88-0.277 89-0.258 88-0.277 89-0.258 80-0.277 89-0.258 80-0.277 89-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.277 80-0.258 80-0.257 80-0.258 80-0.257 80-0.258 80-0.257 80-0.258

Ex. Algal fluorescence after 4h exposure to a gradient of diclofenac measured via Imaging-PAM

Results

Pharmaceuticals concentrations in surface water:

Number and concentrations of pharmaceuticals were higher downstream than upstream.



Pharmaceuticals concentrations in surface water in upstream (Up) and downstream (Down) sites

Conclusions

rici responses:

 At day 28 the main differences in tolerance between Upstream and Downstream biofilms were observed with ofloxacin (toxicity tests on βglucosidase, data not shown), diclofenac and atenolol (toxicity tests on photosynthesis)

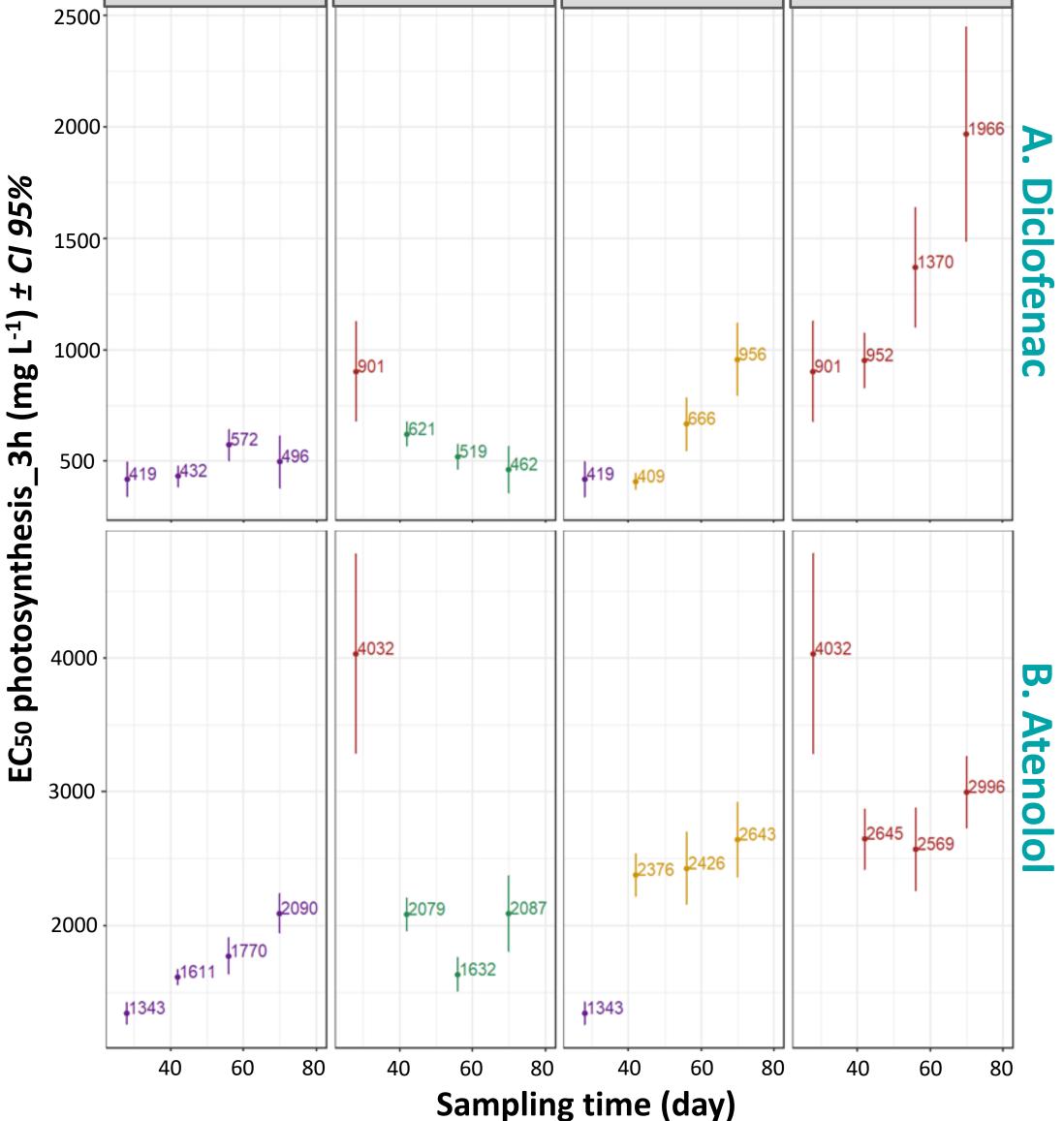
EC50 upstream < EC50 downstream

 Biofilms moved from upstream to downstream progressively increased sive their tolerance to both diclofenac and atenolol

EC50 ex-upstream 7

 Biofilms moved from downstream to upstream progressively decreased their tolerance to both diclofenac and atenolol

EC50 ex-downstream 凶



• As expected, pharmaceutical concentrations in river were significantly higher downstream than upstream.

- After the 28-Day colonization period, periphytic communities from the downstream site were more tolerant to 3 of the 7 tested substances (ofloxacin, diclofenac, atenolol), in accordance with our first hypothesis.
- The temporal evolution of tolerance levels to diclofenac and atenolol during the post-translocation period was also in line with our second hypothesis since biofilms moved from upstream to downstream progressively increased their tolerance while opposite changes were observed with biofilms moved from downstream to upstream.
- The results suggest that **PICT can be a relevant in situ diagnostic tool** to evaluate the chemical pressure by pharmaceuticals.

Further analyses will be conducted to

better characterize the *in situ* microbial exposure to pharmaceuticals (concentration measurements within periphytic biofilms)
 evaluate whether temporal changes in tolerance reflect temporal changes in microbial diversity (bacterial & algal metabarcoding)









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