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

Hélène Rogue, Cécile Miège, Chloé Bonnineau, Bernadette Volat, Christophe Rosy, Bernard Motte, Amandine Daval, Emilie Lyautey, Stéphane Pesce

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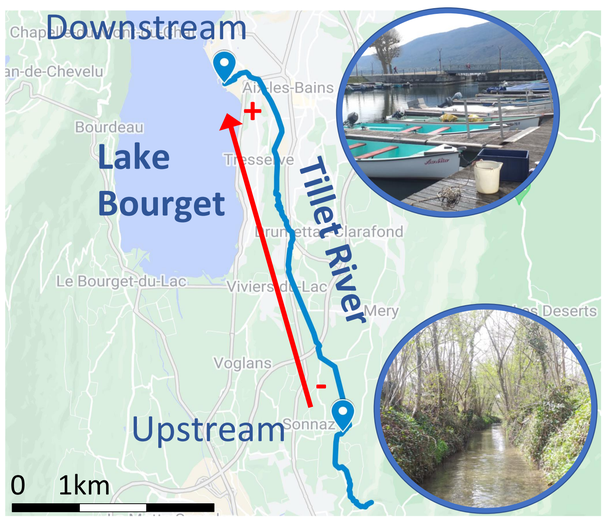
Rogue Hélène [1,2], Miège Cécile [2], Bonnineau Chloé [2,3], Volat Bernadette [2], Rosy Christophe [2], Motte Bernard [2], Daval Amandine [2], Lyautey Emilie [1], Pesce Stéphane [2]

1. University Savoie Mont Blanc, UMR CARRTEL, le Bourget-du-Lac, France ; 2. INRAE, UR RiverLy, Lyon-Villeurbanne, France ; 3. INRAE, UR EABX, Cestas, France

Introduction



Gradient of pharmaceutical concentrations

Map of the study site: the Tillet River

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:
1) Chronic exposure to pharmaceuticals increases periphytic community tolerance to these substances
2) 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).

Materials and methods

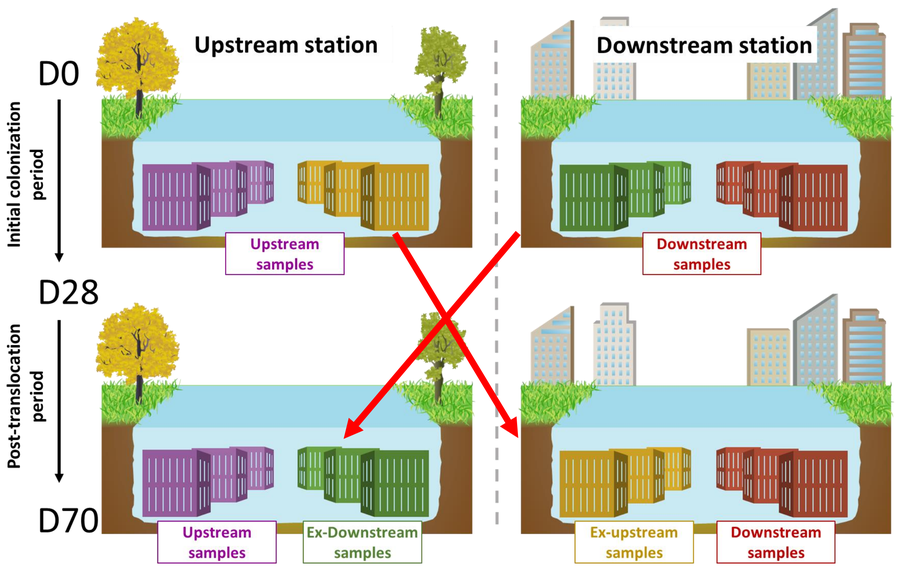
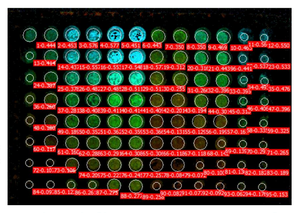


Illustration of the *in situ experimental strategy*

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.



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

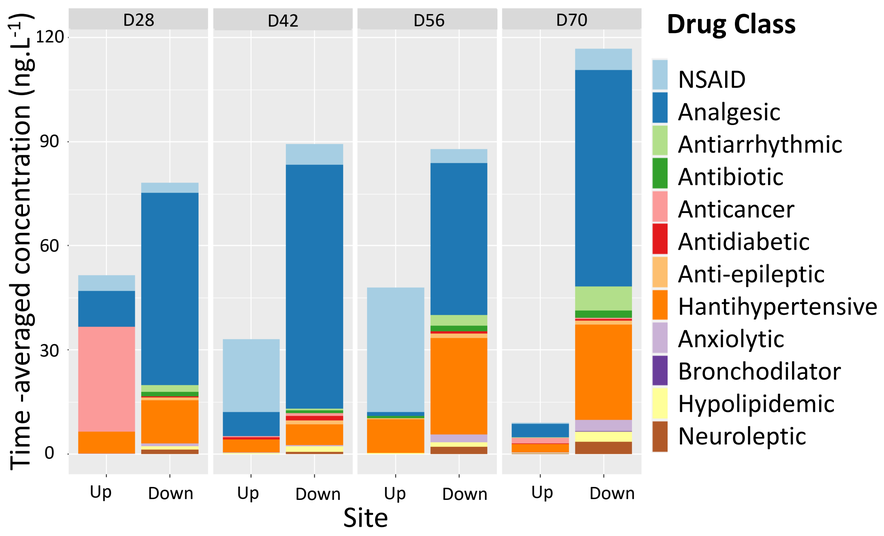
PICT measurements and tolerance comparisons:

- Acute toxicity tests on bioflms were performed with **7 pharmaceuticals**:
  atenolol, diclofenac, erythromycin, paracetamol, ofloxacin, sulfamethazine, and sulfamethoxazole
- **One of the three following activities** was used as toxicity endpoint:
  ß-glucosidase (4 hours), photosynthesis (4 h) or growth (72 h)
- **Tolerance comparisons** were based on estimated median effective concentrations (EC_{50}).

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

PICT responses:

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

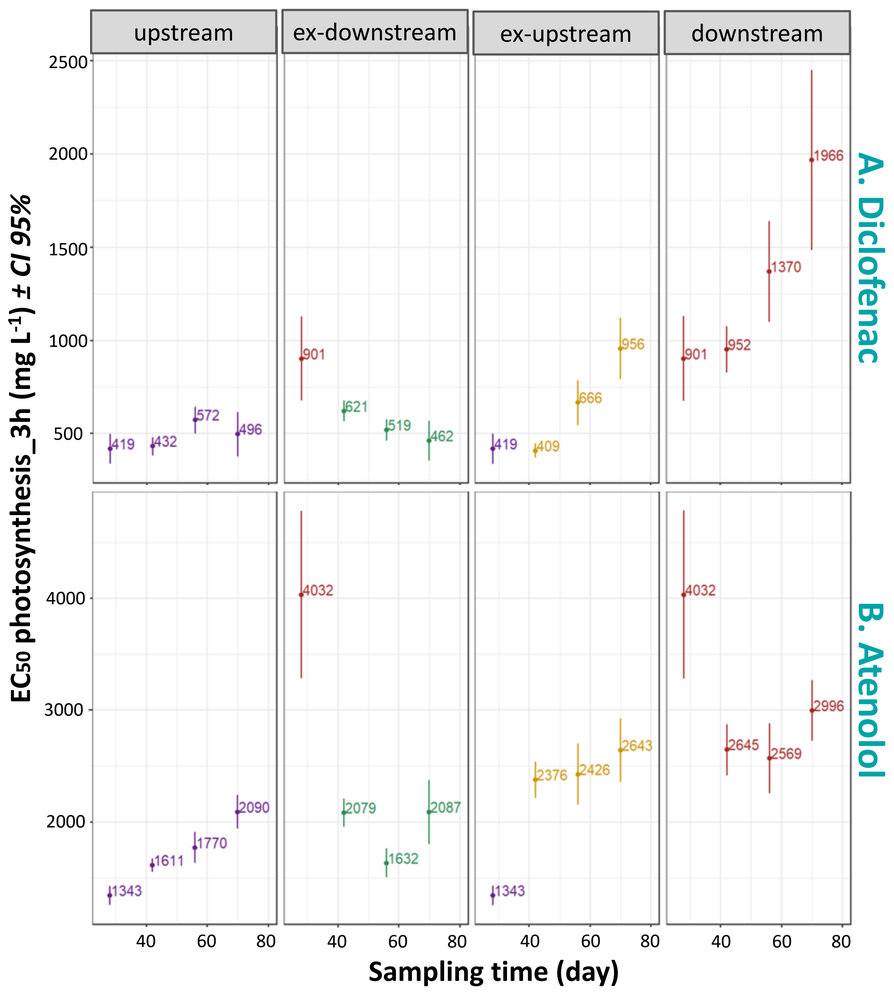
EC_{50} upstream < EC_{50} downstream

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

EC_{50} ex-upstream ↗

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

EC_{50} ex-downstream ↘



Conclusions

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

References [1] Wilkinson et al., 2022 doi: 10.1073/pnas.2113947119
[2] Tlili et al., 2016, doi: doi:10.1111/fwb.12558

Contact: helene.rogue@inrae.fr