Aquatic Toxicology 102 (2011) 123–133

Contents lists available at ScienceDirect

Aquatic Toxicology

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

# Effects of flow regime and pesticides on periphytic communities: Evolution and role of biodiversity

Aurélie Villeneuve[a,b], Bernard Montuelle[a], Agnès Bouchez[a,*]

[a] INRA, UMR CARRTEL, 75 avenue de corzent, 74200 Thonon-les-Bains, France
[b] Institut Pasteur, Unité des Cyanobactéries, 75015 Paris, France

ARTICLE INFO

*Article history:*
Received 19 November 2010
Received in revised form 13 January 2011
Accepted 17 January 2011

*Keywords:*
Periphyton
Flow regime
Pesticide
Mesocosm
Biodiversity
Ecological function

## ABSTRACT

The effects of chemical and physical factors on periphyton structure, diversity and functioning were investigated in an outdoor mesocosm experiment. Stream biofilms were subjected to a pesticide mix (diuron and azoxystrobin) under two different hydraulic regimes. The hydraulic regimes differed by spatial variations of flow conditions (turbulent with high variations vs. laminar with low variations). The effects of the hydraulic regime and pesticides were assessed at the level of the periphytic communities. We focused on the change in the biodiversity of these communities under the two hydraulic regimes, and on the role of these biodiversity changes in case of pesticide contamination. Changes in structural (biomass, cell density), diversity (community composition assessed by PCR-DGGE and microscopic analysis) and functional (bacterial and algal production, sensitivity to the herbicide) parameters were monitored throughout a 2-month experiment.

The results showed that exposure to pesticides affected the phytobenthic community targeted by the herbicide, impacting on both its growth dynamics and its primary production. Conversely, the impact of the flow regime was greater than that of pesticides on the non-target bacterial community with higher bacterial density and production in laminar mesocosms (uniform regime). An interaction between flow and pollution effects was also observed. Communities that developed in turbulent mesocosms (heterogeneous regime) were more diversified, as a result of increased microhabitat heterogeneity due to high spatial variations. However, this higher biodiversity did not increase the ability of these biofilms to tolerate pesticides, as expected. On the contrary, the sensitivity of these communities to pesticide contamination was, in fact, increased.

## 1. Introduction

Benthic microbial communities growing as a biofilm (or periphyton) play an important role in the ecological functioning of watercourses (Hansson, 1992; Munoz et al., 2001; Pusch et al., 1998; Romani and Sabater, 1999; Woodruff et al., 1999). The development, structure, taxonomic diversity, and functioning of the periphyton are heavily dependent on both physical and chemical environmental factors (Biggs, 2000; Hillebrand and Sommer, 2000; Sabater et al., 1998; Villeneuve et al., 2010). Thus any modification of these various factors, whether of natural origin or related to human activity, can impair the ability of biofilms to play their part in aquatic ecosystems.

One of the main physical factors controlling the development of the periphyton is the flow regime. Numerous studies have shown that the hydraulic regime controls the algal biomass, and alters the species composition of periphytic communities (Battin et al., 2003a,b; Biggs and Thomsen, 1995; Francoeur and Biggs, 2006). However, many human interventions (dredging, canalization, containment, damming, etc.) can alter the hydraulic characteristics of watercourses. These modifications can, for example, reduce the diversity of the physical habitats in watercourses (microhabitats), thus resulting in a loss of biodiversity (Licursi and Gomez, 2009; Nakano and Nakamura, 2008; Negishi et al., 2002; Poff et al., 2007). Indeed, Rainey and Travisano (1998) showed that spatial variability (in our particular case, variations in the hydraulic regime) is crucial for both the emergence of biodiversity, and its restoration after a disturbance. This relationship between habitat heterogeneity and diversity has been observed for many organisms living in a variety of ecosystems. In aquatic ecosystems, Buckling et al. (2000) have studied the diversity of *Pseudomonas aeruginosa* in mesocosms as a function of a more or less regular distribution of nutrients. In "static" mesocosms, in which the distribution of nutrients was not uniform, the genetic diversity of *P. aeruginosa* was greater than that observed in "agitated" mesocosms, where it was uniform. The link between spatial heterogeneity and biodiversity has also been

---

* Corresponding author.
*E-mail address:* bouchez@thonon.inra.fr (A. Bouchez).

0166-445X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2011.01.004

assessed by Singer et al. (2010) in the case of a bacterial community growing in lotic biofilm subjected to flow variations. Spatial flow heterogeneity increased both bacterial diversity and resource use by the bacteria present. The first hypothesis that we advance here is that microhabitat heterogeneity created by flow variations leads to the development of a more diversified community, due to the presence of more diverse "ecological niches" that can accommodate species with preferences for a variety of habitats.

Another factor controlling the development of biofilms is the chemical quality of the water, whether in terms of nutrients (Ludwig et al., 2008; Luttenton and Lowe, 2006) or of toxic pollutants (Guasch et al., 2003; Pesce et al., 2008). The levels of organic compounds found in surface waters have increased in recent decades as a result of human activities. Pesticides (insecticides, herbicides, fungicides, etc.) are the organic compounds most commonly detected in flowing waters (Azevedo et al., 2000; Sáenz and Di Marzio, 2009), and are mainly used for agricultural purposes. Structural and functional homology with the initial targets of herbicides with organisms that compose periphyton together with trophic interactions in periphyton means that toxicants may affect primary producers through disruption of the food web from its base. Periphyton may therefore be used to detect the potential early effects of herbicides on aquatic ecosystems (Sabater et al., 2007). Many authors have already highlighted the impact of herbicides on the species richness and taxonomic composition of autotrophic organisms, as a result of the selection of resistant species and the detriment of susceptible species (Dahl and Blanck, 1996; De Lorenzo et al., 1999; Pérès et al., 1996). An indirect effect due to heterotrophic use of algal material released from decaying algae after pesticide exposure has also been observed (Ricart et al., 2009), and resulted in changes in the bacterial density, production and composition. The main objectives of our study were to evaluate the direct and indirect effects of pesticides on the structure, composition and function of both algae and bacteria, and to investigate if these effects are modulated by changes in flow regime.

Finally, the response of the periphyton to chemical stress is known to depend on the pattern of species present when pollution occurs (Guasch et al., 1997; Gurney and Robinson, 1989; Pesce et al., 2006; Tlili et al., 2008). However, this pool of species can be modified by changes in river flow regime, which can subsequently lead to a change in ecological responses. McCann (2000) introduced the "biodiversity-stability hypothesis", according to which the resistance and resilience of the ecosystem increase along with the number of trophic interactions within a community. This hypothesis is also known as the "biological insurance hypothesis" (Yatchi and Loreau, 1999), and suggests that more diversified ecosystems are more likely to contain species that are able to resist the disruption, and that this enables them to offset the functional impairment resulting from the disappearance of other species. According to Yatchi and Loreau (1999), the second hypothesis of this work is that a more diversified biofilm would be less sensitive to pesticide exposure.

Briefly, the main objective of our study was to find out how the response of benthic microbial communities to the presence of a realistic mix of pesticides depends on the hydraulic regime of watercourses. To do this, we used outdoor lotic mesocosms, with artificial substrates colonized by natural periphyton communities under two contrasting hydraulic regimes (one turbulent with heterogeneous flow, and the other laminar with uniform flow). After being allowed to colonize the substrate for one month, the periphytic community was exposed to a mix of pesticides (diuron and azoxystrobin). The hydraulic changes and pesticide contaminations were designed to correspond to realistic changes in the factors being investigated, i.e. changes consistent with *in situ* observations. The structure, function, diversity and sensitivity to pesticides of the communities were monitored throughout the 2 months of the experiment, in order to assess the effects of both the hydraulic regime and the pesticides, and the potential interactions between them. Diversity was evaluated by microscopy (micro-algae) and by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) on the prokaryotic and eukaryotic communities. Finally, sensitivity towards diuron was assessed by primary production measurements in short-term bioassays.

## 2. Materials and methods

### 2.1. Lotic mesocosms

We used a set of four outdoor lotic mesocosms made of stainless steel that had been designed by Volatier (2004). Fig. 1 shows the diagram of a mesocosm. Each mesocosm (channel length: 4 m, width: 0.4 m and depth: 0.35 m) was supplied with water pumped from a depth of 36 m in Geneva Lake, and was operated as a semi-open system regulated by an overflow. The entire volume of water (2.2 $m^3$ per mesocosm) was replaced continuously (with a complete turnover on average 4 times a day), in order to allow seeding to occur for the colonization of a natural periphyton on artificial substrates placed at the bottom of each channel, and to ensure that the periphyton had an adequate supply of nutrients. The outflow was decontaminated by activated-carbon filtration before it was discharged. Two types of artificial substrates were used: frosted glass slides (7.9 $cm^2$) and frosted glass discs (1.2 $cm^2$). All four mesocosms were exposed to the same outdoor conditions (light intensity, day length and air temperature) and the same incoming water supply (pH, nutrients, temperature, cell seeding and renewing rate).

Two different flow regimes were applied: a heterogeneous turbulent flow (0.1 to >1 $m\,s^{-1}$) in two of the mesocosms, and a

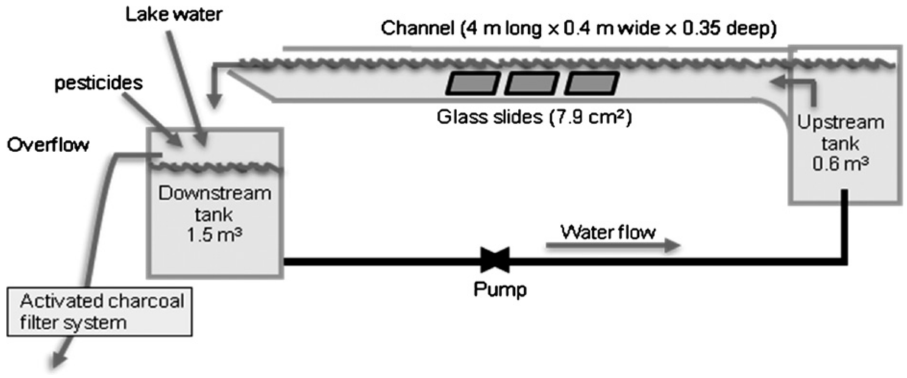

**Fig. 1.** Schematic description of a lotic mesocosm (side view). The experimental platform is equipped with 4 mesocosms.