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Environmental effects of realistic pesticide mixtures on natural biofilm communities with different exposure histories

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HIGHLIGHTS

- We assessed the effects of pesticides exposure on biofilms using passive samplers.
- The sensitivity of biofilm to pesticides revealed the past history of communities.
- Pesticides had significant effects on growth-related and structural endpoints.
- History exposure had a crucial role in biofilm responses to pesticides exposure.
- POCIS extracts were highly relevant for assessment of chronic effects of mixture.

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ABSTRACT

This study deals with the use of Polar Organic Chemical Integrative Sampler (POCIS) extracts to assess the impact of low-dose pesticide mixtures on natural biofilm communities originating from either a chronically contaminated or a reference field site. To investigate how natural biofilm communities, pre-exposed to pesticides in situ or not might respond to environmentally realistic changes in pesticide pressure, they were exposed to either clean water or to POCIS extracts (PE) in order to represent toxic pressure with a realistic pesticide mixture directly isolated from the field. The impacts of PE were assessed on structure, physiology and growth of biofilms. Initial levels of tolerance of phototrophic communities to PE were also estimated at day 0.

PE exposure led to negative effects on diatom growth kinetics independently of in-field biofilm exposure history. In contrast, the impacts observed on dry weight, ash-free dry mass and algal fluorescence-related parameters followed different trends depending on biofilm origin. Exposure to PE induced changes in diatom assemblages for the biofilm originating from the reference field site with higher relative abundance of *Eolimna minima* and *Nitzschia palea* with PE exposure. Initial tolerance of phototrophic communities to PE was 8-fold higher for the biofilm originating from the chronically contaminated site compared to the reference field site.

The use of POCIS extracts allowed us to highlight both chronic impacts of low doses of a mixture of pesticides on natural communities with regard to biofilm exposure history as well as initial levels of tolerance of phototrophic communities.

1. Introduction

The traditional approach to environmental risk assessment consists of evaluating the toxicity of a single compound on single species. But, this reasoning lacks ecological relevance because organisms are grouped in complex biological communities exposed to chemical mixtures in the environment. The development of new tools is thus needed to reach a more environmentally realistic and integrative approach in risk assessment studies. Attached microbial communities could respond to such needs as they play a fundamental role in the ecological functioning of river systems, owing to their key position in the trophic web and their important contribution as primary producers and microbial decomposers. Moreover, such communities interact strongly with dissolved substances such as pesticides present in water and are likely to respond quickly to contaminant pressures making river biofilms useful early warning systems for the detection of the effects of toxicants (Montuelle et al., 2010). Among the variety of methods available, physiological approaches may be appropriate for the detection of acute effects whereas persistent or chronic effects should act on other biofilm indicators, for example growth or

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biomass-related factors, or community composition (in particular the diatom community) (Sabater et al., 2007). It is for these qualities that attached microbial communities have been used as biological models in the study of various toxicants in controlled laboratory conditions (Laviale et al., 2011; Corcoll et al., 2012a,b; Tlili et al., 2008). Nevertheless, most of these studies dealt with single compounds or binary mixtures. On the other hand, translocation experiments with attached microbial communities have been used to reach more realistic exposure conditions (in particular with regard to toxicant exposure) (Morin et al., 2010a; Arini et al., 2012). Reference biofilms transferred to contaminated sites generally presented, after some weeks, the characteristics of communities from polluted sites. Conversely, recovery of communities after translocation from a contaminated to a less contaminated site is observed after a few weeks to a few months. Nevertheless these approaches do not allow clear identification of the extent to which the toxicant(s) of interest is (are) responsible for the observed effects on biofilms, since abiotic (e.g. light, temperature, current velocity) and biotic (e.g. species drift) parameters are not controlled and multiple contamination often occurs.

Passive sampling devices like the Polar Organic Chemical Integrative Sampler (POCIS) are useful tools for monitoring trace levels of chemicals in aquatic environments since they concentrate many organic chemicals from large volumes of water (Mazzella et al., 2010). This high concentration of compounds makes the POCIS a powerful instrument for the assessment of extract toxicity via biological testing. The use of the POCIS in combination with bioassays has the advantage of being more relevant from an ecotoxicological perspective because of the pollutant mixtures it provides. Moreover, this approach gives an estimation of an integrative measure of the toxic potential of a group of compounds including unknown toxicants (a non a priori approach). While POCIS extracts in combination with short-term toxicity tests have been successfully used in particular in order to reveal the exposure history of biofilms (Pesce et al., 2011b), evaluation of potential extract toxicity in long-term studies is still in a challenge. The first and only study dealing with chronic low-dose effects of passive sampler extracts on biofilms was conducted by Morin et al. (2012b). The experiment was a first attempt to evaluate whether the chronic effects of pesticides in a mixture could be approached by the use of POCIS extracts. The study highlighted the methodological issues of dealing with low contaminant doses in long-term experiments, particularly the difficulty of monitoring the concentration of contaminants in large volumes of water. It nevertheless reported the promising perspectives of the approach for further ecotoxicology studies.

The objective of the present study was to evaluate the efficiency of the use of PE in order to study the impact of changes in pesticide pressure on biofilms. In order to reach this goal, biofilm communities were collected from a reference and from a chronically contaminated site of a small river located in a French vineyard area, and then communities were chronically exposed. Initial levels of tolerance of phototrophic communities to pesticide mixtures were also estimated using a short-term photosynthetic bioassay with the PE. Chronic effects were assessed by exposing biofilms either to clean water or to low doses of PE. Diatom growth kinetics, dry weight, ash-free dry mass, algal fluorescence-related parameters, effective quantum yield of Photosystem II and diatom community structure and composition were determined after 0, 3, 7 and 13 days of exposure in experimental channels. Exposure to PE was expected to provoke structural and/or functional changes in the communities originating either from the chronically contaminated or from the reference field site.

2. Material and methods

2.1. Study site and sampling procedure

The study was carried out on the Morcille River, located in the Beaujolais vineyard watershed of eastern France. The Morcille River has been extensively studied over the past decades (Dorigo et al., 2007; Montuelle et al., 2010; Rabiet et al., 2010). The area is subjected to strong agricultural pressure – essentially exerted by vineyards – and is characterized by an increasing multi-contaminant gradient. Pesticides overall and to a lesser extent metal and nutrient concentrations increase from upstream to downstream.

Glass slides fixed in perforated plastic boxes were used as artificial substrates to allow biofilm colonization for 4 weeks (from 24th May to 21st June 2011) at the 2 stations located upstream (reference site) and downstream (the more contaminated site). The minimum number of slides to run the analyses considered here was estimated at 120 slides of 14 cm^2 (60 at each station). Consequently, 100 slides were immerged at each site (in order to have sufficient biofilm taking into consideration possible breakage of glass slides in the river).

"Quantitative" POCIS were used for pesticide quantification of the majority of the compounds found in the water (Mazzella et al., 2010; Lissalde et al., 2011), while grab sampling was used to determine the concentration of compounds that were not calibrated by POCIS in the present study (average of the 5 grab samples taken from 24th May–21st June 2011).

A Performance and Reference Compound (PRC) was introduced in a "Quantitative" POCIS. The devices were immersed in the current at upstream and downstream stations for two weeks and then replaced by new ones for two extra weeks for pesticide quantification and characterization of biofilms in situ past exposure. After collection, all POCIS were kept at −4 °C until extraction and chemical analysis.

"Accumulative" POCIS used for the toxicity tests (Morin et al., 2012b) were immersed at the downstream station during the biofilm colonization period to concentrate pesticides. After 4 weeks in the river, the glass slides and POCIS were brought back to the laboratory. The biofilms were put in aquariums filled with water from their respective sites supplemented with nutrients following the composition of WC culture medium (Guillard and Lorenzen, 1972) given in Table 1 for one week before the beginning of the experiment (corresponding to the time required to carry out POCIS extraction).

2.2. Laboratory experimental conditions

2.2.1. Acute toxicity testing

In order to characterize the initial tolerance of upstream and downstream biofilms to pesticides, acute toxicity tests were carried out after one week in the lab. Two conditions were tested (upstream and downstream biofilms) in triplicate (10 glass slides for each replicate) leading

Table 1
Composition of the "WC" culture medium from Guillard and Lorenzen (1972).

Freshwater "WC" medium	
Major nutrients	
$CaCl_2$, $2H_2O$	36.76 mg/L
$MgSO_4$, $7H_2O$	36.97 mg/L
$NaHCO_3$	12.6 mg/L
K_2HPO_4	8.71 mg/L
$NaNO_3$	85.01 mg/L
Na_2SiO_3, $9H_2O$	28.42 mg/L
Traces	
Na_2EDTA	4.36 mg/L
$FeCl_3$, $6H_2O$	3.15 mg/L
$CuS0_4$, $5H_2O$	0.01 mg/L
$ZnSO_4$, $7H_2O$	0.022 mg/L
$CoCl_2$, $6H_2O$	0.01 mg/L
$MnCl_2$, $4H_2O$	0.18 mg/L
Na_2MoO_4, $2H_2O$	0.006 mg/L
H_3BO_3	1.0 mg/L
Vitamins	
Thiamin, HCl	0.1 mg/L
Biotin	0.5 μg/L
B12	0.5 μg/L