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Responses of chronically contaminated biofilms to short pulses of diuron An experimental study simulating flooding events in a small river

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ABSTRACT

An experimental study was undertaken to highlight the potential ecotoxicological impact of the herbicide diuron on biofilms during flooding events in a small river (Morcille) in the Beaujolais vineyard area (France). We investigated the responses of chronically contaminated biofilms exposed to short-term pulses (3 h) of diuron. Biofilms were grown in indoor microcosms that were either non-contaminated or exposed to low-level chronic contamination, and not exposed, or exposed to single or double pulses of two environmental concentrations (7 and 14 $\mu g\,L^{-1}$) of diuron. Exposure to pollution and its impact on biofilms were assessed by measuring pesticide concentrations in biofilms, biomass parameters (chl *a*, AFDW), community structure (using 18S and 16S rDNA gene analysis by DGGE, and HPLC pigment analysis to target eukaryotes, bacteria and photoautotrophs, respectively) and by performing a physiological test. Control biofilms displayed very low diuron concentrations, whereas the herbicide was found in the contaminated biofilms. Nevertheless, diuron concentrations were not higher in the pulsed biofilms than in the non-pulsed ones. AFDW and chl *a in vivo* fluorescence increased in both microcosms during the experiment and biomass was higher in chronically exposed biofilms than in control ones. The impact on biomass was higher for the control double-pulsed biofilms than for the non-pulsed ones. Carbon incorporation by the chronically exposed biofilms was greater during the first 28 days of growth than during the first 28 days of growth in the control biofilms. Both single and double pulses inhibited carbon incorporation of all biofilm communities, especially of the control ones. Short-term inhibition of photosynthesis was never significantly different in exposed and non-exposed biofilms. Few differences in the pigment structure were found between chronically exposed and control biofilms, but pulses impacted on the pigment structure of all biofilm communities. Bacterial structural differences were observed between single-pulsed and non-pulsed biofilms, but not between double-pulsed and non-pulsed biofilms. The different pulses affected the eukaryotic community structures of the control biofilms, but not of the chronically exposed ones. Unlike the bacterial communities, the control eukaryotic communities were structurally different from the chronically exposed ones. This preliminary experimental study indicates that exposure to environmental concentrations of diuron and other agricultural contaminants and further exposure to diuron can have measurable effects on small river biofilm communities. The effects of a pulsed acute exposure to diuron on biofilms depended on whether the biofilms had previously been exposed to the same stressors or not.

1. Introduction

The European Water Framework Directive (WFD) and the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) programme aims that non-target communities inhabiting water bodies should not be affected by the presence of chemical substances. Pesticides are one of the main groups of chemicals that could impair aquatic ecosystems (INRA-Cemagref, 2005), and so they have been included in the frame of reference of both WFD (one of the strategies against water pollution is the setting of environmental quality standards (EQS) for pesticides) and REACH. Small lotic ecosystems (Strahler order below 3) are very sensitive to this type of pollution, because little dilution can occur, and because of the proximity of the sources of contamination (spraying of pesticides, run off . . .) (Dorigo et al., 2003). In these ecosystems, microbenthic biofilms (an attached community of autotrophic and heterotrophic, eukaryotic and prokaryotic populations) play a

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fundamental role in the trophic web and in the geochemical cycles within aquatic ecosystems (Battin et al., 2003). In rivers, epilithic microbial communities are one of the first organisms exposed to pesticides that run off from fields, and their structure and function can be affected. Structural responses to environmental changes occur within a few weeks (Sabater et al., 2007).

Studying stress effects by investigating changes in community composition is attractive, since the presence and density of different species integrate all the ecological drivers, and reflect inter-species interactions (Bérard et al., 1999). The meta-analysis on 446 measures of biodiversity effects by Balvanera et al. (2006) confirms the importance of biodiversity for ecosystem function and services. This means that it is essential to develop ecotoxicological studies that take into account both the biodiversity and functioning of microbial communities.

Diuron (*N*-(3,4-dichlorophenyl)-*N*,*N*-dimethyl-urea, MW = 233.10 g) is an herbicide (phenylurea) that inhibits photosynthesis by blocking the chloroplast electron transport chain in Photosystem-II (PSII) of phototrophic microorganisms and higher plants (Moreland, 1967). Diuron is considered to be a model herbicide with regard to physiological studies about PSII inhibition in higher plants and algae, but few studies have investigated its ecological impact on the microbial biofilms (Mølander and Blanck, 1992; Pesce et al., 2006). This compound has been used to control a wide variety of annual and perennial broadleaf and grassy weeds, and is used in vineyard husbandry. Due to its persistence in the natural environment (half-life from 56 to 231 days in soil), diuron has been found in streams running through agricultural areas: for example in 2005 in France, diuron was found in 34.6% of surface waters and its concentration in low quality sites ranged from 2.1 to 36 $\mu g\,L^{-1}$ (IFEN, 2008).

In small watersheds, the environmental exposure of stream organisms to agricultural pesticides increases very rapidly during rainfall events, and reaches relatively high concentrations (example: 14 $\mu g\,L^{-1}$ of diuron during the flood event of 27/07/06 in the Morcille river, France, Rabiet et al., 2008): floods typically last only a few hours, with a discharge that increases and then decreases very rapidly. Pesticide concentrations are usually nearly synchronous with discharges (Rabiet et al., 2007), leading to marked changes in exposure levels. Recent studies have investigated the effects of short and environmentally realistic pulsed toxicant exposures on survival, development, reproduction, latent mortality, and the different life stages of aquatic invertebrates (Cold and Forbes, 2004; Forbes and Cold, 2005; Reynaldi and Liess, 2005; Zhao and Newman, 2006). These studies reflect that increasing attention is being paid to pulsed-exposures resulting from episodic run off events after the application of agrochemicals, and to ecotoxicological questions about how to predict the lethal and sublethal consequences of such exposure on biological populations.

Most experimental studies of aquatic microbial communities have been done using chronically high concentrations of pesticides (see the reviews of Bérard and Pelte, 1999 and DeLorenzo et al., 2001), and only few have attempted to assess the effect of environmentally realistic concentrations of pesticides on microbenthic biofilms (Dorigo et al., 2004, 2007; Mølander and Blanck, 1992; Nyström et al., 2000). To our knowledge, only one study has made any attempt to determine the impact of flooding by testing short-term pulses of herbicides (atrazine) on autotrophic biofilms (Jurgensen and Hoagland, 1990) without having previously exposed the biofilms to chronic herbicide contamination. Nevertheless, in this study pulses lasted for 24 h, which is too long to mimic flood events in small watersheds, which generally last a few hours.

In interpreting the biological impacts of such changing exposure levels, complex and variable interactions between the chemical and the organisms must be taken into account (Guasch et al., 2003). For example, the bioavailability of pesticides to microbenthic biofilms should be determined, and this can be done by measuring the concentration of the pesticide within the biofilm matrix (Lawrence et al., 2001). The choice of biological endpoints is also a matter of concern, as the effect of a pesticide depends on the type of molecule involved (specific mode of action), and because, as a result of a cascading effect, various populations within the aquatic trophic web could be affected indirectly (Aubertot et al., 2005).

The purpose of this study was to investigate the ecotoxicological impact of the herbicide diuron on river biofilms (autotrophic communities, named as periphyton, and heterotrophic communities), and especially to study the sublethal and long-term effects of diuron pulses on an indoor microcosm mimicking different exposure scenarios resulting from flood events. The impact of diuron on microbenthic biofilm composition and function was observed. To simplify this first study, our lentic microcosms did not take the possible impacts of scouring on the biofilms into account.

The specific questions investigated by this study were: (i) What are the consequences of chronic diuron contamination on biofilms? (ii) What are the consequences of the various different acute diuron exposure scenarios on long-term responses of biofilms? (iii) What influence does previous chronic diuron contamination have on responses to subsequent acute exposures to diuron?

2. Material and methods

2.1. Sampling site

This study was carried out in a small river (the Morcille). The Morcille river is situated in the Beaujolais vineyard area (eastern France, latitude 46.150N; longitude 4.600E), which is a region with high wine-producing activity (70% of the 8 km^2 of the catchment area), which contributes to the observed gradient of pesticide contamination along the river (Gouy and Nivon, 2007; Rabiet et al., 2007). Diuron is one of the herbicides most often detected in this stream. Water and stones carrying biofilms were collected from the Morcille River at the upstream, unpolluted reference site known as St. Joseph (physical parameters ranged from 3 to 5 °C for temperature, 49 to 66 $\mu mol\,m^{-2}\,s^{-1}$ for irradiance and 0.20 to 0.33 $m\,s^{-1}$ for stream current). Sampling was performed during January and February 2007, before the pesticide application period. The acute diuron concentrations used in our microcosm study were similar to those at the polluted downstream site of Versauds in late spring or early summer (diuron is usually applied in April) (Rabiet et al., 2008). For experimental and analytical convenience, we used a chronic diuron concentration of 1 $\mu g\,L^{-1}$ (on average 1–10 times higher than the concentrations found at the polluted downstream site of the river Morcille during low water periods, which ranged between 0.15 and 1.4 $\mu g\,L^{-1}$ in 2006 (Rabiet et al., 2008), which was a realistic chronic concentration for low water periods, especially in the spring and early summer in small rivers in agricultural areas (Pesce et al., 2006).

2.2. Experimental schedule and long-term contamination

Two 35-L aquariums were filled with river water from the reference site, which had been filtered through a 500-μm mesh to remove most of the grazers. Two stones from the same site, which were coated with biofilms, were brought to the laboratory, and one was placed in the bottom of each aquarium to act as a natural biofilm inoculum in the experimental systems. Initially, the aquariums were connected to each other by a system of PVC pipes in order to ensure the homogeneity of the microbial communities in