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# Role of the hyporheic heterotrophic biofilm on transformation and toxicity of pesticides

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**Abstract –** The role of heterotrophic biofilm of water–sediment interface in detoxification processes was tested in abiotic and biotic conditions under laboratory conditions. Three toxicants, a herbicide (Diuron), a fungicide (Dimethomorph) and an insecticide (Chlorpyrifos-ethyl) have been tested in water percolating into columns reproducing hyporheic sediment. The detoxification processes were tested by comparing the water quality after 18 days of percolation with and without heterotrophic biofilm. Tested concentrations were 30 $\mu g.L^{-1}$ of Diuron diluted in 0.1% dimethyl sulfoxide (DMSO), 2 $\mu g.L^{-1}$ of Dimethomorph and 0.1 $\mu g.L^{-1}$ of Chlorpyrifos-ethyl. To characterise the detoxification efficiency of the system, we performed genotoxicity bioassays in amphibian larvae and rotifers and measured the respiration and denitrification of sediments. Although the presence of biofilm increased the production of *N*-(3,4 dichlorophenyl)-*N*-(methyl)-urea, a metabolite of diuron, the toxicity did not decrease irrespective of the bioassay. In the presence of biofilm, Dimethomorph concentrations decreased compared with abiotic conditions, from 2 $\mu g.L^{-1}$ to 0.4 $\mu g.L^{-1}$ after 18 days of percolation. For both Dimethomorph and Chlorpyrifos-ethyl additions, assessment of detoxification level by the biofilm depended on the test used: detoxification effect was found with amphibian larvae bioassay and no detoxification was observed with the rotifer test. Heterotrophic biofilm exerts a major influence in the biochemical transformation of contaminants such as pesticides, suggesting that the interface between running water and sediment plays a role in self-purification of stream reaches.

**Key words:** detoxification / hyporheic zone / diuron / dimethomorph / chlorpyriphos-ethyl

## Introduction

Water quality assessment is strongly related to the global dimension of aquatic ecosystems including the interactions between ecology, hydrology and geomorphology (Everard and Powell, 2002). Among the ecosystem functions relative to water quality, the self-purifying capacity of a river is defined as its ability to improving water quality and to induce a chemical change in the dynamic equilibrium of the system (Marmonier *et al.*, 2012). In the context of nutrient increase in surface waters, the study of this self-purifying capacity associated with nutrient retention in sediment is a relevant research domain. A focus on nitrogen and carbon uptake capacities of rivers leads to identification of river compartments that actively participate in nutrient transformation (Brugger *et al.*, 2001; Battin *et al.*, 2003; Peyrard *et al.*, 2011; Navel *et al.*, 2012). The hyporheic zone, a transition zone between groundwater and streams (Orghidan, 1959), is now recognised as a site of high biogeochemical activity (Pusch *et al.*, 1998) that participates in stream ecosystem functioning by changing water quality (Stanford and Ward, 1993; White, 1993; Storey *et al.*, 1999; Marmonier *et al.*, 2012). Stream nutrient retention depends on several physical conditions such as water residence time (*i.e.*, hydrological retention) (Lefebvre *et al.*, 2006), as well as on biological and chemical processes affecting nutrient transport and transformation (House *et al.*, 2001) and the interactions between these processes (Mermillod-Blondin *et al.*, 2003).

In stream self-purification is largely controlled by the microbial communities that represent a large part of the

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biomass that develop in the hyporheic zone and are, for the most part, responsible for respiration, nutrient cycling and organic matter transformations (Grimm and Fisher, 1984; Pusch and Schwoerbel, 1994; Findlay, 1995) such as degradation of dissolved and particulate organic (Brunke and Gonser, 1997; Schindler and Krabbenhoft, 1998; Sánchez Pérez *et al.*, 2003; Peyrard *et al.*, 2011). In this sediment, organic matter is oxidised in aerobic or reduced in anaerobic conditions through different metabolic pathways, such as aerobic mineralisation, denitrification, iron and manganese reduction, sulfato-reduction and methanogenesis (*e.g.* Hunter *et al.*, 1998; Baker *et al.*, 2000). If the hyporheic compartment of rivers is often regarded as a significant bio-reactor with the potential to transform and reduce organic pollution (Gavrilescu, 2005; Jekel and Gruenheid, 2005; Wyss *et al.*, 2006; Ifabiyi, 2008), but quantitative demonstration is still required for pesticides.

This observation leads to the framework hypotheses of the present study: if the hyporheic compartment is active as a bio-reactor at reducing natural dissolved and particulate matter loads, it may be possible that the same compartment performs transformation of some pesticides. Understanding these bioremediation processes requires one to closely integrate physical and biotic functioning of river systems at a variety of scales (Janauer, 2000; Sumpono *et al.*, 2003), and the present paper is an attempt to simulate the hyporeic condition in laboratory microcosms.

There is little information on the retention of pesticides in field or laboratory conditions. In field assessments, pesticide load is demonstrated to decrease for all major families of pesticides (s-triazines, substituted ureas and anilides) in water along river reaches or in reservoir (Devault *et al.*, 2009). In the field, concentration of pharmaceutical micropollutants decreased with depth in the hyporheic zone, but the reason for this reduction was not clear (Lewandowski *et al.*, 2011). In bank filtration studies, Gruenheid *et al.* (2005) demonstrated that redox conditions and travel time significantly influence DOC degradation kinetics and the efficiency of absorbable organic halogens and trace compound removal.

If this self-purification capacity of natural systems also affects more resistant pollutants such as pesticides, it becomes pertinent to test in the laboratory the biological factors that influence their degradation. In laboratory microcosms investigations, Jekel and Gruenheid (2005), Gruenheid *et al.* (2005) and Baumgarten *et al.* (2011) demonstrated that in bank filtration, as in long retention soil column the major factors that influence the degradation of bulk and trace organics are redox conditions and retention times. In laboratory columns degradation of the antibiotic sulfamethoxazole takes months (Baumgarten *et al.*, 2011) and led to the conclusion that it is essential to provide several weeks or even months of travel time in bank filtration to allow the degradation of this toxicant. The most efficient conditions to give evidence of this retention capacity must consider the physical, chemical and biological conditions of the natural that is to say microbial community of interstitial biofilm and reproduce it in artificial hyporheic sediment. Permanent water flow through the hyporheic zones explains why the biogeochemical processes in this transition zone are essential in mediating the water quality (Boulton *et al.*, 1998). The experimental design adopted for the microcosms series is fixed to maintain vertical water circulation through the column of macroporous sediment with interstitial water flow in the range of *in situ* condition.

The effects of the heterotrophic biofilm on retention capacity of the hyporheic sediment are tested. From the literature, we just begin to understand the influence of microbiological organisms in transformation of organic micropollutants (Bogaerts *et al.*, 2000; Bonnemoy *et al.*, 2001). The ability of microbial diversity to participate in the bioremediative capacity of river beds applied to organic micropollutants is neither widely known in rivers nor in the hyporheos (Gifford *et al.*, 2007). Therefore, there is a strong need for methodologies to estimate the hyporheic biodegradation of these pollutants that flow in the interstitial water (Williams *et al.*, 2007; Landmeyer *et al.*, 2010) with and without microbiological activity to give evidence of biofilm retention rate.

The objective of the work is to characterise the potential impact of microbial activity on water quality at the river subsurface–surface interface. Specifically, this paper focuses on (1) measuring the role of heterotrophic biofilm on retention and transformation of pesticides in water and (2) characterising the influence of this biofilm on potential toxicity of sediment water.

## Materials and methods

We performed experiments in sediment columns mimicking river hyporheos, with water recirculating through the columns. To test the role of biofilm on the transformation of pesticides, some of the columns were previously treated to allow for biofilm development, some were not.

### Experimental design

We set 24 Plexiglas columns (20 cm in height, 6.8 cm internal diameter), and filled them with sand and gravel in successive layers, 2 cm thick, of 4 size ranges ($L_1 = 0.5$–1 mm, $L_2 = 1$–2 mm, $L_3 = 2$–10 mm and $L_4 = 10$–20 mm). Each gravel and sand layer was sieved manually with corresponding mesh before being autoclaved (20 min at 121 °C). The total mass of sediment in each column was equal to $1000 \pm 50$ g. Mean porosity, measured as the difference in weight of five columns dried and filled with water, corresponded to $34 + 3\%$. A 300 µm filter was positioned at the exit and entrance of the microcosm to both retain sedimentary particles within the column and to avoid modifying the porosity. Each microcosm was connected by silicone tubing (internal diameter = 3.2 mm) to a high-density polyethylene tank containing 15 L of filtered water (90 microns mesh size) from the Garonne River (France),

collected some days before the beginning of the experiment. The water of different Plexiglas columns ($n = 24$) was put in circulation by peristaltic pumps (323Du Watson Marlow). The peristaltic pumps ensured a constant infiltration flow rate of 7–8 mL.min$^{-1}$ (Darcian velocity = 1.39–1.59 m.day$^{-1}$) similar to the *in situ* range of water flow in hyporheic sediments (Weng *et al.*, 2003; Peyrard *et al.*, 2008). Supplied water was aerated in tanks to maintain oxygen saturation. The global experimental device consisted of 24 microcosms with tanks and was installed in a dark room to avoid phototrophic biofilm development. Twelve sterile microcosms were set up without biofilm (biofilm is not developed during the experimentation in this condition) and 12 microcosms were set up with biofilm, with the three different toxicants being tested separately under two conditions. Room temperature was fixed at $15 \pm 0.5$ °C.

## Experimental sampling and analysis

The effects of biofilm were tested by comparison of two experimental conditions without biofilm (called abiotic condition) and with biofilm (called biotic condition) developed previously in the macroporous sediment.

Four replicates per condition were used to evaluate intra-condition variability compared with inter-condition variability. Sediment and water for the abiotic condition was autoclaved just before the beginning of water circulation to limit biofilm development in these microcosms. In the biotic columns, water circulation began two months before water circulation in abiotic columns.

## Experimental planning

For biotic microcosms, the experiment lasted 90 days. After ten days of water circulation for stabilising hydraulic functioning in microcosms, in each reservoir, the water was enriched with $KNO_3$ in order to reach a final concentration of $N\text{-}NO_3^-$ equal to 10 mg.L$^{-1}$ and with $CH_3COONa, 3H_2O$ in order to reach a final concentration of DOC equal to 30 mg.L$^{-1}$. Nutrients inputs were chosen to be high enough to prevent total consumption between two nutrient additions and allow constant biofilm development. Nutrient injections were added once a week throughout the experiment to adjust the same final concentrations. To limit biofilm development under the abiotic condition, these microcosms were started only one week before addition of pesticides.

Tested concentrations for pesticides were 30 µg.L$^{-1}$ of Diuron (CAS number: 330-54-1; log Kow = 2.78) diluted in 0.1% DMSO, 2 µg.L$^{-1}$ of Dimethomorph (CAS number = 110488-70-5, log Kow Isomère E = 2.63) and 0.1 µg.L$^{-1}$ of Chlorpyrifos-ethyl (CAS number 2921-88-2 and log Kow = 4.96). Diuron is solid at ambient temperature (melting point = 160 °C), hence we dissolved it in DMSO 0.1% before injection.

## Biomass measurement

The weight of the interstitial biofilm was determined at the end of the experiment by ash free dry measurements. Two samples of sediment (50 g) collected at the top and bottom of each column were dried in an incubator at 105 °C for 48 h and then burned-off at 500 °C for 5 h. Averaged values between top and bottom samples of each microcosm were used.

## Physico-chemical analyses

Microcosms were kept recirculating for one month for biofilm to reach equilibrium. Afterwards, water was analysed weekly during the 18 days of the experiment. Dissolved oxygen was measured at the outlet of the columns and additional water samples were taken from the reservoir containing the recirculated water to measure nitrate, nitrite, ammonium and dissolved organic carbon. Nitrate, nitrite and ammonium were measured using a high performance ion chromatographic analyser (DIONEX, DX500 and DX120). Water samples were filtered through cellulose acetate membranes (25 mm diameter, 0.2 µm pore size and VWR). For dissolved $O_2$ measurements, a measuring chamber containing an electrode WTW CellOx 325 (pre-calibrated) was incorporated into water circulation at the outlet of the column. For DOC measurements, water samples were filtered through Whatman GF/F glass-fiber filters (0.7 µm pore size, 25 mm diameter) pre-combusted at 500 °C for 4 h. Filtrates were acidified with concentrated hydrochloric acid (6N) until pH < 2 (10 µL HCl per mL of filtrate) and kept in 8 mL glass tubes in the refrigerator pre-combusted at 500 °C. DOC was measured using a Total Organic Carbon Analyzer (Shimadzu TOC-5000A).

Dimethomorph, Chlorpyriphos-ethyl, Diuron and two of its main metabolites (3-(3,4-dichlorophenyl)-1-methyl urea (DCPMU) and 3,4-dichloroaniline (DCA)) were measured in water by ESI-LC–MS/MS (API 4000, Applied Biosystems) at the end of the experiment, 18 days after injection. Detection limit is 0.5 µg.L$^{-1}$ for Dimethomorph, 0.08 µg.L$^{-1}$ for Chlorpyriphos-ethyl, and 0.01 µg. L$^{-1}$ for Diuron and its main metabolites.

## Biological tests

Ecotoxicological investigations were performed using the standardised (ISO, 2006) amphibian micronucleus test (MNT) on *Xenopus laevis* larvae to assess the potential detoxification of the three toxicants by the biofilm. The MNT allows the evaluation of potential genotoxicity of pure substances or mixed substances of the exposure media (Mouchet and Gauthier, 2013). The sensitivity and reliability of the MNT applied to amphibians as biomarker, to detect chromosomal and/or genomic mutations makes it a sensitive method to analyse the potential cytogenetic damage caused by various contaminants.

This method has been standardised on *Xenopus laevis*, in French (AFNOR, 2000) and International (ISO, 2006) recommendations. It consists of measuring the number of erythrocytes with micronucleated cells in the circulating blood of *Xenopus laevis* larvae. Larvae were obtained after *in vivo* fertilisation and maintained for about three weeks in aquariums at 20 °C ± 2 °C until the appropriate developmental stage (*i.e.* st. 50) (Nieuwkoop and Faber, 1956). The MNT was conducted as described in the ISO guideline 21427-1 on water samples from various conditions to be tested. Larvae were exposed for 12 days at 22 °C ± 2 °C under normal photoperiod in groups of 15 (100 mL per larva) in 5-L glass flasks containing 1.5 L of water, including the negative and positive controls. The negative control (NC) was the reconstituted water alone (294 mg.L$^{-1}$ $CaCl_2{\cdot}2H_2O$, 123.25 mg.L$^{-1}$ $MgSO_4{\cdot}7H_2O$, 64.75 mg.L$^{-1}$ $NaHCO_3$, 5.75 mg.L$^{-1}$ KCl). The positive control (PC) was cyclophosphamide monohydrate (a well-known mutagenic agent) added to the reconstituted water at 20 mg.L$^{-1}$. The experiments were conducted in semi-static conditions over the 12 days of exposure. Media were renewed daily. After exposure, the number of micronucleated cells per thousand present in a blood smear sampled by cardiac puncture of each animal were scored under a photonic microscope.

Rotifer test was made according to the French AFNOR norm (NF T 90-377 December 2000). Female *Brachionus calyciflorus* (Toxkit Cysts Microtests Inc.) reproduction is measured 48 h after exposure to the pollutant. Before exposure females were isolated in a box with a nutritional supply (*Chlorella vulgaris*). Reproduction rate was calculated on the basis of the number of eggs produced during exposure. Data were expressed in CE 50 (inhibition of 50% of the reproduction rate) and calculated with RegTox code (http://eric.vindimian.9online.fr).

### Micro-organisms activities

Aerobic respiration and denitrification were measured at the end of the experiment following the slurry technique (Furutani *et al.*, 1984). About 10 g of wet mixed sediment collected both from the top and the bottom layers was placed in 150 mL flasks supplemented with a feeding solution, in order to optimise microbial activity. For the measurement of $CO_2$ production (respiration), incubation was realised under aerobiosis with 5 mL of a feeding solution of glucose (7.5 g.L$^{-1}$) and glutamic acid (7.3 g.L$^{-1}$). For the measurements of $N_2O$ production (denitrification), the incubation was performed under anaerobic conditions with an $N_2$ atmosphere. The feeding solution was a mixture of 5 mL of $KNO_3$ (2.2 g.L$^{-1}$), glucose (7.5 g.L$^{-1}$) and glutamic acid (7.3 g.L$^{-1}$). The incubation flasks were then filled with helium atmosphere. The sequence was repeated three times and internal pressure was adjusted to a pressure of 1 atmosphere. After removal of 15 mL of He from the incubation flasks, 15 mL of $C_2H_2$ (10% v/v final volume) was added to inhibit $N_2O$ reductase. All incubations were carried out at 20 °C, in the dark and gently shaken. At $t = 3$ h and $t = 6$ h, gases (C–$CO_2$ and N–$NO_2$) were measured by gas chromatography model on an MTI 200 microcatharometer and dry weights of sediment were determined after drying at 60 °C to express the results as µg of C or N per hour and per gram of dry weight sediment (µg.h$^{-1}$.$g^{-1}_{sed\ DW}$).

In order to understand the effects of DMSO addition in the diuron experiment in a better manner, complementary experiments were run to compare the effect on microbial activities (glucosidase and amino-peptidase, denitrification rates) and respiration of DMSO alone at 0.1%, Diuron alone at 30 µg.L$^{-1}$, a mixture of Diuron 30 µg.L$^{-1}$ and DMSO 0.1%, a control without toxicant.

### Statistics

Differences in bioassay were considered significant at $P < 0.05$. All statistical calculations, including ANOVA, were performed using a Minitab computer package (Minitab Inc., USA).

Since the micronuclei were not distributed normally, median values and confidence intervals were calculated instead of means, using the statistical method described by McGill *et al.*, (1978). The results of each test were (i) considered to be negative for genotoxicity if the confidence intervals determined from the negative control and the test condition shared common values (ii) considered to be positive if the confidence intervals determined for the negative control and the test condition did not share common values, and the median value of the test condition was twice that of the negative control.

## Results

Microbial biomass averaged 0.22 ± 0.05 g per microcosm in the abiotic condition and 0.24 ± 0.03 g per microcosms in the biofilm condition. The occurrence of a significant AFDM in both the conditions is due to detritus residues fraction included in the sediment that were collected in natural river bed before sterilisation. The slight difference between conditions is interpreted by the occurrence of a growing biofilm in the sediment column that reach 0.025 mg of dried biomass.g$^{-1}$ of sediment in some of the columns at the end of the experiment.

Oxygen concentration (mean ± SD) at the outlet of the microscosms was respectively in conditions without and with biofilm 3.40 ± 1.02 and 1.97 ± 0.43 for microcosms with diuron and DMSO; 4.86 ± 2.3 and 3.23 ± 1.62 with Chlorpyrifos-ethyl, and 5.19 ± 2.3 and 4.18 ± 1.14 with Dimethomorph. Oxygen consumption with diuron was estimated at 72.97 ± 17.47 and 89.55 ± 8.55 mg.d$^{-1}$ in each experimental unit (15 l) without and with biofilm respectively, with Chlorpyrifos-ethyl 55.37 ± 27.48 and 74.04 ± 35.00, and with Dimethomorph 53.20 ± 25.89 and 62.88 ± 30.10. These changes in consumption are

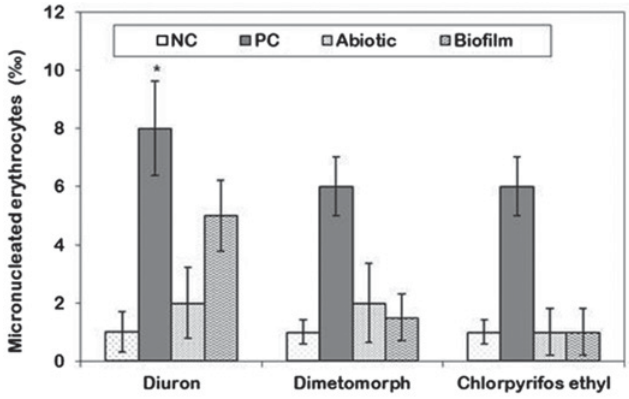


**Fig. 1.** Results of micronucleus test (genotoxicity) for Diuron, Dimethomorph and Chlorpyrifos-ethyl additions. NC, negative control; PC, positive control. Error bars are confidence interval (95%). *Indicates genotoxicity compared with NC.

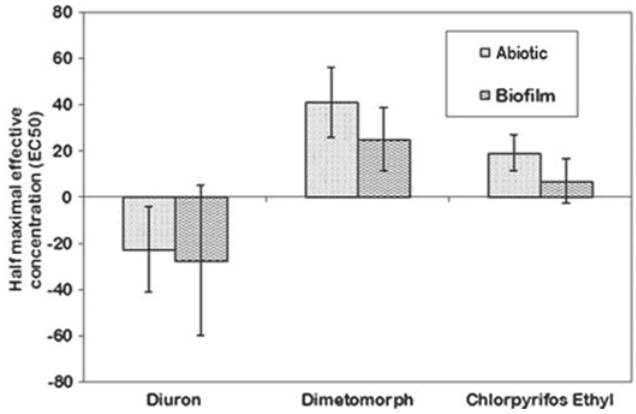


**Fig. 2.** Rotifer test for Diuron, Chlorpyriphos-ethyl and Dimethomorph additions measured in water 18 days before addition.

summarised by an average increase of 20% of aerobic respiration with interstitial biofilm in the sediment units.

The pesticides did not affect the consumption of carbon and nitrogen, which ranged from 1.91 to 2.17 mg $N.day^{-1}$ and from 30.7 to 31.2 mg $C.day^{-1}$ in the biofilm experiment.

One day after addition, the Diuron concentrations were $25.0 \pm 2.7$ $\mu g.L^{-1}$ (mean $\pm$ SD). Eighteen days after addition in abiotic and biofilm conditions, Diuron concentrations were $21.8 \pm 1.7$ $\mu g.L^{-1}$ and $22.0 \pm 1.3$ $\mu g.L^{-1}$, respectively, and DCPMU concentrations were $0.06 \pm 0.01$ $\mu g.L^{-1}$in the abiotic and $0.54 \pm 0.15$ $\mu g.L^{-1}$ in the biofilm conditions. The 3–4 DCA molecules were under detection limit in all cases. Four days after addition, the Dimethomorph concentration was $1.44 \pm 0.06$ $\mu g.L^{-1}$in both conditions. After 18 days, Dimethomorph concentrations were $1.17 \pm 0.06$ $\mu g.L^{-1}$ in the abiotic conditions and below detection limit (0.5 $\mu g.L^{-1}$) in the biofilm condition. Chlorpyrifos-ethyl concentrations were found under the detection limit of the apparatus (0.08 $\mu g.L^{-1}$) during the whole period after addition. These concentrations lower than 0.08 $\mu g.L^{-1}$ attest to a Chlorpyrifos-ethyl degradation at least equal to 20% of the initial loads in all conditions.

The genotoxic response obtained in larvae exposed to PC (positive control), compared with negative control (NC), validates the *Xenopus laevis* larvae strain used in this experiment. Larvae exposed to effluents flowing through abiotic conditions did not show any genotoxic response compared with negative control, whatever the nature of the toxicant used. Larvae of *Xenopus laevis* showed no genotoxic response when subject to either Dimethomorph or Chlorpyriphos-ethyl, but showed significant response when subject to Diuron/DMSO contamination. This response was stronger in the "biofilm" treatment (Fig. 1).

Rotifer tests performed with the Diuron/DMSO showed that the presence of biofilm did not affect toxicity of the effluent, whereas it decreased significantly the toxicity of Dimethomorph and Chlorpyriphos-ethyl (Fig. 2). About microbial processes, no significant differences were observed between biotic and abiotic conditions for potential respiration for Dimethomorph (ANOVA $P < 0.001$; Fig. 3a) and for denitrification for Dimethomorph and Chlorpyriphos-ethyl (Fig. 3b). The potential respiration and potential denitrification were less affected in the Diuron/DMSO treatment for both conditions than with the two other pesticides.

## Discussion

### The heterotrophic biofilm influence

The biofilm effect on water quality was measured with direct measurements of toxic concentrations and indirect tests of ecotoxicity. Toxic concentrations gave evidence of initial toxic molecules decreased or metabolite production that is interpreted as enhanced transformation under biofilm influences in this experiment. The Diuron concentration did not significantly change with time and conditions but the first metabolite DCPMU significantly increased with biofilm effects. Under the same biofilm influence Dimethomorph concentration dropped by 60% in 18 days. These results obtained over a short period experiment suggest increased effects of biofilm over a longer period of time. It also suggests that biofilm could have an important detoxifying effect in natural conditions.

Performed ecotoxicological tests attest to these changes in water quality in the displayed experimental conditions. Although responses vary greatly depending on the test performed, when a change exists in water quality under biofilm activity with positive or negative effects on water toxicity, this change attests to a chemical transformation in the toxic composition of water. The increase or decrease of water toxicity under biofilm effects is codified in Table 1. These results were obtained in the final water of the recycled reservoir that is a mixture of initial molecule and its metabolites. The difference between ecotoxicological test obtained in both conditions (with and without biofilm) is interpreted as a consequence of initial toxicant

**Table 1.** Pesticide concentrations used in different additions and comparison of responses of different tests. For each test the toxicity measured with biofilm is compared with toxicity in condition without biofilm. Codes are " − " for increased toxic effect *e.g.* lower water quality with biofilm; " + " for decreased toxic effect, *e.g.* better water quality with biofilm; "O" when no biofilm effects are measured.

| Family<br>Name | Herbicide<br>Diuron | Fungicide<br>Dimethomorph | Insecticide<br>Chlorpyrifos-ethyl |
|---|---|---|---|
| Initial concentration ($\mu g.L^{-1}$) | 30 | 2 | 0,1 |
| Tests | | | |
| Genotoxicity *Xenopus* | + | O | O |
| Rotifer test | O | +[a] | +[a] |
| Aerobic respiration | O | O | − |
| Denitrification | O | O | O |

[a]Indicate a significant difference with admitted error = 0.05% with ANOVA for rotifer and microbial activities and McGill method (McGill *et al.*, 1978) for genotoxicity tests. + , increased toxic effect; − , decreased toxic effect; O, no effect.

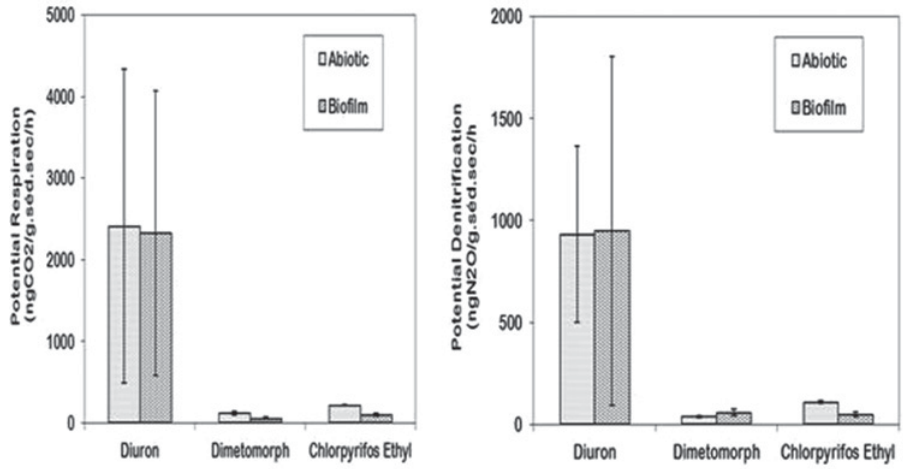


**Fig. 3.** Respiration (a) and denitrification (b) rates (mean ± SE, $n = 4$–6) in biofilm condition ($n = 4$) 18 days after Diuron, Dimethomorph and Chlorpyrifos-ethyl addition, expressed in microgram per hour and per gram of dry weight sediment ($\mu g.h^{-1}.g_{sed\ DW^{-1}}$).

concentration and metabolites creation with different levels of toxicity and concentrations.

The occurrence of different metabolic pathways explains how the same compartment may be efficient at transforming such complex molecules as organic contaminants, and in this way reducing initial pesticides charges. The degradation of Diuron exhibits alternative steps of aerobic, anaerobic and toxic conditions. The efficiency of water purification after filtration through a sediment column with biofilm developed in macroporous media may be explained by the occurrence of different redox conditions along the columns. Aerobic respiration enhanced with 20% in the biofilm condition demonstrates a more developed aerobic community activity. Water flow and recirculation through these different porous media should permit contact with micro-organisms that perform different degradation steps with various redox potential of a single toxicant. Concerning redox conditions, Baumgarten *et al.* (2011) outline that bank filtration under aerobic conditions should be suitable to remove SMX to levels below 0.12 $mg.L^{-1}$. However, previous field studies suggested that organic pollutants were removed more effectively under anoxic (97%) and anaerobic conditions than under aerobic (64%) conditions (Schmidt *et al.*, 2004; Jekel and Gruenheid, 2005). The present study suggests that all the required conditions possibly exist in adjacent spots of sediment in a slow filtration column to enable the degradation of organic micropollutants.

## Metabolites arrival

The effects of pesticide transformation due to biofilm activity are not clear, since it includes the production of a mixture of metabolites with various toxicity levels. Ecotoxicological investigations showed different effects depending on the bioassay and the contaminant. Using amphibian larvae, no genotoxic effect was found in abiotic conditions whenever the toxicant and genotoxicity was only highlighted under biofilm influence in Diuron treatment. These results may be related to the measured DCPMU concentration in biotic condition than in abiotic

one. According to the microtox test in the literature, the metabolite *N*-(3,4-dichlorophenyl-*N*-methylurea and *N*-3,4-dichlorophenylurea presented three times higher toxicity that that of diuron (Bogaerts *et al.*, 2000; Bonnemoy *et al.*, 2001). The observed genotoxicity in amphibian larvae would then come from DCPMU, rather than Diuron/DMSO. To our knowledge, there are no published genotoxicity studies in the literature concerning amphibian larvae exposed to this metabolite, except the works of Bogaerts *et al.* (2000) who measured diuron fungal degradation in soil. Schuytema and Nebeker (1998) reported LC50 (The concentration of a substance estimated to be lethal to 50% of the tested organisms) of 15.2, 12.7, 22.2 and 11.3 $mg.L^{-1}$ of Diuron after chronic exposure of 12 or 14 days by amphibian embryos and larvae (Pacific treefrog, bullfrog, red-legged frog and Xenopus).

The affect of DMSO shows contrasted effects: 0.1% of DMSO is able to change bacterial activity and associated enzymatic process. It is assumed to be possible to affect global microbial metabolism of the interstitial biofilm. Ecological test (not included in the paper) at 48 h demonstrated no effect of DMSO, alone or mixed with Diuron. Ninety-six hours of expositions (standard period for algae) led to different conclusions with the evidence of a slight DMSO effect of our experimental concentration (10% decrease of algae growing rate). However, if the literature (Gordeliy *et al.*, 1998; Griebler and Slezak, 2001) and our tests converge to demonstrate potentiality of DMSO effects, and the change in toxicity of a DMSO–diuron mixture, in the present paper it is the change in the toxicity of DMSO + diuron that is discussed. In the present paper, it is stated that it is the change in the toxicity of the mixture DMSO 0.1% and diuron that is followed in our experimental condition. If it is demonstrated that there exists a change in the toxicity of water containing the same initial mixture of diuron + DMSO in our experimental condition, and after percolation with and without biofilm effect, the demonstration of biofilm activity in biotransformation of the initial mixture is possible.

In the case of Chlorpyrifos-ethyl, one of its primary metabolites, 3,5,6- trichloro-2-pyridinol (TCP), is less toxic than the parent compound towards aquatic organisms (Giesy *et al.*, 1999). The combination of a minimum of 20% depletion of initial toxic concentrations and the lower toxicity attested by the rotifer test with this molecule, suggest that biofilm microbial degradation is also active on this type of molecule. The decrease in concentration under the detection limit in all experimental conditions also demonstrates that the sediment matrix should participate in this purification with adsorption processes.

To our knowledge, no information is available concerning metabolites of Dimethomorph and their relative toxicity. However, according to rotifer tests, it is emphasised that microbial activity may also be able to influence this type of toxicant, with metabolite production at lower toxicity than the primary molecule.

## Conclusion

From direct and indirect assessment of the water quality in this short term experiment, it is demonstrated that the presence of a heterotrophic biofilm in a slow filtration column enhances the transformation of the three studied organic micropollutants.

The affects may be enlarged to river conditions with longer residence times due to spiralling functioning and larger spatial conditions with the hyporheic compartment of a natural reach. Since the biomass in sediments with biofilm colonisation influenced the extent of toxic transformation in our laboratory column experiments, further measurements of this transformation are essential to confirm this process in *in situ* condition. These previous results also suggest that further research on bioremediation of pesticide-contaminated water should for better results include the contribution of heterotrophic biofilm as a biological factor largely involved in degradability. The developed ecological tests applied on residual water suggest that the toxicity of water depends on metabolites production as well as initial molecule concentrations and may vary a lot depending on the initial toxic molecule. This observation should be an encouragement for a more precise assessment of metabolites concentrations in water, through experiments, in order to emphasise in a better manner toxicity variations. Also, this work provides new information to extend self-purification capacity by the benthic boundary layer to a more refractory molecule such as micro-organic toxicants in river waters.

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