Sensitivity of freshwater periphytic diatoms to agricultural herbicides

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A B S T R A C T

The biomonitoring of pesticide pollution in streams and rivers using algae such as diatoms remains difficult. The responses of diatom communities to toxic stress in stream water are disturbed by the variations of environmental parameters. In this study, periphytic algae collected *in situ* were exposed under controlled conditions to two major herbicides used in French agriculture (isoproturon and s-metolachlor). Three exposure regimes were tested: 5 and 30 $\mu g\,L^{-1}$ for 6 days and 30 $\mu g\,L^{-1}$ for 3 days followed by a recovery period of 3 days. The algal biomasses were assessed from pigment concentrations (chlorophyll a and c) and from live cell density. The highest concentration (30 $\mu g\,L^{-1}$) of isoproturon inhibited the biomass increase statistically significantly. In periphyton exposed to 5 and 30 $\mu g\,L^{-1}$ of s-metolachlor, chlorophyll c concentration and live cell density were also statistically significantly lower than in the control. Periphyton left to recover after reduced exposure duration (3 days) showed higher growth rates after treatment with s-metolachlor than with isoproturon. Taxonomic identifications showed that species like *Melosira varians*, *Nitzschia dissipata* and *Cocconeis placentula* were not affected by the herbicide exposure. Other species like *Eolimna minima* and *Navicula reichardtiana* were more sensitive. Studying diatoms according to their trophic mode showed that facultative heterotroph species were statistically significantly favoured by isoproturon exposure at the highest concentration. Results obtained with s-metolachlor exposure showed a disturbance of cell multiplication rather than that of photosynthesis. These results suggest that photosynthesis inhibitors like isoproturon favour species able to survive when the autotroph mode is inhibited.

1. Introduction

Benthic diatoms are efficient bio-indicators of water pollution (Stevenson and Pan, 1999). Based on the evolution of freshwater diatom community structure, several monitoring tools like the diatom biological index (DBI), the Rott saprobic index (ROT), the trophic diatom indexes (TDI), the Sladecek index (SLA), the eutrophication pollution index (EPI) and the European index (CEE) have been developed to assess the water quality of rivers (Lenoir and Coste, 1995; Prygiel and Coste, 1996; Potapova and Charles, 2007). The ROT and the SLA are relevant to detect organic pollution whereas the DBI, the TDI, the EPI and the CEE are more sensitive to trophic pollution. These indexes do not have the same degree of integration and monitor the effects of different stresses (Rimet et al., 2005). In the context of the Water Framework Directive (WFD), no single index appears more relevant than the others to assess river water quality. Moreover, these indexes, and especially the DBI, are not well suited for detecting the effects of water pollution by pesticides on diatom communities (Dorigo et al., 2004). Indeed, this last tool takes into account the sensitivity of 649 taxa to 7 standard physico-chemical parameters: pH, conductivity, percent saturation oxygen, BOD and nutrient concentration (NH_4^+, NO_3^-, and PO_4^{3-}). Other tools like PICT (pollution-induced community tolerance) or the PAM (pulse-amplitude modulated) have been proposed to measure the effects of pesticide pollution on diatoms (Molander and Blanck, 1992; Berard and Benninghoff, 2001; Dorigo and Leboulanger, 2001; Blanck, 2002; Schreibera et al., 2007; McClellan et al., 2008; Schmitt-Jansen and Altenburger, 2008). These tools have been tested for a restricted number of pesticides, especially PS II inhibitors. Moreover, environmental parameters, like light exposure and nutrient concentration may interfere with the response of these tools to pesticide exposure (Guasch et al., 1997, 1998; Berard and Benninghoff, 2001).

To study the effects of herbicide water pollution on diatoms, a field survey (18 sites) was carried out in rivers located in agricultural watersheds (intensive crops). Reduced growth of the algal biomass in agricultural sites was observed during the main periods of herbicide treatments (in May for the spring crops) (Debenest,

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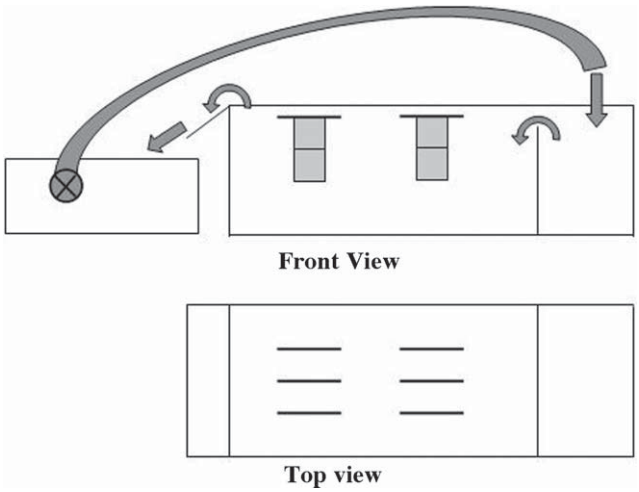

Fig. 1. Schema of the microcosm with a main tank containing the glass slides and a secondary tank with pump.

2007). Nevertheless, no relation was established between these variations and the herbicide concentrations measured in stream water. The isolated monitoring of the pesticides in water except during flooding does not make it possible to properly estimate the real exposure of diatoms to pesticides. Indeed, numerous authors have reported that stream pollution by pesticides increases during high water (Spalding and Snow, 1989; Schulz, 2001; Ferenczi et al., 2002; Neumann et al., 2003). In our study, a surface water sample taken during a flood period was found to contain very high total concentrations of pesticides (14.4 $\mu g\,L^{-1}$). In addition, the high variability of environmental parameters, especially light exposure, between the sites did not allow comparison of growth results. This parameter is known to influence the response of diatoms to pesticide exposure (Guasch et al., 1998; Guasch and Sabater, 1998).

To study the impact of pesticides on algae, on diatom biomass growth and on diatom community species structure, an experiment was carried out under controlled conditions. Freshwater periphytic diatoms fixed on glass slides and immersed for 2 weeks in a field survey site with good water quality, were transferred to microcosms and exposed to two agricultural herbicides (isoproturon and s-metolachlor) simultaneously used for the major crops of the study area (wheat and irrigated maize). We chose agricultural formulations which are more toxic for plants than the active substance alone according to Dimitrov et al. (2006).

2. Materials and methods

2.1. Microcosm system

The microcosm systems were composed of a main glass tank (650 mm × 270 mm × 210 mm *l*/*w*/*h*) and a secondary glass tank (50 mm × 270 mm × 210 mm *l*/*w*/*h*) (Fig. 1). Each microcosm was filled with 51.5 L of stream water in order to avoid any stress for benthic diatoms. The water fell from the main tank into the secondary tank by overflowing. A pump (NJ 2300, Aquarium Systems-NEWA, Loreggia, Italy) immersed in the secondary tank sent the water back to the main tank to give continuous circulation of water in the microcosm. To limit disturbance of uniform conditions by the flow of water into the aquarium, the slides were separated from the inlet by a baffle which reduced turbulence in the main tank. The pump was set to provide an overall water velocity of 2.1 ± 0.1 cm/s which favoured the growth of periphytic diatoms (Biggs, 1990; Watanabe et al., 1998; Othoniel, 2006). The microcosms were placed in a temperature-regulated room (18.8 °C ± 0.7). Each microcosm was lit for algal growth by eight neon tubes (Biolux 18W, Osram GmbH, Munich, Germany) positioned 40 cm above each system and operated by timer switches. The experimental system operated with 14 h light cycles and a light intensity of 108 $\mu mol\,s^{-1}\,m^{-2} \pm 9.8$.

2.2. Freshwater periphytic diatom collection

Following Hoagland et al. (1982), plastic racks with glass slides (70/60 mm) were immersed (flow velocity = 2.5–4.0 $cm\,s^{-1}$, light intensity = 80–90 $\mu mol\,s^{-1}\,m^{-2}$ at 12 h a.m. and depth of sampling = 30–40 cm below the surface) for 2 weeks (January and February, 2007) in a site on the river Save (Southwest France). This site was chosen due to its low concentrations of herbicides (≈0.2 $\mu g\,L^{-1}$) and nutrients (5.53 $mg\,L^{-1}$ NO_3, 0.08 $mg\,L^{-1}$ PO_4 and 3 $mg\,L^{-1}$ Si) monitored during the field survey (Debenest, 2007). At the end of the immersion, the racks were transferred to the laboratory in cool boxes containing stream water. Water used (100 L) for the experiment was sampled in the field.

2.3. Growth conditions

Nutrients were introduced in the stream water to reach trophic conditions (25 $mg\,L^{-1}$ of NO_3, 1.3 $mg\,L^{-1}$ of PO_4 and 9 $mg\,L^{-1}$ of Si) typical of those in rivers affected by agricultural pollution. Water was not compensated for nutrient loss during the experiment since a previous experiment showed that nutrient concentrations were sufficient for diatom growth (16.9 $mg\,L^{-1}$ of NO_3, 1 $mg\,L^{-1}$ of PO_4 and 5 $mg\,L^{-1}$ of Si) at the end of the experiment (t = 6 days). Six stainless-steel racks each containing two glass slides with benthic diatoms were immersed in the main tank of each microcosm. The large size of the tanks used enabled replicates to be made: three parallel blocks of two racks were placed in each tank (Fig. 1). The glass slides were introduced vertically into the rack. After the transfer, benthic diatoms were allowed to grow for 6 days. Finally, four slides were sampled at each step of the experiment.

2.4. Herbicide contamination

Benthic diatoms were exposed to a single concentration of the agricultural herbicides isoproturon (commercial grade, 500 $g\,L^{-1}$, flowable concentrate) or s-metolachlor (commercial grade, 960 $g\,L^{-1}$, emulsifiable concentrate). For each herbicide, three exposures were carried out: 5 and 30 $\mu g\,L^{-1}$ for 6 days and 30 $\mu g\,L^{-1}$ for 3 days followed by a recovery period of 3 days. The last exposure was chosen to observe the ability of the diatom community to recover after an acute toxic stress.

2.5. Periphyton samples in microcosms

Four slides were taken at 0, 3 and 6 days. The periphyton was scraped off the slides and the solution obtained was filled up to 40 mL. From this solution, aliquots were taken: 8 mL were filtered (GF/C, Waterman, London, UK) for chlorophyll a and chlorophyll c measurement according to standardized method (French standard NF T90-117) and to the method of Strickland and Parsons (1968), and 12 mL were removed and fixed with formaldehyde [CAS No.: 50-00-0] for live cell density assessment and diatom species identification. Chlorophyll a was assessed to study the global algal biomass and chlorophyll c to study the biomass of some algae (Bacillariophyceae, Chrysophyceae, Raphidophyceae and Phaeophyceae). Chrysophyceae, Raphidophyceae and Phaeophyceae were not observed in the samples most likely because the glass slides were immersed in lotic conditions whereas these algae prefer lentic ecosystems (Van Den Hoek et al., 1995). Therefore, chlorophyll c level could be used to estimate diatom biomass.

2.6. Solid phase extraction and herbicide concentration assessment

Preconcentration of the water samples for analysis used solid phase extraction (SPE) with Oasis HLB cartridges (Waters Corporation, Milford, MA, USA). Prior to SPE, 200 mL water samples (pH adjusted to 7) were filtered using GF/F glass microfibre filters (0.7 μm pore size). Afterwards, 10 μL of a stock solution (acetonitrile) containing 100 ng μL^{-1} of atrazine d5 (surrogate), was added to the water samples, resulting in fortification level of 5 $\mu g\,L^{-1}$. SPE was conducted using a VisiPrep 12-port manifold (Supelco, France). The conditioning, extraction and rinsing steps were carried out under a 53.33 kPa vacuum. The SPE cartridges were successively washed with 10 mL of methanol, conditioned with 10 mL of HPLC-grade water, loaded with 200 mL water samples, then rinsed with 20 mL of HPLC-grade water and dried under a stream of nitrogen for 30 min. The samples were then eluted with 5 mL of methanol. The 5 mL extracts were blown dry under a gentle stream of nitrogen and dissolved in 1 mL of ethyl acetate prior to the GC–MS analysis. The final concentration of the surrogate was about 1 $mg\,L^{-1}$ after SPE extractions. Recovery was $100 \pm 12\%$ and $93 \pm 16\%$ for s-metolachlor and isoproturon, respectively. Herbicide concentrations were also measured using a Trace GC 2000 gas chromatograph (Thermo Electron Corporation, MA, USA) equipped with a Zebron ZB-5 (Phenomenex, Le Pecq, France) capillary column (60 m, 0.25 mm and 0.25 μm) and an AS 800 autosampler (Thermo Electron Corporation, MA, USA). The Trace GC 2000 gas chromatograph was coupled to a GCQ/Polaris ion trap mass spectrometer (Thermo Electron Corporation, MA, USA). The temperature of the transfer line was held at 280 °C and that of the source at 240 °C. Electron impact mass spectra were acquired at 70 eV. Quantitative analyses were performed in full scan mode from 100 to 350 amu. S-metolachlor was quantified with the $m/z = 162$ ion and isoproturon was analyzed as the 4-(isopropyl) phenyl isocyanate breakdown product and quantified with the $m/z = 146$ ion. The internal standard (atrazine d5) was quantified with the $m/z = 205$ ion. The total scan time was set to 0.68 s (6 microscans) and the max ion time was kept constant at 25 ms. A volume of 2 μL (samples dissolved in ethyl acetate) was injected on a splitless injector (270 °C, 138 kPa pressure pulse for 1.2 min). Helium was used as carrier gas at a constant flow rate of 1 mL min^{-1}. The temperature program was 40 °C for 1.2 min, then 15 °C min^{-1} up to 160 °C and 4 °C min^{-1} to 270 °C followed by a 3.3 min isotherm (total running time: 40 min).

2.7. Physical and chemical characterisation

Temperature, pH and conductivity were measured by probes from WTW GmbH (Weilheim, Germany) everyday from the beginning of the experiment. Water samples, taken during the laboratory experiment (0, 3 and 6 days), were used to determine the nitrate, phosphate and silica concentrations (French standards NF EN ISO 13395, NF T90-023 and T90-007).

2.8. Assessment of cell density and identification of diatom species

The growth of the diatom community was followed by live cell density assessment based on the score of cells with chloroplasts determined with a Nageotte counting chamber. Following the French standard for the determination of the Diatom Biological Index (DBI) (AFNOR NF T90-354) samples were treated with hydrogen peroxide [CAS No.: 7722-84-1] to digest organic cell content and subsequently centrifuged ($2571 \times g$ for 10 min) in demineralised water four times to eliminate the hydrogen peroxide. An aliquot of 200 μL was dried on a cover slip. Siliceous diatom frustules fixed on the cover slip were mounted on a microscope slide with Naphrax (Brunel Microscope Ltd.), a resin with a high refractive index (1.74) dissolved in toluene. The slides were scanned with a light microscope (Leica DMRD Microsystems GmbH, Wetzlar, D) at a magnification of 1000× and about 400 frustules were identified as recommended by French standard NF T90-354. On each slide, between 10 and 15 parallels were run to identify and count diatom cells.

Table 1
Concentrations of isoproturon and s-metolachlor measured in the microcosms at $t = 0$ and $t = 6$ days.

Time (days)	Concentration expected ($\mu g\,L^{-1}$)	Concentration measured ($\mu g\,L^{-1}$)	
		Isoproturon	s-metolachlor
0	5	6.3	5.1
	30	38.0	26.5
	30 (3 days exp.)	30.1	24.2
6	5	5	1.6
	30	20.7	6.2
	30 (3 days exp.)	<D.L.	1.8

D.L.: detection limit (0.1 $\mu g\,L^{-1}$).

2.9. Data analysis

A descriptive analysis calculated the mean and standard deviation of the datasets. Histograms were used to study the variations of total algal and diatom biomasses, abundances for the main species and for species groups according to their trophic mode. Biomass inhibition (I) values were calculated according to:

$$I = \frac{B_{control} - B_{treated}}{B_{control}}$$

"$B_{control}$" was the biomass concentration or cell density in the control and "$B_{treated}$" the biomass concentration or cell density in the treated microcosm at the same date.

The growth rate was calculated according to:

$$\mu = \frac{B_{t''}/B_{t'}}{t}$$

where $B_{t''}$ and $B_{t'}$ represent biomass concentrations or cell densities at the start (t') and at the end (t'') of the growth period considered, and t represents the duration of this period ($t = t'' - t'$) in hours. The growth rate is expressed per day. The results of the quantitative data were statistically analyzed with either ANOVA and Tukey test or Kruskal–Wallis analysis and Dunn test (Sigmastat, SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Variation of physico-chemical parameters

Assessment of the physico-chemical parameters showed alkaline conditions (pH 8.5 ± 0.1). The mean value of water conductivity was $367 \pm 48\ \mu S\,cm^{-1}$ and the mean water temperature was 20.1 ± 0.4 °C. The mean nutrient concentrations at $t = 6$ days were $25.4 \pm 6.5\ mg\,L^{-1}$ for nitrate, $1.0 \pm 0.1\ mg\,L^{-1}$ for phosphate and $6.1 \pm 2.2\ mg\,L^{-1}$ for silica in the microcosms regardless of the exposure conditions. Concentrations of herbicides measured in the microcosms are shown in Table 1. The values at $t = 0$ were close to the nominal concentrations. After 6 days in the microcosms, the herbicide concentrations decreased on the average by about 30% for isoproturon and 70% for s-metolachlor.

3.2. Variation of algal and diatom biomasses

The results of chlorophyll a, chlorophyll c and density of live diatom cells (Fig. 2a) showed a clear inhibition of biomass growth

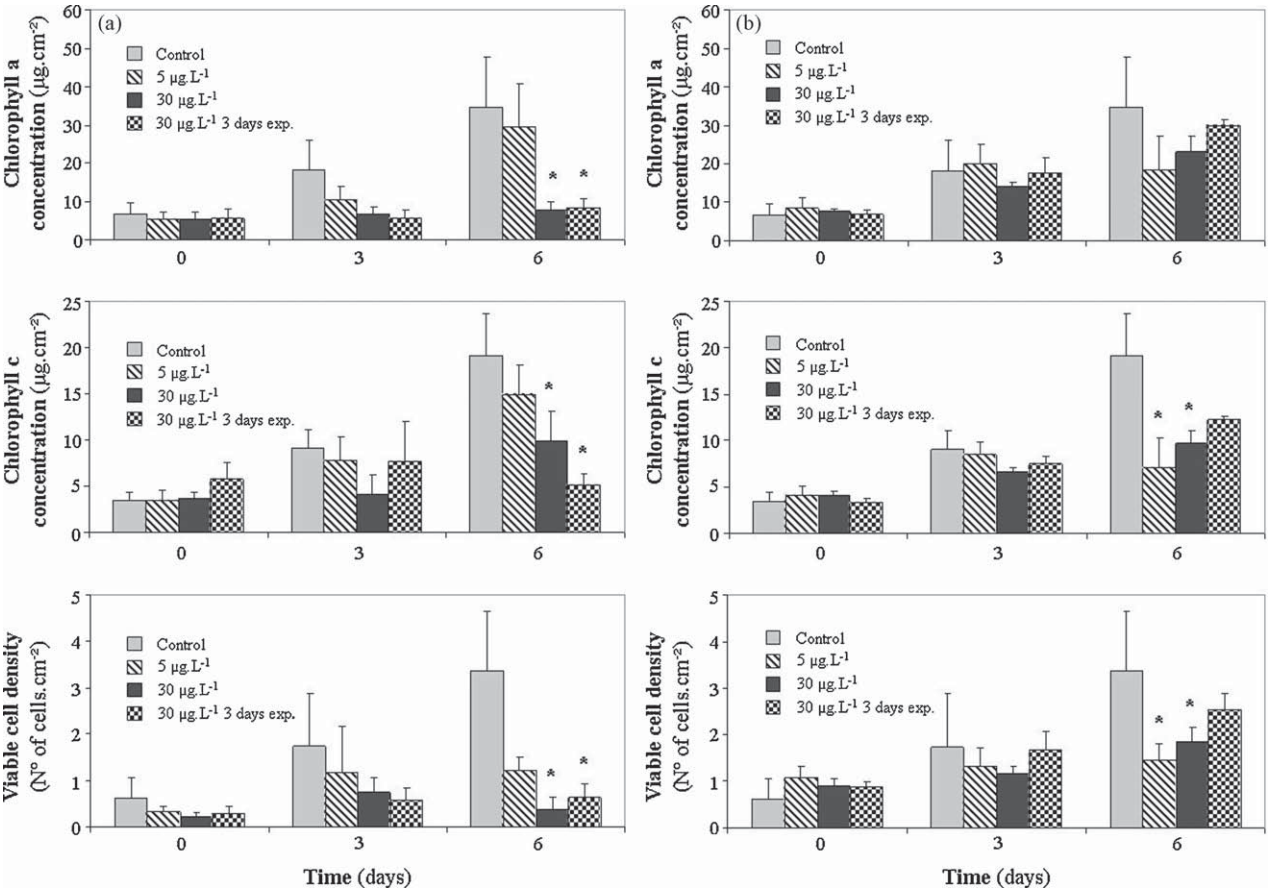

Fig. 2. Histograms of algal biomasses (chlorophyll a and c) and live diatom cell density from periphytons exposed to (a) isoproturon and (b) s-metolachlor (*statistical difference with the control; ANOVA followed by Tukey test, $P < 0.05$, for algal biomasses and Kruskal–Wallis analysis and Dunn test, $P < 0.05$, for cell density; $N = 4$).

in microcosms treated with isoproturon. Statistical differences at $t = 6$ days were observed between the control and the highest isoproturon concentrations (30 µg L^{-1}) ($P < 0.05$). Reduced growth rates, calculated between $t = 0$ and $t = 6$ days, were noted for these parameters ($\mu = 0.25$ for chlorophyll a, $\mu = 0.44$ for chlorophyll c and $\mu = 0.26$ for cell density) in the microcosms treated with this concentration in comparison with the control ($\mu = 1.09$, $\mu = 1.16$ and $\mu = 1.7$, respectively). The inhibition values were remarkable for chlorophyll a (78%) and for cell density (89%) while chlorophyll c was less affected (49%). No significant effects were observed at 5 µg L^{-1} even if the calculated growth rate was low ($\mu = 0.53$ versus $\mu = 1.7$ for the control) and marked inhibition (64%) was noted for cell density at this concentration. Periphyton exposed to 5 and 30 µg L^{-1} s-metolachlor showed reduced values for chlorophyll c and cell density (Fig. 2b) ($P < 0.05$). The growth rates in the periphyton exposed to these two concentrations were lower ($\mu = 0.31$ and $\mu = 0.4$, respectively, for chlorophyll c; $\mu = 0.23$ and $\mu = 0.35$, respectively, for cell density) than in the control. Close growth inhibition values were found for these two parameters (63% and 49% for 5 µg L^{-1} and 30 µg L^{-1}, respectively, for chlorophyll c and 57% and 45% for 5 µg L^{-1} and 30 µg L^{-1}, respectively, for cell density). Differences to the control were not significant for chlorophyll a even though reduced growth rates ($\mu = 0.39$ and $\mu = 0.49$, respectively) and growth inhibition (76% and 48%, respectively) tended to be observed at these concentrations (5 and 30 µg L^{-1}).

In the periphyton communities allowed to recover after 3-d exposure to 30 µg L^{-1}, growth did not resume in the microcosms treated with isoproturon. The difference to the control was statistically significant ($P < 0.05$). In contrast, the periphytons exposed to s-metolachlor for 3 days had higher biomass values than the periphytons treated with this herbicide for 6 days. The growth rates calculated between 3 days (end of the exposure) and 6 days (end of the experiment; 3-d exposure + 3-d recovery) had higher values in the microcosms treated with s-metolachlor ($\mu = 0.57$ for chlorophyll a, $\mu = 0.54$ for chlorophyll c and $\mu = 0.51$ for cell density) than those treated with isoproturon ($\mu = 0.48$ for chlorophyll a, $\mu = 0$ for chlorophyll c and $\mu = 0.37$ for cell density).

3.3. Changes in species community structure

Between the beginning of the exposure ($t = 0$ days) and the end of the experiment ($t = 6$ days) the community sampled in the control retained its structure. Three main species *Nitzschia palea*, *Nitzschia dissipata* and *Melosira varians*, represented 25%, 20% and 13% of the community, respectively.

Isoproturon favoured the growth of *M. varians* (Fig. 3a) at all concentrations and durations of exposure. The abundance of this species increased in comparison with the control. The abundance of *N. dissipata* remained stable during the treatment. On the other hand, the abundance of some species like *Eolimna minima* and *N. palea* decreased with increasing isoproturon concentrations. Communities exposed to s-metolachlor presented the same pattern of species structure as communities exposed to isoproturon. *M. varians* abundance increased (Fig. 3b), *N. dissipata* was not affected. Its abundance remained stable between the control and the treated microcosms and even increased after the 3 days of exposure. Higher abundances of *Cocconeis placentula* were also observed in communities exposed for 6 days to s-metolachlor. Some species were more sensitive to s-metolachlor (*E. minima* and *Navicula reichardtiana*).

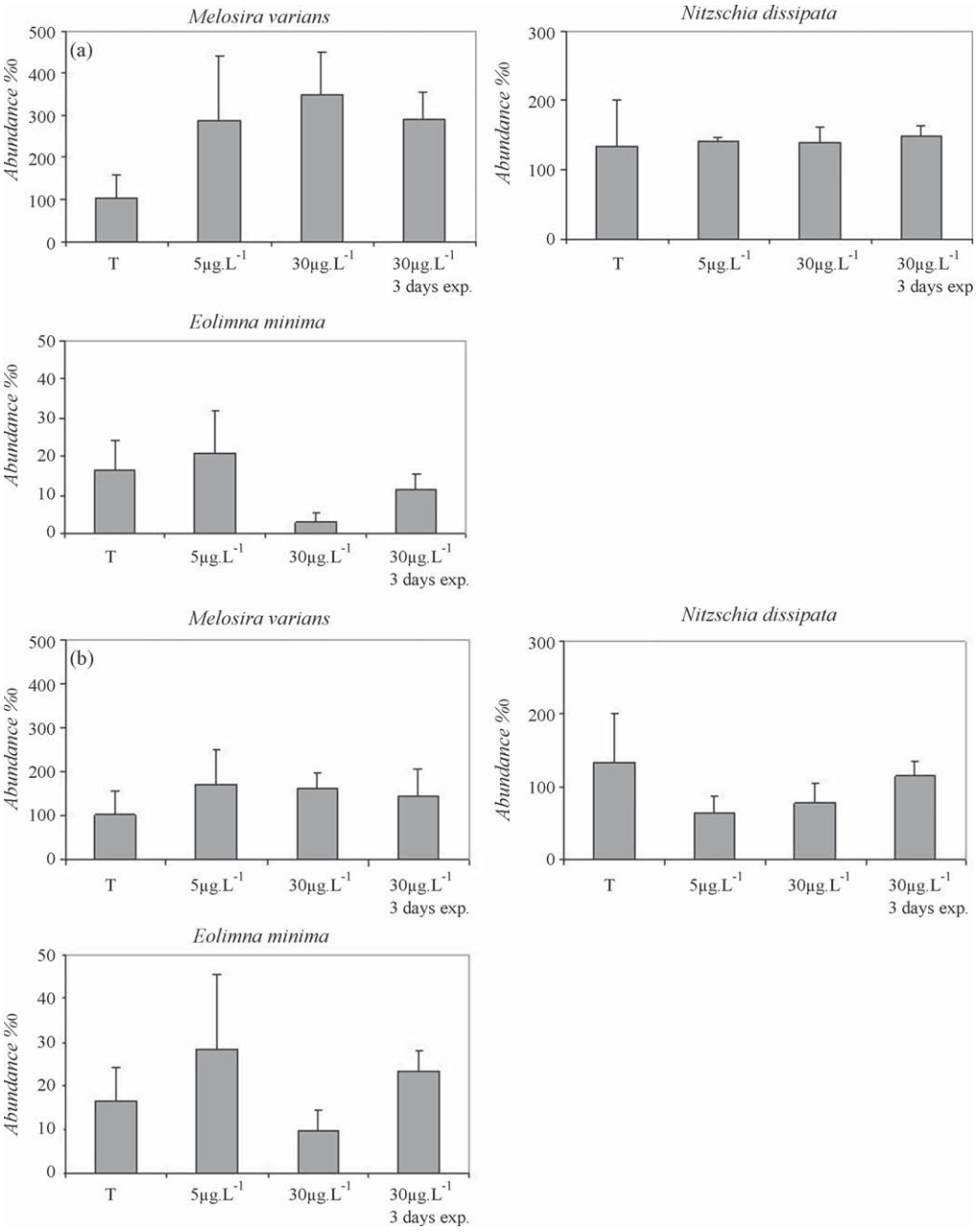

Fig. 3. Histograms of diatom species abundance in microcosms exposed to (a) isoproturon and (b) s-metolachlor at the end of the experiment ($t = 6$ days).

After 3 days of exposure, the abundances of these species increased again.

The study of species structure according to their trophic mode (tolerant or sensitive; facultative or obligate autotrophs; heterotrophs) showed a marked evolution in the communities exposed to isoproturon. The abundance of facultative heterotrophs increased with isoproturon exposure (Fig. 4). The difference with the controls was statistically significant at the highest concentrations ($30\,\mu g\,L^{-1}$) ($P < 0.05$). This evolution was limited to communities exposed to isoproturon. No such result was observed with s-metolachlor (Fig. 4).

4. Discussion

Based on the methodology developed in numerous other studies to allow the transfer of periphytic diatom communities from the field to the laboratory, the algae were collected on artificial substrata (glass slides) immersed in the river (Dorigo and Leboulanger, 2001; Navarro et al., 2002; Guasch et al., 2003). Algal communities collected *in situ* were preferred to the use of unialgal cultures. Genetic selection in *in vitro* culture alters the sensitivity of algae to chemicals (Peterson et al., 1997). Thus, culture responses to herbicide exposure are not representative of the community responses in rivers due to the different sensitivities of species (Badr and Abouwaly, 1997). To collect diatoms, glass slides were immersed for 2 weeks in winter (January and February). This duration is sufficient for the growth of algal biomass on artificial substrata (Hoagland et al., 1982; Biggs, 1990) and, moreover, diatoms are dominant in periphyton collected in winter (Ghosh and Gaur, 1998). In addition, Guasch et al. (1997) showed that the sensitivity of periphyton to a herbicide was higher in winter than other seasons. Benthic diatom communities were transferred into the laboratory to limit the variations of environmental parameters (illumination and current velocity) which may interfere with the response of diatoms to

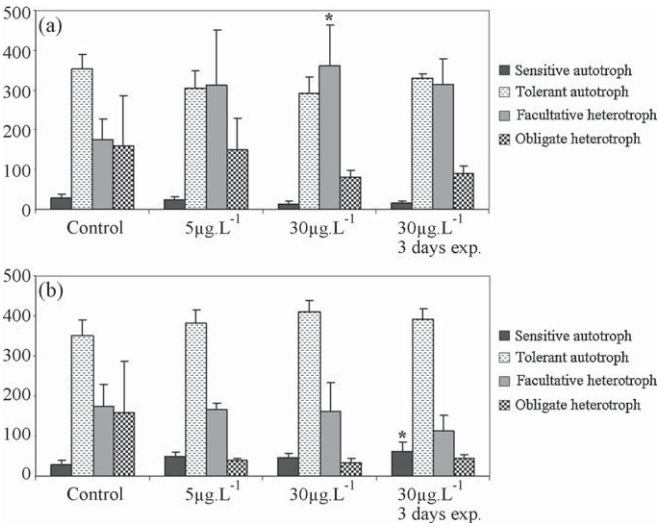

Fig. 4. Histograms of different diatom species group abundance according to their trophic mode (sensitive autotroph, tolerant autotroph, facultative heterotroph and obligate heterotroph) in communities exposed to isoproturon (a) and s-metolachlor (b) at $t = 6$ days (*statistical difference with the control ANOVA followed by Dunnett test, $P < 0.05$).

pesticide exposure (Guasch et al., 1997, 1998). In this experiment, high nutrient concentrations were applied to reproduce the trophic conditions of rivers contaminated by fertilisers (20–30 mg/L of NO_3, 1 mg/L of PO_4). At the end of experiments ($t = 6$ days), the concentrations of the major nutrients (NO_3, PO_4 and Si) in the microcosms were close to those measured during the field survey in rivers located in agricultural watersheds (Debenest, 2007; Perrin et al., 2008).

In our experiment, the herbicides applied disturbed the periphyton development. Higher growth inhibition was observed in microcosms treated with isoproturon especially at the highest concentration (30 $\mu g\,L^{-1}$) in comparison with s-metolachlor. With the 3-day exposure protocol, diatoms treated with isoproturon did not recover, whereas higher biomasses were observed in microcosms treated with s-metolachlor in the same conditions. In the presence of isoproturon, pigment concentrations and cell density did not follow the same pattern. Cell density was more affected by isoproturon exposure even at lower concentrations (5 $\mu g\,L^{-1}$). The pigment concentration response was independent of diatom cell density. Numerous authors (Peres et al., 1996; Schmitt-Jansen and Altenburger, 2005) have reported effects of isoproturon on diatom cell density at a concentration of 5 $\mu g\,L^{-1}$. These results contrasted with the findings of Seguin et al. (2001) who observed an increase of chlorophyll concentration at low concentrations of the photosynthesis inhibitors nicosulfuron and atrazine. The use of pigment concentrations to study the effects of herbicides on diatom biomass growth may be discussed. Algae may be differentiated according to their pigment composition (Van Den Hoek et al., 1995). In some conditions, the concentration of chlorophyll c may be used as a marker for diatom biomass and the one of chlorophyll b for the biomass of green algae. In the presence of Chrysophyceae, Raphidophyceae and Phaeophyceae, which also contain chlorophyll c, the assessment of more specific pigments like xanthophyll pigments (diadinoxanthin) would be more efficient to study the growth of diatom biomass. Porsbring et al. (2007) suggested using shifts in pigment composition as ecotoxicological tools. They proposed a test (SWIFT test) based on the study of pigment shift to assess toxicant effects on the succession of natural communities in periphyton. They also reported that ecological features such as species sensitivity profiles are crucial to determine the succession of communities under toxicant stress. The three parameters highlighted similar variations with s-metolachlor exposure. This difference could be explained by the target of this herbicide. According to Carder and Hoagland (1998), chloroacetamides such as s-metolachlor disrupt fatty acid synthesis and thus inhibit cell division. In this case, inhibition of diatom cell multiplication may be assessed from the chlorophyll concentration. These results highlighted that the sensitivity of algal biomass parameters (pigment concentrations or live cell density) depended on the mode of action of the herbicide.

In view of these results, a relationship can be assumed to occur between the inhibition of algal biomass growth observed during the main period of herbicide spring crop treatments (May) in the field survey carried out prior to this experiment (Debenest, 2007) and herbicide stream water pollution. Indeed, isoproturon is applied on winter crops (wheat, barley, etc.) whereas s-metolachor is used for spring crops.

The study of diatom community species composition in periphyton showed that some species (*M. varians*, *N. dissipata*, and *C. placentula*), tolerant to eutrophic conditions, were not affected by herbicide exposures. Similar results have been reported in diatom communities exposed to s-triazines (Hamala and Kollig, 1985; Goldsborough and Robinson, 1986; Berard and Pelte, 1996; Guasch et al., 1998; Munoz et al., 2001; Berard et al., 2003). Whereas a 6-day exposure to s-metolachlor stopped the development of species like *N. reichardtiana* and *Amphora pediculus*, they were able to grow again after a 3-day recovery period. This tolerance could be related to the trophic conditions. Berard et al. (1998) mentioned that in rivers located in agricultural watersheds and contaminated by high levels of fertiliser, the impact of pesticides on algal communities are difficult to discern from those due to trophic pollution. Indeed, a wide panel of parameters such as light exposure or nutrient concentration can interfere in the response of diatoms to pesticide (Guasch et al., 1997, 1998). For these reasons, the use of diatom indexes alone for toxicity evaluation remains quite problematic. These tools were developed to assess trophic and organic water quality. Their efficiency to detect pesticide effects in diatom communities has not yet been clearly demonstrated (Dorigo et al., 2004). In this context, the PICT concept is probably the most suitable tool to assess toxic pollution in rivers (Molander and Blanck, 1992; Blanck, 2002).

In communities exposed to isoproturon, facultative heterotroph species able to switch trophic mode from autotrophy to heterotrophy were favoured. These results are consistent with numerous other publications that mention the tolerance of these species to photosynthetic inhibitor herbicides like isoproturon (Goldsborough and Robinson, 1986; Peres et al., 1996). Hamala and Kollig (1985) also revealed that exposure to another well-known photosynthetic inhibitor (atrazine) increased the heterotrophic activity of periphytic algal communities. Changing the trophic mode is a known survival processes for some diatoms in unfavourable conditions (darkness or organic pollution) (Hellebust and Lewin, 1977). According to Hamilton et al. (1988), this adaptation explains the tolerance of these species to herbicides which inhibit photosynthesis (autotrophic mode). Our isoproturon results were consistent with those obtained for s-metolachlor exposure. The target site of this herbicide differs from that of isoproturon. No similar evolution of the diatom community structure was observed with this herbicide. When photosynthesis is inhibited, the organic matter in the microcosm may represent an alternative source of carbon for cell development. In addition, several authors have mentioned of the possibility that herbicides such as isoproturon (Peres et al., 1996) and 2–4 D (Okay and Gaines, 1996) can be metabolised by algae. This could explain the decrease in herbicide concentrations observed in the present microcosm experiments. Other physico-chemical processes (photolysis and hydrolysis) could also be involved in the degradation of these molecules. The marked decrease noted for s-metolachlor could be also related to its physico-chemical properties. This substance is

hydrophobic ($\log K_{ow} > 3$) and adsorbs onto glass, suspended matter and biofilm. Assessments of pesticide levels in biofilms have revealed their potential to act as storage compartments (Margoum et al., 2006).

The present work has shown the effects of commercial formulations of two major agricultural herbicides in France on benthic diatom community growth and structure. Overall, the data obtained highlight, for the first time, the direct effects of a herbicide (isoproturon) on species community structure according to the trophic mode used by the diatoms.

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