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Short Communication

Short term recovery of periphyton photosynthesis after pulse exposition to the photosystem II inhibitors atrazine and isoproturon

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ABSTRACT

Aquatic organisms are exposed to fluctuating concentrations of herbicides which contaminate rivers following their use for agricultural or domestic purposes. The development of sensitive bioanalytical tests enabling us not only to detect the effects of those pollutants but to take into account this pattern of exposure should improve the ecological relevance of river toxicity assessment. In this respect, the use of chlorophyll fluorescence measurements is a convenient way to probe the effect of photosystem II (PSII) inhibitors on primary producers. This study was devoted to validate the combined use of two fluorescence parameters, the effective and the optimal quantum yields of PSII photochemistry (Φ_{PSII} and F_v/F_m), as reliable biomarkers of initial isoproturon (IPU) or atrazine (ATZ) toxicity to natural periphyton in a pulse exposition scenario. Φ_{PSII} and F_v/F_m were regularly estimated during a 7 h-exposure to each pollutant (0–100 μM) and also later after being transferred in herbicide-free water (up to 36 h). Our results showed that IPU was more toxic than ATZ, but with effects reversible within 12 h. Moreover, these two similarly acting herbicides (i.e. same target site) presented contrasted short term recovery patterns, regarding the previous exposure duration.

1. Introduction

Contamination of rivers by herbicides has been widely reported in agricultural as well as urbanized watersheds (Holvoet et al., 2007). Once in the aquatic environment, those substances may affect non-target photosynthetic organisms. In lotic systems, attached microalgae (i.e. periphyton), are generally exposed to low herbicides exposure, with transient peaks related with changes in hydrology (Holvoet et al., 2007). Several authors stressed out that more environmental realism, regarding both patterns of exposure (i.e. pulsed vs chronic) and biological models (i.e. single species vs community level based tests) is essential (e.g. Sabater et al., 2007). In particular, the literature investigating the relevance of the recovery time between pulses of herbicides on algal sensitivity is scarce (Gustavson et al., 2003; Tlili et al., 2008; Vallotton et al., 2008a,b, 2009). In this respect, the development of tools based on periphyton physiological response should be of great help to refine our understanding on the initial herbicides effect at the community level (Sabater et al., 2007).

The Pulse Amplitude Modulated (PAM) chlorophyll fluorescence technique is a convenient way to study in vivo the early adverse effects of herbicides on photosynthetic activity and related physiological activities (Juneau et al., 2007). Of the fluorescence parameters already validated for toxicological purposes, the effective quantum yield of the photosystem II (PSII) photochemistry (Φ_{PSII}) gives a measure of the proportion of the PSII absorbed light that is used in photochemistry and integrates all the processes downstream of PSII which are dependant of the actual test conditions, e.g. light and temperature (Baker, 2008). In comparison, the optimal quantum yield (F_v/F_m) reflects the number of functional PSII, thereby illustrating the sample physiological state (Baker, 2008). Although it offers a more accurate view than Φ_{PSII} regarding the pollutant toxicity, it is rarely used, especially for river biofilms (Dorigo and Leboulanger, 2001; Laviale et al., 2010).

In this context, the aim of this study was to validate the combined use of Φ_{PSII} and F_v/F_m for a reliable assessment of periphyton sensitivity to herbicides and its potential recovery in a pulse exposition scenario. For this purpose, we focused on the effects of the triazine atrazine (ATZ) and of the phenylurea isoproturon (IPU), two herbicides specifically designed to alter photosynthesis by

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inhibiting the photosystem II (PSII) activity (Rutherford and Krieger-Liszkay, 2001) and which are among the 10 pesticides most frequently quantified in French rivers (Agences de l'Eau). A short-term ecotoxicological bioassay was carried out to monitor the photochemical response to IPU and ATZ of natural stream biofilms. Φ_{PSII} and F_v/F_m were regularly estimated during herbicide exposure (up to 7 h) and then after transfer in herbicide-free water (up to 36 h).

2. Materials and methods

2.1. Biofilms collection and incubation conditions

As previously described (Laviale et al., 2009, 2010), natural stream periphyton was regularly sampled from glass substrata immersed in early Spring in the Stream Sensée (Nord-Pas de Calais, France) at a site slightly influenced by herbicides (<0.2 μg L^{-1} over the last 12 months, Agence de l'Eau Artois-Picardie). After 2–3 weeks of colonization, the substrata were transported to the laboratory within 1 h in cool-boxes filled with site water and transferred in dark climate (20 °C) chamber. Preliminary microscopic observations indicated that these communities were dominated by diatoms. Six randomly selected slides were used to estimate the total diatom density (cells cm^{-2}). Each biofilm was collected with a razor blade in a preservative 5% formalin solution (Formol 37%) and observed under stereomicroscope using a Nageotte counting chamber (VWR, Fonteney sous Bois, France) at 200 magnification.

Within 1 d, slides were incubated horizontally under gentle agitation in polycarbonate vessels (1 slide per vessel) containing 60 mL of filtered stream water (Whatman GF/F, VWR, Fontenay sous Bois, France) and placed in continuous light (50 μmol m^{-2} s^{-1}) provided by fluorescent tubes (36 W, Grolux, Sylvania, VWR). Incubation media containing IPU ([3-(4-isopropylphenyl)-1,1-dimethylurea], Dr. Ehrenstorfer GmbH, Augsburg, Germany) or ATZ ([2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine], Sigma Aldrich, St. Quentin Fallavier, France) were prepared as Laviale et al. (2010).

2.2. Chlorophyll fluorescence parameters

The fluorescence signals were measured with a PAM 2100 fluorometer (Walz, Effeltrich, Germany) by means of home-made systems which were consistently described elsewhere (Laviale et al., 2009, 2010). Φ_{PSII} was evaluated on several slides under ambient light (50 μmol m^{-2} s^{-1}) according to Genty et al. (1989):

$$\Phi_{PSII} = (F'_m - F_t)/F'_m \quad (1)$$

where F_t is the fluorescence steady-state level under ambient light and F'_m is the maximum level of fluorescence measured during a saturating white light pulse (0.8 s).

Other slides were transferred to complete darkness for 10 min. The minimum fluorescence (F_0) was determined after a weak (5 s) far red modulated light (735 nm). Then the maximum fluorescence (F_m) was reached by exposing the biofilm to a saturating light pulse (0.8 s). F_v/F_m was then calculated using the Genty et al. (1989) equation:

$$F_v/F_m = (F_m - F_0)/F_m \quad (2)$$

2.3. Experimental design

The biofilms were exposed for 7 h to four nominal concentrations (0.1, 1, 10 and 100 μM) of each herbicide, i.e. 20.6–20.6 × 10^3 μg IPU L^{-1} or 21.6–21.6 × 10^3 μg ATZ L^{-1}. At the end of each time of exposure, the biofilms were gently rinsed and transferred for 36 h in uncontaminated filtered stream water which was regularly replaced to limit nutrient depletion and herbicide reuptake by passive diffusion through the biofilm. Chlorophyll fluorescence was measured on independent samples: (i) at the beginning of the experiment; (ii) after 1, 3 and 7 h (Φ_{PSII}) or 3 and 7 h (F_v/F_m) of herbicide exposure and (iii) after 1, 12 and 36 h in herbicide-free water for each condition of exposure.

All experiments were performed twice and each algal sample was analyzed in triplicates. Analyses of variance (ANOVA) and Tukey's honestly significant difference (HSD) tests were performed using the *R* statistical computing environment (v 2.8.1, Ihaka and Gentleman, 1996) after checking data normality and homoscedasticity of the residuals. Φ_{PSII} and F_v/F_m were expressed as % of response of the mean value obtained from the control community at the same time of exposure or recovery; letters indicate statistically homogenous groups.

3. Results and discussion

3.1. Short-term effects of herbicides

Fluorescence measurements were carried out on periphytic communities before contamination and then after 1, 3 and 7 h of exposure. F_v/F_m and Φ_{PSII} estimated on the controls were stable all along the experiment with mean values of 0.69 ± 0.03 (95% Confidence Interval) and 0.65 ± 0.01 respectively, indicating that the diatoms, which dominated communities (1.4 × 10^5 ± 0.1 × 10^5 cells cm^{-2}), were in good physiological state (Baker, 2008; Laviale et al., 2009, 2010).

In treated biofilms, the established concentration–effect relationships indicate that both herbicides strongly inhibited these parameters ($p < 0.001$, Fig. 1). Effects on Φ_{PSII} were significant within 1 h ($p < 0.001$) and remained stable thereafter ($p \geqslant 0.07$), whatever the concentrations tested (Fig. 1 A and B). IPU was 10-fold more toxic than ATZ with a total inhibition of Φ_{PSII} at 1 μM IPU and 10 μM ATZ, respectively (Fig. 1A and B). This falls within the range of values reported for periphyton in comparable short term bioassays based on chlorophyll fluorescence measurements (Guasch et al., 2003; Schmitt-Jansen and Altenburger, 2007,

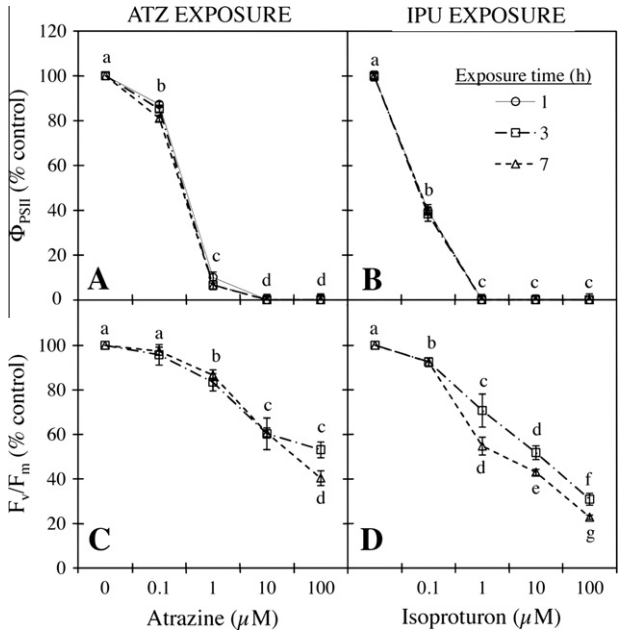

Fig. 1. Mean values (±95% CI, $n = 6$) of Φ_{PSII} (A and B) and F_v/F_m (C and D) estimated after 1 (○), 3 (□) or 7 h (△) of exposition to 0–100 μM of ATZ (A–C) or IPU (B and D).