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# Variations in periphytic diatom tolerance to agricultural pesticides in a contaminated river: An analysis from community to population level

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## Abstract

Periphytic diatoms are an important phototrophic component of river biofilm and are used in situ for the bioindication of pollution as well as in laboratory ecotoxicological tests to assess the toxicity of contaminants. In spring 2009, phototrophic biofilm samples composed mostly of diatoms were collected in a small river and their sensitivity to the herbicide diuron was estimated via photosynthesis bioassays. A large difference in tolerance to diuron was demonstrated between two periphytic communities from an upstream unpolluted site and a downstream site subjected to high seasonal contamination by pesticides. The comparison of diatom community structure between sites revealed important variations of the relative abundance of some species which could explain this difference. Consequently, some of these species were isolated from the river in autumn when toxic pressure was low, and kept in culture for more than six months in uncontaminated water. Acute toxicity tests of diuron based on growth inhibition were then performed on each species. Surprisingly the sensitivities of the species as estimated by EC50 were almost the same. However two strains of another species that could be isolated from each site of the river showed significant differences in tolerance to diuron and copper, another contaminant of the river. These results suggest the importance of adaptation at the intraspecific level in the induction of periphytic community tolerance to toxicants and the probably low sensitivity of bioindication methods to assess river contaminations.

*Keywords: diatoms; tolerance; pesticides; adaptation; bioindication.*

## 1. INTRODUCTION

Pesticides contaminate rivers draining agricultural areas via spray drift and runoff and can generate toxic stress to aquatic organisms. According to the PICT concept [1], periphytic communities exposed to one toxicant may increase their tolerance to this toxicant. Thus, the increase in tolerance of a community to a pesticide should reveal the contamination of the river. Tolerance acquisition in communities results either from the replacement of sensitive species by more tolerant ones or by the development of more tolerant populations in the communities without necessary change in species composition.

Diatoms are a major component of lotic periphyton and are commonly used as bioindicators of river eutrophication. Although little is known about their response to toxicants, diatom communities may be altered by exposure to pesticides and therefore also used for the bioindication of river contamination. Ecotoxicological studies with diatom communities have resulted in the identification of rather tolerant or sensitive species. In a community under toxic pressure, tolerant species may develop at the expense of sensitive ones. However, the capacity of adaptation at the species level has been little investigated and may contribute to a large extent to community tolerance acquisition [2,3,4].

From the observation of a high increase in periphytic community tolerance to the herbicide diuron in a contamination gradient (Morcille River, France), the possible ways communities can adapt

were examined in this study using ecotoxicological tests on monoclonal diatom cultures. Tolerance variations between species were compared to variations between genetically differentiated populations of the same species and within the same monoclonal population.

## 2. MATERIALS AND METHODS

### 2.1 Study area

Periphyton samples were collected at two sites along the Morcille River (Beaujolais, France) which presents a clear streamward contamination gradient by pesticides used on vineyards [5]. The upstream site (St Joseph) is preserved from pollution and can be used as a reference while high concentrations of pesticides are usually recorded at the downstream site (St Ennemond). The most common contaminants are herbicides, especially diuron (phenylurea) and fungicides including copper.

### 2.2 Tolerance assessment of periphytic communities to diuron

Periphytic community tolerance to diuron was assessed via short-term photosynthesis bioassays [6]. Biofilm samples were collected on artificial substrates at both sites on 16<sup>th</sup> June 2009. On return to the lab, the samples were suspended in triplicates (3 samples per site) in 7 diuron solutions of concentrations ranging from 1 to 1000  $\mu\text{g.L}^{-1}$ . After 3.5h exposure in a climatic chamber at constant temperature (23°C) and light (1400 lux) followed by 30 minutes in the dark, the effect of diuron on photosynthesis was assessed through PSII quantum yield estimation using a Phyto-PAM fluorometer. 50% Effect Concentrations (EC50) were determined for each sample triplicate after non-linear regression on the following model:

$$\frac{Y}{Y_0} = \frac{(Y_0 + \delta \cdot x)}{\left(1 + \frac{x}{g}\right)^\beta} \quad (1)$$

Where  $Y$  is the measured endpoint ( $Y_0$  the control value),  $x$  is diuron concentration and  $\beta$ ,  $\delta$  and  $g$  are model parameters.

The proportion of diatoms in periphyton biomass was assessed by fluorescence analysis using Phyto-PAM. The specific composition of diatom communities was determined under microscope ( $\times 1000$ ) after oxidation of periphyton samples in boiling  $\text{H}_2\text{O}_2$  solution.

### 2.3 Monospecific ecotoxicological tests

Acute toxicity tests based on growth inhibition were performed on monoclonal diatom cultures in order to assess sensitivity to pesticides.

One clone of each of the species *Navicula lanceolata* (NLAN), *Eolimna minima* (EOMI), *Achnanthydium minutissimum* (ADMI), *Nitzschia linearis* (NLIN) and *Encyonema neomesianum* (ENME) was isolated from the upstream (ADMI and NLIN), the downstream site (NLAN and EOMI) or from both sites (ENME) of the Morcille River in November 2009. The diatoms were then grown in a culture chamber during more than 6 months in filtrated pesticide-free river water enriched with nutrients [7].

For the tests, the cultures were exposed in triplicates (+ 5 controls) to five concentrations of pesticide (diuron or copper) in 50 mL glass bottles placed on a rotative panel at 23°C and a 150  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$  illumination. Growth was assessed after 4 to 6 days (according to specific growth rate) from chlorophyll-*a* concentration measured at the beginning and the end of test using a bbe Fluoroprobe with its cuvette holder. The same toxicological model as for communities (equation 1) was used to fit growth data and to derive EC50 values. A bootstrap analysis on weighted residuals was finally carried out to determine 95% confidence intervals associated to CE50.

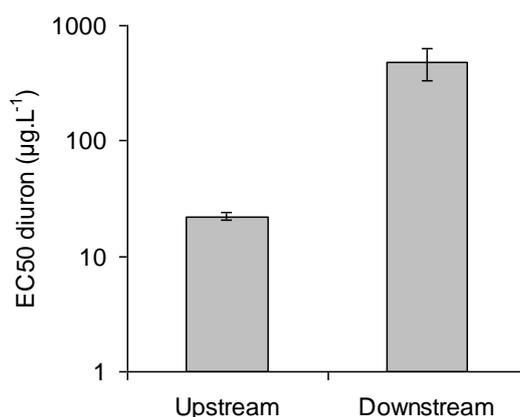
The last pair of tests consisted in the assessment of diatom potential for acclimation to toxic stress at a time scale of a few weeks. They were done with the species *Surirella angusta* (SANG) isolated from a unpolluted site (Gèze River at Organ) in a region where diuron and copper are not used

(Coteaux de Gascogne, France). A monoclonal culture of SANG was divided into two sub-cultures: one was used as a control and the other was contaminated with diuron at a concentration of  $1 \mu\text{g.L}^{-1}$ . After 15 days, the toxicity of diuron was tested on each culture separately as described above.

### 3. RESULTS AND DISCUSSION

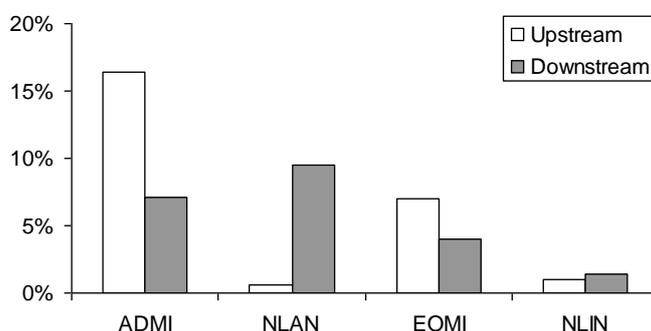
#### 3.1 Variations in community tolerance

The phyto-PAM analysis of periphyton samples revealed that diatoms represented about 80% of algal biomass at each site. Photosynthesis bioassays resulted in a much higher tolerance to diuron of the downstream periphytic community as shown by the EC50 values (Figure 1). This tolerance increase (PICT) is consistent with the high concentration of diuron measured in the river over the month before sampling [6].



**Figure 1.** Difference in the tolerance to diuron of natural periphytic communities sampled upstream and downstream the river.

The analysis of diatom communities in periphyton samples revealed large variations in relative abundance of some species from up to downstream. ADMI, EOMI and NLAN were among the species presenting the highest variations (Figure 2). The species NLIN had low and similar abundance at the two sites. From the results of community tolerance it can be deduced that the species having higher abundance upstream are sensitive to diuron (ADMI and EOMI) whereas the others are rather tolerant to the herbicide (NLAN and NLIN). The high variations of the three first species may then contribute to explain the difference observed in community tolerance.

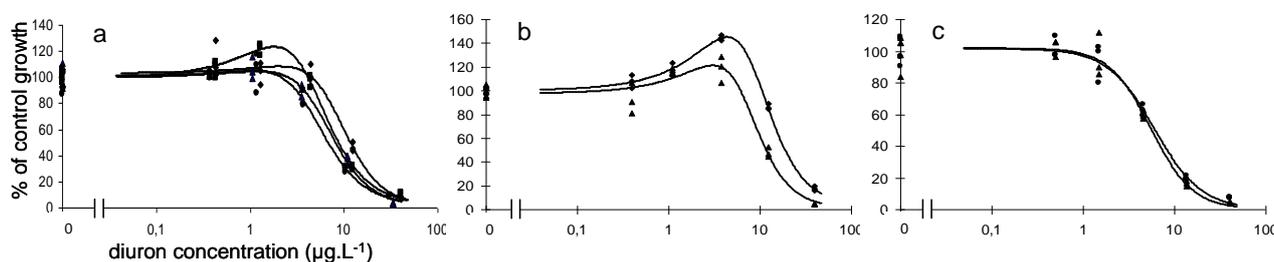


**Figure 2.** Variations in relative abundance of some diatom species in the diatom communities present in the periphyton samples used for the experiment.

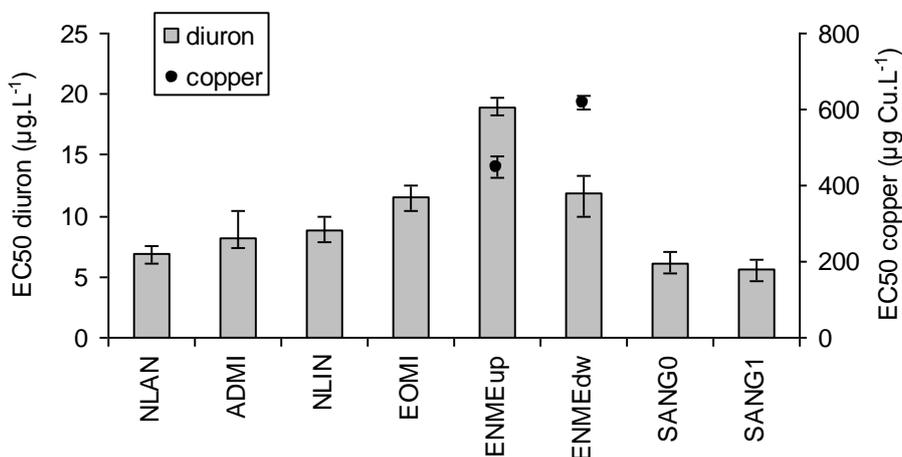
#### 3.2 Variations between species

The isolates of the four diatom species tested showed surprisingly similar sensitivities to diuron (Figure 3a). The EC50 values were not significantly different (Figure 4) and much lower than the value estimated for the downstream community. Therefore the hypothesis that the species NLAN

and NLIN are generally more tolerant to diuron than the two others has to be rejected. It is more likely that community induced tolerance was due to the development of adapted populations in the downstream periphyton and that the replacement of sensitive species by more tolerant ones was not as important.



**Figure 3.** Results of the ecotoxicological tests of diuron performed on monoclonal diatom cultures: (a) comparison of 4 isolates of the species ADMI, EOMI, NLAN and NLIN, (b) comparison of 2 clones of the species ENME isolated from the upstream (ENME<sub>up</sub>, ●) and downstream (ENME<sub>dw</sub>, ▲) sites and (c) comparison of two cultures of the same clone of SANG with and without chronic pre-exposure to a low concentration of diuron.

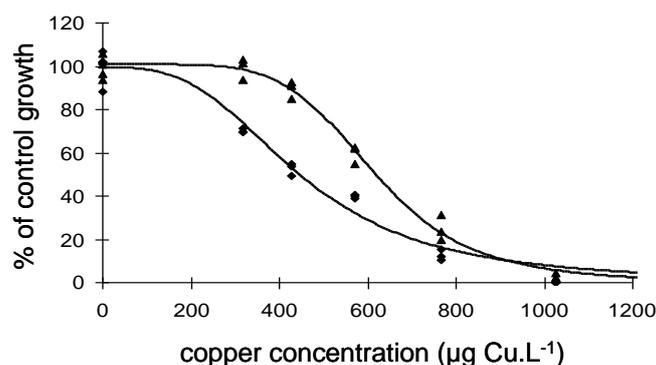


**Figure 4.** Summary of the EC50 values with their confidence interval determined from the ecotoxicological tests of diuron and copper on monospecific diatom cultures.

### 3.3 Intraspecific variations

The tests performed on the two clones of the species ENME isolated from the two sites along the river resulted in significant differences as regard to their sensitivity to diuron and copper (Figure 3b and 5). The downstream clone was more tolerant to copper but more sensitive to diuron than the upstream one. A 40% change in the EC50 was measured from the upstream to the downstream clone (Figure 4) for each contaminant. These differences might result from an adaptation of the population at the downstream site since copper had higher concentration than diuron when the clones were isolated (in November). They have probably a genetic basis as the clones were grown for long time in pesticide-free water prior to the tests. The opposite variations of sensitivity to the 2 contaminants within clones may reflect the cost of adaptation to a toxicant [3,8].

As illustrated in Figure 3c, the long-term chronic exposure to a low concentration of diuron did not increase the tolerance of the clone of SANG as estimated via acute toxicity tests. This means that diatoms are probably not capable to acclimate to such an herbicide as diuron at a time scale of 2 weeks.



**Figure 5.** Results of the ecotoxicological tests of copper on the two clones of ENME isolated from the upstream (▲) and downstream sites (●).

#### 4. CONCLUSIONS

The comparison of diatom tolerance to diuron at the community, species and population level via ecotoxicological tests showed the importance of intraspecific variations to explain tolerance induction of a community. The increase of community tolerance to diuron in spring at the downstream site of the Morcille River was probably the consequence of the development of genetically adapted populations. The replacement of sensitive species by more tolerant ones and the acclimation of clones seem to be less important in the tolerance acquisition process. Thus, bioindication may not be a sensitive tool to assess water contamination unless diatom species have not all the same potential for adaptation to toxicants.

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