Aquatic Toxicology 158 (2015) 202–210

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Use of diatom motility features as endpoints of metolachlor toxicity

Nathalie Coquillé[a,b,c], Gwilherm Jan[a], Aurélie Moreira[a], Soizic Morin[a,*]

[a] Irstea, UR EABX, 50 avenue de Verdun, 33612 Cestas Cedex, France
[b] Université de Bordeaux, UMR EPOC 5805 CNRS, Laboratoire de Physico Toxico Chimie de l'environnement, 351 Cours de la Libération, CS 10004, 33405 Talence Cedex, France
[c] Ifremer, Laboratoire d'écotoxicologie, rue de l'île d'Yeu, BP 21105, 44311 Nantes Cedex 03, France

ARTICLE INFO

Article history:
Received 26 August 2014
Received in revised form
19 November 2014
Accepted 21 November 2014
Available online 28 November 2014

Keywords:
Diatoms
Physiology
Behavior
Pesticide exposure
Motility
CASA

ABSTRACT

Many recent ecotoxicological studies suggest a relationship between freshwater contamination and increasing abundances of motile diatoms (potentially able to move). The capacity to escape would present advantages to species in polluted environments. However, actual motility as a response to toxicants had not been described and required experimental validation.

We designed a specific experiment to assess how a field-isolated diatom (*Gomphonema gracile*) distributes energy to in situ resistance (increased population growth or photosynthesis) and escape (behavioral changes), when exposed to increasing concentrations of the herbicide metolachlor.

We report here the dose–time dependent responses of *G. gracile* populations. They coped with low contamination by resisting in situ, with early hormetic responses highlighted by stimulation of chlorophyll-*a* fluorescence. At a higher dose, harmful impacts were observed on growth after a few days, but an earlier behavioral response suggested that higher motility (percentage of motile individuals and mean distance crossed) could be involved in escape.

Our findings bring new arguments to support the implementation of real measurements instead of motility traits in toxicity assessment. Specifically, motion descriptors have been used as early-warning indicators of contamination in our study. Further works should address the reliability of these endpoints in more complex conditions (interspecific variability, behavior in the field).

1. Introduction

Motility has been described in many pennate diatoms (e.g. Bertrand, 1999; Harper, 1977), and migration plays a role in photoregulation, described as a major behavioral response in intertidal environments (Ni Longphuirt et al., 2006; Serôdio et al., 2012). Freshwater diatom motility, also observed in response to light intensity (Cohn, 2001; Cohn et al., 2004), may be affected by environmental factors other than light (Cohn and Disparti, 1994; Cohn et al., 2003). The decrease in the speed of the diatom *Craticula cuspidata* has been proposed to assess toxicity of sediment elutriates (Cohn and McGuire, 2000). Ahmed and Häder (2010) demonstrated inhibition of the percentage of motile cells and of upward swimming in *Euglena gracilis* exposed to heavy metals. When dealing with community ecotoxicology, the motility trait is preferred to the effective behavioral descriptors in toxicity assessment, and is calculated based on the structural composition (and more specifically, growth forms) of communities and ability of some species to move (i.e. species belonging to the "motile guild", as defined by Passy, 2007). Indeed, the structure of the biofilm is made up of very diverse species, with various growth forms controlling its thickness. The layers of cells closest to the substrates are dominated by prostrate tightly attached diatoms. Other diatom growth forms (clumps, filaments) compose a complex three-dimensional structure. Species not fixed have the ability to move within the biofilm matrix. According to Roubeix et al. (2011b) or Paule et al. (2013), the abundance of potentially motile taxa is expected to increase with toxic contamination. Indeed, the motile species are assumed to be able to control their refuge within the biofilm (Larras et al., 2012), or regulate the balance between access to environmental resources (light, nutrients) and exposure stress (Fore and Grafe, 2002; Laviale et al., 2009). But, the real motility response to toxicants has been, to date, overlooked and requires experimental validation.

A toxicant entering the cell generates a dramatic accumulation of reactive oxygen species (ROS) in the cytosol (Wang et al., 2004). Excess ROS are cytotoxic and have different cellular targets (nucleus, mitochondria and chloroplasts). Adverse effects in diatoms are most often: damage to the photosynthetic

* Corresponding author. Tel.: +33 557892726.
E-mail address: soizic.morin@irstea.fr (S. Morin).

http://dx.doi.org/10.1016/j.aquatox.2014.11.021
0166-445X/

apparatus (Knauert and Knauer, 2008) affecting D1 protein resulting in photoinhibition (Cartaxana et al., 2013); damage to the electron transfer chain in mitochondria, causing a decrease in ATP production (Stohs and Bagchi, 1995); damage to DNA affecting vegetative growth (Stohs and Bagchi, 1995). The way to cope with sublethal contamination would rather be linked to temporary physiological adaptation: low concentrations of toxicants promote hormesis (compensatory stimulation), as a mechanism to counteract the stress induced by the toxicant. In microalgae, an increase in chlorophyll-*a* synthesis ("greening effect") can be observed (e.g. Tlili et al., 2011) as a short term response to maintain an efficient photosynthetic processes. In contrast, exposure to higher doses may provoke more overwhelming responses (even death). The capacity to escape may thus constitute a serious advantage for cells experiencing contamination.

Metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide] is a pre-emergent and early post-emergent chloroacetanilide herbicide widely used in agriculture and detected at high concentrations in rivers in particular during the field application period and after rainfall (e.g. Liu and Xiong, 2009; Roubeix et al., 2012). It inhibits growth by suppressing synthesis of chlorophyll, proteins, fatty acids and lipids, isoprenoids, and flavonoids (Fuerst, 1987; Rivard, 2003). Assessing metolachlor toxicity is challenging, because current diatom indicators of toxic impact (mainly growth and photosynthesis) fail to properly assess metolachlor toxicity, except when it is at extremely high concentrations (Debenest et al., 2009; Roubeix et al., 2012, 2011a).

We designed a specific experiment to determine behavior changes (motility parameters) of a pennate diatom isolated in the field (*Gomphonema gracile* Ehrenberg) when exposed to three environmentally realistic concentrations of metolachlor. In our experiment, we hypothesized that physiology and behavior would be modified by exposure to metolachlor. More specifically, we expected the responses to preferentially be in situ resistance at low doses (increased reproduction or photosynthetic processes) and escape (increased motility) at higher concentrations. Over a certain level of exposure, and thus stress for the organisms, impacts on population dynamics (reproduction and mortality) were also expected to occur. A delay before the detection of an impact, and the potential non-linearity of the dose–response relationships, could complicate toxicity assessment. Here we aimed to determine whether motility parameters, obtained with a rapid method commonly used in animal biology (an ImageJ plug-in), would provide a finer assessment of metolachlor toxicity earlier than commonly used descriptors.

2. Materials and methods

2.1. Gomphonema gracile

Gomphonema gracile Ehrenberg (1838) is a benthic species preferentially found in slightly acidic waters (Germain, 1981). Its length and width range from 20 to 100 μm and from 4 to 11 μm, respectively (Krammer and Lange-Bertalot, 1986). This species is considered to be very sensitive to various types of pollution (according to the Indice de Polluosensensibilité Spécifique: Coste in Cemagref, 1982). It is a pennate diatom and has a bidirectional movement, like most raphid species (Cohn, 2001).

The specimen used was from a field sample (December 2013) collected from an upstream section of the river Leyre, the main tributary of Arcachon Bay (South West France) by micromanipulation under the inverted microscope, and cultured in Dauta medium (Dauta, 1982). The culture was incubated in a chamber with a light/dark cycle of 12 h:12 h at a mean temperature of 20 °C. The culture was regularly (every 1–2 weeks) transplanted into freshly prepared medium. Diatom length in our cultures was mainly in the range of 20–40 μm. The effective quantum yield (see Section 2.3.1) of the culture was periodically checked prior to the experiment and found to be stable over time (0.36 ± 0.00, $n = 70$).

2.2. Experimental design

Thirteen experimental units (EUs) of 40 mL (final volume) were filled with culture medium autoclaved at 121 °C for 21 minutes and with the culture of *G. gracile*, at an initial cell concentration of around 30,000 cell mL^{-1}. Four EUs were used as control replicates (named C0), and the three concentrations of increasing exposure (C1, C2 and C3) were performed in triplicate. The cultures in exponential growth phase were exposed by pouring metolachlor in solution (racemic metolachlor at 100 mg L^{-1}, 99.5% purity, Dr. Ehrenstorfer, Germany) into the EUs to reach the following nominal concentrations: 1, 10 and 100 μg L^{-1} (i.e. 4 nM, 35 nM, 352 nM). Three additional experimental units, used as abiotic positive controls, were filled with culture medium contaminated with the highest concentration of metolachlor. The three concentrations correspond to (i) environmentally realistic exposure concentrations (1 μg L^{-1}) in the Leyre watershed (Roubeix et al., 2012) or other rivers of the area (Fauvelle et al., 2014), and (ii) for the higher ones, concentrations of the same order of magnitude as those used in other ecotoxicological studies (Debenest et al., 2009; Roubeix et al., 2011a). The cultures were thus exposed to these four treatments over 7 days, during which samples were taken for chemical and biological measurements.

2.3. Biological endpoints

Biological endpoints were determined on day 0 (d0, immediately after contamination), d1, d2, d3, d4 and d7. First, measurements of yield and chlorophyll-*a* fluorescence were performed directly on the intact biofilms (in their benthic "form"). Then the cultures were gently shaken to homogenously suspend the cells before sampling aliquots for the other endpoints measured: 20 μL were used for the motility measurements and image acquisition; 375 μL were preserved with a 25 μL drop of formalin solution for growth and mortality analyses; 300 μL were immediately frozen for further ATP content determination.

2.3.1. Chlorophyll-a fluorescence derived parameters

Measurements were performed on the intact biofilms, using a PHYTO-PAM (Heinz Walz, GmbH) equipped with an emitter-detector unit (PHYTO-EDF). Using a home-made system for reproducible direct measurements on bottom of the EUs, ten randomly selected benthic measurements of the effective quantum yield ($\Delta F/Fm'$) and chlorophyll-*a* content estimated by chlorophyll-*a* fluorescence (Chl-*a*) were performed to account for the patchy pattern of benthic settlement (especially in the initial phase of colonization). The median of 10 values per sample was then used for statistical analyses.

2.3.2. Motility

The motility parameters (percentage of motile cells, velocity) were determined using the ImageJ plug-in, CASA (Computer-assisted sperm analysis). This plug-in, initially developed to measure the motility of human spermatozoa, was adapted to animal biology (zebrafish sperm) by Wilson-Leedy and Ingermann (2007) and is commonly used to analyze the movement of trochophore larvae, sea bass spermatozoa, etc. It allows a quick measurement of different motility parameters, characterizing the general state of the cells.

The measurement conditions as well as the CASA plug-in were specifically adapted to *G. gracile*. Briefly, a 20 μL drop of