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Assessment of toxicity thresholds in aquatic environments: Does benthic growth of diatoms affect their exposure and sensitivity to herbicides?

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HIGHLIGHTS

- We assessed influence of diatom growth mode on their sensitivity to herbicides.
- Growth mode and herbicide hydrophobicity modify diatoms sensitivity.
- Most hydrophobic herbicides were more toxic under benthic than planktonic growth.
- Planktonic dataset was adapted for hydrophilic herbicide risk assessment.
- For hydrophobic herbicides, benthic thresholds tended to be more protective.

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ABSTRACT

Benthic diatoms evolved in a biofilm structure, at the interface between water and substrata. Biofilms can adsorb toxicants, such as herbicides, but little is known about the exposure of biofilm organisms, such as benthic diatoms, to these adsorbed herbicides. We assessed the sensitivity of 11 benthic diatoms species to 6 herbicides under both planktonic and benthic conditions using single-species bioassays. The concentration that reduced the growth rate of the population by 10% (EC_{10}) and 50% (EC_{50}), respectively, varied depending on the species, the herbicides, and the growth forms involved. As a general trend, the more hydrophobic the herbicide, the more species were found to be sensitive under benthic growth conditions. Statistical differences (alpha < 5%) were observed between the sensitivities under planktonic and benthic growth conditions for many hydrophobic herbicides. A protective effect of the biofilm against herbicides was observed, and this tended to decrease (at both the EC_{10} and EC_{50} levels) with increasing hydrophobicity. The biofilm matrix appeared to control exposure to herbicides, and consequently their toxicity towards benthic diatoms. For metolachlor, terbutryn and irgarol, benthic thresholds derived from species sensitivity distributions were more protective than planktonic thresholds. For hydrophobic herbicides, deriving sensitivity thresholds from data obtained under benthic growth seems to offer a promising alternative.

1. Introduction

Benthic diatoms play key roles in the environment, for instance constituting the primary production in functioning aquatic ecosystems. With their great diversity and wide-ranging ecological niches, they are found in a variety of habitats and provide reliable indicators of the ecological status of freshwater ecosystems (Lenoir and Coste, 1996; Rimet and Bouchez, 2012; Van Dam et al., 1994). Benthic diatoms in biofilms live embedded in a matrix consisting of an extracellular polymeric substance (EPS), that is made up mainly of proteins, saccharides and nucleic acids (Sutherland, 2001). On the one hand, the biofilm may protect diatom communities against physico-chemical changes and environmental factors. On the other hand, it can also interact with the whole environment, catching and remobilizing dissolved substances, such as nutrients or toxicants, from the water column (Flemming and Leis, 2002). Many authors have studied the biofilm sorption capabilities of herbicides, such as atrazine (Headley et al., 1998; Lawrence et al., 2001; Bohuss et al., 2005), diuron (Tlili et al., 2008), diclofop methyl (Wolfaardt et al., 1995; Lawrence et al., 2001), and various other pesticides such as DDT (Headley et al., 1998; Dong et al., 2011), lindane, chlorpyrifos and carbofuran (Lundqvist et al., 2012). Sorption processes depend partly on the chemical structure and properties of the biofilm involved (thickness, EPS composition, architecture) (Headley et al., 1998; Lawrence et al., 2001). Flemming (1995) identified various sorption sites, with specific affinities, including the EPS, cell

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walls, cell membranes and cytoplasm. Toxicants can be adsorbed onto the biofilm, and so organisms living in it, such as benthic diatoms, may be exposed to concentrations differing from those in the dissolved phase.

Herbicide contamination of water ecosystems is widely reported (Gilliom, 2007; Loos et al., 2009; Wittmer et al., 2010; Dubois and Lacouture, 2011). This contamination is dependent of many parameters such as watershed structure and land use (anthropic activity, surface, slope), soil structure and composition, environmental factor and hydrology (rain event, flow, and wind), aquatic medium conditions (pH, organic matter, suspended particles) and herbicide chemical properties (Leu et al., 2004; Blanchoud et al., 2007; Ulrich et al., 2013). These substances enter aquatic environments via several different pathways, including watershed runoff from fields and urban and peri-urban discharges. Herbicides generally act by inhibiting various vital functions of plants. Triazine (atrazine, terbutryn, irgarol) and phenylurea (diuron, isoproturon) herbicides are known to inhibit photosynthesis by acting on Photosynthetic System II (PSII) at different sites, while chloracetamide herbicides (such as metolachlor) act by inhibiting the biosynthesis of fatty acids and amino acids (Wakabayashi and Böger, 2002). Many studies in Europe have reported the frequent detection of diuron, metolachlor, isoproturon, terbutryn and atrazine (Loos et al., 2009; Wittmer et al., 2010; Meyer et al., 2011; Dubois and Lacouture, 2011; Köck-Schulmeyer et al., 2012). Given their phytotoxic properties, their presence in freshwater ecosystems constitutes a risk for non targeted primary producers, such as benthic diatoms (Akerblom, 2004). The toxic effects of these herbicides on benthic diatoms have been demonstrated in laboratory bioassays by several authors (Magnusson et al., 2008; Roubeix et al., 2011; Larras et al., 2012). Benchmarks, such as the Effective Concentration (EC) show that the sensitivity of the different species varies considerably. This variation of sensitivity within a single assemblage or a community can be described by Species Sensitivity Distribution (SSD) curves (Posthuma et al., 2002), which plot the cumulative distributions of benchmarks (e.g. EC) obtained for a single substance or a mixture from bioassays with different species. They are mainly used as predictive models for risk assessment purposes, with a view to extrapolate a threshold that will protect most of the environmental species (Chèvre et al., 2006). This protective threshold is known as the Hazardous Concentration (HC), and is usually defined as that which affects 5% of the species (HC_5). HC_5 can also be considered to be the concentration at which 95% of species in the environmental assemblage are not affected (Posthuma et al., 2002; Van Straalen, 2002). To be considered as protective, these thresholds have to be based on low level effect data, such as the EC_{10} in our study. Moreover, HC_5 thresholds can be used to derive the Predicted No Effect Concentration (PNEC) to ensure the protection of environmental communities. Larras et al. (2012) have shown that diuron, isoproturon and terbutryn concentrations found in the environment sometimes exceed the protective thresholds extrapolated from SSDs based on the sensitivity of benthic diatoms. However, these SSDs were based on a dataset of sensitivities obtained under planktonic growth conditions, and this protocol was not intended to be representative of the mode of life of these species, which live naturally in biofilm matrices, and under benthic growth conditions. As the risk assessment of chemicals is currently of major concern, we wondered how the safety threshold predictions of these tools could be enhanced.

The aim of this study was to find out whether the exposure and consequently the apparent sensitivity of diatom species to a panel of 6 herbicides are affected by their growing under planktonic or benthic conditions, and so growing either without a biofilm or inside a biofilm. We tested 11 diatom species to find out whether the presence of biofilm significantly modified the extrapolated HC, and consequently, significantly affected the predicted safety threshold used for environmental risk assessment. We focused on the EC_{10} and EC_{50} thresholds because of their regulatory relevance. The EC_{10} is a low level effect threshold, which is relevant in the context of risk assessment to derive protective thresholds, and the EC_{50} is a less variable threshold that is often used in the literature.

2. Material and methods

2.1. Benthic diatoms

We selected eleven diatom species, with a range of life forms and taxonomic diversity that are typical of freshwater benthic ecosystems, and are usually found in the littoral zone of Lake Geneva. As in natural biofilms, there was a higher proportion of pennate diatoms than centric diatoms among tested species, and ten species were pennate while, only one was centric (*Cyclotella meneghiniana*). Most of them are classified as benthic, and all were isolated from benthic biofilms. The species selected for inclusion in the panel were *Fragilaria capucina var vaucheriae* (FCVA), *Fragilaria rumpens* (FRUM), *Ulnaria ulna* (UULN), *Craticula accomoda* (CRAC), *Mayamaea fossalis* (MAFO), *Sellaphora minima* (SEMN), *Nitzschia palea* (NPAL), *Achnanthidium minutissimum* (ADMI), *Cyclotella meneghiniana* (CMEN), *Encyonema silesiacum* (ESLE), and *Gomphonema parvulum* (GPAR), and the strains were all taken from the Thonon Culture Collection (Thonon-Les-Bains, France, http://www.inra.fr/carrtel-collection). Cultures were maintained in the "Diatom medium + Vitamines" (DV) culture medium (http://www6.inra.fr/carrtel-collection_eng/Culture-media/Composition-of-the-culture-media) that had been filtered at 0.22 μm (Millipore, Sigma-Aldrich (St Louis, MO 63103, USA)) and were re-suspended daily. They were grown in 300 mL Erlenmeyer flasks at 21 ± 2 °C and with a 16:8 h light:dark cycle at 66 $\mu mol \cdot m^{-2} \cdot sec^{-1}$.

2.2. Herbicides

The herbicides selected were atrazine, terbutryn, irgarol, diuron, isoproturon and metolachlor (Table 1). These herbicides occur in the littoral zone of Lake Geneva (diuron (≤0.006 μg/L), isoproturon (≤0.002 μg/L), atrazine (≤0.006 μg/L), terbutryn (≤0.002 μg/L), metolachlor (≤0.004 μg/L)) (INRA, unpublished data) and were selected on the basis of their higher level of toxicity, variety of mode of action and range of hydrophobicity. We focused on active substances, obtained from Sigma-Aldrich (St Louis, MO 63103, USA). Stock solutions were prepared in DV culture media. Atrazine and diuron were dissolved in 0.05% dimethyl sulfoxide (DMSO) obtained from Sigma-Aldrich (St Louis, MO 63103, USA) and sonicated for 30 min before adding the DV media. The toxicity of 0.05% DMSO to diatoms was assessed in the DV medium prior to the bioassay implementation and no effect was detected.

2.3. Bioassays

The 11 diatom species were grown as either planktonic or benthic cultures. Planktonic EC_{50} values were derived from Larras et al. (2012) for each of the herbicides, except that of irgarol while planktonic EC_{10} values were derived from dose–response curves also obtained in the context of this previous study. The irgarol/planktonic bioassays and all

Table 1
Chemical properties and mode of action of herbicides.

Family	Herbicide	Mode of action	Log K_{ow}	Purity (%)
Phenylurea	Diuron	Photosystem 2 inhibition	2.87	99.5
	Isoproturon		2.5	99.9
Triazine	Atrazine	Photosystem 2 inhibition	2.7	99.9
	Irgarol		3.95	98.4
	Terbutryn		3.65	99.3
Chloroacetamide	Metolachlor	Inhibition of the synthesis of very long chain fatty acid	3.4	98

Log K_{ow} values from footprint database.