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Nationwide screening of surface water toxicity to algae

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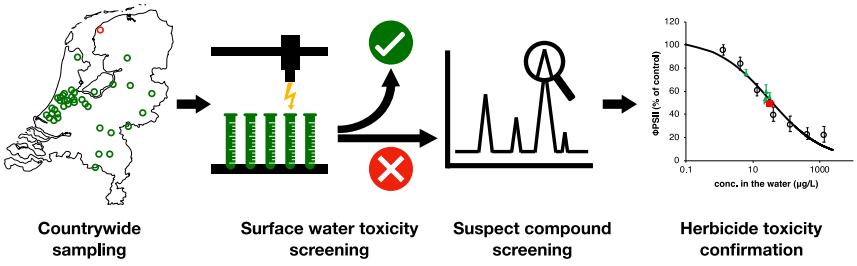
HIGHLIGHTS

- Chemical monitoring of surface water overlooks (mixture) effects of unknown compounds.
- Effect-based analyses offer a valuable tool for toxicity screening in surface waters.
- An algal photosynthesis bioassay was successfully applied to assess toxicity to algae at a nationwide scale.
- Toxicity was observed at one location, and was solely attributable to a single herbicide.
- The algal bioassay allows for efficient and effective screening of herbicide risk in surface waters.

GRAPHICAL ABSTRACT



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ABSTRACT

According to the European Water Framework Directive (WFD), chemical water quality is assessed by monitoring 45 priority substances. However, observed toxic effects can often not be attributed to these priority substances, and therefore there is an urgent need for an effect-based monitoring strategy that employs bioassays to identify environmental risk. Algal photosynthesis is a sensitive process that can be applied to identify the presence of hazardous herbicides in surface water. Therefore, the aim of this study was to employ an algal photosynthesis bioassay to assess surface water toxicity to algae and to identify the compounds causing the observed effects. To this purpose, *Raphidocelis subcapitata* was exposed to surface water samples and after 4.5 h photosynthetic efficiency was determined using PAM fluorometry. In this rapid high throughput bioassay, algal photosynthesis was affected by surface water from only one of 39 locations. Single compounds toxicity confirmation elucidated that the observed effect could be solely attributed to the herbicide linuron, which occurred at 110 times the EQS concentration and which is not included in the WFD priority substances list. In conclusion, applying the algal photosynthesis bioassay enables more efficient and effective assessment of toxicity to primary producers because it: (i) identifies the presence of herbicides that would be overlooked by routine chemical WFD monitoring, and (ii) avoids redundant chemical analyses by focusing only on (non-)target screening in samples with demonstrated effects.

1. Introduction

According to the European Union (EU) Water Framework Directive (WFD) (The European Parliament and the Council of the European

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Union, 2013), chemical water quality is determined by monitoring surface waters for the presence of 45 (groups of) priority substances. However, the use of many of these compounds is restricted or banned, and concentrations of priority substances in European waters are therefore decreasing (Altenburger et al., 2015; Fliedner et al., 2016). Simultaneously, industries have switched to a plethora of thousands of alternative compounds, which potentially enter aquatic environments and can severely impact water quality (Schwarzenbach et al., 2006). Hence, many substances on the priority list are not representative of present day contamination (Busch et al., 2016). Consequently, a large portion of toxic effects observed in surface waters cannot be attributed to compounds measured by water authorities (Altenburger et al., 2015), and toxic risk to freshwater ecosystems is thus caused by myriads of (un) known, unregulated and unmonitored compounds that are present in the environment (Daughton, 2005). Understanding of these risks requires a paradigm shift, that allows for new monitoring methods that do not depend on chemical target analysis of priority compounds, but contrastingly consider adverse biological effects first. Therefore, there is a need for an effect-based monitoring strategy that employs bioassays to identify environmental risk (Wernersson et al., 2015). Bioassay responses to surface water samples are caused by mixtures of all bioavailable (un)known compounds and their metabolites, thereby overcoming the limitations posed by chemical analysis of a limited number of target compounds (Brack et al., 2017). The indication of surface water toxicity by bioassays in turn allows for identification of locations with environmental risks, although the compounds responsible for the observed toxicity are initially unknown. However, these can subsequently be elucidated with targeted or non-target chemical analysis, which will only be necessary for locations with indicated environmental risk (Altenburger et al., 2015).

The success of this approach will rely largely on the ease of use, endpoint specificity and scale of the selected bioassays. *In vitro* or small scale *in vivo* assays with specific drivers of adverse effects allow for focused identification and subsequent confirmation of toxic compounds (Brack et al., 2016). Adequate selection of bioassays employed in water quality monitoring can thus greatly aid in narrowing down the identification of compound(s) that cause environmental risks. Microalgal photosynthesis is an example of a sensitive and well-studied bioassay endpoint that can be applied to identify hazardous effects of herbicides in surface waters (Booij et al., 2014; Muller et al., 2008; Ralph et al., 2007; Ricart et al., 2010; Sjollema et al., 2014a). In these bioassays photosynthesis is often quantified using pulse amplitude modulation (PAM) fluorometry, a rapid measurement technique suitable for quick screening purposes (Escher et al., 2008; Sjollema et al., 2014b). Algal photosynthesis is preferably quantified in light adapted cells as effective photosystem II (PSII) efficiency (ΦPSII). This end point responds most sensitively to herbicide activity (Ralph et al., 2007; Sjollema et al., 2014b), as the most commonly applied herbicides either directly target PSII, or indirectly affect ΦPSII (DeLorenzo et al., 2001; Wood et al., 2016).

Herbicides are the most frequently detected pesticide group in North American and European surface waters, and are hence expected to have a significant effect on aquatic ecosystem functioning (Moschet et al., 2014; Schreiner et al., 2016). Moreover, a wide variety of herbicides often exceed environmental quality standards (EQS) in European surface waters (Moschet et al., 2014; Schreiner et al., 2016; Smit and Kalf, 2014). Herbicides can be phytotoxic to non-target aquatic organisms such as algae, and effects on primary producers can cascade up the food web altering community structure (DeLorenzo et al., 2001; Ralph et al., 2007; Wood et al., 2016). Algae respond quickly to environmental changes (McCormick and Cairns, 1994), thus making identification of locations where algae are affected of great ecological importance, while simultaneously functioning as an early warning system for herbicide induced ecosystem changes (Bae and Park, 2014; Ricart et al., 2010). Triggered by the need to identify these herbicide induced risks to algae in surface waters, the aim of the present study was to employ an algal photosynthesis bioassay that allows for screening of surface water toxicity to algae and subsequent identification of the causing compound(s) on a nationwide scale. To this purpose, the microalga *Raphidocelis subcapitata* was exposed to surface water samples in 96-well plates. After 4.5 h, previously shown to be a sufficient exposure time for stable effect determination (Sjollema et al., 2014b), effective ΦPSII was determined using PAM fluorometry connected to an autosampler, resulting in a rapid high-throughput bioassay. Inhibitory effects on ΦPSII of surface water samples from 39 locations were assessed, and chemical analysis at the location with observed toxicity was performed to elucidate responsible compounds. For accreditation of compound contribution to the observed toxic effect, subsequent toxicity tests with individual suspected compounds were carried out.

2. Materials and methods

2.1. Sample collection

Water grab samples were collected at 39 locations within the Netherlands during May, June and July 2016 (Fig. 1). Locations were provided by the Dutch water boards and only partly originated from their regulatory monitoring networks, resulting in a scattered availability of chemical and ecological quality scores for the sampling sites. The time of sampling was chosen because late spring and early summer are relevant periods for agricultural pesticide application in The Netherlands. Water was collected in 1 L polypropylene (PP) bottles and filtered through pre-combusted (100 °C, to avoid sorption of contaminants to carbon residues on the filters) 1.2 μm glass fiber filters (GF/C Whatman) in the laboratory to eliminate autochthonous microalgae and stored overnight in the dark at 4 °C until bioassay analysis.

2.2. Test species and culturing conditions

The freshwater green microalga *Raphidocelis subcapitata* CCAP 278/4 (form. *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*)

Fig. 1. Surface water sampling locations in The Netherlands.