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Effects of low concentrations of glyphosate-based herbicide factor 540® on an agricultural stream freshwater phytoplankton community

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HIGHLIGHTS

- GBH can modify the structure and functions of phytoplankton communities.
- Physiological impacts occur at concentrations much lower than the Canadian standard.
- Shikimate content can be used as an indicator of GBH exposure in phytoplankton.

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ABSTRACT

Residual glyphosate from glyphosate based herbicides (GBH) are ubiquitously detected in streams draining agricultural fields, and may affect phytoplankton communities present in these ecosystems. Here, the effects of the exposure (96 h) of a phytoplankton community collected in an agricultural stream to various glyphosate concentrations (1, 5, 10, 50, 100, 500 and 1000 μg l^{-1}) of Factor 540® GBH were investigated. The lowest GBH concentration of 1 μg l^{-1} reduced chlorophyll *a* and carotenoid contents. Low glyphosate concentrations, such as 5 and 10 μg l^{-1}, promoted changes in the community's structure and reduced the diversity of the main algal species. At glyphosate concentrations ranging from 50 to 1000 μg l^{-1}, the phytoplankton community's composition was modified and new main species appeared. The highest glyphosate concentrations (500 and 1000 μg l^{-1}) affected the shikimate content, the lipid peroxidation and the activity of antioxidant enzymes (superoxide dismutase, catalase and ascorbate peroxidase). These results indicate that GBH can modify structural and functional properties of freshwater phytoplankton communities living in streams located in agricultural areas at glyphosate concentrations much inferior to the 800 μg l^{-1} threshold set by the Canadian guidelines for the protection of aquatic life.

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1. Introduction

The use of herbicides to control weeds in crops is a major cause of rural aquatic ecosystem contamination (Klöppel et al., 1997), since the high water solubility of herbicides in their commercial formulations facilitates their leaching from fields to nearby streams (Blanchoud et al., 2007). Glyphosate [*N*-(phosphonomethyl) glycine] based herbicides (GBH) are now the most used weed control substances worldwide due to the introduction of transgenic glyphosate-resistant (GR) crops in 1996 (Duke, 2011). Although it has been characterized as slightly mobile in soils and rapidly biodegradable (Schuette, 1998), a fraction of glyphosate and its by-products, especially the aminomethylphosphonic acid (AMPA), ends up in the waterways draining agricultural fields (Kolpin et al., 2006; Borggaard and Gimsing, 2008). Glyphosate's herbicidal effects are due to the inhibition of the shikimate pathway via

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competition with the 5-enolpyruvylshikimate-3-phosphate enzyme's (EPSP) synthase (EC 2.5.1.19), thus preventing the biosynthesis of aromatic amino acids phenylalanine, tyrosine and tryptophan (Helander et al., 2012). It is also known that glyphosate produces secondary effects on cellular metabolism (as reviewed by Gomes et al., 2014). Indeed, photosynthesis, mineral nutrition and pigment biosynthesis are altered by glyphosate. In addition, glyphosate can induce oxidative events (Gomes et al., 2014; Romero et al., 2011), which results in modulation of antioxidant system activities (Sergiev et al., 2006; Miteva et al., 2010; Gomes and Juneau, 2016; Gomes et al., 2016).

Glyphosate toxicity assessments were previously performed to investigate glyphosate toxicity on various phytoplankton species (Wong, 2000; Tsui and Chu, 2003; Cedergreen and Streibig, 2005; Ma et al., 2006; Vendrell et al., 2009; Kaushik, 2010; Lipok et al., 2010; Smedbol et al., 2017. Although these monospecific tests are useful in understanding the physiological impacts of pesticides on various species, they provide limited information related to the effects these substances might have on natural phytoplankton communities (Lewis, 1995). The “community scale approach” was previously used to assess pesticides' impact on microbial or periphytic communities (Séguin et al., 2001; Schmitt-Jensen and Altenburger, 2005; Stachowski-Haberkorn et al., 2008; Vera et al., 2010, 2012; Sura et al., 2012). This approach showed that sensitivity variations among species could modulate the aquatic microorganism communities' responses to pesticides (Bérard et al., 1999). Some of these studies focused on the effects of atrazine, nicosulfuron and isoproturon, on periphyton communities (Bérard et al., 1999; Séguin et al., 2001; Schmitt-Jensen and Altenburger, 2005), as well as the effects of an herbicide mixture (Sura et al., 2012) on microbial wetlands communities. As for glyphosate, Stachowski-Haberkorn et al. (2008) showed that a Roundup® concentration of 1 g l^{-1} can induce structural perturbations in prokaryote and eukaryote marine communities, while Vera et al. (2010) demonstrated that this herbicide can have an influence on periphyton's colonization of outdoor mesocosms by promoting cyanobacteria at the expense of diatoms. Vera et al. (2012) also demonstrated that the addition of GBH at high concentration (3.5 mg l^{-1}) in aquatic systems could decrease water quality. The increasing turbidity and total phosphorus concentrations, led to increased productivity and eutrophication in outdoor microcosms. The increasing in total phosphorus concentrations was also verified by Pizarro et al. (2015), after the addition of 2.5 mg l^{-1} of technical-grade glyphosate acid. However, these concentrations are much higher than those monitored in streams draining agricultural fields in Quebec (Canada) for the 2011–2014 period (0.04 to 18 g l^{-1} (Giroux, 2015)) and Europe from 1993 to 2011 (1.3 to 370 g l^{-1} (Horth, 2012.)), and to our knowledge, no information exists on the effects of environmental concentrations of glyphosate-based herbicide on natural phytoplankton communities from agricultural streams.

The purpose of this study was to evaluate the effects of a commercial formulation of glyphosate (Factor 540®) on a natural phytoplankton assemblage obtained from an agricultural area watercourse, by exposing this community to various concentrations of glyphosate, under controlled laboratory conditions. Species identification was determined by microscopy, to evaluate the possible effects of the herbicide on the phytoplankton's community composition. Moreover, we hypothesised that modifications on phytoplankton community might be associated to the herbicide induced changes on phytoplankton metabolism. Thus, physiological biomarkers of GBH effects, such as the pigment content, the shikimate content, photosynthetic activity as well as the oxidative stress markers (lipid peroxidation and antioxidant enzymes), were evaluated.

2. Material and methods

2.1. Sampling and test conditions

The phytoplankton community used in this study was collected from the Dumontier stream (45°36′41.38″ N and 73°51′38.55″ W), located in southern Quebec (Canada), near the city of Boisbriand, a region characterized by field crop agriculture and pressure from urban sprawl. The agricultural site, adjacent to the sampled stream, was under GR soybean and GR maize rotation. The water sampling was performed in summer (2012 07 21), 42 days after the latest GBH application.

The sampled water was filtered through nylon mesh (250 μm) to eliminate filamentous macroalgae and suspended macroparticles. To avoid nutritional constrain, soluble reactive phosphorus, inorganic nitrogen and silicate were added to the collected water following Bérard (1996). After agitating for several minutes, the pH was checked and corrected to its original value (7.4). To remove the presence of zooplankton, the water was filtered a second time using a nylon mesh (40 μm), and then the filtrate (200 ml) was transferred into 500 ml Erlenmeyer flasks.

A commercial GBH formulation, Factor540® (IPCO, Winnipeg, Manitoba, Canada) was used in the present study. This GBH was the one used in the fields neighbouring the sampling site. Throughout this paper, we refer to glyphosate concentrations of pure active substance present in the Factor 540® formulation. A pesticide stock solution of 54 mg l^{-1} concentration was prepared from the commercial formulation Factor 540®, in 0.22 μm filtered Dumontier stream water. After agitation, the solution was kept at 4 °C and was added directly into the Erlenmeyer flasks containing the phytoplankton samples (three replicates for each concentration). Glyphosate concentrations (0, 1, 5, 10, 50, 100, 500 and 1000 μg l^{-1}) were chosen based on the range of environmental concentrations found in agricultural water streams in Canada and the United States (Scribner et al., 2007; Struger et al., 2008; Giroux, 2015). Phytoplankton samples were then placed in an environmental growth chamber (MTR30, Conviron, Manitoba, Canada), under similar temperature and light conditions as the ones observed on the day before sampling (150 E m^{-2} s^{-1}; light dark cycle of 16:8 and average temperature of 15 °C, determined with a Hobo temp probe and data logger (Onset, Massachusetts, USA)), and agitated manually twice daily. Phytoplankton cell density was the same as the one found in the stream at sampling. The sampling was conducted after a period of 96 h of exposure to GBH. Glyphosate concentration originally present in the stream was evaluated at 1 g l^{-1} (±0.02 g l^{-1}) using an enzyme linked immunosorbent assay (ELISA) (Abraxis LLC, Warminster, Pennsylvania, USA) with a detection limit of 0.05 μg l^{-1}.

2.2. Phytoplankton counting and identification

After a 96 h exposure, 50 ml of each homogenized triplicate was preserved in a lugol iodine solution. Determination and quantification were then carried out at the lowest taxonomic level: subsamples of 5 ml were settled into Utermöhl settling chambers (Dawson, 1960; Edler and Elbrächter, 2010) and counted using an IMT2 inverted phase contrast microscope. Counts were carried out on 15 random fields 600X magnified for the common pico and nanoplankton species, and 150X magnified for a cross over the settling chamber for the remaining rare and microplankton species. Further effort was made when necessary to identify cells using a Leica DM-IRB at 400, 800 or 2000X magnification and different optical microscopy options like bright field, dark field and phase contrast for internal cell structure, and interferential phase contrast for the external cell ultrastructure observation. At least 145 individual cells were counted for each sample to ensure that the

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