Ecotoxicology and Environmental Safety 113 (2015) 214–222

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Modelling the effect of fluctuating herbicide concentrations on algae growth

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ARTICLE INFO

Article history:
Received 28 August 2014
Received in revised form
1 December 2014
Accepted 3 December 2014
Available online 12 December 2014

Keywords:
Isoproturon
Pulse
Recovery
Scenedesmus vacuolatus
Modelling
Cell density inhibition

ABSTRACT

Herbicide concentrations fluctuate widely in watercourses after crop applications and rain events. The level of concentrations in pulses can exceed the water chronic quality criteria. In the present study, we proposed modelling the effects of successive pulse exposure on algae. The deterministic model proposed is based on two parameters: (i) the typical growth rate of the algae, obtained by monitoring growth rates of several successive batch cultures in growth media, characterizing both the growth of the control and during the recovery periods; (ii) the growth rate of the algae exposed to pulses, determined from a dose–response curve obtained with a standard toxicity test. We focused on the herbicide isoproturon and on the freshwater alga *Scenedesmus vacuolatus*, and we validated the model prediction based on effect measured during five sequential pulse exposures in laboratory. The comparison between the laboratory and the modelled effects illustrated that the results yielded were consistent, making the model suitable for effect prediction of the herbicide photosystem II inhibitor isoproturon on the alga *S. vacuolatus*. More generally, modelling showed that both pulse duration and level of concentration play a crucial role. The application of the model to a real case demonstrated that both the highest peaks and the low peaks with a long duration affect principally the cell density inhibition of the alga *S. vacuolatus*. It is therefore essential to detect these characteristic pulses when monitoring of herbicide concentrations are conducted in rivers.

1. Introduction

Herbicides are frequently detected in watercourses (Konstantinou et al., 2006; Muller et al., 2002; Skark et al., 2004). Indeed, they can reach surface waters during rain events by surface transport or drainage (Brown and van Beinum, 2009; Freitas et al., 2008). Thus, they do not contaminate the aquatic environment continuously but rather in pulses. Several authors have described this non-continuous pattern of herbicide concentrations in rivers that is mainly linked with the rain events following application periods. They are characterised by successive short pulses of high concentrations followed by period of low concentrations of various durations (House et al., 1997; Muller et al., 2002; Reinert et al., 2002). The concentrations during pulsed exposures are often above chronic water quality criteria, and even the acute quality criteria, defined to protect aquatic life from the deleterious effects of chemicals such as herbicides (Vallotton, 2007). The effects, and thus the risk of such pulses, are therefore crucial to determine (Boxall et al., 2013).

The effects of pulsed exposures to herbicides on non-target aquatic species, i.e mainly algae and macrophytes, have been subject to question for more than a decade (Reinert et al., 2002). Some authors have tried to depict the effects these pulses may generate. In general, the impact of pulsed exposures on algae and macrophytes seem to be substance dependent (Cedergreen et al., 2005). For example, isoproturon, a photosystem II inhibitor commonly applied on cereal fields, has a lower impact in pulses than in continuous exposure (Boxall et al., 2013). Indeed, photosystem II inhibitors such as triazines and phenylureas induce toxicity during the pulse exposure, but the algae recover totally, i.e. the growth rate is similar to that of non-exposed algae, after the chemical is removed (Reinert et al., 2002; Vallotton et al., 2009). Consequently, the effect of successive pulses is lower than continuous exposure at the same concentration. Similarly, water plants exposed to a 24 h pulse of sulfonylureas seem to recover, usually reaching the same biomass as the control 6 days after the exposure

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http://dx.doi.org/10.1016/j.ecoenv.2014.12.010
0147-6513/

(Rosenkrantz et al., 2012). But this can be different for other compounds and mechanisms of action. For example, Vallotton et al. (2008b) showed that a pulse of the herbicide S-metolachlor induces a delay in recovery after exposure to algae. Along the same lines, the growth of macrophytes seems to be significantly reduced after a 48 h and a 96 h pulse of pentachlorophenol (Boxall et al., 2013). Compound-specific uptake, degradation or dissipation rates in plants, and the potential of recovery between pulses can explain these differences of effects (Boxall et al., 2013; Cedergreen et al., 2005).

The effects of pulse exposure scenarios were also assessed for more complex systems such as periphyton communities. Gustavson et al. (2003) showed that photosynthetic activity of natural periphyton communities can be strongly disturbed by low and environmentally realistic pulse concentrations of isoproturon. Laviale et al. (2011) also showed that a 1, 3 or 7-h peak exposure to isoproturon induces an inhibition of two fluorescence parameters, the effective and the optimal quantum yields of PSII photochemistry, on the periphyton community at environmentally relevant concentrations; however, 12 h after the pulse, the periphyton recovery is complete at these concentrations.

Although the effects of sequential pulses of herbicides on non-target organisms are partially depicted, very few models have been developed to predict these effects (Nagai, 2014). Such latter models, however, are of particular importance due to the large varieties of pulse scenarios. It would also be a first step for risk assessment of pulsed exposures. Recently, Weber et al. (2012) simulated the effects of successive pulse exposure to isoproturon on algae populations in a flow-through system. In that study, the authors modelled the population fluctuations as a function of four parameters: temperature, light intensity, nutrient availability and chemical concentration. But the model proposed is mainly descriptive and therefore difficult to use for effects predictions due to the lack of information on the different variables.

The aim of this study was to develop a simple model, i.e with parameters easily determinate with classical experiments (standard OECD test), able to predict the cell density inhibition of algae exposed to sequential pulses of herbicides. The model was developed to simulate the effects of photosystem II inhibitors, which are widely used in European countries. In Switzerland, they are the most common herbicides found in surface waters such as lakes (Gregorio et al., 2012). Furthermore, as mentioned above, they have the advantage of not inducing a delay in the recovery phase of algae. The model will be validated by comparing the predictions with laboratory measurements obtained with 5 typical scenarios. For the experiments, we chose to test the herbicide isoproturon, which is regularly detected in rivers up to several μg/l in pulses (Garmouma et al., 1998; IFEN, 2007; Muller et al., 2002). The alga selected was *Scenedesmus vacuolatus*, which has already been tested successfully with pulses (Vallotton et al., 2009). As an illustration, the model will also be used to predict the cell density inhibition for a realistic pulse scenario in a river.

2. Materials and methods

2.1. Chemical

Isoproturon, 3-(4-isopropylphenyl)-1,1-dimethylurea, (99% purity) was purchased from Ehrenstorfer GmbH. A stock solution of 3200 μg/l was prepared in an algae medium, in axenic conditions, for pulse exposure testing. This stock solution was kept in the fridge at 6.4 °C. The concentration was checked analytically and the measured concentrations were in the same range as the nominal concentrations (results not shown).

2.2. Algae cultures

Permanent agar culture tubes of green unicellular microalga *S. vacuolatus* (Chlorophyceae; strain 211-15, Shihira and Krauss, Melbourne, Australia) was obtained from the Department of Bioanalytical Ecotoxicology at the Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany. Microalga was cultured in a growth media described in the OECD guideline (OECD, 2011). Microalgae were cultured in the OECD medium by successive transfers in order to maintain exponential growth conditions and to possibly identify signs of abnormal growth (Le Faucheur et al., 2005). The method involves transferring regularly, i.e. every 48 h, a specific volume of algae culture, defined by a calibration curve, into a new 50 ml OECD medium. 50 ml of algal suspension were placed in erlenmeyer flasks of a capacity of 250 ml on a HT Infors shaker table (90 rpm) (Le Faucheur et al., 2005; Vallotton et al., 2008a) at 25 °C and under continuous illumination at a light intensity of 70 μmol/m^2/s provided by cool-white fluorescent lamps.

Algae were inoculated in a new culture medium with an initial optical density of 0.056 at 690 nm ($OD_{\lambda 690}$), which corresponds to a density of 650,000 cells/ml. The optical density was measured with a microplate reader (ELx800™, BioTek® Instruments, Winooski, Vermont) at a wavelength of 690 nm. The cell density (cells/ml) was determined by cell counting using the improved Neubauer Haemacytometer (Optik Labor, Lancing, United Kingdom). The calibration curve was obtained by plotting the cell density as a function of the measured optical density.

A control charter was established to monitor algae growth in growth media. In our laboratory, the average growth rate of the algae was 0.027 h^{-1} with a standard deviation of 0.002 h^{-1} (average of successive 47 cultures).

2.3. Dose–response curve of isoproturon

The dose–response curve of isoproturon, required to parameterise the model and to defined the tested concentrations, was established following a method adapted from the standard OECD procedure (OECD, 2011). The tests were performed in the same conditions as algae cultures (see Section 2.2). Five concentrations ranging from 4.6 to 256 μg/l and a control were tested in octoplicates. The optical density measured at the beginning and at the end of the test was used to evaluate the average specific growth rate for each concentration and for the control. Growth inhibition is the ratio between the growth rates of the different concentrations and that of the control (Eq. (1); see Section 2.5.1).

2.4. Pulse exposure tests

Five pulse exposure scenarios were tested in the laboratory (Fig. 1; Table 1). They differed in the duration and concentration levels of the pulses, and in the duration of the recovery periods. Two cases were considered: short pulse duration and long recovery period, and long pulse duration and short recovery period. These cases can be considered as representative of two extreme scenarios that can be found in rivers. Pulse exposure scenarios differed also in the pulses concentration tested as shown in Table 1. Algae exposed to scenarios, as well as the controls, were tested in triplicates.

The test was started the same way the algae were cultured (initial cell density 650,000 cells/ml, 50 ml in 250 ml flask) and in the same conditions (see Section 2.2). Algae grew for a short period (Fig. 1; V is around 24 h) at the beginning before being exposed to the first pulse. At the end of each pulse exposure, the algae were centrifuged twice for 7 min at 1046*g* and 25 °C. The supernate was removed and the algae were re-suspended in growth media (Vallotton et al., 2008a). These two centrifugations