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Immediate and mid-term effects of pyrimethanil toxicity on microalgae by simulating an episodic contamination

Cândida Shinn [a,b,*], Danieli Delello-Schneider [c], Lucas B. Mendes [c], André L. Sanchez [c], Ruth Müller [d], Evaldo L.G. Espíndola [c], Cristiano V.M. Araújo [a,e]

[a] IMAR-Instituto do Mar & MARE-Marine and Environmental Sciences Centre, Department of Life Sciences, University of Coimbra, Apartado 3046, 3001-401 Coimbra, Portugal
[b] Escuela de Ciencias Agrícolas y Ambientales, Pontificia Universidad Católica del Ecuador - Sede Ibarra, Ibarra, Ecuador
[c] Center for Water Resources and Applied Ecology, University of São Paulo, São Carlos, Brazil
[d] Biodiversity and Climate Research Centre (BiK-F), Department Climate and Adaptation, Frankfurt, Germany
[e] Central Department of Research, Universidad Laica Eloy Alfaro of Manabí (ULEAM), Ciudadela Universitaria, vía San Mateo, Manta, Ecuador

HIGHLIGHTS

- The effects of pyrimethanil on the growth of *Selenastrum capricornutum* were studied.
- Decrease of the pyrimethanil toxicity due to its dissipation in water was assessed.
- Pyrimethanil was applied in mesocosm and samples were taken from 1st and 10th days.
- Toxicity varied if pyrimethanil was diluted with reference water or culture medium.
- The toxicity of pyrimethanil in water was reduced at as short a period as 10 d.

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ABSTRACT

Since pesticides can represent a threat for non-target aquatic communities, including microalgae, we looked at the effects of the fungicide pyrimethanil on the growth of the freshwater green microalgae *Selenastrum capricornutum*. Additionally, attenuation of the toxicity of pyrimethanil due to its dissipation in the water was assessed. Pyrimethanil-contaminated samples were taken from outdoor mesocosms one (1.4 mg L^{-1} of pyrimethanil) and ten (0.78 mg L^{-1} of pyrimethanil) days after pyrimethanil application. Different dilutions were prepared using both nutrient-rich culture medium (LC Oligo) and non-contaminated mesocosm samples, and cell growth inhibition was assessed. Reference mesocosm samples were also diluted with LC Oligo in order to verify how the nutrient concentration in the LC Oligo could improve cell growth. Comparing cell growth of population exposed to pyrimethanil-treated sample taken at day 1 with cells growing in reference sample and LC Oligo, the growth inhibition was 80% (±6.5) and 95% (±2.0), respectively. The toxicity of samples taken from contaminated mesocosms at day 10 was attenuated to 34% (±15) (when compared with reference sample) and 88% (±3.0) (when compared with LC Oligo), as pyrimethanil concentrations in the mesocosms decreased. In conclusion, (i) pyrimethanil can be an environmental disturber for the microalgae; (ii) the toxicity of pyrimethanil in water was reduced almost 2.4 times (when compared with the reference sample) at as short a period as 10 d if assuming that pesticide entrance is not continuous; (iii) toxicity of an environmental sample could be underestimated if the sample/medium used in dilution presents different nutrient levels.

1. Introduction

Contamination by agrochemicals has been an increasing and worldwide problem for aquatic ecosystems due to spray-drift, leaching, run-off or accidental spills that occur during or after application (Verdisson et al., 2001; Dewez et al., 2005). Undesirable effects can be expected to occur directly on organisms as well as on

* Corresponding author at: IMAR-Instituto do Mar & MARE-Marine and Environmental Sciences Centre, Department of Life Sciences, University of Coimbra, Apartado 3046, 3001-401 Coimbra, Portugal. Tel.: + 351 239855781; fax: + 351 239855789.
E-mail address: candida.shinn@gmail.com (C. Shinn).

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the ecosystem functioning by impacting aquatic community structure (Ma et al., 2006). Therefore, the decision to apply a given agrochemical should consider not only its efficiency in treating the pest, but also the environmental impact at a local scale as well as on organisms inhabiting non-target areas (Schreinemachers and Tipraqsa, 2012). In this regard, many non-target aquatic organisms, including phytoplankton, have been used to assess the risk of agrochemicals and provide a broader understanding of the extent at which agricultural activities can disturb aquatic ecosystems (Lewis, 1995; Guida et al., 2008). Unfortunately, due to the resistance developed by pathogens to many agrochemicals available on the market, manufacturers are constantly developing new compounds (Reilly et al., 2012). The fungicide pyrimethanil was recently developed and has shown to be very efficient against resistant fungus strains and, therefore, its use has increased considerably (Sholber et al., 2005; Smilanick et al., 2006; Sugar and Basile, 2008; Xiao and Boal, 2009). Despite the intensive use of this fungicide, its use is not supported by an exhaustive study regarding the effects on the environmental quality of surrounding ecosystems. This is possibly related to the assumption that pyrimethanil has a short half-life, with fast degradation: the degradation half-life of pyrimethanil in sediment–water systems can reach 80 d (PPDB, 2009), while 50% dissipation from the water phase can occur between 9 and 24 d (EFSA, 2006); and recently Shinn et al. (2013) showed that the dissipation half-life of pyrimethanil was 57 and 72 d in warm and cold temperate mesocosms, respectively. However, pyrimethanil has been recently recorded in natural surface and groundwater (Reilly et al., 2012).

Some studies have addressed the toxic effects of pyrimethanil on different organisms and responses. For the cladocera *Daphnia magna* the 96 h-LC_{50} (lethal concentration to 50% of exposed organisms) was 2.9 mg L^{-1}, and the no observed effect concentration (NOEC) on reproduction after 21 d exposure was 0.9 mg L^{-1} (EFSA, 2006); and for *Daphnia pulex* the reproduction EC_{50} was 0.69 mg L^{-1} and the NOEC was 0.015 mg L^{-1} (Scherer et al., 2013). Regarding aquatic insects, the NOEC of pyrimethanil for the non-biting midge *Chironomus riparius* was 4 mg L^{-1} (Müller et al., 2012) and the EC_{50} for the phantom midge *Chaoborus flavicans* was 1.78 mg L^{-1} (Scherer et al., 2013). Within the similar pyrimethanil range, the oligochaete *Lumbriculus variegatus* revealed a NOEC of 4 mg L^{-1} with respect to reproduction (Seeland et al., 2012) and the snail *Physella acuta* presented embryo LC_{50} of 0.402 mg L^{-1} (Seeland et al., 2013). The lethal concentration for the fish *Oncorhyncus mykiss* was between 14 and 35 mg L^{-1} (van Leeuwen and Vonk, 2008) and avoidance of 50% of juvenile fish *Danio rerio* was observed at 1 mg L^{-1} pyrimethanil (Araújo et al., 2014a). The growth of the macrophytes *Lemna minor* and *Lemna gibba* were likewise inhibited by pyrimethanil with a growth EC_{50} of 23 and 7.8 mg L^{-1}, respectively (PPDB, 2009; Seeland et al., 2012).

Although freshwater microalgae are not the target of the chemical products applied in agriculture, the suspicion of the potential impact of these compounds on microalgae has generated a lot of studies which alert to the risk of excessive application and consequent contamination of aquatic environments. Many microalgae species have been used as test organisms in different studies focused on effects of contaminants in aquatic ecosystems (Lewis, 1995; Ma, 2002; Ma et al., 2003; Gómez de Barreda Ferraz et al., 2004; Dewez et al., 2005; Liu et al., 2013). The freshwater green microalgae *Selenastrum capricornutum* (=*Pseudokirchneriella subcapitata*) is an organism widely used in eco-toxicity assays to monitor disrupted ecosystems as well as to predict toxicity of different compounds (Oliveira-Filho et al., 2004; Chung et al., 2007; Rodgher et al., 2012). The sensitivity of *S. capricornutum* has favored its use as test organism in assays with uncountable agricultural trade formulations and their active ingredients (Ma et al., 2006; Guida et al., 2008; Braun et al., 2012). Regarding pyrimethanil, van Leeuwen and Vonk (2008) observed that at a concentration of 8 mg L^{-1} the population growth of *S. capricornutum* was reduced by 50%. In comparison, the growth of *Raphidocelis subcapitata* (=*S. capricornutum*) was affected by pyrimethanil at a lower level with an EC_{50} of 1.2 mg L^{-1} (PPDB, 2009). On the other hand, Seeland et al. (2012) found pyrimethanil effects on the growth of green microalgae *Desmodesmus subspicatus* and *Scenedemus acutus* at higher EC_{50} values: 13 and 23 mg L^{-1}, respectively.

To investigate the ecotoxicological effects of pyrimethanil under more realistic nutrient conditions, we simulated an episodic pyrimethanil contamination via the application of the commercial formulation Mythos® in outdoor mesocosm systems (nominal concentration aimed: 1 mg L^{-1}). The effects of pyrimethanil-contaminated mesocosm water sampled 1 d after the application on the growth of *S. capricornutum* were assessed via laboratory assays. In addition, samples of mesocosm water were taken 10 d after application in order to assess to what extent a potential dissipation of the pesticide in the system lead to a decrease in toxicity. Besides the experiments with microalgae using culture medium (rich in nutrients; OECD, 1998; ABNT, 2011) as the control to compare toxic effects, we included an additional treatment, named reference, containing natural water from oligothophic non-contaminated mesocosm systems. This treatment, used to dilute contaminated mesocosm samples, prevents any interference of the addition of nutrients on the toxicity of the contaminated sample (Müller et al., 2010). Diluting non-enriched contaminated mesocosm water with culture medium (in this case LC Oligo) could lead to an overestimation of toxicity as nutrient levels are not similar (see Table 1). On the contrary, the difference between uncontaminated and contaminated mesocosm samples would be solely due to the presence of the contaminant. Finally, reference mesocosm samples were also diluted with culture medium in order to verify how the cell growth in the non-enriched, non-contaminated sample could increase due to the nutrient levels present in the culture medium.

2. Materials and methods

2.1. Test organism

A strain of the freshwater green microalga *S. capricornutum* was cultured in a 500-mL Erlenmeyer flask containing 250 mL of LC Oligo medium following the ABNT-NBR 12648 guideline (ABNT, 2011). Cultures were maintained in continuous white light, at 22 °C and agitation (100–175 rpm; Shaker Table, Ética). The algal cells used in the assay were three days old (exponential growth phase).

2.2. Mesocosms and water sampling

The mesocosm setup was composed of two treatments: reference (non-contaminated mesocosms) and treated with pyrimethanil. Each mesocosm system consisted of 1500 L cylindrical tanks with 1.43/1.75 m (bottom/top) diameter and 0.83 m height. All mesocosms had a layer of natural sediment and were filled with water pumped from the nearby Lobo Reservoir (Itirapina, SP, Brazil, 22°10′05.60″S, 47°54′10.51″O) which during the same experimental period (October, 2012) showed to be non-toxic to the fish *D. rerio* and tadpoles of the amphibians *Leptodactylus latrans* and *Lithobates catesbeianus* (Araújo et al., 2014a,b) and cladocera *Ceriodaphnia silvestrii* (data not shown).

The commercial formulation Mythos®, which contains 300 g L^{-1} of pyrimethanil as active ingredient, was dissolved in distilled water and added to the treated mesocosms to a final nominal