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Can cyanobacterial biomass applied to soil affect survival and reproduction of springtail *Folsomia candida*?

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ABSTRACT

Biomass of cyanobacterial water blooms including cyanobacterial toxins may enter soils, for example, when harvested water bloom is directly applied as an organic fertilizer or when water with massive cyanobacterial biomass is used for irrigation. In spite of this, no information is available about the potential effects on soil arthropods. The objective of this pilot study was to evaluate the effects of water bloom biomass sampled in five different fresh water lakes on the soil dwelling arthropod, springtail *Folsomia candida* (Collembola). These samples contained different dominant species of cyanobacteria and varied significantly in microcystin content (21–3662 μg/g dw biomass). No adverse effects on survival or reproduction were observed for any tested sample at concentration up to 4 g dw biomass/kg dw soil. Despite the known hazardous properties of water blooms in aquatic ecosystems, our pilot results suggest that cyanobacterial biomass might have no significant impact on arthropods in soil. It remains a question, if this is due to low bioavailability of cyanobacterial toxins in soil.

1. Introduction

Cyanobacterial water blooms potentially containing a wide range of toxic or bioactive compounds commonly occur in eutrophic fresh water lakes all over the world (Zurawell et al., 2005). Prevalent fresh water strains of planktonic genera such as *Microcystis, Aphanizomenon, Anabaena* or *Planktothrix* are able to produce anatoxins or saxitoxins with a neurotoxic effect or lipololysaccharides that are an obligatory part of the outer cell wall of the cyanobacteria (Wiegand and Pflugmacher, 2005). However, the most prominent group within these toxins is hepatotoxic microcystins (MCs), which are more commonly encountered by humans and other animals during an occurrence of cyanobacterial blooms. They are inhibitors of protein phosphatases 1 (PP1) and 2A (PP2A) and are potent liver tumor initiators and promoters (Humpage et al., 2000; Mackintosh et al., 1990). Because of human health concerns, the occurrence of MCs in fresh water bodies is monitored in many countries, including the Czech Republic (Blahova et al., 2008; Blaha et al., 2010).

Water containing these potentially toxic water blooms is also extensively used for irrigation and so it is possible for a large amount of cyanotoxins, including MCs, to be released into cropland (Liu et al., 2008). Furthermore, in many countries harvested water bloom is directly applied as an organic fertilizer (e.g. Chen et al., 2006a, 2006b). The fate of cyanobacterial toxins in soil has not been investigated so far, with the exception of some studies on MCs. Under simulated field conditions, it seems that the major dissipation process for MCs is mainly via microbial degradation (Miller and Fallowfield, 2001; Chen et al., 2006b). The persistence half-lives of three different MCs ranged between 6.0 and 17.8 days in agriculture soils (Chen et al., 2006b). The adsorption mechanism of MCs in soil has not been elucidated clearly because of significant differences in mobility between different MCs (Chen et al., 2006b). However, it seems that the clay content and its quality may be more important for the adsorption than other soil characteristics (Miller et al., 2001; Morris et al., 2000). Chen et al. (2006b) reported that there is a highly mobile (i.e. potentially bioavailable) proportion of MCs that can be transported away from soils. Eynard et al. (2000) suggested that soil was unable to protect groundwater from toxins that originated from rivers and lakes around Riga. Thus, it seems that MCs sorption in soils is low and could potentially result in their high bioavailability to soil organisms.

Research of the effects of cyanobacterial toxins on soil organisms has been limited to several studies with terrestrial plants and one study with nematodes. It is obvious that MCs are able to directly affect the photoautotrophic metabolism by selective inhibition of protein phosphatases (Babica et al., 2006), and they have been shown to induce formation of reactive oxygen species causing oxidative stress (Chen et al., 2004; Pflugmacher et al., 2006). Li et al. (2009) studied microcystin-LR toxicity for nematode *Caenorhabditis elegans*. However, the assay was not conducted in soil, but in solution at sterile culture plates. To our knowledge, there is no information about the potential effects of cyanobacterial toxins on soil dwelling arthropods. Considering high toxicity of

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these toxins to aquatic invertebrates (Wiegand and Pflugmacher, 2005; Marsalek and Blaha, 2004; Kyselkova and Marsalek, 2000), the possible effects to soil organisms should be investigated.

The aim of the present study was to examine the toxicity of cyanobacterial biomass harvested in five different fresh water lakes in the Czech Republic on the soil-dwelling springtail *F. candida* (Collembola). We chose this standard collembolan species as a representative of soil arthropods that are highly ecologically relevant and sensitive to the soil toxicity (Fountain and Hopkin, 2005; Bezchlebová et al., 2007).

2. Materials and methods

2.1. Complex cyanobacterial samples

The origin of the samples, details on dominant cyanobacteria and the concentrations of MCs are given in Table 1. Cyanobacterial water blooms were collected with a plankton net from natural reservoirs and were freeze-dried before storage. The MCs concentrations were determined by HPLC according to the method described by Lawton et al. (1994). Freeze-dried biomass samples were resuspended in deionized water to achieve the required biomass concentrations for the toxicity tests. The suspensions of complex cyanobacterial blooms were homogenized by sonication and the homogenate with cell fragments was used as "biomass" for the experiments.

2.2. Test organisms

F. candida was cultured in plastic food containers (250 ml) with plaster of Paris and activated charcoal mixture as a substrate. Containers were placed in a climatic chamber (20 ± 1 °C; 12:12 h light:dark cycle). Granulated dried baker's yeast were put to the containers as food twice a week.

2.3. Soil

Natural arable soil from non-polluted background site was used for all tests. It was selected to have properties that should cause low sorption of MCs and their high bioavailability. The soil was loamy sand cambisol with the following particle size distribution: sand (>50 μm) 64.4%, silt (2–50 μm) 29.1% and clay (<2 μm) 6.5%. The cation exchange capacity was 16.4 meq/100 g and pH (KCl) was 6.5. Organic carbon and total nitrogen contents were 1.6% and 0.13%, respectively. Water holding capacity (WHC_{max}) of the soil was 0.58 ml/g dw soil. Concentrations of organic pollutants and metals were comparable to the background levels according to the Czech Republic limits. The soil was dried at laboratory temperature, sieved (2 mm) and stored at 4 °C before the experiments.

2.4. Toxicity tests

The toxicity tests with springtail *F. candida* were carried out according to ISO guideline 11267 (ISO, 1999). At the start of the assay, ten synchronised 10–12 day-old springtails were introduced to 30 g of soil (wet weight) in glass vessels. Vessels were closed with polyethylene foil. A few grains of granulated dry baker's yeast (2–10 mg) were added weekly on the soil surface as food. All test vessels were kept at 20 ± 1 °C under a light:dark cycle of 12:12 h for 4 weeks. Adults and juveniles were counted after flotation extraction and coloring the water surface with ink.

In the first experiment demonstrating the concentration dependency, biomass A (Table 1) was tested at three concentrations. At the start of the assay, the dry soil was rewetted to 50% WHC using 0.29 ml/g dw soil of the suspension of the cyanobacterial biomass in water and homogenized thoroughly with a small spatula. The concentrations of the cyanobacterial biomass corresponded to 4.8, 48 and 480 mg/kg dw soil. Counting with the MCs content in biomass A (Table 1), this corresponded approximately to 0.1, 1 and 10 μg/kg dw soil. Each concentration was tested in five replicates. Control soil was moistened only with deionized water and was tested in ten replicates.

Because no effect was observed in the first experiment, four different cyanobacterial biomasses B, C, D and E, including those with high MCs content (Table 1), were chosen for the second experiment. At the start of the assay, the soil was rewetted to 30% WHC using 0.17 ml/g dw soil of the suspension of cyanobacterial biomass in water and homogenized thoroughly with a small spatula. The concentration of cyanobacterial biomass corresponded to 1 g/kg dw soil. Vessels were covered with the foil with openings to enable water evaporation and repeated moistening. One, two and three weeks after the start of the assay, the same amount of the suspension of cyanobacterial biomass was added to the soil surface to simulate repeated irrigation at a field. Hence, the amount of added biomass increased by 1 g/kg dw soil every week, accumulating to a total of 4 g/kg dw soil one week before the end of the assay. With regard to MCs concentrations (Table 1), this corresponded to 0.32–14.4 mg/kg dw soil at the end of the test. Each biomass was tested in four replicates. Seven control vessels were run the same way, receiving deionized water only.

2.5. Statistics

Differences between the treatments and controls were compared using analysis of variance (ANOVA) followed by the Dunnet's post-hoc test. *P*-values less than 0.05 were considered statistically significant. Calculations were performed with the Statistica 8.0 (StatSoft, Tulsa, USA).

3. Results and discussion

All validity criteria defined for the untreated controls in ISO guideline 11267 (ISO, 1999) were fulfilled in both experiments. No significant adverse effects (ANOVA, $p > 0.05$) on survival or reproduction were observed after the exposure of springtails to the concentration range of biomass A in the first experiment (Table 2) or to different biomasses B, C, D and E in the second experiment (Table 3). Biomass D induced weak stimulation of the reproduction, which was statistically significant (Dunnet's test, $p < 0.05$). This effect could be related to the addition of high amount of organic material to the soil, which may serve directly as food for springtails or stimulate microorganisms in the soil, which may be consumed by springtails afterwards. Stimulation of springtail reproduction by nutrients was previously reported by Kaneda and Kaneko (2004),

Table 1
Characterization of the cyanobacterial samples used in the toxicity tests with the springtail *Folsomia candida*.

Biomass	Location	Sampling date	Dominant species	Concentration of microcystins in the biomass
A	Brno reservoir	8/10/2004	*Microcystis wessenbergii* (> 75%) *Microcystys ichthyoblabe* (10%) *Planktothrix* sp. (1%)	21 mg/kg dw
B	Vranov	22/11/1997	*Microcystis aeruginosa* (> 98%)	957 mg/kg dw
C	Loudilka	4/10/2004	*Microcystis aeruginosa* (98%) *Diatoma* sp. (2%)	3662 mg/kg dw
D	Březová	17/7/2004	*Microcystis aeruginosa* (17%) *Anabaena flos-aquae* (30%) *Aphanizomenon flos-aquae* (23%) *Woronichinia naegaliana* (23%)	2442 mg/kg dw
E	Lučina VN	10/10/2005	*Woronichinia naegeliana* (70%) *Microcystis aeruginosa* (15%) *Microcystis flos-aquae* (5%) *Anabaena lemmermanii* (10%)	79 mg/kg dw