Available online at www.sciencedirect.com
SCIENCE DIRECT®
Ecotoxicology and Environmental Safety 64 (2006) 163–170
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Toxicity of a cyanobacteria bloom in Barra Bonita Reservoir (Middle Tietê River, São Paulo, Brazil)

Rosana Barbosa Sotero-Santos[a], Carlos Roberto De Souza E. Silva[a], Nelsy Fenerich Verani[b], Keico Okino Nonaka[c], Odete Rocha[a,*]

[a]*Departamento de Ecologia e Biologia Evolutiva, Centro de Ciências Biológicas e da Saúde, Universidade Federal de São Carlos, Via Washington Luis, km 235, CEP 13.565-905 - São Carlos, SP, Brazil*
[b]*Departamento de Hidrobiologia, Centro de Ciências Biológicas e da Saúde, Universidade Federal de São Carlos, Via Washington Luis, km 235, CEP 13.565-905 - São Carlos, SP, Brazil*
[c]*Departamento de Ciências Fisiológicas, Centro de Ciências Biológicas e da Saúde, Universidade Federal de São Carlos, Via Washington Luis, km 235, CEP 13.565-905 - São Carlos, SP, Brazil*

Received 20 May 2004; received in revised form 17 February 2005; accepted 10 March 2005
Available online 1 July 2005

Abstract

In eutrophic waters during cyanobacterial bloom lysis, a blend of cyanobacterial toxins and other compounds are released into the water, affecting aquatic communities. This research investigated the effect of a simulated cyanobacterial lysis event. For this purpose, intact cells from a natural cyanobacterial bloom from Barra Bonita Reservoir (Tietê River basin, Brazil) were taken, and the cells were broken by repeated freeze/thaw cycles. The toxicity of the crude cyanobacterial extract was investigated using cladocerans (*Daphnia similis* and *Ceriodaphnia silvestrii*), and the hepatotoxicity of the cyanobacterial lyophilized material was confirmed by mouse bioassay. The results obtained using *D. similis* and *C. silvestrii* acute bioassays indicated 24-h LC_{50} values of 186.61 and 155.11 mg L^{-1}, respectively. The 24-h LD_{50} determined by intraperitoneal injection into mice was 445.45 mg dry kg^{-1}. Microcystin content was 311 μg g^{-1} dry wt freeze-dried cyanobacteria. The acute tests with cladocerans were effective in indicating the toxicity of the crude cyanobacterial extract and in prognostiating the toxic effects of cyanobacterial blooms, at least on some usual components of the aquatic community, such as microcrustaceans.

Keywords: Cyanobacteria; Bloom; Toxicity tests; *Microcystis aeruginosa*; Cyanobacterial toxins

1. Introduction

Cyanobacterial blooms are an increasing problem, worldwide, in both freshwater and marine environments. Massive proliferation of these organisms is a clear sign of eutrophication. In Brazil, the most common cyanobacterial genera found in eutrophic freshwaters are *Microcystis*, *Anabaena*, *Anabaenopsis*, and *Cylindrospermopsis* (Tundisi and Matsumura-Tundisi, 1992; Domingos et al., 1999).

Generally, freshwater blooms of cyanobacteria produce toxic secondary metabolites, and most of them are bioactive as hepatotoxins, neurotoxins, or skin irritants (Carmichael, 1996; Fastner et al., 2001; Chorus, 2001). Among the hepatotoxins, the microcystins are the most commonly detected. The chemical structures and mode of action of a number of these cyanotoxins, such as microcystins, nodularin, and anatoxins, have been studied by Codd et al. (1989), Carmichael (1992), and Carmichael and Falconer (1993). Observations of the impact of these pure toxins on various organisms are numerous (e.g., Codd et al., 1989; Codd, 1995; Fastner et al., 1995; Dawson, 1998; Törökne et al., 2000; Wiegand et al., 2002).

Most studies on toxic effects of cyanobacteria have involved the use of isolated toxins (Pietsch et al., 2001), although the effects of cyanobacterial crude extracts on rat

*Corresponding author. Fax: +055 (16) 335 18322.
E-mail address: doro@power.ufscar.br (O. Rocha).

0147-6513/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2005.03.011

hepatocytes, on embryos and eggs of fishes and amphibians, and on microalgae, macrophytes and invertebrates have also been investigated (Fastner et al., 1995; Oberemm et al., 1999; Pietsch et al., 2001; Marsálek and Bláha, 2004). Other studies have also reported the effects of hepatotoxins (microcystins) and other toxic compounds, such as lipopolysaccharides, on fishes (Best et al., 2002, 2003).

In nature, bioactive compounds may threaten all aquatic organisms that coexist with or feed on cyanobacteria. Of particular interest are the effects on the microcrustaceans *Daphnia* spp., which are potential grazers of planktonic cyanobacteria. Daphnids are common representatives of Cladocera, a key group of organisms in freshwater systems, and disturbance of their populations may have effects throughout the aquatic food chain (Rohrlack et al., 2003). Some laboratory studies have examined cyanobacteria–zooplankton interactions, focusing mostly on the effects of cultured strains of cyanobacteria on cladocerans, especially on species from the temperate zone (Lampert, 1981; Gilbert, 1990; DeMott et al., 1991; Reinikainen et al., 1995; Thostrup and Christoffersen, 1999; Lürling, 2003; Rohrlack et al., 2003; Gustafsson and Hansson, 2004). However, Data on the effects of natural blooms of cyanobacteria and their effects on tropical zooplankton communities are lacking. Also, there are not many studies on zooplanktonic organisms exposed to toxic algae and/or their toxins using field material collected during natural bloom events.

In the present study, the effects of toxic cyanobacteria from Barra Bonita Reservoir were investigated by performing, short-term acute toxicity tests on cladocerans (*D. similis* and *Ceriodaphnia silvestrii*), using crude cyanobacterial extract. The main objective was to evaluate the possible effects of cyanobacterial blooms on zooplankton invertebrates.

2. Description of the study site

Barra Bonita is a eutrophic reservoir located in the Tietê River basin, São Paulo State, Brazil (22°29′S and 48°34′W). This reservoir is in the most populous and industrialized region of South America and receives heavy inputs of domestic, industrial, and agricultural effluents. The main morphological features of the reservoir are: area 310 km^2, volume 3.135 $m^3 \times 10^6$, average depth about 10.1 m, and average water retention time of 90 days (CESP, 1998; Barbosa et al., 1999).

3. Material and methods

3.1. Sampling

Material from cyanobacterial scum was collected with a 25-μm-mesh phytoplankton net in November 2002 at Barra Bonita Reservoir. The cyanobacterium *Microcystis aeruginosa* was dominant in the field at this time. The freeze-dried cells were stored at −20 °C.

3.2. Water quality parameters

To evaluate water quality, samples for laboratory analysis were collected in Barra Bonita Reservoir, and simultaneously, in situ, the temperature, pH, conductivity, and dissolved oxygen in the water column were measured with a Horiba U-10 multiprobe (Horiba, Co., Japan). Nutrient concentrations in the water were determined in the laboratory by methods described by Koroleff (1976) for ammonium and total ammoniacal nitrogen; Golterman et al. (1978) for total dissolved and inorganic phosphate and also organic nitrogen; Mackereth et al. (1978) for nitrate and nitrite; and APHA (1995) for total organic phosphorus.

3.3. Preparation of crude cyanobacterial extract

Lyophilized bloom material was dissolved in distilled water, frozen, and thawed at room temperature. This freeze/thaw cycle was repeated four times. After the last cycle the thawed material was ultrasonicated for 10 min (procedure adapted from Oberemm et al., 1999; Pietsch et al., 2001). Finally, debris was removed by centrifuging at 3500 rpm for 30 min. The crude extract concentration was 500 mg L^{-1}.

3.4. Daphnid cultures

Stock cultures, maintained at the Ecotoxicology Lab of the Federal University of São Carlos, Brazil, for several years, were used in acute toxicity tests. *Daphnia similis* and *C. silvestrii* (Cladocera, Crustacea) were cultured in 2-L glass jars in a standard culture medium (modified from CETESB, 1991, 1992) in a temperature-controlled chamber at 25 °C and 12/12-h light–dark cycle. Reconstituted water was used as culture medium (pH 7.2–7.6, conductivity 160 μS cm^{-1}, and hardness between 42 and 48 mg $CaCO_3$ L^{-1}). Daphnids were fed with an algal suspension of *Selenastrum capricornutum* at the concentration of 10^5 cells mL^{-1}) and a mixture of yeast and fermented trout chow (CETESB, 1992; ABTN, 2003).

3.5. Acute toxicity tests

Acute toxicity tests were carried out with a tropical (*C. silvestrii*) and a temperate (*D. similis*) cladoceran, analyzing their sensitivity and survival rate during exposure to aqueous crude extracts of *M. aeruginosa*. The basic design of the experiment was to expose five neonates (< 24 h) of each cladoceran species in a glass tube with 10 mL of aqueous crude extract of *Microcystis*, at concentrations of 0, 25, 50, 100, 200, 300, and