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Ecotoxicological risks of calcium nitrate exposure to freshwater tropical organisms: Laboratory and field experiments

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ABSTRACT

This study aimed to analyze laboratory and field data to assess the ecotoxicological risks of calcium nitrate exposure to freshwater tropical biota. Short-term laboratorial tests resulted in estimated EC_{50} values of 76.72 (67.32–86.12) mg $N\text{-}NO_3^-$ L^{-1} for *C. silvestrii* and 296.46 (277.16–315.76) mg $N\text{-}NO_3^-$ L^{-1} for *C. xanthus*. Long-term laboratorial tests generated IC_{25} values of 5.05 (4.35-5.75) and 28.73 (26.30–31.15) mg $N\text{-}NO_3^-$ L^{-1} for *C. silvestrii* and *C. xanthus*, respectively. The results from *in situ* mesocosm experiments performed in the Ibirité reservoir (a tropical eutrophic urban water body located in SE Brazil) indicated that *C. silvestrii* and *C. xanthus* were not under severe deleterious acute impact due to the treatment because the higher nitrate concentrations determined were 5.2 mg $N\text{-}NO_3^-$ L^{-1} (t=24 h; sediment-water interface) and 17.5 mg $N\text{-}NO_3^-$ L^{-1} (t=600 h; interstitial water). However, an abrupt decrease in the densities of Cyanophyceae members and other benthic taxa was observed. In summary, the present work contributes greatly to the toxicity data linked to two taxonomically distinct organisms that have never been screened for calcium nitrate sensitivity. Furthermore, considering the problem of the management and restoration of eutrophic environments, our study reports a comprehensive field assessment that allows the elucidation of the possible toxic impacts caused by the addition of calcium nitrate (a remediation technique) on aquatic and benthic organisms as well as the implications on the aquatic ecosystem as a whole, which may greatly allow expanding the current knowledgebase on the topic.

1. Introduction

The suitable provision of good-quality water is a crucial factor for social and economic developments. However, aquatic ecosystems around the world have been subjected to a variety of environmental stressors, including the release of pollutants and the inappropriate management of the available resources (Takenaka et al., 2006; Taylor and Owens, 2009). Accordingly, the demand for clean water and the increasing awareness of the adverse effects of pollution on aquatic ecosystems, and consequently human health, highlight the urgency for developing affordable technologies to control degradation and recover water quality (Yuan and Wu, 2007).

Linked to this context, the nitrate ion (NO_3^-) is an important source of anthropogenic nitrogen pollution in the environment (Camargo and Alonso, 2006; Ortiz-Santaliestra and Sparling, 2007; Mann et al., 2009). Although nitrate naturally occurs in surface waters because of natural processes, increasing levels of this nutrient have been determined in groundwater and surface water due to pollution from terrestrial run-off, sewage outfall, wastewater release from industries and soil leaching from agricultural areas (Muir et al., 1991; Scott and Crunkilton, 2000; CCME, 2003; Gheju et al., 2006; Rivett et al., 2008; Mann et al., 2009; Stelzer and Joachim, 2010).

The high concentration of nitrate accumulating in aquatic ecosystems causes great concern, and the main preoccupations are its health and environmental effects once its concentration can reach toxic levels that impair the ability of animals to survive, grow and reproduce (Scott and Crunkilton, 2000; Ortiz-Santaliestra and Sparling, 2007; Rivett et al., 2008). Nitrate toxicity is mainly related with its conversion to nitrite, which is able to convert oxygen-carrying pigments into forms that are incapable of

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carrying the gas. Moreover, nitrite reacts with compounds in the stomach and gut to form products that have been classified as carcinogens (Gheju et al., 2006; Rivett et al., 2008) and also acts as a disrupter of multiple physiological functions, including ion regulatory, respiratory, cardiovascular, endocrine and excretory processes (Guillette and Edwards, 2005; Hannas et al., 2010; Alonso and Camargo, 2013). It has also been speculated that nitrate induces hypothyroidism and is capable of developing estrogenic properties in an individual (Mann et al., 2009).

Despite such undesirable outcomes, the potential toxicity of nitrate to aquatic organisms has been ignored, probably because it has been assumed that ammonia and nitrite are more toxic forms (Scott and Crunkilton, 2000; CCME, 2003; Camargo et al., 2005; Mann et al., 2009) or because other consequences, such as eutrophication, tend to occur at nitrate concentrations that are much lower than those thought to be acutely toxic to aquatic organisms (Soucek and Dickinson, 2012).

In addition to the increase in nitrate concentrations related to the aforementioned anthropogenic activities, many studies have demonstrated the effectiveness of calcium nitrate to control the release of phosphorus from eutrophic sediments, suggesting that elevating the oxidation capacity in a lake through nitrate addition may be an important intervention for preventing eutrophication. The use of calcium nitrate in eutrophic environments was first proposed by Ripl (1976) in a Swedish lake. After the good-recovery results achieved in this pioneer test, calcium nitrate has been successfully used in many other ecosystems to reverse eutrophic conditions (Foy, 1986; Feibicke, 1997; McAuliffe et al., 1998; Murphy et al., 1999; Wauer et al., 2005a, 2005b; Hemond and Lin, 2010; Ripl, 2010; Yamada et al., 2012).

Unfortunately, despite the proposal of using calcium nitrate as a restoration method, very few studies related to this technique have mentioned the effects of calcium nitrate application on the aquatic communities. To the best of our knowledge, the microcosm experiments performed by Janke et al. (2011) and Yamada et al. (2012) were the first attempt to assess the toxicity of calcium nitrate during remediation treatment. These studies demonstrated that this technique presents a transient potential for exerting adverse acute effects in aquatic organisms. The most recent study conducted by Liu et al. (2014) also aimed to assess the potential toxicity induced by calcium nitrate injection, and similarly to the two previously cited works, these researchers stated that the release and accumulation of inorganic nitrogenous compounds may severely influence the ecosystem until they transform into less harmful forms. Such studies are very important because they indicate that precautions must be taken before calcium nitrate application, but they only noted acute effects. Therefore, chronic toxicity investigations are still in strong demand for providing extended information about this technology.

In this scenario, the main objective of the present study was to analyze laboratory and field data in order to assess the ecotoxicological risks of calcium nitrate exposure to freshwater tropical biota. For this purpose, bioassays using planktonic (*Ceriodaphnia silvestrii*) and benthic (*Chironomus xanthus*) organisms were performed to evaluate the sensibility of these species to exposure to calcium nitrate under laboratorial controlled conditions. Acute and chronic endpoints, such as survival rate, brood size and emergence rate, were evaluated. A second goal was to analyze the toxic impacts of calcium nitrate on *C. silvestrii* and *C. xanthus* through *in situ* mesocosm experiments developed in the Ibirité reservoir (a tropical eutrophic urban water body located in SE Brazil). In this field trial, it was deemed important to include analyses of the structures of phytoplanktonic and benthic communities as a way to broaden the understanding of the deleterious effects caused by calcium nitrate on resident organisms.

We believe that addressing the effects of calcium nitrate on tropical organisms, such as *C. silvestrii* and *C. xanthus*, has great potential for obtaining reliable data to guide the management of tropical water bodies and to compare the results and sensitivities obtained for other species and environments, providing support for a better management of global water resources. It is also expected that the results obtained in the present study may be used to support the future implementation of a recovery program in the Ibirité reservoir, which is extremely important to the industrial sector as well as to recreational practices for the population residing in the metropolitan area of Belo Horizonte city, Minas Gerais state. A brief description of this study area is provided in Figs. ESM1–ESM3 Online Resource. More details linked to the deterioration of this aquatic ecosystem were well characterized previously by Garcia et al. (2009), Moreno and Callisto (2006), Lanza et al. (2011), Mozeto et al. (2012) and Mozeto et al. (2014).

2. Materials and methods

2.1. Laboratorial bioassays

The sensibility of *C. silvestrii* and *C. xanthus* to calcium nitrate was assessed through lethal and sub-lethal bioassays, as will be described in the following sections. Both species were obtained from the Center for Water Resources and Applied Ecology (CRHEA), which is part of University of São Paulo (USP), São Carlos, São Paulo, Brazil. Short descriptions of these organisms are presented in Figs. ESM4 and ESM5 Online Resource. Before conducting the bioassays, the scientific information available about nitrate acute toxicity was revised to propose some concentrations to be evaluated in preliminary tests (data not shown). After the preliminary tests, the range of concentrations to be repeatedly tested was defined, as will be described in the subsequent sections. The higher nominal nitrate concentration to be tested in chronic tests was defined as $EC_{50}/2$. To set other concentrations, a dilution factor of 1.5 or 2.0 was used. Nontoxic polystyrene cups served as test chambers for all of the toxicity tests performed in this study. Dilution water and control water were the same as those used in the cultivation. Calcium nitrate was purchased from Synth® (Diadema, São Paulo, Brazil-reagent grade, lot 132083, reported purity: 99%). Determinations of the pH (Micronal B374 potentiometer), electrical conductivity (Orion 145A conductivimeter), dissolved oxygen (YSI OD meter) and hardness (EDTA titration—APHA (1999)) were performed at the beginning and end of all of the exposure periods. The nitrate and nitrite concentrations were determined by flow injection analysis based on gas diffusion and conductometry measurements (Faria and Pasquini, 1991).

2.1.1. C. silvestrii

The culture procedures, bioassays and assurance of the culture's quality were performed following the instructions presented by Fonseca and Rocha (2004a) and ABNT (2004, 2005).

2.1.1.1. Culture. Glass beakers were used as cultivation containers. The organisms were cultured in reconstituted natural water (hardness: 40–48 mg $CaCO_3\ L^{-1}$; pH: 7.0–7.6). The cultures were fed with *Pseudokirchineriella subcaptata* (1×10^5 cells mL^{-1}) supplemented with Fleishman® yeast extract ($1\ mg\ L^{-1}$) and incubated at constant room temperature ($25 \pm 2\ °C$) with a photoperiod of 16 h of light (fluorescent illumination) and 8 h of dark. The culture water was renewed, food was added and the produced offsprings were discarded twice weekly.

2.1.1.2. Acute toxicity tests (water). Neonates aged less than 24 h were exposed to six concentrations of calcium nitrate solution in 48-h survival static tests. Four replicates, each containing five