

Risk assessment of manganese contamination in cyanobacteria and zooplankton resting eggs

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Manganese is an essential micronutrient to the normal growth and development of both vegetables and animals. However, high concentration of this element can become highly toxic to the organism (Pittman, 2005).

A stress agent alters the homeostatic state of the organism which responds in order to restore it. Such responses can be used as a tool to evaluate the environment quality, giving subsidy to bio-monitoring technique

From the growing interaction between the mining-industrial sector and the scientific community, research have concentrated their efforts to provide knowledge and technology to assist the recovering, mitigation and monitoring of the negative impacts caused by exploitative activities of natural resources, including the mining sector.

In this work we evaluate responses of aquatic microorganisms (cyanobacteria strains and resting eggs of zooplankton) exposed to low and supra-optimal supply of manganese. The effects of Mn were evaluated based on cyanobacteria growth and hatching rate of resting eggs of zooplankton. Two distinct strains of cyanobacteria were used as test organisms- *Synechococcus* sp. and *Nostoc* sp while for zooplankton resting eggs of *Daphnia ambigua* were used. All cyanobacteria cultures were placed in Erlenmeyer containing 150 mL of BG-11 culture medium added of increasing doses of Mn (0.5, 1.0, 5.0, 15.0 and 50.0 mg L⁻¹) as MnCl₂·4H₂O. The

control groups were cultivated in BG-11 medium without the addition of Mn. The pH of each solution was kept in 6.0 by using MES buffer. The cultures growth were evaluated by reading absorbance in spectrophotometer every day of exposure for 96 hours. After the period of exposure the cultures were centrifuged and washed in MES-EDTA (20mM and 10mM) and then subsamples were taken to chlorophyll analysis and determination of accumulated Mn content in the cells.

The resting eggs were isolated from sediments by using the sugar flotation method (Onbé, 1978) and decapsulated with hypochlorite solution (Paes, et al., 2016). The isolated eggs were exposed to the same Mn concentrations of the cyanobacteria experiment above mentioned. The experiment was carried out in incubator with a photoperiod of 12 h and temperature of 20°C, adequate condition to hatching resting eggs of *D. ambigua*.

Synechococcus sp. and *Nostoc* sp. showed different responses under stress conditions of Mn. The growth of *Synechococcus* was highly affected under high concentrations of Mn and a small reduction (17 %) in lower concentration after 72 hr of exposure (Figure1). On the other hand, *Nostoc* growth only showed a small reduction (16%) in higher concentration of Mn and after 72hr of exposure.

The excess of Mn in solution decreased the chlorophyll content in *Synechococcus* cultures.

These decline occurred differently among the treatments. Nevertheless, the content of Mn in dry biomass of *Synechococcus* exposed to Mn did not differ compared to control group. This finding shows that *Synechococcus* did not accumulate Mn into the cells. However, the growth reduction in parallel to decreased chlorophyll content suggests that Mn affects negatively the metabolism of *Synechococcus* even at low levels (0.5 mg/L after 96 hr).

The experiment using resting eggs did not show significantly effect of Mn on the hatching rate ($F_{(5, 24)} = 0.79$; $p = 0.57$) (Fig. 2). However, under concentration of 50 mg.L⁻¹ of Mn the individuals did not survive, thus demonstrating a highly toxic effect of the Mn at high concentration.

Analysis of responses at high Mn concentrations are important to establish the tolerance level and selectivity of the species, besides guiding further bioindication and bioremediation studies focusing in contaminated environments at high levels of Mn.

The present work allows to conclude that toxic effects of Mn differ among species and time of exposure which might be very dangerous for some organisms even at low concentration while others it will depend of long-term exposure.

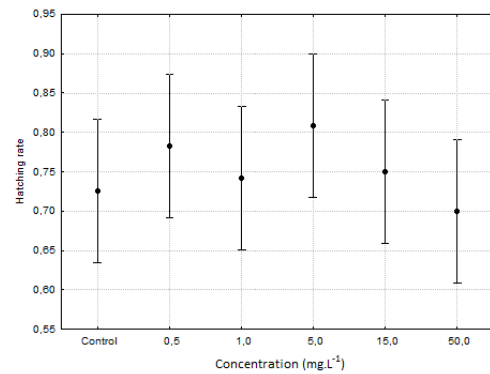


Figure 2. Hatching of *Daphnia ambigua* resting eggs (%) exposed to different concentrations of manganese (n = 120 eggs/treatment). Vertical bars denote 0.95 confidence intervals.

The factors that are involved in toxic effect of Mn are still unknown and these are our challenges for future work.

Reference

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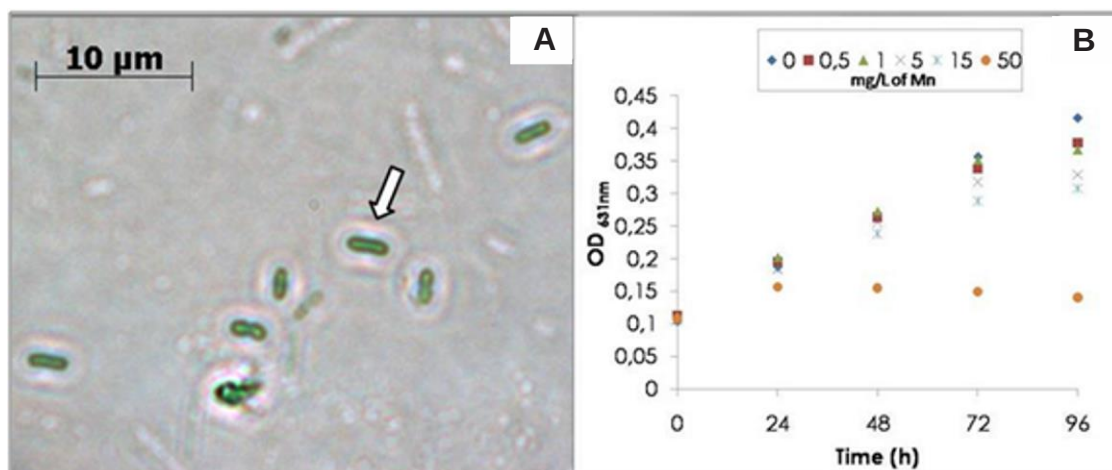


Figure 1. A – *Synechococcus* cells (white arrow). B – Growth behavior of *Synechococcus* subjected to different concentrations of Mn. OD = Optical density