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How do toxic metals affect harmful cyanobacteria? An integrative study with a toxigenic strain of *Microcystis aeruginosa* exposed to nickel stress

Erika Berenice Martínez-Ruiz, Fernando Martínez-Jerónimo *

Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Laboratorio de Hidrobiología Experimental, Carpio y Plan de Ayala S/N, Col. Santo Tomás, Mexico, D.F. 11340, Mexico

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ABSTRACT

Nickel (Ni) is an essential metal for some organisms, but also a common toxic pollutant released into the water. Toxicity of Ni has not been completely established for cyanobacteria; for this reason, we evaluated the effect of sub-inhibitory Ni concentrations on a toxigenic strain of *Microcystis aeruginosa* and on microcystins production. Population growth, photosynthetic pigments concentration, biomarkers, including antioxidant enzymes (catalase [CAT], glutathione peroxidase [GPx], and superoxide dismutase [SOD]), as well as macromolecules (proteins, carbohydrates and lipids) were quantified; SEM and TEM observations were also performed. Population growth was affected starting at 3 $\mu g\ L^{-1}$, and at 24 $\mu g\ L^{-1}$ growth was completely inhibited; the 96-h Ni^{2+} IC_{50} was 3.7 $\mu g\ L^{-1}$. Ni exposure increased pigments concentration, augmented all the macromolecules, and increased activities of CAT and GPx; alterations on the internal cell structure were also observed. The integrated biomarker response revealed that Ni^{2+} augmented the antioxidant response and the macromolecules content. Ni stress also increased microcystins production. *M. aeruginosa* was affected by Ni at very low concentrations, even lower than those established as safe limit to protect aquatic biota. Aside from the toxic effects produced in this cyanobacterium, stimulation to produce toxins could potentiate the environmental risks associated with water pollution and eutrophication.

1. Introduction

Eutrophication is the process where some limiting nutrients, like nitrogen and phosphorus, increase in water bodies and promote primary productivity. This process occurs in a natural way, but in recent years it has been accelerated by some anthropogenic causes (Smith, 2003; Kulikova and Syarki, 2004). One of the main problems related with the increase of nutrients are algal blooms, which frequently include cyanobacteria (Kormas et al., 2011), originating noxious cyanobacterial blooms. Cyanobacteria are one of the first groups that colonized the Earth (Moreira et al., 2013); in water bodies, they are part of the phytoplankton community, responsible for organic carbon production and oxygen release (through photosynthesis); for this reason, they are the basis of all the trophic webs in aquatic environments.

Bloom-forming cyanobacteria produce degradation of the water quality due to oxygen depletion, increase in turbidity, production of nasty odors and tastes, and mainly for the ability of some cyanobacteria to produce biologically-active secondary metabolites (Paerl, 2014; Beversdorf et al., 2015; Wang et al., 2016). These metabolites may include toxins, known as cyanotoxins, that are related to toxic effects on fish, algae, zooplankton, other aquatic organisms (Wiegand and Pflugmacher, 2005), and mammals (Boopathi and Ki, 2014), including humans (Drobac et al., 2013). Different cyanotoxins have been identified and have been classified according to their mode of action in mammals as hepatotoxins, neurotoxins, and dermotoxins (Merel et al., 2013).

Cyanobacteria blooms can thrive in water bodies polluted with domestic and industrial wastes (Polyak et al., 2013; Prasath et al., 2014; Fistarol et al., 2015); the combination of chemical pollution and byproducts of blooms could have higher environmental impact than that expected from each individual event.

The release of chemical pollutants by anthropogenic activities has increased in recent years (Peñuelas and Fillela, 2002), causing toxic effects on the growth, reproduction, and development of aquatic biota (Arunakumara and Zhang, 2008; Dudkowiak et al., 2011; Hermenean et al., 2015). Among the most common toxic contaminants released to the environment are metals, among these, nickel (Ni) is relevant for its environmental impact.

* Corresponding author.
E-mail addresses: ferjeronimo@hotmail.com, fjeroni@ipn.mx (F. Martínez-Jerónimo).

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Worldwide Ni production ranges from 1.4 to 1.933 million tonnes (http://www.insg.org/stats.aspx; https://www.nickelinstitute.org/NickelUseInSociety/AboutNickel/HowNickelIsProduced.aspx), and anthropogenic emissions of this metal to aquatic environments reach 33.1 to 194.2 10^3 t $year^{-1}$ (Tercier-Waeber and Taillefert, 2007). Sources of Ni pollution are mining and all the processes around this activity; Ni-based alloys, combustion of fossil fuels, incineration of municipal wastes, as well as the production and disposal of NiCd and secondary batteries (Cempel and Nikel, 2006; Nnorom and Osibanjo, 2009; Ahmad and Ashraf, 2011; Yu et al., 2014). Specifically, the content of Ni in rechargeable NiCd batteries exceeds 38 times the Toxicity Threshold Limit Concentration established by the California Department for Toxic Substance Control (Nnorom and Osibanjo, 2009). The environmental impact of these common use products has been perfectly described as dangerous (Majeau-Bettez et al., 2011; Yu et al., 2014).

Nickel is an essential metal for the cellular development of some eukaryotes and prokaryotes, including cyanobacteria (Muyssen et al., 2004; Poonkothai and Vijayavathi, 2012). Low concentrations of this metal are essential for phytoplankters, because it is required for some enzymatic activities (Muyssen et al., 2004); nevertheless, at higher concentrations this metal can affect their photosynthetic system (Boisvert et al., 2007) and growth (Manju and Balakrishnan, 2001). Ni is toxic to plants (Ahmad and Ashraf, 2011), microalgae and cyanobacteria (Mohammady and Fathy, 2007; Martínez-Ruiz and Martínez-Jerónimo., 2015), fish (Kubrak et al., 2012), and humans (Das et al., 2008; Poonkothai and Vijayavathi, 2012). This metal can also produce reactive oxygen species (ROS) and cause oxidative stress (Valko et al., 2005; Das et al., 2008) that can damage macromolecules, such as lipids, carbohydrates, proteins, and DNA, leading to cell death; ROS also affect the activity of antioxidant enzymes (van der Oost et al., 2003; Morón and Castilla-Cortázar, 2012).

Microcystis aeruginosa, the most common cyanobacteria found in blooms (Oberholster et al., 2004; Yoshida et al., 2008; Wu et al., 2015), is a toxigenic organism that produces different types of microcystins. These toxins are cyclic heptapeptides, soluble in water, very stable under natural conditions, they affect water quality and produce biological damages in freshwater bodies all around the world (Van Apeldoorn et al., 2007).

Information about the toxic effect of Ni on cyanobacteria and the possible consequences on cyanotoxins production are issues of environmental concern. The eutrophic status of many freshwater environments around the world promotes cyanobacterial blooms and is frequently associated also with chemical pollution conditions. Accordingly, the aim of this study was to determine the toxicity of this metal on one toxigenic strain of *M. aeruginosa*, assessing the effects on population growth, an array of biomarkers, photosynthetic pigments concentration, and internal and external structure; likewise, the effect of Ni stress on microcystins production was studied. We aimed at obtaining information about the toxic effect of this metal in a worldwide-distributed phytoplankter, through an integral study involving the integrated biomarker response (IBR).

2. Material and methods

2.1. Strain and culture conditions

The toxigenic *Microcystis aeruginosa* strain VU5 (Arzate-Cárdenas et al., 2010), obtained from the culture collection of the *Laboratorio de Hidrobiología Experimental* from the *Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional*, was cultured in modified Z-8 liquid medium (467 mg L^{-1} $NaNO_3$, 41 mg L^{-1} K_2HPO_4, 25 mg L^{-1} $MgSO_4$, 59 mg L^{-1} $Ca(NO_3)_2 \cdot 4H_2O$, 21 mg L^{-1} Na_2CO_3 and 2.7 mg L^{-1} $FeCl_3 \cdot 6H_2O$; without EDTA nor micronutrients), at 26 ± 1 °C, under continuous illumination using white cool fluorescent light lamps (55 μmoles photons m^{-2} s^{-1}). This strain was isolated from a Cuemanco's channel, in Mexico City, which is a eutrophic water body without chemical pollution. It is a collection strain, maintained with no interaction with toxic pollutants.

2.2. Median inhibition concentration (IC_{50})

The 96-h IC_{50} was determined using different Ni concentrations (as Ni^{2+}), according to the protocol 201 of the OECD (2011) in liquid AAP medium, pH 7.5, with an initial cell density of 1×10^5 cells mL^{-1}. Cultures were maintained in static conditions at 26 ± 1 °C under continuous illumination. Assays were performed in 20-mL vials containing 5 mL of test solution. Every day, cell density was quantified with a Neubauer improved cell counter chamber. Three different assays, each with three replicates for each of the five established Ni^{2+} concentrations were used to obtain the average IC_{50} value. All the concentrations tested in this assay and in the assays below were prepared from a stock solution of $NiSO_4$ (J. T., Baker, 99.99% purity), with a nominal value of 1 mg L^{-1} (3.8 μM). The actual concentrations were determined using the HACH© Method 8150 (1-(2 Pyridylazo)-2-Napthol (PAN)) in five different samples taken at different times; the average actual value was 0.945 mg L^{-1} (3.6 μM), so the real Ni concentrations in treatments are supposed to be up to 5.5% lower than the nominal values.

2.3. Sub-inhibitory toxicity tests

Once the average IC_{50} was determined, *M. aeruginosa* was exposed for 168 h to four sub-inhibitory Ni concentrations equivalent to IC_{10}, IC_{15}, IC_{25}, and IC_{50}. The control was *M. aeruginosa* in Z-8 medium without EDTA or micronutrients; each treatment and the control series had three replicates. Incubation conditions were 26 ± 1 °C, continuous illumination at 55 μmoles photons m^{-2} s^{-1}, and shaking at 120 rpm. Test vessels were 500-mL Erlenmeyer flasks containing 350 mL of test solution. Every day cell density was determined. At the end of the assay, the cell density was determined in each replicate and the biomass in each flask was concentrated by centrifugation at 13,300 *g* for 5 min at room temperature; the supernatant was discarded, and the pellet was washed with deionized water for pigments, microcystins, and biomarkers quantification (see below). In addition, samples were processed for observations with scanning and transmission electron microscopes (SEM and TEM).

2.4. Pigments

Chlorophyll *a* and carotenoids were extracted with DMSO and absorbance was determined at 665, 649, and 470 nm. Concentrations were determined using the equations proposed by Wellburn (1994).

Chlorophyll *a* (Chl *a*, μg mL^{-1}) = 12.19 A_{665}–3.45 A_{649}.

Carotenoids (μg mL^{-1}) = (1000 A_{470} − 2.86 Chl *a*)/221.

Phycobiliproteins were extracted with deionized water from cells that were physically disrupted with the freeze/thaw method (Oberemm et al., 1999); 3 cycles of freezing (−70 °C) and thawing (40 °C) were made. Absorbance was determined at 562, 620, and 652 nm. Concentrations were determined using the equations proposed by Becker (1994).

Phycocyanin (PC, mg mL^{-1}) = (A_{620} − 0.7 A_{652})/7.38.

Allophycocyanin (AP, mg mL^{-1}) = (A_{652} − 0.19 A_{620})/5.65.

Phycoerythrin (PE, mg mL^{-1}) = (A_{562} − 2.8 PC − .34 AP)/12.7.