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Karen Ann Ferreira Moura

**Efeitos fisiológicos, morfológicos, ultraestruturais e potencial biorremediador de
cianobactérias cultivadas com o fornecimento supra-ótimo de manganês**

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**Efeitos fisiológicos, morfológicos, ultraestruturais e potencial biorremediador de
cianobactérias cultivadas com o fornecimento supra-ótimo de manganês**

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Karen Ann Ferreira Moura

Ao vigésimo quarto dia do mês de fevereiro, do ano de dois mil e dezessete, às 14 horas, na Sala 236 bloco I 3, no Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, teve lugar a defesa de Mestrado no Programa de PG ECMVS, de autoria da Mestranda Karen Ann Ferreira Moura intitulada: "**Respostas fisiológicas e ultraestruturais de cianobactérias cultivadas com o fornecimento supra-ótimo de manganês em solução.**" Abrindo a sessão, o Presidente da Comissão, Dr. Francisco Antônio R. Barbosa, após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho Final, passou a palavra a candidata para apresentação de seu trabalho. Estiveram presentes a Banca Examinadora composta pelos Professores: Dra. Cristiane Freitas de Azevedo Barros (UEMG/Frutal); Dra. Arnola Cecília Rietzler (ICB/UFMG) e demais convidados. Seguiu-se a arguição pelos examinadores, com a respectiva defesa da candidata.

Após a arguição, apenas os Srs. Examinadores permaneceram na sala para avaliação e deliberação acerca do resultado final, sendo a decisão da banca pela:

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Nada mais havendo a tratar, o Presidente da Comissão encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora.

Belo Horizonte, 24 de fevereiro de 2017.

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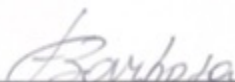
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"Quase tudo é possível quando se tem dedicação e habilidade. Grandes trabalhos são realizados não pela força, mas pela perseverança."

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RESUMO

Embora o manganês (Mn) seja um micronutriente essencial, é tóxico em altas concentrações. Este elemento é também um poluente proveniente de rejeitos de atividades industriais e de mineração. Os efeitos tóxicos de Mn são bem conhecidos em plantas terrestres, mas mal compreendidos em comunidades aquáticas, especialmente em cianobactérias. Os objetivos deste trabalho foram analisar os efeitos tóxicos desse elemento e analisar o potencial de remoção de Mn do meio de cultivo por cianobactérias, visando sua aplicação na biorremediação do Mn de ambientes aquáticos contaminados. Foram selecionadas duas cepas, devido a distinção morfológica dos grupos, sendo uma cepa unicelular, colonial (*Microcystis novacekii* – BA005); e uma heterocitada, filamentosa (*Nostoc paludosum* – BA033). Aqui, os efeitos da exposição ao Mn nas duas cepas de cianobactérias (BA005 e BA033) foram avaliados através de análises fisiológicas, morfológicas e ultraestruturais. As concentrações de Mn de 7.0, 10.5, 15.7, 23.6 e 35.4 mg · L⁻¹ para BA005 e 15.0, 22.5, 33.7, 50.6 e 76.0 mg · L⁻¹ para BA033 foram utilizadas para análises. Os efeitos do Mn foram avaliados pelo teste de inibição da taxa de crescimento (EC₅₀), teor de clorofila *a* e quantificação da acumulação de Mn na biomassa de cianobactérias, após 96 h de exposição. BA005 mostrou-se mais sensível ao Mn do que BA033, com EC₅₀ de 16 mg · L⁻¹ para BA005 e 39 mg · L⁻¹ para BA033. Além disso, redução de 56% e 54% no conteúdo de clorofila *a*, associada a alterações ultraestruturais foram observadas em concentrações de Mn maiores que 23.6 mg · L⁻¹ e 33.7 mg · L⁻¹ para BA005 e BA033, respectivamente. Danos nas camadas do envoltório celular (aumento do espaço periplásmico), surgimento de espaços intratilacóides e grânulos de polifosfato, bem como aumento do número de carboxissomos foram observados em altas concentrações de Mn. Apesar da cepa BA033 ter mostrado mais resistente ao metal, sobrevivendo em concentrações mais elevadas, BA005 mostrou-se mais eficiente na remoção do metal, atingindo remoção de aproximadamente 100% na concentração 10.5 mg · L⁻¹ Mn. Em conjunto, nossos resultados demonstram a importância dos estudos toxicológicos para uma melhor compreensão dos efeitos do Mn em cepas de cianobactérias, além de aferir sobre potencial biorremediador de Mn por cianobactérias.

Palavras-chave: biorremediação, ecotoxicologia, metal, *Microcystis*, *Nostoc*, taxonomia, ecotoxicidade, ultraestrutura.

ABSTRACT

Although manganese (Mn) is an essential micronutrient, it is toxic at high concentrations. This element is also a pollutant from waste from industrial and mining activities. The toxic effects of Mn are well known in terrestrial plants, but poorly understood in aquatic communities, especially in cyanobacteria. The objectives of this work were to analyze the toxic effects of this element and to analyze the potential of removal of Mn from the culture medium by cyanobacteria, aiming its application in the bioremediation of the Mn of contaminated aquatic environments. Two strains were selected, due to the morphological distinction of the groups, being a unicellular, colonial strain (*Microcystis novacekii* - BA005); and a heterocyte filamentous (*Nostoc paludosum* - BA033). Here, the effects of exposure to Mn in the two strains of cyanobacteria (BA005 and BA033) were evaluated through physiological, morphological and ultrastructural analyzes. The concentrations of Mn of 7.0, 10.5, 15.7, 23.6 and 35.4 mg · L⁻¹ for BA005 and 15.0, 22.5, 33.7, 50.6 and 76.0 mg · L⁻¹ for BA033 were used for analysis. The effects of Mn were evaluated by the growth inhibition test (EC50), chlorophyll *a* content and quantification of Mn accumulation in cyanobacteria biomass after 96 h of exposure. BA005 was more sensitive to Mn than BA033, with EC50 from 16 mg · L⁻¹ to BA005 and 39 mg · L⁻¹ to BA033. In addition, 56% and 54% reduction in chlorophyll *a* content associated with ultrastructural changes were observed at concentrations of Mn greater than 23.6 mg · L⁻¹ and 33.7 mg · L⁻¹ for BA005 and BA033, respectively. Damage to the cell envelope layers (increase of the periplasmic space), appearance of intrathylakoidal spaces and polyphosphate granules, as well as increase in the number of carboxy-enzymes were observed at high concentrations of Mn. Although the BA033 strain showed to be more resistant to the metal, surviving in higher concentrations, BA005 showed to be more efficient in the removal of the metal, achieving a removal of approximately 100% in the concentration 10.5 mg · L⁻¹ Mn. Together, our results demonstrate the importance of toxicological studies for a better understanding of the effects of Mn on cyanobacteria strains, as well as on the bioreactor potential of Mn by cyanobacteria.

Keywords: bioremediation, ecotoxicology, metal, *Microcystis novacekii*, *Nostoc paludosum*, taxonomy, toxicity, ultrastructure.

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1. INTRODUÇÃO GERAL

Minas Gerais extrai mais de 180 milhões de toneladas/ano de minério de ferro; o estado é responsável por aproximadamente 53% da produção brasileira de minerais metálicos e 29% de minérios em geral; sendo que a atividade de mineração está presente em mais de 400 municípios mineiros (IBRAM 2015). A produção brasileira de minério de ferro em 2014 foi de 411,8 Mt (milhões de toneladas), deste montante c. 80 milhões de toneladas de minério de ferro foram originados em Minas Gerais (DNPM 2015).

Os principais bens minerais produzidos no estado de Minas Gerais são: bauxita, ferro, manganês, ouro, paládio, prata, dolomito, filito, quartzo, calcário, chumbo, zinco, fosfato, feldspato, granito, zircônio, cobalto, enxofre, níquel, barita, manganês e nióbio (IBRAM 2015). O manganês (Mn) está presente nas rochas ígneas, sedimentares e metamórficas, sendo amplamente distribuído na litosfera, hidrosfera e atmosfera. O Mn é um dos metais mais abundantes na crosta terrestre, geralmente ocorrendo com ferro. É componente de mais de 100 minerais, mas não é encontrado naturalmente em sua forma pura (elementar) (WHO 2011). Apesar dos inúmeros benefícios trazidos pela mineração, atividades relacionadas à exploração e beneficiamento de minério alteram drasticamente a área explorada e áreas vizinhas, além de causar impactos ambientais e sociais relacionados aos elementos usados nestes processos, contaminando solo, subsolo e cursos d'água.

De acordo com o Serviço Geológico do Brasil, CPRM (2002), os principais problemas oriundos da mineração podem ser divididos em cinco categorias: poluição da água, poluição do ar, poluição sonora, subsidência do terreno e rejeitos radioativos. Diante dos inúmeros impactos e ameaças a que os ambientes aquáticos estão sujeitos, há preocupação mundial com a preservação deste recurso, sendo considerado um ecossistema vulnerável (Allan & Flecker 1993, Leidy & Moyle 1998, Malmqvist & Rundle 2002, Saunders *et al.* 2002).

Além do setor mineral, efluentes oriundos de diversas fontes industriais contendo metais tóxicos têm sido descartados no meio ambiente, sendo estes os principais contribuintes da contaminação por metais pesados das águas. Uma vez lançados no ambiente, os cátions de alguns metais pesados podem ser acumulados através da cadeia alimentar mesmo que presentes em baixas concentrações nos efluentes, pois não são biodegradáveis e assim são facilmente incorporados, tornando-se fatores de

1 concentração perigosos em peixes e animais que, posteriormente, são consumidos pelo
2 homem (Sato 2005).

3 O Mn pode existir em 11 estados oxidativos. Os compostos de Mn mais
4 ambientalmente e biologicamente importantes são aqueles que contêm Mn^{2+} , Mn^{4+} ou
5 Mn^{7+} (USEPA 1994). E este micronutriente pode ser encontrado nos sistemas biológicos
6 em três níveis de oxidação: Mn^{2+} , Mn^{4+} (formas estáveis) e Mn^{3+} (forma instável)
7 (Marschner 1995, Bartsevich & Pakrasi 1996, Citak *et al.* 2010). As propriedades físicas e
8 químicas de diferentes compostos de Mn variam substancialmente. Essas características,
9 por sua vez, determinam o comportamento e destino ambiental, o potencial de exposição e
10 o potencial toxicológico subsequente de cada composto (WHO 2011). Isso devido ao fato
11 de que o Mn é um elemento essencial para o metabolismo de plantas e animais, uma vez
12 que é necessário para o funcionamento de muitas enzimas celulares tais como, superóxido
13 dismutase de manganês, piruvato carboxilase e pode servir para ativar muitas outras,
14 exemplos destas são as quinases, descarboxilases, transferases, hidrolases (IPCS 2002).

15 Embora o Mn esteja naturalmente presente nos ambientes terrestres e aquáticos, sua
16 distribuição está também diretamente relacionada às ações antrópicas. A contaminação dos
17 cursos d'água por Mn geralmente está associada ao despejo de resíduos domésticos,
18 mineração, processo de manufatura papéis, agentes químicos além de resíduos de esgotos
19 (Thornton 1995). O Mn é utilizado principalmente na fabricação de ligas de ferro e aço e
20 compostos e como ingrediente em vários produtos (IPCS 1999, ATSDR 2000). Dióxido de
21 manganês e outros compostos de manganês são usados em produtos de baterias, vidro e
22 fogos de artifício. O permanganato de potássio é utilizado como oxidante para fins de
23 limpeza, branqueamento e desinfecção (ATSDR 2000, HSDB 2001). Mn também é usado
24 em alguns locais para tratamento de água potável (ATSDR 2000). Um composto orgânico
25 de Mn, metilciclopentadienil manganês tricarbonil (MMT), é utilizado como agente de
26 aumento de octanas na gasolina sem chumbo no Canadá, Estados Unidos da América,
27 Europa, Ásia e América do Sul (Lynam *et al.* 1999). Outros compostos de manganês são
28 utilizados em fertilizantes, vernizes e fungicidas e como suplementos alimentares para
29 animais (HSDB 2001). Na atividade mineradora, o manganês é amplamente encontrado na
30 água de drenagem das minas (Banks *et al.* 1997).

31 Os compostos de Mn podem estar presentes na atmosfera sob a forma de partículas
32 em suspensão resultantes de emissões industriais, erosão do solo, emissões vulcânicas e
33 queima de gasolina contendo MMT (IPCS 1999). As águas subterrâneas anaeróbicas

1 geralmente contêm níveis elevados de Mn dissolvido. A forma divalente (Mn^{2+}) predomina
2 na maior parte da água a pH 4-7, mas formas mais altamente oxidadas podem ocorrer a
3 valores de pH mais elevados ou resultam da oxidação microbiana (ATSDR 2000). O Mn
4 pode ser adsorvido no solo, a extensão da adsorção depende do conteúdo orgânico e da
5 capacidade de troca catiônica do solo. Nas águas de superfície, o Mn ocorre tanto em forma
6 dissolvida quanto em suspensão, dependendo de fatores como pH, ânions presentes e
7 potencial de oxidação-redução (ATSDR 2000). Podendo haver bioacumulação em
8 organismos inferiores (por exemplo, fitoplâncton, algas, moluscos e alguns peixes)
9 (ATSDR 2000).

10 Mesmo este elemento sendo um micronutriente essencial tanto para os animais
11 quanto para os vegetais, em altas concentrações pode tornar-se tóxico (Boucher & Watzin
12 1999; Doyle *et al.* 2003). Estudos em humanos demonstram que a exposição a níveis
13 elevados de Mn podem resultar em efeitos neurológicos e neuropsiquiátricos, como
14 alucinações, instabilidade emocional, fraqueza, distúrbios de comportamento e da fala, que
15 culminam em uma doença, semelhante ao Mal de Parkinson, denominada Manganismo
16 (Ladou 2016). Apesar disso, pouco ainda é sabido em relação aos efeitos do Mn sobre as
17 comunidades aquáticas, sendo conhecidos seus efeitos adversos apenas nos vegetais
18 (Marschner 1995, Lizieri *et al.* 2012). Os efeitos adversos associados à toxidez de Mn
19 incluem: deformações nas paredes celulares, necrose do caule e das folhas, degradação do
20 aparato fotossintético, crescimento retardado, queima das pontas de folhas e flores e
21 encarquilhamento das folhas (Foy 1984, Lindon *et al.* 2004).

22 Afim de evitar intoxicação humana por Mn aquoso, o CONAMA estabelece valores
23 máximos permitidos de Mn para diferentes classes de água: águas doces: $0,1 \text{ mg} \cdot \text{L}^{-1}$
24 (classes 1 e 2 – água destinada ao consumo humano, após desinfecção) e $0,5 \text{ mg} \cdot \text{L}^{-1}$
25 (classe 3 – água destinada ao consumo humanos, após tratamento convencional); $1,0 \text{ mg} \cdot$
26 L^{-1} lançamentos de efluentes (manganês dissolvido). Desta forma, ainda que a análise das
27 características físicas e químicas retratem o “status” da coluna d’água, é importante a
28 associação destes métodos com a comunidade biológica, permitindo uma caracterização
29 mais completa, muitas vezes necessária para o manejo, conservação ou restauração do
30 ambiente (Atlas & Bartha 1997, Boopathy 2000, Andreazza *et al.* 2010, 2011, Andreazza
31 2013). Neste contexto, grupos específicos de microrganismos têm sido potencialmente
32 utilizados em diferentes métodos de avaliação ambiental devido a apresentarem tempo de
33 vida relativamente curto e respostas rápidas às alterações do ambiente (Rosemberg & Resh
34 1993).

1 Neste contexto, microrganismos aquáticos têm sido estudados não somente como
2 bioindicadores, mas também como biorremediadores de ambientes impactados seja por
3 poluentes orgânicos ou inorgânicos (Scott *et al.* 1992, Bonaventura & Johnson 1997, Vidali
4 2001, Camargo *et al.* 2007, Andreazza 2013).

5 Entre os grupos de microrganismos aquáticos destaca-se o grupo das cianobactérias,
6 as quais vêm sendo potencialmente indicadas como agentes promissores para mitigação de
7 áreas contaminadas (Scott *et al.* 1992, El-Bestawy 2008, Campos 2009, Andreazza 2013).

8 Cianobactérias, também conhecidas como algas azuis, são organismos cosmopolitas,
9 procariontes, unicelulares, fotoautotróficos (Campos 2009, Herrero 2010), podendo ser
10 encontradas na forma unicelular cocóide, filamentos ou colonial (Campos, 2009; Komárek
11 1998, 2005 e 2013). A parede celular é constituída de peptidoglicano (mureína), recobertas
12 por uma membrana lipopolissacarídica, comum de bactérias gram-negativas. A parede
13 celular corresponde a uma estrutura complexa formada por várias camadas (Fig. 1). Além
14 da membrana citoplasmática, a parede da célula apresenta uma fina camada formada por
15 peptídeoglicanos (cerca de 10%) e uma membrana externa. Os peptídeoglicanos
16 formadores da camada interna são compostos por dois derivados de açúcares
17 (N-acetilglicosamina e ácido N acetilmurâmico) e um pequeno grupo de
18 aminoácidos. Já a membrana externa da parede é composta por lipídeos,
19 fosfolipídeos e polissacarídeos. Entre as camadas da parede, está o espaço periplasmático.
20 Esse espaço contém vários tipos de proteínas, incluindo enzimas hidrolíticas (promovem a
21 degradação inicial dos nutrientes), proteínas de ligação (iniciam os processos de
22 transporte de substratos) e quimiorreceptores (correspondem a proteínas envolvidas
23 na resposta quimiotática) e principalmente as porinas. Tais porinas são proteínas
24 transmembrânicas que se associam formando canais que permitem a entrada e a saída de
25 substâncias hidrofílicas de baixo peso molecular, tornando a membrana externa permeável
26 a moléculas pequenas (Madigan *et al.* 2004). Outra característica típica das cianobactérias
27 é que a parede celular pode não ser a camada mais externa, uma vez que pode ser
28 coberta por uma superfície externa de polissacarídeos e proteínas, como cápsula,
29 bainha e camada limosa (Phoenix *et al.* 2002).

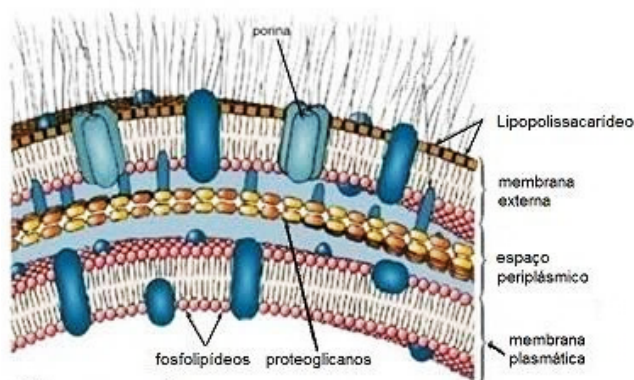


Figura 1: Representação das camadas de uma parede celular Gram negativa, mostrando as diferentes camadas constituintes da parede. Retirado de Prescott *et al.* 1996.

Segundo o sistema de classificação de Komárek e Anagnostidis (1998, 2005, 2013) as cianobactérias pertencem ao domínio Bacteria e à divisão Cyanobacteria. Estas podem apresentar morfolologicamente na forma unicelular livre (Ex. *Synechococcus*), unicelular colonial (Ex. *Microcystis*), filamentosa sem ramificações (Ex. *Pseudanabaena*), filamentosa falsamente ramificada (Ex. *Tolypothrix*), filamentosa com ramificação verdadeiramente ramificada (Ex. *Stigonema*) e filamentosa heterocitada (Ex. *Nostoc*) (Komárek e Anagnostidis 1998, 2005 e 2013). Ao longo de sua história evolutiva as cianobactérias têm se adaptado a ambientes extremos, sendo encontradas em ambientes de águas doces, salobras ou marinhas, ambientes terrestres, bem como desertos, águas termais e ambientes árticos (Franceschini *et al.* 2010). Os ambientes dulcícolas são os mais propícios para o crescimento das cianobactérias, pois a maioria das espécies se desenvolve melhor em águas neutro-alcálinas (pH de 6.0 a 9.0), temperaturas em torno de 25°C e alta concentração de nutrientes (Madigan *et al.* 2004). Esta adaptação a ambientes extremos é decorrente de suas adaptações celulares e fisiológicas, obtendo mecanismos para tolerar a incidência de raios ultravioletas, elevadas concentrações de metais pesados, baixas concentrações de oxigênio, diferentes amplitudes de temperaturas e pH (Whitton & Potts 2000, Molica & Azevedo 2009; Franceschini *et al.* 2010). Algumas das adaptações celulares ou estruturas que permitem a sobrevivência em diversos ambientes são: heterócitos, células vegetativas diferenciadas que promovem a fixação de nitrogênio atmosférico. Estas células apresentam parede espessada, sem conteúdo fotossintético e com nódulos polares que permitem a comunicação seletiva com as células vizinhas; acinetos, esporos de resistência em condições adversas. Acinetos são células vegetativas aumentadas devido a estocagem de substâncias de reserva e espessamento da parede celular, podendo

1 permanecer vivas no ambiente e germinarem quando as condições ambientais se tornarem
2 favoráveis ao seu desenvolvimento. Além destas estruturas, alguns grupos apresentam
3 aerótopos, que permitem a migração vertical na coluna d'água. Estes vacúolos gasosos são
4 inclusões citoplasmáticas, estruturas fusiformes ocas e rígidas, que permitem a regulação
5 da flutuabilidade, facilitando sua sobrevivência conforme as condições ambientais
6 (Komárek 1998 e 2005, Brandão & Domingos 2006). Estas características lhe conferem
7 vantagens competitivas sobre outros grupos fitoplanctônicos.

8 Estes microrganismos ocupam um grupo taxonômico único, uma vez que combinam
9 o modo autotrófico de crescimento, com um sistema metabólico considerado bacteriano
10 (Shaheen *et al.* 2007). Essa característica denominada mixotrofia permite às cianobactérias
11 um crescimento não estritamente dependente de luz, permitindo o uso de variadas fontes
12 de carbono (Chojnacka & Noworyta 2004, Pinho *et al.* 2010). Além disso, para melhor
13 aproveitamento da amplitude luminosa as cianobactérias possuem dois tipos de sistemas
14 para captação, um desses sistemas é realizado por pigmentos fotossintéticos (moléculas de
15 clorofila a e carotenóides) que estão situados nas membranas dos tilacóides e o outro
16 sistema são as ficobiliproteínas que são cromoproteínas solúveis em água arranjadas em
17 agregados denominados de ficobilissomos ligado à superfície exterior das membranas dos
18 tilacóides (Lee 2008) (Fig 2). A energia luminosa é capturada pelos pigmentos nos
19 tilacóides e transferida para clorofilas dentro do núcleo reativo dos fotossistemas. O
20 fotossistema II (PSII) é responsável pela quebra da molécula de água que resulta na
21 produção de elétrons e prótons, enquanto o fotossistema I (PSI) eleva o nível da energia
22 dos elétrons (Ducat 2011).

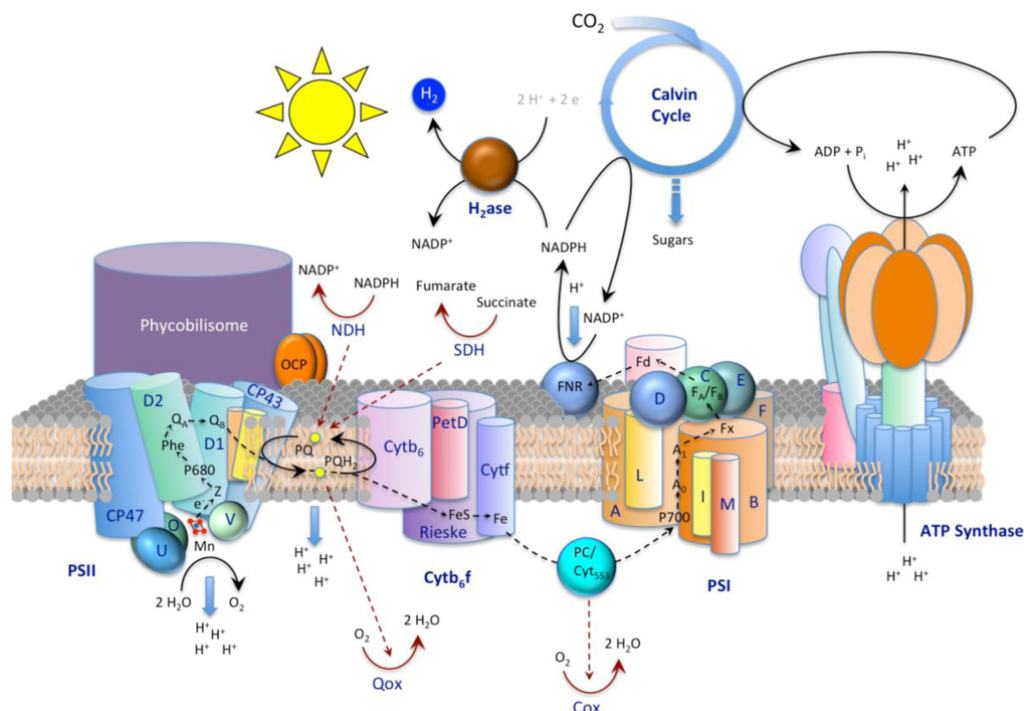


Figura2: Mecanismo fotossintético em cianobactérias. Maquinaria fotossintética, transporte de elétrons e síntese de ATP nos tilacóides. Retirado de Kamennaya *et al.* 2012.

Dentre as cianobactérias, o gênero *Microcystis* Kützinger ex Lemmermann 1907, é representante das cianobactérias unicelulares coloniais, tipicamente planctônico, com *c.* 50 espécies. Este gênero apresenta colônias recobertas por mucilagem e células de formas variadas, as células apresentam-se numerosas, dispondo-se de modo irregular, frouxa ou densamente na mucilagem. Apresentam aerótopos, mas nunca células acessórias como acinetos e heterócitos (Komárek & Anagnostidis 1998).

O gênero *Nostoc* Vaucher ex Bornet et Flahault 1888, representante das cianobactérias filamentosas heterocitadas, apresenta tricomas isopolares, em geral constritos, não atenuados nas extremidades, frouxa ou densamente aglomerados dentro da colônia, sempre envoltos por mucilagem. As células são cilíndricas, em forma de barril ou quase esféricas, às vezes mostrando variabilidade de forma e tamanho dentro da mesma espécie. Neste grupo não há aerótopos e podem ser observados heterócitos solitários, localizados na porção terminal ou intercalar no tricoma. Os acinetos são arredondados, elípticos ou cilíndricos, dispostos em série entre heterócitos (Komárek & Anagnostidis 2013).

Cianobactérias tem grande importância ecológica e econômica, uma vez que espécies desse grupo são comumente responsáveis por extensas florações em diferentes tipos de

ambientes aquáticos, sendo capazes de crescer em corpos d'água eutrofizados. O enriquecimento das águas com nutrientes provenientes de esgotos urbanos, atividades agropecuárias e industriais, com grandes quantidades de nitrogênio e fósforo, é considerado a principal causa da ocorrência de florações de cianobactérias (Molica & Azevedo 2009). Os principais problemas ambientais ocasionados por essas florações são mortandade de peixes, aves e mamíferos que tem como principal consequência a perda da biodiversidade nesses ecossistemas aquáticos. Ainda, as florações de cianobactérias podem causar gosto e odor desagradáveis nas águas afetando os serviços de abastecimento de água (Azevedo & Sant'Anna 2006). Além desses problemas, algumas das cianobactérias é capaz de produzir toxinas que podem ser acumuladas na cadeia alimentar e produzir diversos tipos de intoxicações, que podem afetar outros organismos além da comunidade aquática (Bittencourt- Oliveira *et al.* 2001).

Apesar de causar grandes transtornos ambientais quando em florações pode-se destacar que as cianobactérias atuam como importantes produtoras primárias, desempenhando papel importante nos ciclos biogeoquímicos de nitrogênio, carbono e oxigênio, sendo responsáveis por cerca de 30% da produção anual de oxigênio da Terra, além de compreender a base da cadeia alimentar nos ecossistemas aquáticos (Whitton & Potts 2000, Sharma *et al.* 2011).

Micro-organismos possuem a capacidade de adsorver e/ou absorver íons metálicos dissolvidos. Entre eles estão bactérias, microalgas e fungos (Volesky 1986). Historicamente, a aplicação mais comum do uso de cianobactérias tem sido na alimentação humana, envolvendo várias espécies e explorado por empresas em todo mundo (Habid *et al.* 2008, Nuhu 2013). Entretanto, atualmente vêm sendo estudadas quanto ao seu potencial para acumular metal pesado num processo de biorremediação (bioacumulação) e descontaminação ambiental (Pokrovsky *et al.* 2008, Pereira *et al.* 2011, Kumar *et al.* 2012, Ferreira 2013, Franco *et al.* 2015).

As capacidades de remoção, assim como os mecanismos de acumulação, variam de acordo com a espécie microbiana, ou até mesmo com a linhagem. As células microbianas têm capacidade de remoção ou captação de metais de soluções, empregando metabólitos excretados, parede celular e polissacarídeos. Fatores externos como pH, temperatura, ausência ou presença de nutrientes e outros metais também influenciam no mecanismo atuante e, consequentemente, na eficiência e seletividade da captação (Bolton & Gorby 1995, Gadd 1992, Mullen *et al.* 1989, Galun *et al.* 1987).

1 Devido à complexidade da estrutura dos microrganismos, o processo de acúmulo ou
2 captação implica na existência de muitos caminhos para as células capturarem o metal.
3 Segundo Veglió & Beolchini (1997), os mecanismos de captação de metal podem ser
4 classificados de duas formas distintas: através da dependência do metabolismo celular
5 (bioacumulação) ou de acordo com a localização do sítio de captação eletrostática do metal
6 (biossorção). A remoção neste último caso pode ocorrer tanto em células vivas quanto em
7 células liofilizadas (Gadd 1992).

8 De acordo com o sítio onde o metal é captado, a captação pode ser classificada como
9 um processo de acumulação extracelular, sorção na superfície da célula acompanhada de
10 precipitação e acumulação intracelular. Este último processo depende do metabolismo da
11 célula e normalmente está associada com o sistema de defesa de micro-organismos, os
12 quais reagem na presença de metais tóxicos (Veglió & Beolchini 1997).

13 Já para o caso de ocorrerem interações entre o metal e os grupos funcionais presentes
14 na superfície celular, baseadas em fenômenos físicos e químicos (adsorção química, troca
15 iônica e complexação), tem-se uma sorção na superfície da célula onde às vezes não
16 depende do metabolismo (biossorção com células liofilizadas, por exemplo). O fenômeno
17 físico-químico na captação metálica é um mecanismo não dependente do metabolismo
18 celular, que é relativamente rápido e pode ser reversível. Quando ocorre a precipitação, a
19 classificação não é única, a precipitação do metal pode ocorrer na solução ou na superfície
20 celular (Veglió & Beolchini 1997).

21 Diferentemente, na bioacumulação o transporte de íons de metais pesados é feito
22 através da membrana celular e sua acumulação intracelular é dependente do metabolismo,
23 ou seja, ocorre somente em células vivas, capazes de gerar energia. Ou ainda, a
24 bioacumulação é um processo de acúmulo ou captação de metal em condições de
25 crescimento celular (Davis *et al.* 2003). Quando há dependência do metabolismo celular a
26 captação envolve o transporte ativo de metais através do envoltório celular para o interior
27 da célula, ocorre a bioacumulação fazendo com que o contaminante fique biologicamente
28 disponível para níveis tróficos superiores (Camargo *et al.* 2007, Campos 2009).

29 Os mecanismos de transporte envolvidos na acumulação de metais pelos micro-
30 organismos são pouco conhecidos. Uma das suposições seria a de que esses elementos
31 podem ser captados pelos sistemas de transporte de metais essenciais para o
32 desenvolvimento microbiano (Gadd 1988). Uma vez dentro da célula, os íons metálicos
33 podem se localizar em organelas, ou podem estar ligados a proteínas, deslocando os íons
34 adequados de suas posições, prejudicando as funções metabólicas (Gadd 1992).

Grandes “acidentes” ecológicos decorreram do processo de bioacumulação de metais como mercúrio (Hg) acumulado em peixes em Minamata (Japão) e a doença de Itai-itai, identificada como consequência da incorporação de cádmio (Cd) na água e acumulada nas espécies comestíveis no Japão (Vidotti & Rollemberg 2004).

Dessa forma, esta dissertação foi proposta a fim de se conhecer as respostas eco-fisiológicas e potencial biorremediador de cianobactérias em condições adequadas de cultivo com o fornecimento supra-ótimo de Mn em solução. Apresentando como hipótese: Cianobactérias são capazes de remover Mn do meio aquoso e podem apresentar efeitos tóxicos em elevadas concentrações. Com as predições que: Até um certo limite (diferente para cada espécie), com o aumento da concentração de Mn no meio, maior a quantidade biorremovida pelas cianobactérias. E quanto mais elevada a concentração de Mn no meio, mais efeitos tóxicos serão observados. Assim, os objetivos principais desta presente dissertação foram analisar os efeitos tóxicos do Mn em cianobactérias, além de investigar o potencial biorremediador desses microrganismos, visando sua aplicação na biorremediação deste metal em ambientes aquáticos contaminados.

Para tanto, focou-se nos seguintes objetivos específicos:

1. a. Avaliar as curvas de crescimento;
b. Conteúdo de clorofila *a*;
c. Efeitos tóxicos morfológicos e ultraestruturais;
- em duas espécies de cianobactérias morfológicamente distintas (*Microcystis novacekii*-BA005, *Nostoc paludosum*- BA033) sob exposição à contaminação por Mn.
2. Avaliar a capacidade de remoção de Mn da solução aquosa das espécies em estudo.

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2. TOXIC EFFECTS AND POTENTIAL BIOREMEDIATION OF MANGANESE BY CYANOBACTERIA

2.1 ABSTRACT

Although manganese (Mn) is an essential micronutrient it is toxic at high concentrations. The toxic effects of Mn are well known in plants but poorly understood on aquatic communities, especially in cyanobacteria. Here, the effects of Mn exposure on two cyanobacterial strains (*Microcystis novacekii* BA005 and *Nostoc paludosum* BA033) were evaluated through physiological, morphological and ultrastructural analysis. The Mn concentrations of 7.0, 10.5, 15.7, 23.6 and 35.4 mg · L⁻¹ for BA005, and 15.0, 22.5, 33.7, 50.6 and 76.0 mg · L⁻¹ for BA033 were further used for analysis. These concentrations were determined by previous experiments. From the EC₅₀ value we used the multiplication factor 1.5 to determine the final concentrations. Mn toxicity was assessed by growth rate inhibition test (EC₅₀), chlorophyll *a* content, quantification of the Mn accumulation in biomass and morphological and ultrastructural toxic effects in cyanobacteria after 96 h of exposure. The EC₅₀ was 16 mg · L⁻¹ for BA005 and 39 mg · L⁻¹ for BA033. Reduction of 56 % and 54 % in chlorophyll *a*, coupled with ultrastructural changes were observed in Mn concentrations higher than 23.6 mg · L⁻¹ and 33.7 mg · L⁻¹ for BA005 and BA033, respectively. Damages in cell wraps (increased periplasmic space), appearance of intrathylakoid spaces and polyphosphate granules as well as increased number of carboxysomes were also observed at the highest tested concentrations of Mn. On the other hand, BA005 showed to be more efficient in the removal of metal from the aqueous medium, reaching approximately 100% removal in the 10.5 mg L⁻¹ Mn concentration, while BA033 presented removal of approximately 38% in the concentration 22.5 mg · L⁻¹ Mn, both after 96 h of exposure. However, BA033 was more efficient when placed in high concentrations (above 20.0 mg L⁻¹ Mn), due to its high tolerance, compared to BA005. The results demonstrated the importance of toxicity studies for a better understanding of Mn effects in cyanobacterial strains which can be used as a model for evaluating the negative impact of Mn in aquatic environments and potential bioremediator recovery of aquatic environments contaminated by Mn.

Key words: bioremediation, ecotoxicology, metal, *Microcystis novacekii*, *Nostoc paludosum*, taxonomy, toxicity, ultrastructure.

2.2 INTRODUCTION

Aquatic ecosystems have suffered severe changes in its composition, function and water quality over the last decades, mostly due to intensive activities in both agriculture and mining, which have steadily increased in order to supply human needs (Moreno and Callisto 2004). In Brazil, the mining activities play a significant and growing role on economic development. As such, around 70 % of the country's trade surplus came from the mining sector (IBRAM 2015).

Although mining areas have enjoyed stronger poverty reduction and social development performance than non-mining areas, the contamination of natural waters by mining activities can be a consequence of malfunctioning, nonexistence or break of tailings containment dams. Completely, chemical effluents used in the ore processing may spread over large areas beyond the mines (Sengupta 2000). The increased concentrations of heavy metals and non-metals such as mercury (Hg), arsenic (As), cadmium (Cd) and manganese (Mn) have been assumed as one of the main impacts from mining in natural waters bodies (Kabata-Pendias & Pendias 1986, Nriagu & Pacynat 1988, Sengupta 2000). This increment in heavy metals and non-metals concentration might lead to serious environmental health issues as consequence of their high toxicity and persistence in the environment (Kabata-Pendias & Pendias 1986, Toppi & Gabrielli 1999).

Mn is an essential micronutrient that may become toxic if present at high concentration. In humans, the exposure to high levels of Mn result in neurological and neuropsychiatric effects such as hallucinations, emotional instability, weakness, behavior and speech disorders, a disease similar to Parkinson's disease named Manganism (Siqueira 1985). Nevertheless, little is currently known concerning the effects of Mn on aquatic communities with the majority of the available studies focusing on its adverse effects on plants (Marschner 1995, Lizieri *et al.* 2012). Notably, plants usually display cell wall deformation, stem and leaf necrosis, reduction of photosynthetic apparatus, stunting, leaf burn tips and flowers, as well as shriveling leaves are symptoms associated with Mn toxicity (Foy 1984, Lindon *et al.* 2004).

To establish water quality standards, to avoid prolonged human contamination, the National Environmental Council (CONAMA) defines the maximum permissible limit (MPL) of toxic elements according to a water classification. The MPL for Mn is 0.1 mg ·

1 L^{-1} in waters for human consumption and simple treatment, $0.5 \text{ mg} \cdot L^{-1}$ in waters for
 2 human consumption and conventional treatment $1.0 \text{ mg} \cdot L^{-1}$ in effluent discharges.
 3 However, little is known about the effects of Mn on planktonic communities, being found
 4 in the environments, higher values established by CONAMA.

5 Recently, a huge environmental disaster occurred as consequence of a collapse in a
 6 tailings dam in a local mining company at Mariana city ($20^{\circ} 22' 40'' \text{ S}$; $43^{\circ} 24' 58'' \text{ W}$),
 7 Minas Gerais state, in southeastern Brazil which called the attention of the world. One of
 8 the most important rivers of southeastern Brazil, Rio Doce (Doce river), which runs
 9 through the states of Minas Gerais and Espírito Santo was overflowed by mud containing
 10 mining waste, reaching the coastal areas of the Espírito Santo state. Since this tragic event
 11 occurred (November 2015) several studies have been performed to verify and mitigate the
 12 impact on the Rio Doce basin. Despite many studies are still in progress, water and
 13 sediment analysis from the affected areas have shown high concentration of total Mn, iron,
 14 aluminum and other metals and toxic compounds (IGAM 2016). Remarkably, in some parts
 15 of the river it has been reported values of $857 \text{ mg} \cdot L^{-1}$ of Mn, which largely extrapolate
 16 the allowed concentration (0.5 and $1.0 \text{ mg} \cdot L^{-1}$) following CONAMA resolutions. The
 17 persistence of these metals in contaminated environments has stimulated the development
 18 of clean technologies for decontamination and a possibility of using microorganisms,
 19 cyanobacteria, to immobilize such metals has been widely reported (Farag *et al.* 1998,
 20 Fisher *et al.* 2000, de-Bashan & Bashan 2003 e 2004, Ji *et al.* 2012). Therefore,
 21 phytoplankton organisms, because they act in the process of remineralization of nutrients,
 22 are subject to incorporate pollutants, being able to becoming bioreactors or
 23 bioaccumulators of toxic organic or inorganic elements (Scott *et al.* 1992, Bonaventura &
 24 Johnson 1997, Vidali 2001, Camargo *et al.* 2007, Andreazza 2013, Franco *et al.* 2015).

25 The vast majority of research performed to date investigating Mn effects has been
 26 focusing on crop plants (El-Jaoual & Cox 1998, Reichman 2002, Lizieri *et al.* 2012,
 27 Carrasco-Gil *et al.* 2015). Although these reports have clearly improved our knowledge
 28 concerning the toxic impact of Mn, these data are not useful to directly assess the negative
 29 impact of Mn on aquatic environments. Given the diverse biota present in waterbodies
 30 cyanobacteria are usually assumed as an excellent model for understanding aquatic
 31 environmental problems, due to some safety factors and tolerant in several contaminated
 32 environments than the other phytoplankton groups. This is due to the fact that, during
 33 evolution, these organisms have developed diverse strategies to maintain an equilibrated

relation with heavy metal ions present and available in the surrounding medium (Perales-Vela *et al.* 2006).

Cyanobacteria, members of *Bacteria* domain, are widespread over diverse ecosystems. These microorganisms exhibit great morphological diversity, perform oxygenic photosynthesis, and use energy from sunlight to transform carbon dioxide (CO₂) from the atmosphere into biomass, as conducted by plants (Flores *et al.* 2006). However, some cyanobacterial strains might combine the autotrophic mode of growth with mixotrophic metabolic system which allows cyanobacterial growth using various organic carbon sources (Chojnacka & Noworyta 2004, Shaheen *et al.* 2007). Additionally, the combination of cellular and structural adaptations allows its survival under a wide range of environmental conditions (Flores & Herrero 2010, Campos *et al.* 2013, Komárek & Anagnostidis 1998, 2013). Due to its biochemical, physiological and anatomical characteristics, cyanobacteria have been used in many research proposals. It has been used as supplements and nutraceuticals (Nuhu 2013), in the fertilization of crop plants (Venkataraman 1972, Hashem 2001) and as possible fuel sources (Parmar *et al.* 2011). Cyanobacteria are also known to be a rich source of novel bioactive metabolite, comprising many cytotoxic, antifungal and antiviral compounds (Sudha *et al.* 2011, Silva-Stenico *et al.* 2012, Silva-Stenico *et al.* 2013). Studies have also demonstrated the potential of cyanobacteria to be used in bioremediation process (Pokrovsky *et al.* 2008, Pereira *et al.* 2011, Kumar *et al.* 2012), and to assess metal toxicity as arsenic (Pandey *et al.* 2012, Franco *et al.* 2015), lead (Arunakumara & Xuecheng 2009), and other metals (Hudek *et al.* 2012).

The bioaccumulation process occurs in two moments: i) considered the fast phase and without energy expenditure, consists of the electrostatic attraction between the metal cations and the cell wall anions adsorption; (ii) the slow phase, with energy expenditure, consists of the active uptake of the substance, promoting the internalization of the metal through membrane proteins absorption (Davis *et al.* 2003).

In this study, we investigated the effects of Mn on the growth rate, physiological, ultrastructural characteristics and the bioremediation capacity of two distinct cyanobacterial strains (*Microcystis novacekii*-BA005 and *Nostoc paludosum*-BA033) following exposure to increasing Mn concentrations. By doing this we were able to both shed light on cyanobacterial responses following Mn exposure and to further enhance our understanding of Mn toxicity on aquatic organisms.

2.3 MATERIALS AND METHODS

2.3.1 Cyanobacterial strains

Seeking to select different morphotypes within the cyanobacteria group, two morphologically distinct strains were selected: (i) a unicellular strain (*Microcystis novacekii*-BA005) and (ii) a filamentous heterocytous strain (*Nostoc paludosum*-BA033). The strain BA033 was obtained from a stream located downstream of a gold processing plant in the municipality of Nova Lima (19° 58' 74.8" S; 43° 49' 25.9" W), Minas Gerais state, Brazil. The strain BA005 was obtained from Dom Helvécio lake in the Parque Estadual do Rio Doce (19° 46' 55" S; 42° 35' 28" W), Minas Gerais state, Brazil. Micropipeting, plating and serial dilution were used to isolation of monospecific cyanobacterial cultures. After isolation, these strains have been maintained at the Microalgae and Cyanobacteria Culture Collection of the Laboratory of Limnology, Ecotoxicology and Aquatic Ecology of the Institute of Biological Sciences, Universidade Federal de Minas Gerais. Cultures of the strain BA005 and BA033 are maintained in Erlenmeyer flasks (500 mL) in ASM-1 medium (Gorham *et al.* 1964) and BG-11₀ (Allen 1968), respectively. The differentiated culture media were given as nutritional requirements of the strains, selected according to a better acclimatization and growth of the species. These strains are kept in a growth chamber at 21.0 ± 1.0 °C under constant illumination from white cold fluorescent lamps, with intensity of 110 μmol·photons·m⁻²·s⁻¹ (OSBRAM, lumilux-cool, 16 W, Brazil).

2.3.2 Morphological description

The cyanobacterial strains analyzed in this study were characterized applying the diacritical morphological traits for genera described by Komárek & Anagnostidis (1998, 2013). The classification system from Hoffmann *et al.* (2005) was used for Family and Order designation. The strains were morphologically evaluated under optical light microscope coupled with a digital image system (Zeiss AxioCam ICc1).

As morphologically distinct strains of cyanobacteria selected for this study were: (i) one unicellular, *Microcystis novacekii*-BA005 (Order Chroococcales, Family Microcystaceae) and (ii) a filamentous heterocytous, *Nostoc paludosum*-BA033 (Order Nostocales, Family Nostocaceae). According to morphological inspection the strain BA005 was assigned to the morphospecies *Microcystis novacekii* (Komárek) Compère

1974 which presents free floating colonies with individual cells forming thick aggregates in the center of the colony and with very few isolated cells in the periphery; the cells are spherical or hemispherical (after division) (Komárek and Anagnostidis 1998) (Fig. 1A). The strain BA033 was morphologically identified as *Nostoc paludosum* Kützinger ex Bornet et Flahault 1888 and has aggregated filaments, slightly straight, unbranched, with intercalary and terminal heterocytes, production of mucilage and sometimes forming colonies. The trichomes are isopolar, uniseriate and usually constricted (Komárek and Anagnostidis 2013) (Fig. 1B). In previous analysis, both strains showed no toxicity.

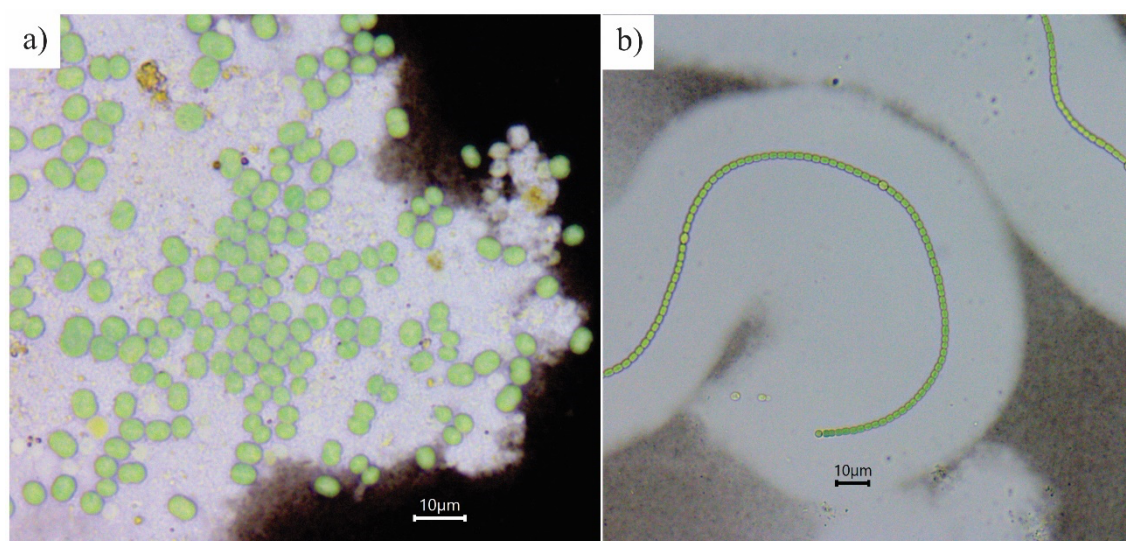


Fig. 1. Light micrographs of the cyanobacterial strains. (a) *Microcystis novacekii* BA005 and (b) *Nostoc paludosum* BA033. H = Heterocyte. The mucilaginous sheath (light patches) is evident, not stained by Indian ink.

2.3.3 Mn acute toxicity test

Mn toxicity tests were performed according to OECD 201 (2006). Briefly describing cyanobacterial cultures were prepared in 250 mL Erlenmeyer flasks containing 150 mL of culture medium, BA005 in ASM-1 and BA033 in BG-11₀ (pH = 7.0 ± 0.2). Initial cell concentration was 10⁵ cells · mL⁻¹. The experiment was conducted in same conditions of light and temperature previously described. Once exponential growth (called the log growth phase) was reached, different for each species, as cultures (in triplicate) were exposed to increasing concentrations of Mn. A stock solution of 15 g · L⁻¹Mn was prepared from the salt MnCl₂·4H₂O and was added to the cultures, obtaining the following Mn concentrations 7.0; 10.5; 15.7; 23.6 and 35.4 mg · L⁻¹Mn for BA005 and 15.0; 22.5; 33.7; 50.6 and 76.0 mg · L⁻¹ Mn for BA033 and negative controls without biomass addition.

These concentrations were defined by preliminary experiments to give an inhibition growth range of 10 to 100%, values above 100% growth reduction, corresponds to cell death. The concentrations were determined from the value of EC50 using a multiplication factor of 1.5, two below and two above this value. The cyanobacterial growth under these treatments was daily monitored up to 96 h by measuring optical density at 680 nm (DO_{680nm}). The equation $\%I = [(\mu_c - \mu_t) / \mu_c] \times 100$ was used to calculate the percentage of growth inhibition (%I), in which μ_c is the average growth rate of the control group and μ_t , the average growth rate for the Mn concentrations. The equations $y = 5.2381x - 35.402$ ($r^2 = 0.94$) for BA005 and $y = 1.2588x - 0.1512$ ($r^2 = 0.97$) for BA033 were obtained by linear regression of the growth inhibition percentage as a function of Mn concentrations and used to calculate the Mn concentration that effectively reduced the growth rate by 50 %. The high r^2 values obtained for both strains demonstrate that the linear fit is adequate for the calculation of EC 50 % as recommended by OECD 201 protocol.

2.3.4 Chlorophyll *a* content

To determine a total chlorophyll *a* (Chl *a*) content after 96 h of Mn exposure in toxicity experiments aliquots (10 mL) were taken from each Erlenmeyer flask and centrifuged at $12\,000 \times g$ for 10 min at 25 °C. The supernatant was discharged and to the pellet was added 3.5 mL of methanol (90 %), stirred vigorously in a manual shaker (Vortex) for 30 s and left for 25 min in the dark at room temperature. The absorbance (UV/Visible Hitachi U-200) was measured at 665 nm and the Chl *a* concentration was determined by the equation: Chlorophyll *a* ($mg \cdot L^{-1}$) = $\alpha \times$ Absorbance 665 nm, where α is the absorption coefficient for Chl *a* extracted with methanol ($\alpha = 12.7$) (Meeks & Castenholz 1971, modified by Fiore 2000).

2.3.5 Mn determinations

After 96 h of exposure to Mn, 30 mL aliquots were centrifuged for quantification of Mn in the biomass. In order to distinguish a fraction of adsorbed Mn on the cell surface and absorbed intracellular, as biomasses in each replicate were separated into two subsamples and prepared as follows: (i) biomass was washed three times with Mes-EDTA buffer (pH = 5.0) to remove or adsorbed Mn on the cell surface and only quantified the intracellular Mn (absorbed) and (ii) biomass washed three times in ultrapure water (Milli-Q) to evaluate the total fraction of Mn, absorbed and adsorbed, in cells. After knowledge of the absorbed Mn (biomass a) it is possible to gauge the quantification of the adsorbed Mn. The samples were dried at room temperature and weighed. The microwave digestion

was performed with 3.0 mL of concentrated HNO₃ (96 %) and 1 mL of H₂O₂ (30 %) (Truus *et al.* 2007). The teflon tubes were heated in a microwave accelerated reaction system for 30 min at 200 °C, power of 45 bar. The final volume was adjusted to 25 mL with ultrapure water. The supernatant was evaluated by subsamples (10 mL) of all replicates, after 2 h addition of Mn in the medium, estimated time for adsorption; and after 96 h of exposure, estimated time for absorption. These samples were centrifuged at 3000 rpm for 10 min at 25 °C, the supernatant was filtered with glass fiber membrane (1.2 µm) in order to remove any biomass still dispersed in the medium. Since the negative control does not have biomass, the quantification of Mn was performed only in the supernatant to confirm that the nominal concentration corresponds to the final concentration. These subsamples were acidified with concentrated HNO₃ (96%) and kept under refrigeration until analysis. Quantification of Mn in the biomass and supernatant were performed by inductively coupled plasma optical emission spectrometry (ICP-OES).

2.3.6 Transmission Electron Microscopy (TEM)

After 96 h of Mn exposure biomass from 10 mL aliquots of all treatments in the Mn acute toxicity test were fixed in glutaraldehyde (2.5 %), phosphate buffer (0.05 M), pH 7.0 during 24 h and washed three times in phosphate buffer (0.1 M). The fragments were post-fixed in osmium tetroxide (1 %) prepared in the same buffer and left standing for 4 h. Then they were dehydrated in ascending ethanol series before embedding in Spurr's resin (Spurr 1969) and the blocks were polymerized at 650°C for 20 h. Ultra-thin sections were obtained with the aid of an ultramicrotome (Sorvall Model MT-2). The post-staining was made in uranyl acetate and lead citrate (Reynolds 1963). The samples were examined in a transmission electron microscope in the Center of Microscopy of the Universidade Federal de Minas Gerais.

2.3.7 Statistical analysis

Cyanobacterial growth was analyzed by two-way analysis of variance (ANOVA) and the means were compared using the Tukey post hoc test ($P < 0.05$) within two factors namely Mn concentration and time. The average Chl *a* content and Mn concentration in the biomass and supernatant were compared between groups (n=3) by ANOVA and the means compared using the Tukey post hoc test ($P < 0.05$).

2.4 RESULTS

2.4.1 Acute toxicity test

The EC_{50} values obtained for BA005 and BA033 were $16 \text{ mg} \cdot \text{L}^{-1}$ and $39 \text{ mg} \cdot \text{L}^{-1}$ Mn, respectively (Fig. 2). Mn affected growth as a function of its concentrations and exposure time. At $7.0 \text{ mg} \cdot \text{L}^{-1}$ no effect was observed after 96 h of exposure on the growth of BA005 ($p=0.34$). However, at concentration of $35.4 \text{ mg} \cdot \text{L}^{-1}$ negative effects were detected after the first 24 h ($p=0.0015$). Behind 48 h of exposure was observed significant growth inhibition at concentration 23.6 and $35.4 \text{ mg} \cdot \text{L}^{-1}$ ($p=2.19^{-5}$ and 9.40^{-5} , respectively). After 96 h of exposure growth inhibition was significant for the concentrations 10.5, 15.7, 23.6 and $35.4 \text{ mg} \cdot \text{L}^{-1}$ ($p=0.004$; 1.7^{-5} ; 1.37^{-11} and 5.0^{-11} , respectively) (Fig. 3). In BA033, growth inhibition was initially detected after 48 h of exposure to the higher concentrations: 50.6 and $76.0 \text{ mg} \cdot \text{L}^{-1}$ ($p=1.24^{-5}$; 8.15^{-5} , respectively) (Fig. 3b); with increasing exposing time (96 h), negative effects in all Mn concentrations were observed ($p=0.019$; 0.016; 6.21^{-7} ; 6.18^{-9} and 5.36^{-11} , respectively).

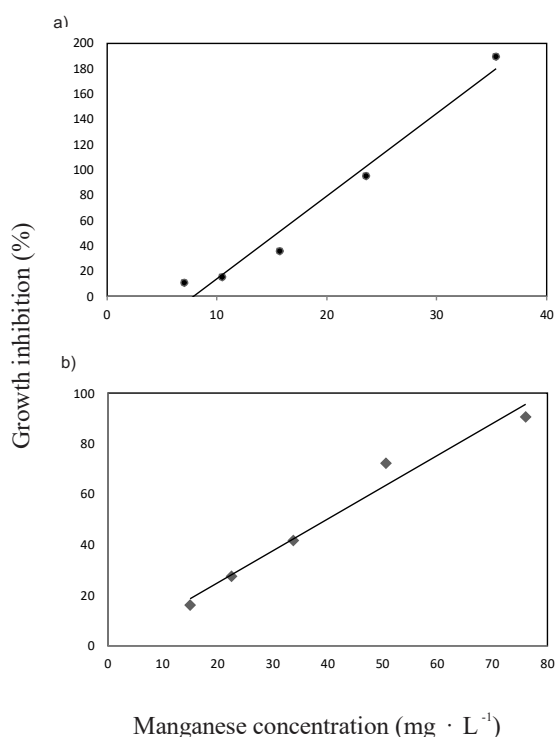


Fig. 2. Percentage growth inhibition after 96 h of Mn exposure. (a) *Microcystis novacekii* BA005, $y = 5,2381x - 35,402$ ($r^2= 0,94$); (b) *Nostoc paludosum* BA033, $y = 1,2588x - 0,1512$ ($r^2= 0,97$).

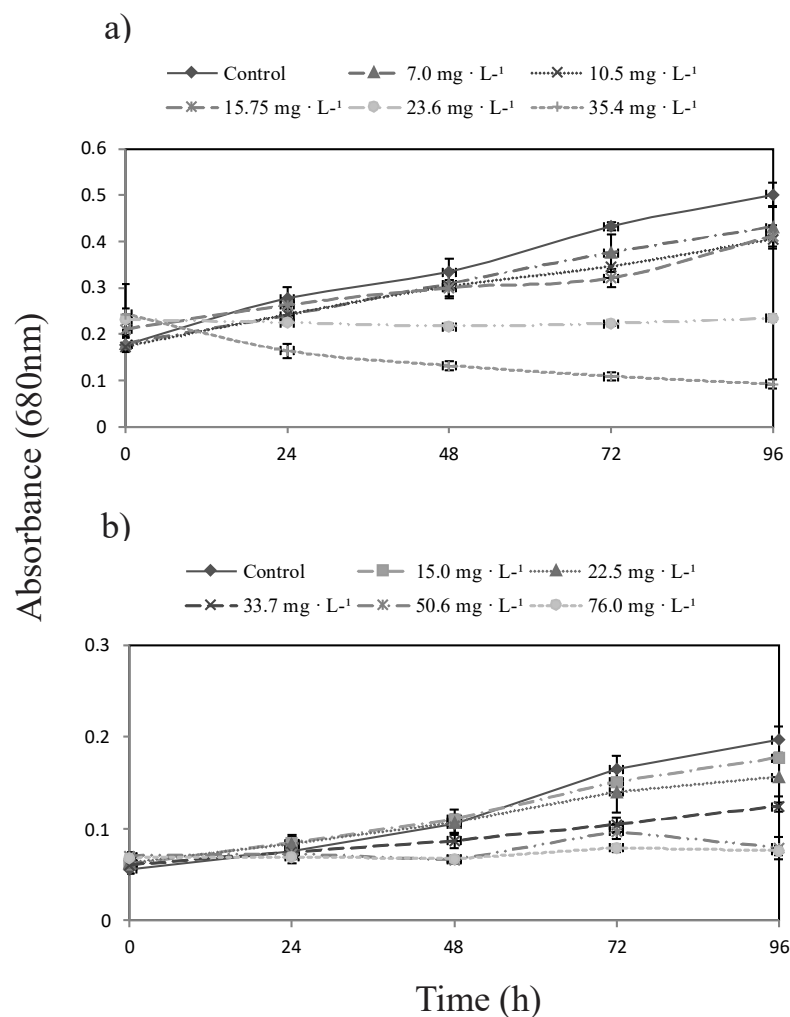


Fig. 3. Growth curves of *Microcystis novacekii* BA005 (a) and *Nostoc paludosum* BA033 (b) during 96 h of exposure to increasing Mn concentrations.

2.4.2 Chlorophyll *a* content

In the BA005 strain, a concentration of Chl *a* increased in the concentration 15.7 mg · L⁻¹ Mn ($p=0.008$) when compared to the control group. In the higher concentrations, 23.6 and 35.4 mg · L⁻¹, there was a significant reduction in the levels of Chl *a* ($p=0.003$ and 0.0002, respectively). In the BA033 strain there was a significant Chl *a* reduction at all concentrations tested, 15.0, 22.5, 33.7, 50.6 and 76.0 mg · L⁻¹ ($p=0.005, 0.01, 0.0001, 0.0001$ and 0.0001, respectively).

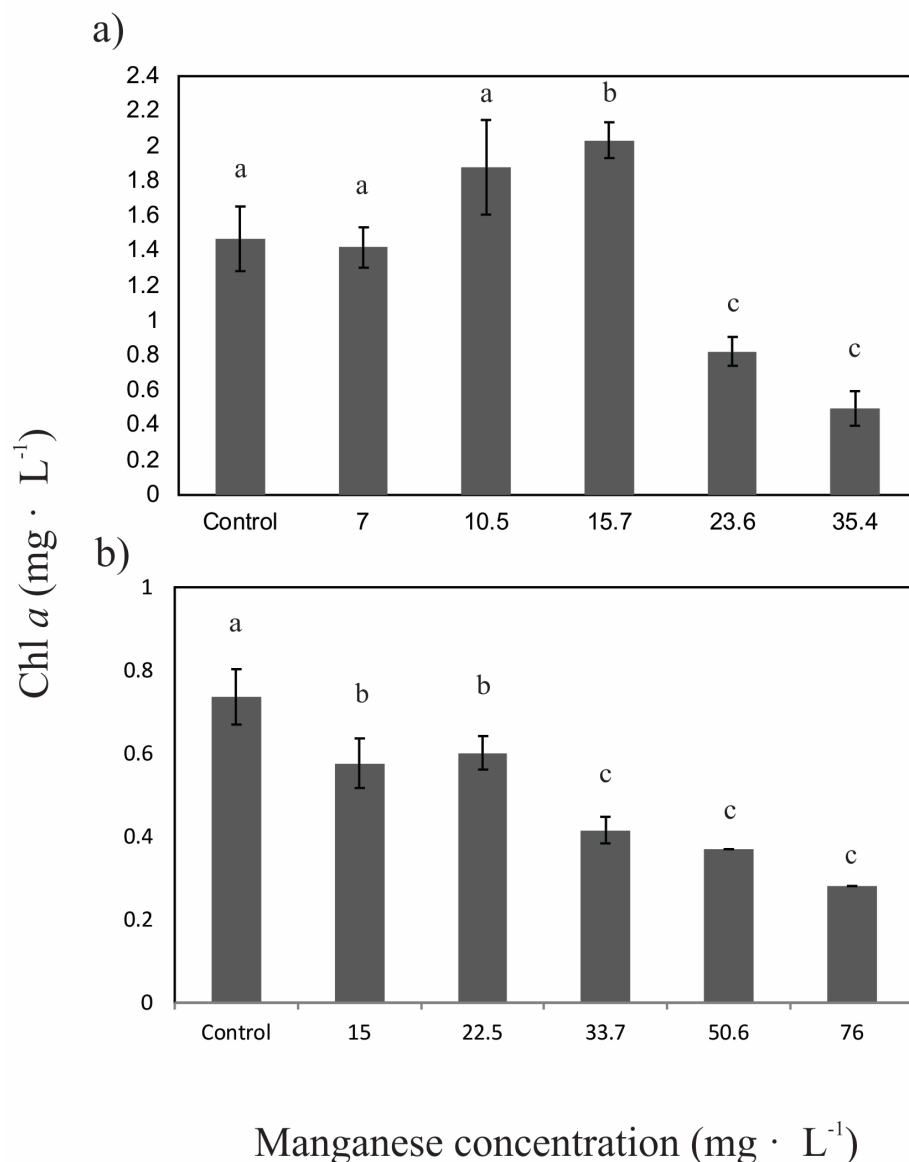
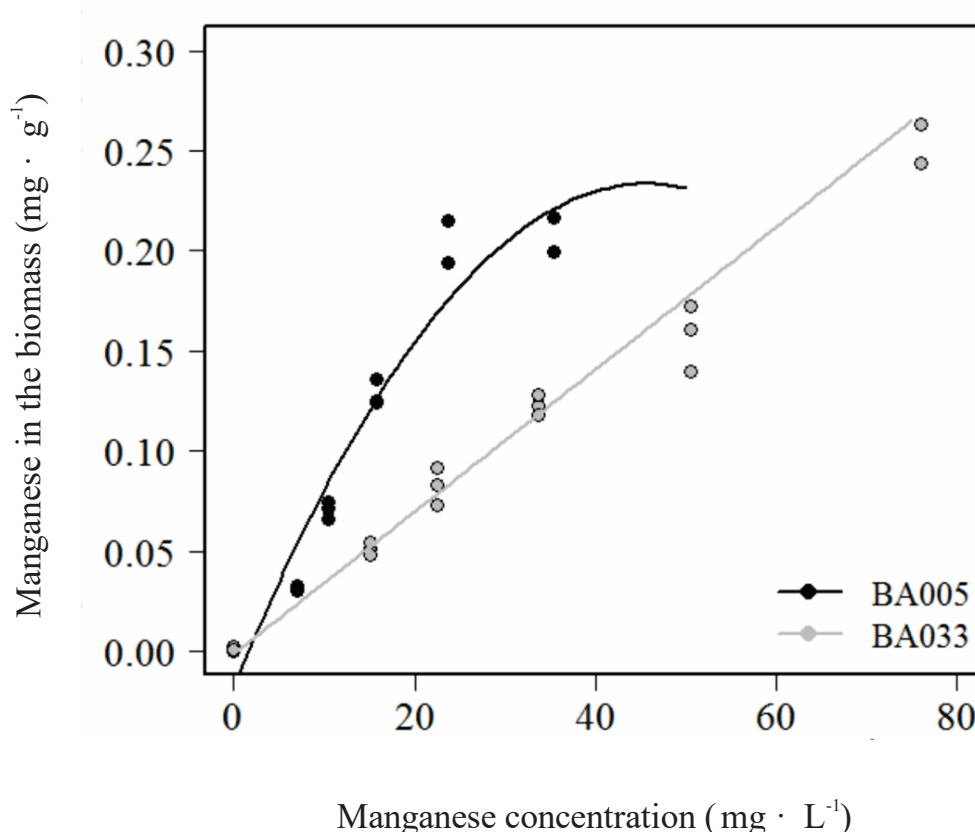


Fig. 4. Chlorophyll *a* content in cyanobacteria biomass after 96 h exposure to Mn. (a) *Microcystis novacekii* BA005; (b) *Nostoc paludosum* BA033. Bars with standard deviation and significant differences between groups.

2.4.3 Mn sorption by biomass

In both strains the quantification of Mn absorption, measure in the biomass washed with MES-EDTA, was less than 0.005 mg · g⁻¹ (limit of detection). On the other hand, the amount of adsorbed Mn quantified in the biomass washed with ultrapure water in BA005 ranged from 0.0 to 0.20 mg · g⁻¹; and BA033 varied between 0 and 0.25 mg · g⁻¹. BA005 at low concentrations (7.0, 10.5 mg · L⁻¹) adsorbed approximately 0.031 and 0.074 mg · g⁻¹ Mn; in the intermediate concentration (15.7 mg · L⁻¹) there was adsorption of approximately 0.12 mg · g⁻¹ Mn; at the highest concentrations (23.6 and 35.4 mg · L⁻¹) there was

1 adsorption of approximately $0.20 \text{ mg} \cdot \text{g}^{-1}$ Mn, suggesting a possible saturation effect on
 2 binding sites. The cianobacterium BA033 showed adsorption of approximately 0.048 and
 3 $0.092 \text{ mg} \cdot \text{g}^{-1}$ in the lower concentrations (15.0 and $22.5 \text{ mg} \cdot \text{L}^{-1}$, respectively); while in
 4 the intermediate concentration ($33.7 \text{ mg} \cdot \text{L}^{-1}$ Mn) the adsorption was approximately 0.12
 5 $\text{mg} \cdot \text{g}^{-1}$; and in the highest concentrations (50.6 and $76.0 \text{ mg} \cdot \text{L}^{-1}$) there was adsorption of
 6 0.14 and $0.27 \text{ mg} \cdot \text{g}^{-1}$ Mn (Fig. 5).



7
 8 Fig. 5. Manganese (Mn) adsorption by biomass at different concentrations. Dark
 9 Line: *Microcystis novacekii* - BA005. Clear line: *Nostoc paludosum*- BA033.

10 2.4.4 Mn concentration in the supernatant

11 After 2 h of exposure there was a decrease in the concentration of Mn in the
 12 supernatant in both species tested, due to the effect of adsorption by the biomass. BA005
 13 showed to be more efficient in the concentrations 7.0 and $10.5 \text{ mg} \cdot \text{L}^{-1}$ Mn, with removal
 14 of approximately 78 and 76%, respectively. While BA033 showed approximately 10%
 15 removal at concentrations 22.5 and $33.7 \text{ mg} \cdot \text{L}^{-1}$. In the highest concentrations tested in
 16 both species (BA005 - $35.4 \text{ mg} \cdot \text{L}^{-1}$; BA033 - $76.0 \text{ mg} \cdot \text{L}^{-1}$ Mn), there was a reduction in
 17 the percentage of removal, 31% and 14%, respectively.

After 96 h of exposure, BA005 showed approximately 100% removal of the Mn at the concentration $10.5 \text{ mg} \cdot \text{L}^{-1}$. At concentrations 7.0 and $15.7 \text{ mg} \cdot \text{L}^{-1}$ Mn showed removal of approximately 94 and 96%, respectively. While BA033 showed removal of 29, 38 and 31% of the Mn at concentrations 15.0 , 22.5 and $33.7 \text{ mg} \cdot \text{L}^{-1}$ Mn, respectively. In the highest concentrations tested in both species (BA005 - $35.4 \text{ mg} \cdot \text{L}^{-1}$; BA033 - $76.0 \text{ mg} \cdot \text{L}^{-1}$ Mn), there was a reduction in the percentage of removal, 31% and 14%, respectively.

Table 1. Concentration of Mn on supernatant after 2 h and 96 h for *Microcystis novacekii* and *Nostoc paludosum*

<i>Microcystis novacekii</i> – BA005					
Treatments (mg L^{-1})	Supernatant (-) (mg L^{-1})	Supernatant 2h (mg L^{-1})	Removal percentage (%)	Supernatant 96h (mg L^{-1})	Removal percentage (%)
control		0.21 ± 0.0		0.18 ± 0.0	
7.0	7.2 ± 0.0	1.57 ± 0.0	78	0.43 ± 0.3	94
10.5	11.4 ± 0.6	2.78 ± 0.0	76	$< 0.06 \pm 0.0$	~100
15.7	15.7 ± 0.0	6.52 ± 0.3	59	0.56 ± 0.4	96
23.6	23.4 ± 0.2	12.96 ± 1.8	45	5.01 ± 2.2	79
35.4	34.3 ± 0.6	24.25 ± 0.9	29	23.69 ± 0.8	31
<i>Nostoc paludosum</i> – BA033					
Treatments (mg L^{-1})	Supernatant (-) (mg L^{-1})	Supernatant 2h (mg L^{-1})	Removal percentage (%)	Supernatant 96h (mg L^{-1})	Removal percentage (%)
control		0.28 ± 0.0		0.25 ± 0.0	
15.0	14.6 ± 0.4	13.38 ± 0.4	8	10.30 ± 0.7	29
22.5	21.9 ± 0.7	19.62 ± 0.2	11	13.68 ± 0.8	38
33.7	32.8 ± 1.0	29.68 ± 0.3	10	22.56 ± 0.3	31
50.6	49.7 ± 2.0	45.23 ± 2.8	9	39.09 ± 0.2	21
76.0	72.9 ± 3.4	69.53 ± 1.9	5	62.70 ± 0.8	14

7

2.4.5 Visual, morphological and ultrastructural effects

Visual effects in both strains were observed during growth. It is important to mention that the control group of BA005 remained homogeneous while in cultures exposed to Mn concentrations higher than $7 \text{ mg} \cdot \text{L}^{-1}$, it was observed the formation of agglomerated visual clumps on the surface of the culture medium. Whilst the color of the cultures was bright green in the control it was slightly yellow following Mn exposure. Notably, cell aggregation in the center of the culture flasks was also observed for cultures of BA033 after exposure to concentrations above $15 \text{ mg} \cdot \text{L}^{-1}$ Mn (Fig. 6 a-d).

Mn precipitation in the medium in both cyanobacteria cultures were observed under light microscope. In cultures of BA005 the cells were aggregated around the precipitate (Fig. 6 e). In BA033 Mn precipitation around vegetative cells was observed (Fig. 6 f). Morphological damage was not detected in BA005 (Fig. 6 g) whereas in BA033 cell

1 disruption, intense granule formation, cell chlorosis, and retraction of the cytoplasm were
2 observed (Fig. 6 h).

3 Ultrastructural changes were observed in BA005 and BA033 under Mn exposure. At
4 lower Mn concentrations (below $20.0 \text{ mg} \cdot \text{L}^{-1}$) an increase in the number of carboxysomes,
5 polyphosphate granules and the appearance of intrathylakoidal spaces were observed (Fig.
6 7b, c, e). At concentrations higher than $20.0 \text{ mg} \cdot \text{L}^{-1}$ an increased periplasmic space was
7 also observed (Fig. 7f).

8

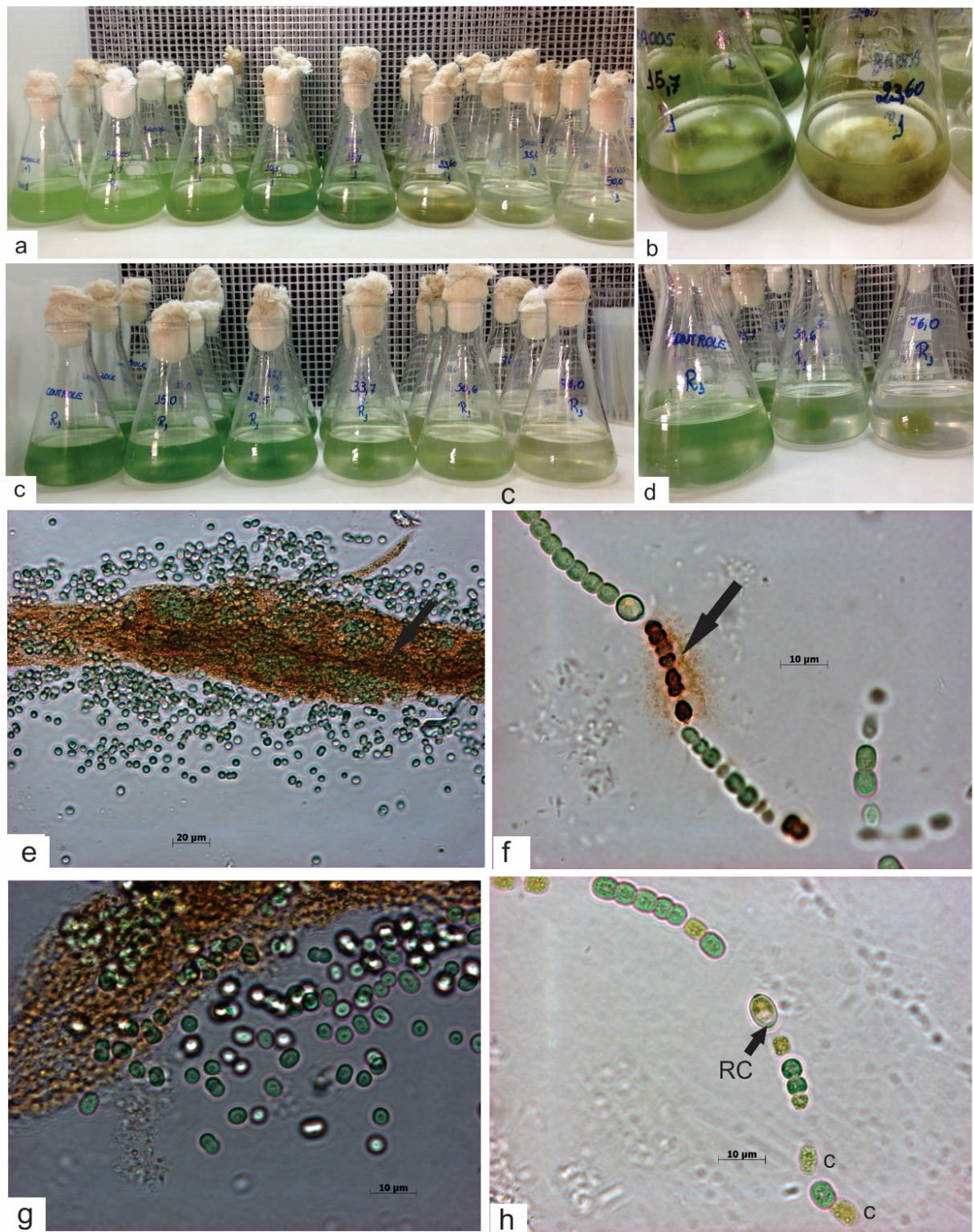
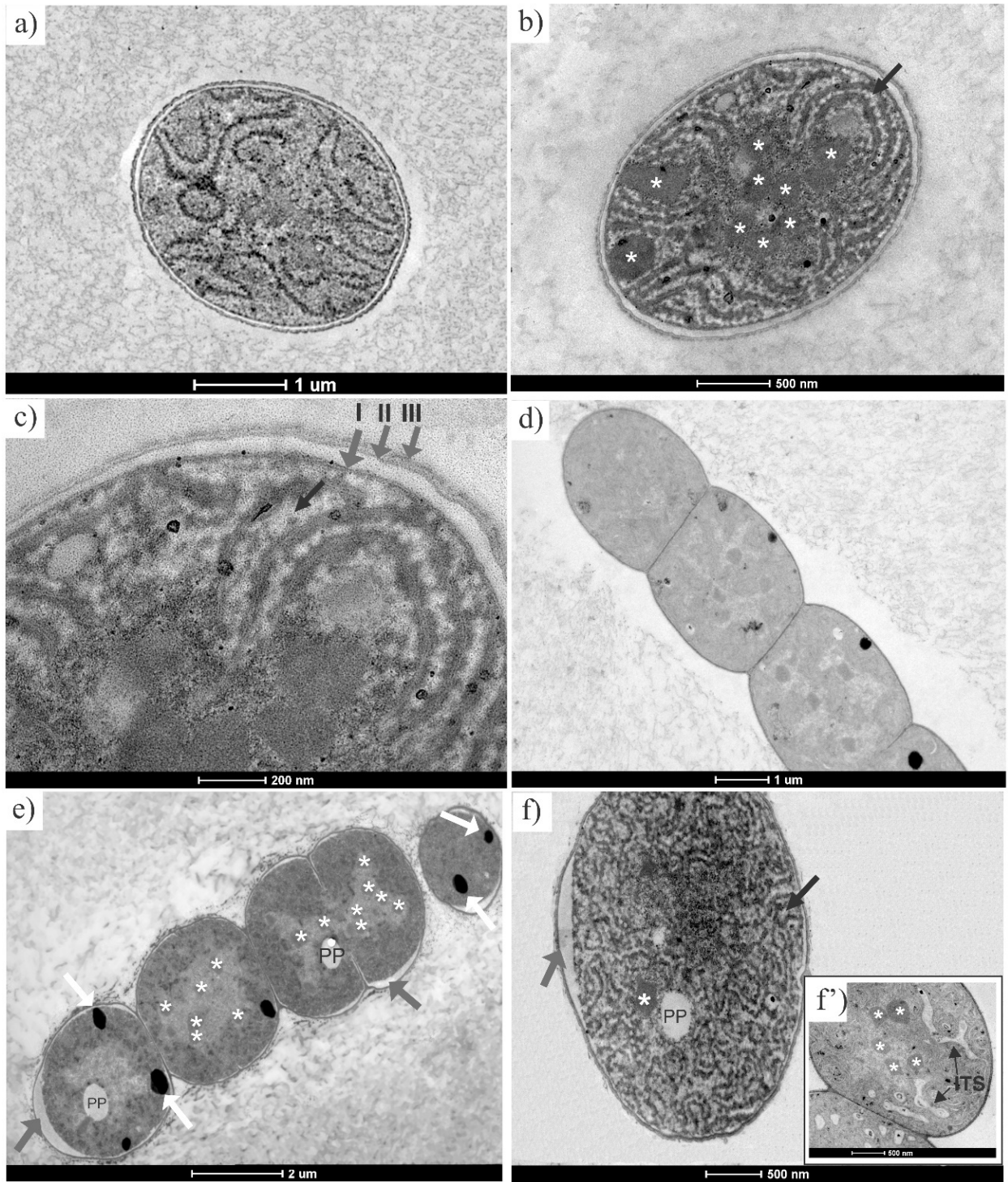


Fig. 6. (a-b) *Microcystis novacekii*; (c-d) *Nostoc paludosum* during the experimental period at different concentrations of Mn, demonstrating visual alterations at the highest concentrations. (b) a brown-green colour was obtained at concentrations above 15.7 mg·L⁻¹ Mn. (d) visible at the center of the flask at concentrations higher than 33.7 mg·L⁻¹ Mn. (e) *Microcystis novacekii* possibly metallic deposit (black arrow) and cellular agglomerate

1 to the environment. (f) *Nostoc paludosum* possibly metallic deposit (black arrow)
 2 extracellular. (g) morphological damage was not detected in *M. novacekii*. (h) *N.*
 3 *paludosum* cell disruption, intense granule formation, cell chlorosis, and retraction of the
 4 cytoplasm were observed in concentrations above $33.7 \text{ mg} \cdot \text{L}^{-1} \text{ Mn}$.

5



6

Fig. 7. Transmission electron microscopy (TEM) micrographs of cyanobacterial strains: (a) *Microcystis novacekii* BA005 – control; (b) *Microcystis novacekii* BA005 after 96 h of Mn exposure, showing the carboxysomes (white asterisks) and intrathylakoidal spaces (black arrow); (c) Details of the cellular shell layers: the outer membrane (gray arrow III), peptidoglycan layer (gray arrow II) and plasma membrane (gray arrow I) and intrathylakoidal spaces (black arrow); (d) *Nostoc paludosum* BA033 – control; (e) *Nostoc paludosum* BA033 showing increased periplasmic spaces (gray arrows), carboxysome (white asterisks) and polyphosphate granules (white arrows), PP = polyphosphate granules (electron-transparent region); (f) *Nostoc* cell showing the intrathylakoidal spaces (black arrow), carboxysome (white asterisk), increased periplasmic spaces (gray arrow), PP: polyphosphate granules (electron-transparent region); (f') Detail of intrathylakoidal spaces (ITS) and carboxysome (black asterisks).

2.5 DISCUSSION

According to our results, it is possible to infer that both strains of cyanobacteria studied have the capacity to remove Mn in solution, with adsorption being the main means of removal. This result was found for all concentrations tested. However, a more promising Mn adsorption result was found for strain BA005 as compared to strain BA033. On the other hand, BA033 showed to be more resistant, surviving in high concentrations ($> 35 \text{ mg} \cdot \text{L}^{-1}$ of Mn), suggesting that the lower adsorption performed by BA033 guarantees a better survival strategy for this species. This characteristic is important in the bioremediation process, since the BA005 strain presents a higher Mn removal capacity of the medium at concentrations below $30 \text{ mg} \cdot \text{L}^{-1}$ Mn, and the BA033 strain has the capacity to survive in concentrations higher than $30 \text{ mg} \cdot \text{L}^{-1}$, with the ability to remove Mn from the medium.

The concentrations of toxic Mn can affect cell metabolism by decreasing the chlorophyll *a* content, reducing the growth of cyanobacteria and significantly altering the cellular ultrastructure. Although these effects were common to both strains tested, the values of the toxic concentrations of Mn differed from each other. BA033 shows a higher resistance to Mn (EC_{50} of $39 \text{ mg} \cdot \text{L}^{-1}$) compared to BA005 (EC_{50} of $16 \text{ mg} \cdot \text{L}^{-1}$) (Fig 2). Growth reductions in BA005 were observed in the first 24 h after exposure to Mn, while BA033 showed effects after 48 h (Fig. 3). The toxic levels of Mn obtained here are in good agreement with previous studies. For example, Hudek et al. (2012) found a significant reduction in *Nostoc punctiform* growth after 24 h exposure to the Mn concentration of $1000 \mu\text{M}$ ($54.9 \text{ mg} \cdot \text{L}^{-1}$).

The effect of Mn on the biomass production of the strains was strongly correlated with the concentration. At high concentrations of Mn not only reduced biomass growth but also led to the death of cyanobacteria, observed in BA005 at concentration $35.4 \text{ mg} \cdot \text{L}^{-1}$ Mn. Percentages of growth inhibition in both species are shown in Table 2.

Table 2. Growth inhibition percentage of after 96 h exposure to Mn for *Microcystis novacekii* and *Nostoc paludosum*

BA005		BA033	
Treatments (mg L ⁻¹)	Percentage inhibition of growth (%)	Treatments (mg L ⁻¹)	Percentage inhibition of growth (%)
Controle (0)		Controle (0)	
7	10	15	16
10.5	14	22.5	27
15.75	35	33.7	41
23.6	95	50.6	72
35.4	189	76	90

1

2 The higher tolerance conferred to BA033 cyanobacteria can be explained by the
3 presence of exopolysaccharides which is more expressive in this species (Fig. 1).
4 Exopolissaccharides are extracellular polymeric substances (EPS) mainly of
5 polysaccharide nature. EPS may remain associated with the cell surface or be released into
6 the surrounding environment as released polysaccharides (PSRs). In the culture medium,
7 EPS plays a defense role causing excess solution ions not to enter the cell. The negative
8 charge of cyanobacterial EPS may be essential to sequester metal cations participating in
9 the process of bioaccumulation of metals of the medium (Pereira *et al.* 2011), thus being a
10 favorable feature for bioremediation, through the immobilization of this metal. At the
11 morphological level, it is possible to observe that BA033 presented an extracellular
12 precipitation (Fig. 6f), facilitated by the mucilage sheath. In BA005 the exopolysaccharide
13 sheath is less evident, making it impossible to visualize extracellular precipitate.

14 It has been reported that the presence of a structured envelope (EPS) at least partially
15 prevents the entry of this metal into the cells (Pereira *et al.* 2011). Consistent with this
16 report, the present study demonstrated that the strain with the highest amount of EPS
17 (BA033) is more tolerant to high concentrations of Mn than the strain with the lowest
18 amount of EPS (BA005). Hazakira *et al.* (2014) reported that growth inhibition in *Nostoc*
19 *muscorum* is dose-dependent for different metals (Cu (II), Zn (II), Pb (II) and Cd (II)) as
20 observed in this work. However, BA005 was more efficient in the removal process,
21 reaching values for removal near to 100% of the total Mn (Table 1). The results obtained
22 here corroborate with previous work, e.g. Bender *et al.* (2009) which demonstrated

adsorption up to 99% of the total Mn and Zinc present in the medium by cyanobacterial biomass columns with cyanobacteria mats without specifying species. Meanwhile, Hazarika *et al.* (2015) demonstrated the ability of *Nostoc muscorum* to remove 98% silver, 88% copper, 82% cadmium and 67% zinc from an initial contamination of $5 \text{ mg} \cdot \text{L}^{-1}$ of each metal. El-Sheekh *et al.* (2005) found approximately 30% of Mn removal from aqueous medium by *Nostoc muscorum* species. The adsorption curves of both species show that there was possibly a saturation in BA005, however, it is necessary to test the adsorption at concentrations higher than $35.4 \text{ mg} \cdot \text{L}^{-1}$ to confirm this event. The same was not observed for BA033, suggesting that in higher concentrations possibly there would be an increasing continuity of the adsorption (Fig. 5). The adsorption saturation occurs because the binding sites of the metals have already been occupied and, although there is an increase in concentration, there is no availability of binding sites (Raungsomboon *et al.* 2008; Kumar *et al.* 2012).

The EC_{50} values obtained for the strains analyzed here demonstrated that both are resistant to high Mn levels (Fig. 2), when compared to other metals. For instance, Rangsayatorn *et al.* (2002) found in *Spirulina (Arthrospira) platensis* EC_{50} of $18.35 \text{ mg} \cdot \text{L}^{-1}$ for cadmium; Franco *et al.* (2015) showed in *Synechococcus* sp. BA015 an EC_{50} of $6.64 \text{ mg} \cdot \text{L}^{-1}$ for arsenic (III). Furthermore, a lower EC_{50} was previously demonstrated by Hudek *et al.* (2012) for the strain *Nostoc punctiforme* ($27.0 \text{ mg} \cdot \text{L}^{-1} \text{ Mn}^{2+}$). Cyanobacteria resistance to high levels of Mn leading to relatively low toxicity of this metal in moderate concentrations may be related to the requirement of Mn ions on whole cell metabolism given that Mn is an important cofactor for enzymes and play an essential role in the photosynthetic process (Bartsevich & Pakrasi 1996, Cao *et al.* 2011, Hudek *et al.* 2012, Fischer *et al.* 2015). By sharp contrast, the harmful effects observed at high Mn concentrations are most likely related to nutritional imbalance caused by excess of Mn in solution. Mn is one of the metals normally associated with iron deficiency due to competition for the site (porphyrin ring) in which iron is necessary for the synthesis of chlorophyll (Csatorday *et al.* 1984). Thus, we cannot formally exclude that reduction of chlorophyll *a* observed in this study for both tested strains may be a result of iron deficiency. The reduction of Chl *a* followed the same pattern observed for growth in presence of Mn: the reduction was prominent in BA005 compared to BA033 possibly due to the presence of an abundant mucilaginous sheath in the cells of BA033. Therefore, according to our results, it seems reasonable to assume that toxic Mn concentrations affect

primary productivity through its interference on the Chl *a* content (Fig. 4). In BA005, Chl *a* content increased following exposure to lower concentrations of Mn coupled with slightly inhibition in growth (Fig. 3). Growth stimulation at low concentration of the contaminant is expected (OECD 201 2006). In a similar vein this effect was previously observed in *Phaseolus vulgaris* as tested organism under Mn stress (Teixeira *et al.* 2004).

The concentration of Mn in the biomass washed with ultrapure water, that is, the amount of Mn adsorbed and absorbed, was significant at the concentrations tested. However, as the biomass absorbed (washed with MES-EDTA) was infema, we consider that the quantification of Mn in the biomass washed with ultrapure water represents the adsorbed portion (Fig. 5). It is possible that at lower pH the H⁺ ions of the Mes-EDTA buffer solution competed for the binding sites in cyanobacteria cell wall and removed the Mn adsorbed. A support for this assumption is obtained by the characteristics of the surface structure of the cyanobacterial cell envelope layers with unique functional molecular groups with diverse metal-binding capacity (Yee *et al.* 2004) that favor adsorption of Mn on the surface. Furthermore, it is known that the higher Mn fraction is deposited in cell outer membrane and the Mn fraction transported into the cell is dependent on specific trans-membrane transporters (Rengstl *et al.* 2012, Fischer *et al.* 2015). Accordingly, Keren *et al.* (2002) demonstrated that the concentration of Mn is 10 times higher in the outer membrane than in the cytoplasmic membrane of *Synechocystis* sp. The adsorption of metals by cyanobacteria can be facilitated by the presence of a mucilaginous sheath. Thus, it has been demonstrated that microorganisms with more evident exopolysaccharide layer have greater potential for adsorption (Parker *et al.* 2000). In agreement with this finding, the higher Mn concentration detected was presented in BA033 a strain that produces abundant mucilaginous sheath.

To our knowledge this is the first study reporting the ultrastructural effects of Mn on cyanobacteria (Fig. 7). In both species, after exposure to Mn concentrations above 15.0 mg · L⁻¹ it was observed a swelling of intrathylakoidal spaces, increased number of carboxysomes and polyphosphate granules, and increased periplasmic space. Notably, all the effects above mentioned were more intense with increasing Mn concentration.

The increase in intrathylakoidal spaces is, since Mn is temporarily stored in these sites, due to its central role in the reactions of photosystem II (PS II) (Sherman *et al.* 1994, Parker *et al.* 2000). Okada & Asada (1983) reported that the Mn oxidized in PS II with the

dehydrogenation of water is transferred to the intrathylakoidal spaces reinforcing the hypothesis that these spaces result from the accumulation of Mn ions in this region after excessive water oxidation in PSII. This process of Mn accumulation in the intrathylakoidal spaces can provide greater acclimatization of cell following stress conditions. Gorelova & Baulina (2009) have investigated cyanobacterial growth under stress caused by absence of light and suggested that the swelling of intrathylakoidal spaces is related to the acclimatization of these microorganisms. Furthermore, intrathylakoidal spaces are a result of the shutdown of respiratory electron transport chain and the photosynthetic system in the membranes of thylakoids (Baulina 2012). It is worth to mention that damages to the thylakoid results in a decrease in photosynthetic activity and growth (Rangsayatorn *et al.* 2002), which could be confirmed in our study in both species. Heng *et al.* (2004) demonstrated that heavy metal ions (such as Pb^{2+}) are capable of binding to thylakoid membrane resulting in the alteration of the ultrastructure of thylakoids, which would eventually deteriorate the routine functions of thylakoids.

Carboxysomes are polyhedral bodies composed mainly by the enzymes carbonic anhydrase (CA) and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), responsible for CO_2 fixation and carboxylation in the Benson-Calvin cycle, respectively (Codd & Marsden 1984, Badger & Price 2003, Kupriyanova *et al.* 2013). The results obtained here indicated that the increased number of carboxysomes observed in both species following exposure to increasing Mn concentrations is most likely due to high energetic requirements for post endogenous use to overcome the toxic effects of Mn. Molecular details concerning the connections between growth and energetic requirements following Mn exposure in cyanobacterial strains still needs to be revealed in order to fully understand how these species adjust their whole metabolism to support growth being important and strategic research avenues to be pursued.

Polyphosphates granules (PP) are a primary source of phosphorus. However, they are also composed of other materials such as magnesium, sodium, potassium, iron, and copper (Baulina 2012). It has been previously suggested that PP are responsible for the uptake of heavy metals in cyanobacteria. The highly negatively charged lipids, and proteins on the surface of PP act as an absorption site for the metal ions, contributing to cellular detoxification, since the metal remains immobilized and unavailable within the granules. (Jensen *et al.* 1982). Further studies had suggested the role of PP in cell detoxification under exposure to various metal ions in different groups of cyanobacteria. For instance,

Rachlin *et al.* (1985) found an increase in PP as an effect of zinc in *Anabaena flos-aquae* and *A. variabilis*, while Rangsayatorn *et al.* (2002) found the same effect in *Spirulina* (*Arthrospira*) *platensis* after exposure to cadmium. Similarly, Baxter & Jensen (1980) observed the presence of magnesium, strontium, barium and Mn in PP in cyanobacterial cells exposed to these metals. In accordance with several studies demonstrating that PP rapidly respond to environmental alterations, the increased number of PP observed in BA005 and BA033 can be assumed as a direct response to Mn and that it further contributes to cellular detoxification. It remains to be tested in which extension this might impact cyanobacterial tolerance to the presence of heavy metal.

2.6 CONCLUSIONS

Our results demonstrated that cyanobacterial strains of different morphologies (*M. novacekii*- BA005 and *N. paludosum*- BA033) suffered acute toxicity due to Mn excess in solution. The high level of this metal promoted metabolic impairments in both strains which were evidenced by a reduction in chlorophyll *a* content and growth rates coupled with morphological and structural changes as well as with the extracellular deposition of Mn, increments in periplasmic space, the appearance of intrathylakoidal space together with higher number of carboxysomes and polyphosphate granules.

Despite toxicity, cyanobacterial strains tested are tolerant to high concentrations of Mn, based on the common values found in contaminated water resources. These lines demonstrate different adsorption profiles depending on the concentration of the metal in the medium. Thus, depending on Mn concentration both strains of cyanobacteria studied demonstrate the potential of bioremediation for contaminated water. That is, the associated toxic effects, the BA005 strain presents a greater bioremediation potential at concentrations lower than 30.0 mg · L⁻¹, since a BA033 strain presents a greater bioremediation potential at concentrations above this mentioned value, presenting a biological alternative for decontamination environmental protection.

It is important to mention that toxicity studies involving excess of Mn in cyanobacteria and other aquatic organisms are barely found in the literature. Thus, the results discussed here may be used as biological foundation for measuring Mn toxicity, especially in aquatic ecosystems as well as it provides an improvement of our current knowledge concerning the physiology and morphology of cyanobacteria following excess of Mn. In addition to inferring about the possibility of cyanobacteria acting as

bioremediation agents of contaminated environments. Although the results are clearly showing the importance of studies involving Mn toxicity it remains to be elucidated how exactly high levels of Mn are able to interfere in cyanobacteria physiology. It will therefore be important to expand this approach to a great number of strains, with even more distinct morphologies maintained in other culture collections.

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3. CONSIDERAÇÕES FINAIS

Neste estudo, verificou-se que ambas as espécies de cianobactérias estudadas apresentaram efeitos tóxicos causados pelo Mn. E que apesar dos efeitos tóxicos apresentados, ambas as espécies apresentaram potencial biorremediador de Mn.

No que tange os efeitos tóxicos do Mn em ultraestruturas de cianobactérias, podemos evidenciar que houve alterações em ambas as espécies. De forma dependente da concentração, foi visualizado o surgimento de espaços intratrilacóides, espaços periplasmáticos, grupos de polifosfatos e carboxissomos. Estes grupos endógenos estão sendo estudados como possíveis desintoxicantes celulares, que podem estar exercendo o papel de aprisionamento do metal imobilizando-o na célula, minimizando os efeitos tóxicos do mesmo. O Mn por se tratar de um co-fator essencial no processo da fotossíntese, em excesso, causou toxicidade no maquinário fotossintético, havendo surgimento dos espaços intratrilacóides gradativos com a concentração de Mn, e redução da concentração de clorofila *a* em ambas as espécies.

No que tange o potencial de biorremediação, este trabalho demonstrou que dependendo da concentração de Mn, ambas as cepas de cianobactérias estudadas demonstraram o potencial da biorremediação para a água contaminada. A cepa BA005 apresentou maior potencial de biorremediação em concentrações menores que $30,0 \text{ mg} \cdot \text{L}^{-1}$, uma vez que uma cepa BA033 apresentou maior potencial de biorremediação em concentrações acima do referido valor, apresentando uma alternativa biológica para descontaminação ambiental. Considerando a quantificação de Mn adsorvido, em BA005 observou-se uma possível saturação dos sítios de ligação, mesmo esta cepa sendo considerada a mais eficiente e removendo aproximadamente 100% do Mn. Enquanto BA033 permaneceu crescente dependente da concentração, mas retirando, aproximadamente 40% do total de Mn. Fato relevante no processo de biorremediação, já que cada cepa apresenta-se mais eficiente em diferentes concentrações.

O processo de biorremediação, ou seja, incorporação do Mn na biomassa das cianobactérias, tem como finalidade o aproveitamento total deste metal. O Mn por se tratar de um micronutriente é amplamente utilizado em enriquecimento de nutrição animal, bem como fertilizantes naturais. Assim, a biomassa com Mn incorporado, seria reutilizada nestes processos, a fim de haver a reciclagem total do metal.

1 É importante mencionar que os estudos de toxicidade envolvendo excesso de Mn em
2 cianobactérias e outros organismos aquáticos são pouco encontrados na literatura. Assim,
3 pode-se concluir, que os resultados discutidos aqui podem ser utilizados como base
4 biológica para medir a toxicidade do Mn, especialmente em ecossistemas aquáticos, bem
5 como proporciona uma melhoria dos nossos conhecimentos atuais sobre a fisiologia e
6 morfologia de cianobactérias após o excesso de Mn. Além de inferir sobre a possibilidade
7 de as cianobactérias atuarem como agentes de biorremediação de ambientes contaminados
8 através de tecnologias a serem desenvolvidas utilizando sua biomassa.

9

4. SUGESTÕES PARA TRABALHOS FUTUROS

- Conhecer os mecanismos de remoção do Mn;
- Conhecer os efeitos bioquímicos envolvidos da bioacumulação de Mn;
- Determinar a localização do Mn no interior da célula;
- Desenvolver tecnologias biológicas para remediar ambientes contaminados por Mn;