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RESEARCH PAPER

Influence of calcium on cadmium uptake and toxicity to the cyanobacterium *Nostoc muscorum* Meg 1

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Abstract It is well known that Ca^{2+} interferes with uptake and expression of toxicity of Cd^{2+} in numerous organisms. This study demonstrated that the morphological alterations expressed in the cyanobacterium *Nostoc muscorum* Meg 1 cells exposed to 0.5 mg L^{-1} Cd^{2+} for 7 days were prevented when 10 mg L^{-1} Ca^{2+} was included in the experimental set-up. The spectroscopic analysis confirmed a severe reduction in contents of chlorophyll *a*, phycocyanin, allophycocyanin, phycoerythrin, carotenoids and total protein at the end of 168 h (7 days) in presence of Cd^{2+}; inclusion of Ca^{2+} significantly lessened these effects. Heterocyst frequency, nitrogenase and, glutamine synthetase activities were similarly improved in the presence of Ca^{2+}. Uptake studies showed a reduction in 94% biosorption of supplied Cd^{2+} to 50% in the presence of Ca^{2+} indicating its competition with Cd^{2+} binding onto the cyanobacterial cell surfaces.

Introduction

Concentrations of different metal ions are increasing in the environment because of various anthropogenic activities and are becoming a serious concern due to their toxicity and non-degradability (Gupta & Rastogi, 2008). Among these, Cd^{2+} is one of the extremely toxic metals with potent carcinogenic and mutagenic properties (Awofolu, Mbolekwa, Mtshemla, & Fatoki, 2005). It has been shown that Cd^{2+} being an analogue of the essential ion Ca^{2+} can use Ca^{2+} transporters to travel into or in between plant cells leading to accumulation in edible parts and increasing the possibility of bio-concentration in higher trophic levels, e.g. humans (Antosiewicz & Hennig, 2004; Jacobson & Turner, 1980; Zorrig, Shahzad, Abdelly, & Berthomieu, 2012).

Cd^{2+} has been shown to produce adverse effects not only in higher organisms but also in various microbes such as cyanobacteria and algae that are the primary producers in many ecosystems (Nongbri & Syiem, 2012; Singh, Kumar, Rai, & Singh, 2016).

Amongst microbes, cyanobacteria are highly versatile and resilient occurring ubiquitously in diverse habitats including rice fields, extreme environments and contaminated sites (Abed, Dobretsov, & Sudesh, 2009; Dubey, Dubey, Mehra, Tiwari, & Bishwas, 2011). A large number of cyanobacteria have been reported from different ecological niches of the state of Meghalaya which boast of

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unique climate and topography (Nongbri & Syiem, 2012). Meghalaya receives an average annual rain of 1200 cm making it wettest place on earth. The state has 70% forest coverage and is primarily agrarian with 80% of its population depending on farming. The state also has substantial coal, lime stone and uranium deposits of which coal and lime are regularly mined for commercial purposes. Many rice fields adjacent to the coal and limestone mining areas receive mining effluents that contain high amounts of Cu^{2+}, Fe^{2+}, Ca^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, and Cr^{6+} during monsoon season (Ahad, Goswami, & Syiem, 2017). Zeng, Tang, Liu, & Jiang (2012) and Gibbons et al. (2011) recently have reported that among these ions, accumulation of Cd^{2+} in microbes upset their cell physiology by producing reactive oxygen species (ROS) and by disrupting respiratory proteins.

In the scenario of Meghalaya, Cd^{2+} along with other metal ions gets added to the environment due to extensive coal mining in the state. In addition, lime stone mining aids in enriching soil and water bodies with excess Ca^{2+} ions. With the understanding that Cd^{2+} is an analogue of Ca^{2+}, it may be rationalized that presence of excess Ca^{2+} competes with Cd^{2+} for surface binding and transport into the cells thus reducing effective concentration of Cd^{2+} that the organism gets exposed to. This may explain the ability of various cyanobacteria to grow and proliferate in these areas. There are reports in the literature of Ca^{2+} interfering with Cd^{2+} induced changes in microbes. Fernandez-Piñas, Mateo, & Bonilla (1995) have reported that Cd^{2+} toxicity to *Nostoc* UAM208 could be reduced by increasing Ca^{2+} in the culture medium. Mateo, Fernandez-Pinas, & Bonilla (1994) found that the nitrogenase and photosynthetic activities were remarkably increased after addition of Ca^{2+} to Cd^{2+} treated *N.* UAM208 cultures suggesting an antagonistic relationship between Ca^{2+} and Cd^{2+}. Probably, when Ca^{2+} is present along with Cd^{2+}, it competes for the transporters due to their similarity in ionic radii and thus reducing Cd^{2+} accumulation in the cells (Gipps & Coller, 1982).

Although there are number of reports of protection by Ca^{2+} against Cd^{2+} toxicity in plants and algae, not many reports are available on cyanobacteria (Hayakaway, Tomiokay, & Takenaka, 2011; Lavoie, Campbell, & Fortin, 2014). As *Nostoc muscorum* Meg 1 is a common cyanobacterium in various rice fields in coal and lime stone mining areas of Meghalaya that receive excess of both Ca^{2+} and Cd^{2+}, this study was taken up to evaluate in detail Ca^{2+} mediated regulation of Cd^{2+} toxicity to carbon and nitrogen fixations and to analyze if Ca^{2+} can interfere with Cd^{2+} uptake and intracellular accumulation. Various morphological, physiological and biochemical characters under Cd^{2+} stress as well as in presence of both Cd^{2+} and Ca^{2+} were evaluated. Growth in terms of chlorophyll *a* and total protein, accessory pigment concentrations (carotenoids, phycocyanin, allophycocyanin and phycoerythrin), percent heterocyst frequency, nitrogenase and glutamine synthetase activities, and the rates of photosynthesis and respiration were assessed. Changes in cell morphology were studied under bright field and scanning electron microscopy. Apart from these, percent Cd^{2+} removal by the cyanobacterium from the growth medium, its distribution within the cells in absence and presence of Ca^{2+} were also estimated.

Materials and methods

Growth and maintenance of the cyanobacterium

N. muscorum Meg 1 (NCBI GenBank Accession No. KM596855) was earlier isolated and purified from a metal contaminated rice field in Sohra, Meghalaya, India (25.27° N, 91.73° E) (Ahad, Goswami, & Syiem, 2017). The organism was grown in a culture room under aseptic conditions in BG-11_0 culture medium [macronutrients: K_2HPO_4 (40 g L^{-1}); $MgSO_4{\cdot}7H_2O$ (75 g L^{-1}); $CaCl_2{\cdot}2H_2O$ (36 g L^{-1}); citric acid (6 g L^{-1}); ferric ammonium citrate (6 g L^{-1}); Na_2CO_3 (20 g L^{-1}); EDTA (1 g L^{-1}); micronutrients: H_3BO_3 (2.86 g L^{-1}); $MnCl_2{\cdot}2H_2O$ (1.81 g L^{-1}); $ZnSO_4{\cdot}7H_2O$ (0.22 g L^{-1}); $Na_2MoO_4{\cdot}2H_2O$ (0.39 g L^{-1}); $CuSO_4{\cdot}5H_2O$ (0.079 g L^{-1}); $Co(NO_3)_2{\cdot}6H_2O$ (0.0494 g L^{-1}) (Rippka, Dereulles, Waterbury, Herdman, & Stanier, 1979)] at pH 7.5, with an initial inoculum size of 3 μg mL^{-1} chlorophyll *a*, at a temperature of 25 ± 2 °C, and under photosynthetically active radiation (PAR) with photon fluence rate of 50 μmol m^{-2} s^{-1} (Goswami, Diengdoh, Syiem, Pakshirajan, & Kiran, 2015).

Cd^{2+} and Ca^{2+} treatment

$3CdSO_4{\cdot}8H_2O$ (Sigma Aldrich, India) and $CaCl_2{\cdot}2H_2O$ (HiMedia Laboratories Pvt. Ltd.) were used for preparation of stock solutions of Cd^{2+} (100 mg L^{-1}) and Ca^{2+} (100 mg L^{-1}). Experimental Cd^{2+} (0.5 mg L^{-1})/Ca^{2+} (10 mg L^{-1}) solutions were prepared by diluting the respective stock solutions with BG 11_0 medium. The final Cd^{2+} and Ca^{2+} concentrations in the experimental flasks were measured using graphite furnace atomic absorption spectrophotometer (GF-AAS) (Vario 6, Analytik Jena, Jena, Germany). Cd^{2+} removal efficiency was studied after 24 h of exposure by following the procedure described in section 'Assay of cadmium removal and its distribution in the cells'.

Selection of Cd^{2+} and Ca^{2+} concentrations for the experiments

For selection of Cd^{2+} and Ca^{2+} concentrations for the study, a range of concentrations (0.1, 0.2, 0.3, 0.5 and 1 mg L^{-1} for Cd^{2+} and 5, 10 and 15 mg L^{-1} for Ca^{2+}) of both these metals were initially tested on the cyanobacterium by growing them for seven days in the presence of different concentrations of these metal ions. Since chlorophyll *a* concentration is a measure of growth parameter in cyanobacteria, increase/decrease in growth of the organism was studied by estimating the changes in the chlorophyll *a* content following metal treatment. Cd^{2+} concentration of 0.5 mg L^{-1} produced 92% reduction in chlorophyll *a* and hence this concentration was chosen for all further experiments. Among 5, 10 and 15 mg L^{-1} of Ca^{2+} concentration tested, the best protection against Cd^{2+} toxicity was noted with 10 mg L^{-1} of Ca^{2+}. Thus in all experiments done thereafter Ca^{2+} concentration was maintained at 10 mg L^{-1}.

Estimation of chlorophyll *a*

Three millilitre cyanobacterial culture was centrifuged at 2500 rpm for 3 min, supernatant was discarded and 3 mL

methanol was added to the pellet. The solution was kept at 4 °C overnight for chlorophyll *a* extraction. Chlorophyll *a* was calculated after the incubation period taking absorbance at 663 nm (Bio Rad Smart Spec Plus) of the supernatant following centrifugation at 2500 rpm for 3 min (Mackinney, 1941).

Estimation of total protein

Aliquot of 3 mL culture was centrifuged at 2500 rpm for 3 min and the pellet was resuspended in 3 mL distilled water. The cells were disrupted by ultrasonication (VC-505, Sonics Vibra cell sonicator, Newtown, USA) fitted with a microprobe. 0.5 mL sonicated solution was taken and the volume was raised to 1 mL by adding distilled water. Protein was estimated in this solution following the method described by Lowry, Roserbough, Farr, & Randall (1951). Reference protein used to make the calibration curve was bovine serum albumin.

Phycobiliproteins

Cyanobacterial culture (5 mL) was centrifuged at 2500 rpm for 3 min and to the pellet 5 mL of phosphate buffer saline (PBS), pH 7 was added. This step was followed by ultrasonication and centrifugation at 13,000 rpm for 45 min. Absorbance of the supernatant was measured at 615, 562 and 652 nm by taking PBS as blank. The amounts of phycocyanin, allophycocyanin and phycoerythrin were calculated by using formulae developed by Bennett & Bogorad (1973).

Carotenoids estimation

Three millilitre culture was centrifuged and the pellet was resuspended in 95% N,N-dimethyl formamide and incubated in dark for 5 min. Incubation was followed by centrifugation (3000 rpm) for 5 min and absorbance was read at 461 and 664 nm. Carotenoids concentration was estimated according to the method developed by Morgan (1967).

Measurement of photosynthetic PSII activity and rate of respiration

Photosynthetic PSII activity and respiration rate were measured using a Clark-type oxygen electrode (Rank Brothers Ltd., Cambridge, England) as described by Robinson, Deroo, & Yocum (1982). Cyanobacterial culture (3 mL) was added to the sample chamber of the non-polarized electrode and allowed to stabilize for 3 min with continuous stirring. PSII was activated by providing PAR light using 100 W Tungsten filaments bulb; electrode was polarized and O_2 evolution was monitored over a period of 3 min. For measurement of respiratory O_2 consumption, the sample chamber was wrapped in aluminium foil and light was switched off creating dark condition. For next 3 min, rate of O_2 consumption was measured for the sample. The rate of oxygen evolution and consumption were expressed as nmol O_2 evolved or consumed min^{-1} μg^{-1} Chlorophyll *a*.

Heterocyst frequency

Heterocyst frequency of the test organism was calculated under Olympus light microscope BX-53 (Tokyo, Japan) (Wolk, 1965). At least 600 cells were counted for calculation of percent heterocyst frequency.

Nitrogenase activity

Measurement of nitrogenase activity was carried out following acetylene reduction assay method (Stewart, Fitzgerald, & Burris, 1967). Acetylene gas at a concentration of 10% (v/v) in air phase was injected into a 15 mL vial containing 5 mL of the culture. This was followed by incubation of the vials at 25 ± 2 °C for 1 h in light with continuous shaking. A gas chromatograph (model 3900, Varian, Walnut Creek, USA) fixed with a Porapak T column (stainless steel column 6′ × 1/8″, Porapak T of mesh size 80/100) and flame ionization detector was used to estimate acetylene in the vials. The activity of the enzyme nitrogenase was calculated as nmol C_2H_2 reduced μg^{-1} Chlorophyll *a* h^{-1}.

Glutamine synthetase activity

Three millilitre culture was centrifuged and the pellet was washed twice with 50 mmol L^{-1} Tris–HCl buffer, pH 7.5 and resuspended in the same buffer. This step was followed by cell disruption using ultrasonicator to release the enzyme into the buffer. The assay mixture was prepared containing 40 mmol L^{-1} Tris–HCl buffer (pH 7.5), 3 μmol L^{-1} $MnCl_2$, 20 μmol L^{-1} of potassium arsenate, 0.4 μmol L^{-1} of ADP (sodium salt), 60 μmol L^{-1} of hydroxylamine and 30 μmol L^{-1} of glutamine. 0.5 mL of assay mixture was added to 0.5 mL of the sonicated cells and incubated in dark at 30 ± 2 °C for 10 min followed by addition of 2 mL of stop mixture (10% $FeCl_3$, 24% TCA, 6 mol L^{-1} HCl in distilled water) to arrest the enzymatic reaction. The solution was centrifuged at 2000 rpm for 5 min and absorbance of the supernatant was read at 540 nm. The γ-glutamyl hydroxamate produced in the reaction was estimated by plotting the absorbance value in a calibration curve as described by Sampaio, Rowell, & Stewart (1979). GS activity was expressed as nmol γ-glutamyl hydroxamate produced μg^{-1} protein min^{-1}.

Bright field microscopy

For bright field microscopic observation, 1 mL each cyanobacterial sample from different experimental sets was taken in an eppendorf tube and centrifuged at 3000 rpm for 3 min. The pellet was washed thrice by adding 1 mL of PBS, pH 7. Following this, the sample was mounted on slide and viewed in 100× under fluorescence microscope using EMP TL-BF filter (Leica Microsystems, SFL 4000, Wetzlar, Germany).

Scanning electron microscopy

Cell morphology was studied using scanning electron microscope (SEM) (INCA Penta FETX3, SEM, JEOL-JSM-6360; JEOL, Tokyo, Japan). The samples were pre-treated with 4%

glutaryldehyde and kept for 24 h in a refrigerator at 4 °C for fixation followed by washing in 0.1 mol L^{-1} sodium cocodylate buffer thrice at an interval of 15 min. Dehydration was carried out in acetone (30%, 50%, 70%, 80%, 90%, 95% and 100%) for two changes at 4 °C. The dehydrated samples were kept in tetramethyl silane for 5 min; mounted on brass stubs and gold-coated before viewing under SEM.

Assay of cadmium removal and its distribution in the cells

Cd^{2+} removal and its distribution in the cells were analyzed using GF-AAS (Vario 6, Analytik Jena, Jena, Germany). Twenty mL of cultures treated for 24 h with Cd^{2+} (0.5 mg L^{-1}) in absence and in presence of Ca^{2+} (10 mg L^{-1}) were centrifuged at 2500 rpm for 10 min and the amount of Cd^{2+} present in the supernatant was estimated. Percent Cd^{2+} removal was calculated according to Nongrum & Syiem (2012).

To the pellet, 20 mL of milliQ water was added, vortexed for 2 min and the culture was centrifuged at 2500 rpm for 3 min and the supernatant was analyzed using GF-AAS to determine the amount of metal precipitated on the cell surface. Following this, 20 mL of 0.1 mol L^{-1} HCl solutions was added to the pellet and left for 2 min before vortexing vigorously to desorb surface bound Cd^{2+} ions. Amount of desorbed Cd^{2+} ions was determined in the supernatant collected after centrifugation. After this step, the residual pellet was resuspended in 20 mL milliQ water, ruptured by ultrasonication for 3 min and the resulting solution was centrifuged and supernatant was collected to measure the amount of Cd^{2+} accumulated intracellularly.

Statistical analysis

Two different types of statistical analyses were done. Data were taken in triplicate and independent *t*-test and one way analysis of variance (ANOVA) using Tukey's *t*-test were performed to determine the level of significance among the treatments. All values were mentioned in mean ± SD. Significance level was expressed as: ns, no significance; $*p < 0.05$; $**p < 0.001$ and $***p < 0.0001$. All analyses were performed using IBM SPSS Statistics 24 programme (IBM Corporation, New York, USA).

Results

Physiological and biochemical studies

Growth in terms of chlorophyll *a* was severely restrained (~92%) in cultures treated for seven days with 0.5 mg L^{-1} Cd^{2+}. Inclusion of 5 mg L^{-1} Ca^{2+} in the experiment containing 0.5 mg L^{-1} Cd^{2+} showed a 2% reduction in the inhibitory effect of Cd^{2+} on chlorophyll *a* concentration. ~34% improvement in chlorophyll *a* was achieved in presence of 10 mg L^{-1} Ca^{2+} and this concentration was chosen for rest of the study.

Exposure to 0.5 mg L^{-1} Cd^{2+} severely compromised the total protein content (↓~88%) in the organism. However, there was 31% improvement in total protein content when

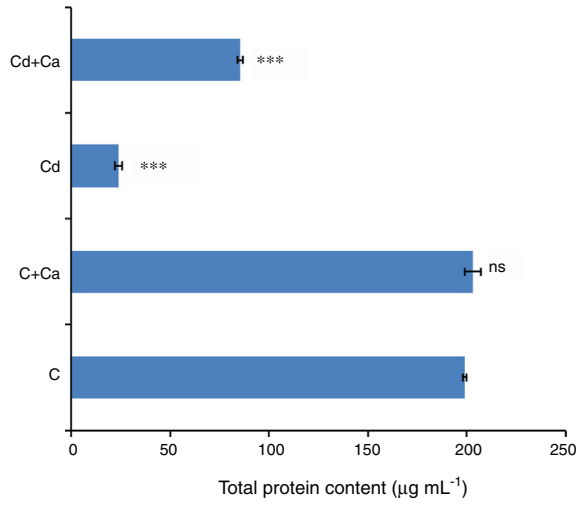

Figure 1 Measurement of total protein content after seven days: C = control; C + Ca = control + Ca^{2+} (10 mg L^{-1}); Cd = Cd^{2+} (0.5 mg L^{-1}) and Cd + Ca = [Cd^{2+} (0.5 mg L^{-1}) + Ca^{2+} (10 mg L^{-1})]. Means ± SD ($N = 3$, ns = no significance; $***p < 0.0001$).

10 mg L^{-1} Ca^{2+} was included along with Cd^{2+} in the experiment indicating antagonistic effect of Ca^{2+} on Cd^{2+} induced toxicity (Fig. 1).

Accessory pigments capture light in different wavelengths extending photosynthetic efficiency of the organisms (Mishra, Shrivastav, Maurya, Patidar, Haldar, & Mishra, 2012). On exposure to 0.5 mg L^{-1} Cd^{2+}, phycocyanin content was compromised by almost 96% (Fig. 2a); the reduction was 98% in case of allophycocyanin (Fig. 2b) and 96% in case of phycoerythrin (Fig. 2c). Although, addition of 10 mg L^{-1} Ca^{2+} to control cultures resulted in no significant changes in phycocyanin and allophycocyanin contents, its inclusion in the medium containing 0.5 mg L^{-1} Cd^{2+} showed percent decrease in these pigment concentrations to be lower than that was seen in absence of Ca^{2+}: 79% for phycocyanin and 87% for allophycocyanin. Contrary to this, there was a significant increase (7%) in phycoerythrin content of the control cells upon inclusion of 10 mg L^{-1} of Ca^{2+}. At the same time, highest reduction (22%) in the inhibition was also seen in phycoerythrin content (74% as against 96%) upon inclusion of Ca^{2+} in Cd^{2+} supplemented medium (Fig. 2a–c). Addition of Ca^{2+} resulted in an increase of 4% in carotenoids content in control cells whereas presence of Cd^{2+} reduced carotenoids content by 77%. Addition of Ca^{2+} in Cd^{2+} supplemented medium improved the carotenoids content by ~5% (Fig. 2d).

Photosystem II activity is crucial for ATP and NADPH production in light reaction of photosynthesis. Similarly, respiration leads to generation of energy during catabolic metabolism in living organisms. Photosynthetic O_2 evolution mediated by PSII activity and respiratory O_2 consumption was significantly reduced (73% and 75%, respectively) in Cd^{2+} treated cells (Table 1). On inclusion of 10 mg L^{-1} Ca^{2+} this decrease was found to be much lesser (57% and 53%, respectively).

Atmospheric nitrogen fixation and its assimilation is a highly important aspect of cyanobacteria (Meeks, 2007). The three important components of nitrogen metabolism in cyanobacteria: (1) total heterocyst number where the

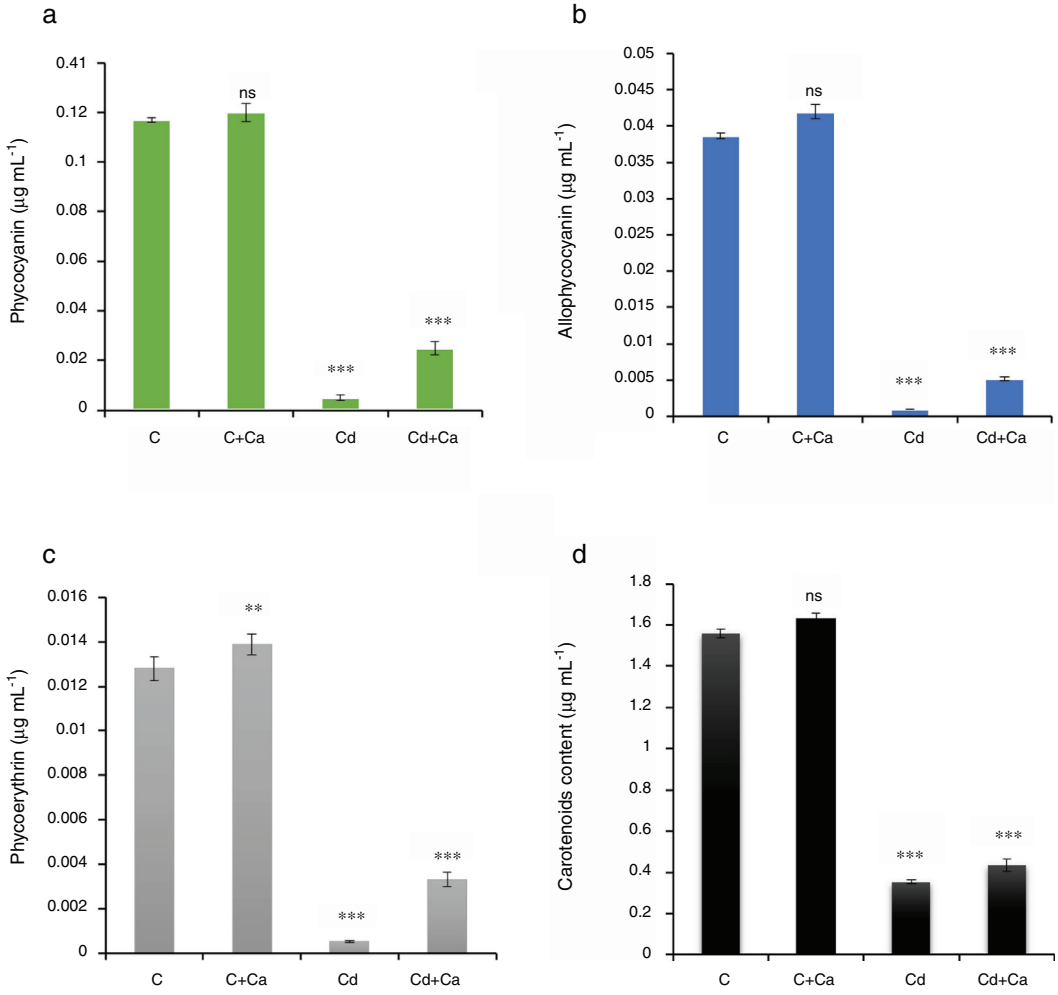

Figure 2 Estimation of accessory pigment concentrations at the end of seven days of treatment: C = control; C + Ca = [control + Ca^{2+} (10 mg L^{-1})]; Cd = Cd^{2+} (0.5 mg L^{-1}) and Cd + Ca = [Cd^{2+} (0.5 mg L^{-1}) + Ca^{2+} (10 mg L^{-1})]. (a) Phycocyanin; (b) allophycocyanin; (c) phycoerythrin and (d) carotenoids contents. Means ± SD ($N = 3$, ns = no significance; $^{**}p < 0.001$; $^{***}p < 0.0001$).

Table 1 O_2 evolution and O_2 consumption of the cyanobacterium *N. muscorum* Meg 1 cells on day seventh post treatment. C = control; C + Ca = [control + Ca^{2+} (10 mg L^{-1})]; Cd = Cd^{2+} (0.5 mg L^{-1}) and Cd + Ca = [Cd^{2+} (0.5 mg L^{-1}) + Ca^{2+} (10 mg L^{-1})]. Control values are taken as 100%. Values are mean ± SD ($N = 3$, ns = no significance; $^{***}p < 0.0001$).

	O_2 evolution (PSII activity)		O_2 consumption (respiratory activity)	
	nmol of O_2 evolved µg^{-1} Chl *a* h^{-1}	% ↑/% ↓	nmol of O_2 consumed µg^{-1} Chl *a* h^{-1}	% ↑/% ↓
Control	449 ± 5	100	368 ± 3	100
Control + Ca^{2+}	461 ± 4^{ns}	↑3	379 ± 5^{ns}	↓3
Cd^{2+}	108 ± 3***	↓73	93 ± 4***	↓75
Cd^{2+} + Ca^{2+}	193 ± 4***	↓57	172 ± 4***	↓53

N_2-fixing enzyme nitrogenase is hosted, (2) the nitrogenase enzyme that fixes atmospheric N_2 and (3) the glutamine synthetase enzyme that assimilates fixed N_2 into organic molecules, were all compromised significantly upon Cd^{2+} exposure for seven days. Percent heterocyst frequency was down by 81% whereas nitrogenase and glutamine synthetase activities were reduced by 74% and 68% (Fig. 3). The presence of Ca^{2+} along with Cd^{2+} brought down Cd^{2+} toxicity on heterocyst frequency by 19% (Fig. 3a) and on nitrogenase and glutamine synthetase activities by 17% and 21% (Fig. 3b and c).

Morphological studies

Under bright field microscopy, cyanobacterial filaments of the control culture grown for a period of seven days showed active, healthy and intact heterocysts and vegetative cells

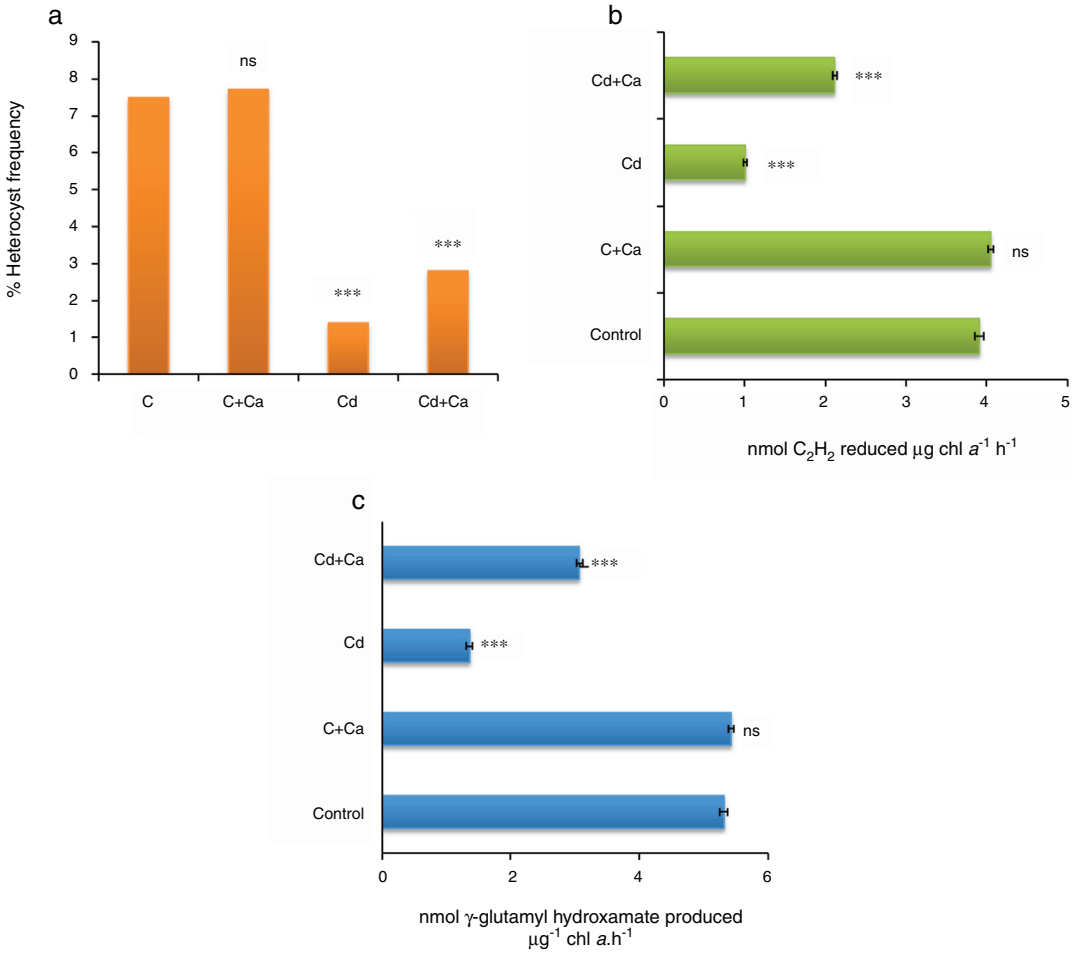

Figure 3 Percent heterocyst frequency, nitrogenase and glutamine synthetase activities in the experimental cultures within seven days: C = control; C + Ca = control + Ca^{2+} (10 mg L^{-1}); Cd = Cd^{2+} (0.5 mg L^{-1}) and Cd + Ca = [Cd^{2+} (0.5 mg L^{-1}) + Ca^{2+} (10 mg L^{-1})]. (a) Percent heterocyst frequency; (b) nitrogenase activity and (c) glutamine synthetase activity. All the values are mean ± SD ($N = 3$, ns = no significance; ***$p < 0.0001$).

(Fig. 4a). When the control culture was supplemented with only Ca^{2+}, no visible changes were observed (Fig. 4b). However, seven days of incubation in presence of 0.5 mg L^{-1} Cd^{2+} showed pronounced changes in terms of extensive filament breakdown and cell damage (Fig. 4c). Detached heterocysts and remnants of disintegrated vegetative cells were also visible under the microscope. The visible change of green colour in filaments to blue colour indicated breakdown of major photosynthetic pigment chlorophyll *a* within the cells (Fig. 4c). Addition of 10 mg L^{-1} Ca^{2+} in the experiment containing 0.5 mg L^{-1} Cd^{2+} showed much milder effect of Cd^{2+} on the cyanobacterial culture. Although some amount of disintegration of cells, heterocyst detachment from the filaments and change in colour of the filaments were seen in the culture, the effects were not as drastic as was in the case of cultures treated with Cd^{2+} in absence of Ca^{2+} (Fig. 4d).

While bright field microscopic analysis presented an overall picture of a general effect of metal treatment on the cell morphology, scanning electron microscopic study confirmed these morphological changes on the individual cells of the filaments with greater details. Continuous filaments with round and healthy cells were visible in control cultures (Fig. 4e). Under same magnification, other than slight elongation, no morphological changes were noticed in the cells treated with 10 mg L^{-1} Ca^{2+} (Fig. 4f). Fig. 4g established the toxic nature of Cd^{2+} where cells were highly extended, irregular and deflated in presence of Cd^{2+} in their surroundings. Stress was also seen in the connections between the cells that ultimately led to filament breakage. These effects were minimized when Ca^{2+} was included in the experiment along with Cd^{2+} (Fig. 4h).

Metal removal

A GF-AAS analysis done to study Cd^{2+} removal in absence and in presence of Ca^{2+} revealed that 94% (~0.47 mg L^{-1} Cd^{2+}) of the 0.5 mg L^{-1} Cd^{2+} supplemented in the growth medium was biosorbed on the cyanobacterial cells within 24 h of treatment. Biosorption reached a plateau within 2 h of exposure and remained constant thereafter indicating saturation limit of the exposed cells. The same cells removed 50% (~0.25 mg L^{-1} Cd^{2+}) of 0.5 mg L^{-1} Cd^{2+} when

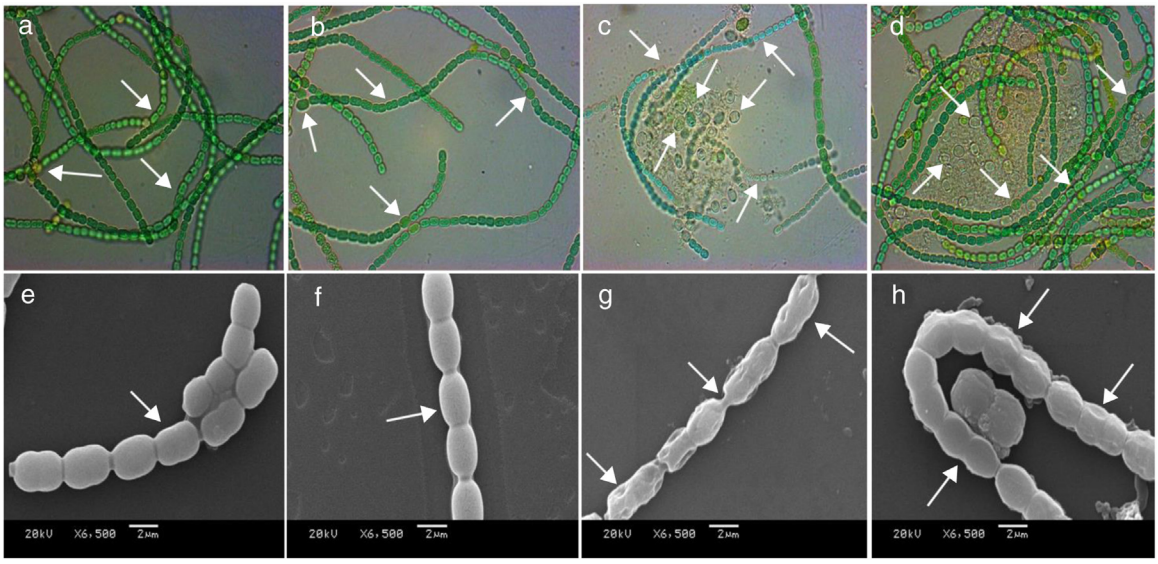

Figure 4 Bright field and scanning electron micrographs of *N. muscorum* Meg 1 cells in different treatments at the end of seven days: bright field microscopic images – (a) control cells; (b) cells treated with Ca^{2+} (10 mg L^{-1}); (c) cells treated with 0.5 mg L^{-1} Cd^{2+}; and (d) cells treated with 0.5 mg L^{-1} Cd^{2+} in combination with Ca^{2+} (10 mg L^{-1}). Scanning electron microscopic images – (e) control cells; (f) cells treated with Ca^{2+} (10 mg L^{-1}); (g) cells treated with 0.5 mg L^{-1} Cd^{2+} and (h) cells treated with 0.5 mg L^{-1} Cd^{2+} in combination with Ca^{2+} (10 mg L^{-1}). The arrows in (a; b) indicate heterocysts in the filaments; in (c; d) arrows indicate disintegrated heterocysts, vegetative cells and change in filament colour from green to blue; in (e; f) arrows represent healthy cells; in (g) arrows represent elongated and shrivelled cells; in (h) arrows indicate round, less damaged cells.

10 mg L^{-1} Ca^{2+} was included in the experiment (Fig. 5a). This value was significantly lesser (↓44%) than Cd^{2+} removed in absence of Ca^{2+}. A closer look into the distribution of the total removed Cd^{2+} in absence and presence of Ca^{2+} showed that in both cases the sorption was strongly a surface phenomenon with 92% (∼0.43 mg L^{-1} Cd^{2+} of 0.5 mg L^{-1} Cd^{2+}) and 94% (0.23 mg L^{-1} Cd^{2+} of 0.25 mg L^{-1} Cd^{2+}) of Cd^{2+} staying bound on the cell surfaces (Fig. 5b and c). However, the scenario involving internally accumulated Cd^{2+} ions were significantly different in the two cases. When the cells were treated only with Cd^{2+}, the internal accumulation of Cd^{2+} was 4% (0.02 mg L^{-1} Cd^{2+}) as against only 2% (0.005 mg L^{-1} Cd^{2+}) in case of cells treated with Cd^{2+} in presence of Ca^{2+} indicating that in the presence of Ca^{2+}, the transport of Cd^{2+} ions into the cells was hampered.

Discussion

The cyanobacterium *N. muscorum* Meg 1 showed fast growth rate and high resilience towards various metal ions and thus presents an intriguing prospect towards understanding different aspects of its metal biosorption potential from water containing single and/or multiple metal add-ons. Many metal ions are vital for organisms' survival but at higher concentrations they can participate in unwanted redox reactions and can bind unsuitably to macromolecules resulting in toxic effects (Gaballa & Helmann, 2003). Thus, in living organisms intracellular concentrations of metal ions are firmly controlled (Nies, 1999; Outten & O'Halloran, 2001). It has been established that metal uptake from the surrounding with multi-metal composition and their retention within the cells are multi-faceted with both influx and efflux systems working towards metal homeostasis (Chandrangsu, Rensing, & Helmann, 2017; Hudek & Ackland, 2017; Huertas, López-Maury, Giner-Lamia, Sánchez-Riego, & Florencio, 2014; Shcolnick & Keren, 2006).

Not many references are available in literature on influence of Ca^{2+} on Cd^{2+} toxicity in cyanobacteria. However, Liorti, Crease, & Heyl (2017) and Chen et al. (2012) had reported in detail the impact of Ca^{2+} on Cu^{2+} toxicity in *Daphnia pulex* and in *Pelteobagrus fulvidraco* respectively where they provided evidences that presence of increasing concentrations of Ca^{2+} can counter Cu^{2+} toxicity.

Researchers in recent times have shown that Ca^{2+} act as a competitive inhibitor of divalent metal ions such as Cd^{2+}, Cu^{2+} and Pb^{2+} (Choong, Liu, & Templeton, 2014; Marchetti, 2012). In Meghalaya, coal and lime stone mining releases both Ca^{2+} and Cd^{2+} among other metal ions (Cu^{2+}, Zn^{2+}, Fe^{2+}, Pb^{2+}, Ni^{2+}, Cr^{6+}, etc.) in mining effluents that find their way into low laying crop fields and water bodies (Ahad, Goswami, & Syiem, 2017; Warjri & Syiem, 2018). Several cyanobacteria are found in these metal contaminated environments although chronic metal exposure is known to adversely affect these microorganisms. Thus their survival in these metal contaminated surroundings is intriguing and therefore we undertook the present study in order to evaluate the influence of Ca^{2+} – an essential metal ion on the Cd^{2+} uptake (a non-essential and toxic metal) and on appearance of its toxicity in a cyanobacterium that is ubiquitously found in many crop fields and metal contaminated wastewaters in mining areas of Meghalaya.

To begin with various physiological, biochemical and morphological changes were assessed in absence and in presence of Ca^{2+} in 0.5 mg L^{-1} Cd^{2+} supplemented medium. The Cd^{2+} mediated toxicity on chlorophyll *a*, phycobiliproteins

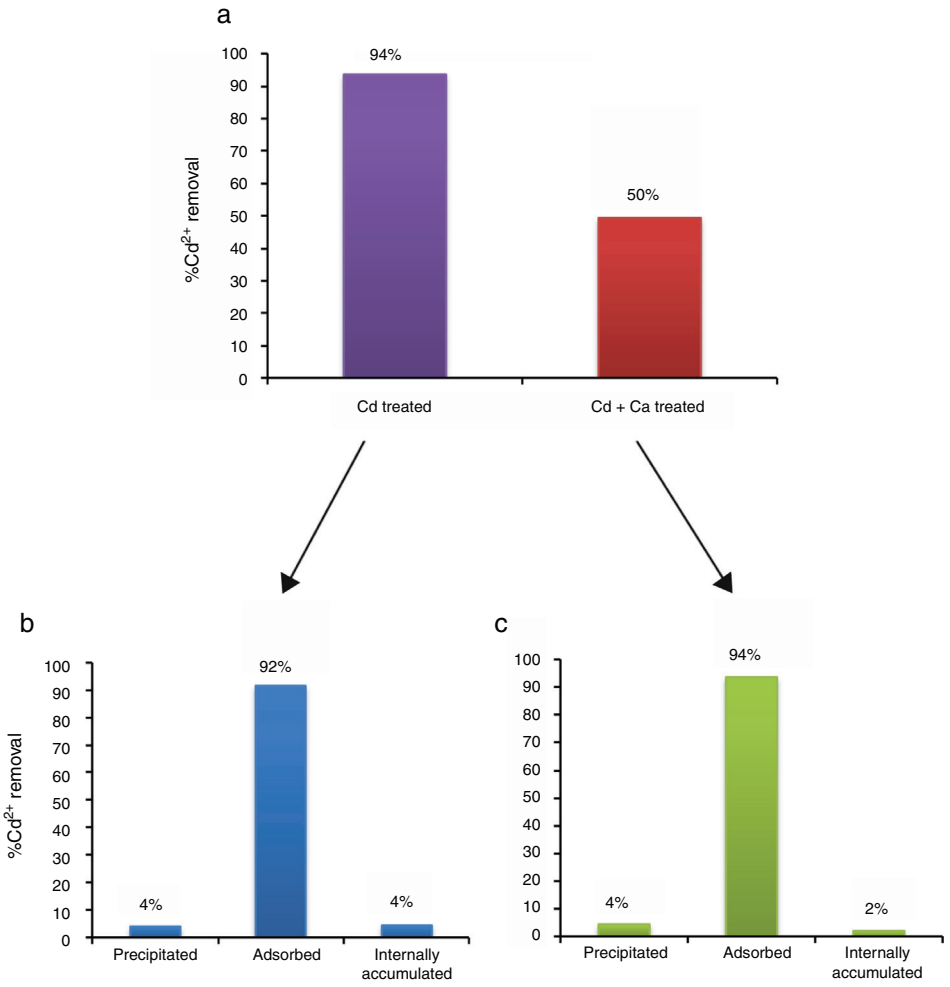

Figure 5 (a) Percent Cd^{2+} removed by *N. muscorum* Meg 1 within 24 h from the medium supplemented with 0.5 mg L^{-1} Cd^{2+} in absence and presence of 10 mg L^{-1} Ca^{2+}; (b) cellular distribution of Cd^{2+} in absence of Ca^{2+} and (c) cellular distribution of Cd^{2+} in presence of Ca^{2+}.

and carotenoids presented in Fig. 2 showed improvements in their percent reduction upon Ca^{2+} supplementation. Both photosynthetic and respiratory electron transport chains registered reduced toxic effects in presence of Ca^{2+} (Table 1). Other parameters such as heterocyst frequency, nitrogenase and glutamine synthetase activities crucial for nitrogen fixation and assimilation showed similar reduction in Cd^{2+} induced toxicity when Ca^{2+} was present in the medium (Fig. 3). That Ca^{2+} had regulatory influence on Cd^{2+} toxicity was also visible from the morphological study done under bright field and scanning electron microscopy where filament breakage, cell distortion and elongation, etc. were far more moderate in presence of Ca^{2+} (Fig. 4).

Metal removal study revealed that there was 44% reduction in Cd^{2+} removal by the organism in presence of 10 mg L^{-1} Ca^{2+}, of which only 2.1% (0.005 mg L^{-1}) Cd^{2+} was internally accumulated as against 4.3% (0.02 mg L^{-1}) internal accumulation of Cd^{2+} in absence of Ca^{2+} (Fig. 5). Comparatively this value is 4 fold lower in presence of Ca^{2+}. As pointed out earlier by Lu et al. (2010) and Kim, Yang, & Lee (2002), this can be explained from the fact that Ca^{2+} and Cd^{2+} are similar in ionic size and Ca^{2+} competes with Cd^{2+} for binding sites on the cell surfaces. Further, their uptake and transport into the cells are dependent on concentrations. Zorrig, Shahzad, Abdelly, & Berthomieu (2012), Clemens (2006) and Clemens, Antosiewicz, Ward, Schachtman, and Schroeder (1998) had reported that increase in Ca^{2+} concentration blocks Cd^{2+} transport into rice roots as a result of competition of Cd^{2+} with Ca^{2+} for transporters. Consequently, less number of Cd^{2+} ions gets transported inside. There are reports of various Cd^{2+} and Ca^{2+} transporters in microbes including in cyanobacteria (Ahad, Kynshi, & Syiem, 2018; Checcetto et al., 2013; Kanamura, Kashiwagi, & Mizuno, 1993; Kaneko, Kaneko et al., 1996; Pandey, Singh, Mishra, & Bisen, 1996; Sulaymon, Mohammed, & Al-musawi, 2013). In absence of Ca^{2+}, Cd^{2+} could also use Ca^{2+} channels to enter cyanobacterial cells. However, when present, Ca^{2+} competes with Cd^{2+} for their dedicated Ca^{2+} channels leading to reduced entry and accumulation of Cd^{2+} in the cells. The lowered amount of internal accumulation of Cd^{2+} found in our study indicated reduced transport of Cd^{2+} into the cells in presence of Ca^{2+}.

Our findings of overall improvement in every physiological and biochemical parameter upon Ca^{2+} supplementation corroborated the earlier findings of different researchers that Ca^{2+} plays a crucial role in regulating various metabolic processes (Inouye, Franceschini, & Inouye, 1983; Pitta, Sherwood, & Kobel, 1997; Torrecilla, Leganés, Bonilla, & Fernández-Piñas, 2004; Watkins, Knight, Trewavas, & Campbell, 1995; Werthén & Lundgren, 2001). Cd^{2+} on the other hand has been implicated in generation of ROS which in turn adversely effects chlorophylls, phycobiliproteins, PSII activity, rate of respiration, total protein, nitrogenase and GS activities, antioxidant concentrations (GSH, ascorbate, vitamin A, E) and various anti-oxidative enzymes (SOD, CAT, Prx) (Cassier-Chauvat & Chauvat, 2015; Goswami, Syiem, & Pakshirajan, 2015). These effects of Cd^{2+} are almost complete reversal of the positive influence Ca^{2+} supplementation. Further, this study provided evidence that when both ions are present, Cd^{2+} internalization is competitively reduced by Ca^{2+} leading to lowered toxic effects on the cyanobacterium.

Conclusion

The present study showed that noxious effects on various physiological, biochemical and morphological characteristics of the cyanobacterium *N. muscorum* Meg 1 seen under seven day exposure to 0.5 mg L^{-1} Cd^{2+} treatment could be abridged by inclusion of 10 mg L^{-1} Ca^{2+} in the Cd^{2+} supplemented medium. Ca^{2+} ions seem to compete with Cd^{2+} for binding and internalization resulting in reduced Cd^{2+} uptake. The significance of the study is the generation of information towards reducing toxicity of metal ions to microbes such as cyanobacteria by including competitive harmless ions in their vicinity. Addition of Ca^{2+} fertilizer in crop fields especially the ones that get industrial wastewater and mining effluents may decrease bio-concentration of toxic metal ions in the grains thus reducing the risk factor of accumulation of harmful metal ions in humans. The findings may be relevant in designing and formulating conditions for better management of biological organisms that can act as bioremediators of metal ions from wastewater where inclusion of an essential metal ion in the media formulation would reduce the toxicity of harmful metal ions and aid in their survival.

Conflict of interest

The authors declare no conflict of interest.

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References

Abed, R. M. M., Dobretsov, S., & Sudesh, K. (2009). Applications of Cyanobacteria in biotechnology. *Journal of Applied Microbiology*, *106*(1), 1–12. http://dx.doi.org/10.1111/j.1365-2672.2008.03918.x

Ahad, R. I. A., Kynshi, B. L., & Syiem, M. B. (2018). Protective role of Ca^{2+} towards Cu^{2+} induced toxicity on photosynthetic pigments, morphology and ultra-structures of the cyanobacterium *Nostoc muscorum* Meg 1. *Biosciences Biotechnology Research Asia*, *15*(3), 591–601. http://dx.doi.org/10.13005/bbra/2665

Ahad, R. I. A., Goswami, S., & Syiem, M. B. (2017). Biosorption and equilibrium isotherms study of cadmium removal by *Nostoc muscorum* Meg 1: Morphological, physiological and biochemical alterations. *3 Biotech*, *7*(2), 104. http://dx.doi.org/10.1007/s13205-017-0730-9

Antosiewicz, D. M., & Hennig, J. (2004). Overexpression of LCT1 in tobacco enhances the protective action of calcium against cadmium toxicity. *Environmental Pollution*, *129*(2), 237–245. http://dx.doi.org/10.1016/j.envpol.2003.10.025

Awofolu, O. R., Mbolekwa, Z., Mtshemla, V., & Fatoki, O. S. (2005). Levels of trace metals in water and sediment from Tyume river and its effects on an irrigated farmland. *Water SA*, *31*(1), 87–94. http://dx.doi.org/10.4314/wsa.v31i1.5124

Bennett, A., & Bogorad, L. (1973). Complementary chromatic adaption in a filamentous blue-green alga. *The Journal of Cell Biology*, *58*, 419–435. http://dx.doi.org/10.1083/jcb.58.2.419

Cassier-Chauvat, C., & Chauvat, F. (2015). Responses to oxidative and heavy metal stresses in cyanobacteria: Recent advances. *International Journal of Molecular Sciences*, *16*(1), 871–886. http://dx.doi.org/10.3390/ijms16010871

Chandrangsu, P., Rensing, C., & Helmann, J. D. (2017). Metal homeostasis and resistance in bacteria. *Nature Reviews Microbiology*, *15*, 338–350. http://dx.doi.org/10.1038/nrmicro.2017.15

Checchetto, V., Formentin, E., Carraretto, L., Segalla, A., Giacometti, G. M., Szabo, I., & Bergantino, E. (2013). Functional characterization and determination of the physiological role of a calcium-dependent potassium channel from cyanobacteria. *Plant Physiology*, *162*, 953–964. http://dx.doi.org/10.110429./pp.113.2151

Chen, Q. L., Luo, Z., Zheng, J. L., Li, X. D., Liu, C. X., Zhao, Y. H., et al. (2012). Protective effects of calcium on copper toxicity in *Pelteobagrus fulvidraco*: Copper accumulation, enzymatic activities, histology. *Ecotoxicology and Environmental Safety*, *7*, 126–134.

Choong, G., Liu, Y., & Templeton, D. M. (2014). Interplay of calcium and cadmium in mediating cadmium toxicity. *Chemico-Biological Interactions*, *211*, 54–65. http://dx.doi.org/10.1016/j.cbi.2014.01.007

Clemens, S., Antosiewicz, D. M., Ward, J. M., Schachtman, D. P., & Schroeder, J. I. (1998). The plant cDNA LCT1 mediates the uptake of calcium and cadmium in yeast. *Proceedings of the National Academy of Sciences*, *95*(20), 12043–12048. http://dx.doi.org/10.1073/pnas.95.20.12043

Clemens, S. (2006). Toxic metal accumulation responses to exposure and mechanisms of tolerance in plants. *Biochemie*, *88*(11), 1707–1719. http://dx.doi.org/10.1016/j.biochi.2006.07.003

Dubey, S. K., Dubey, J., Mehra, S., Tiwari, P., & Bishwas, A. J. (2011). Potential use of cyanobacterial species in bioremediation of industrial effluents. *African Journal of Biotechnology*, *10*(7), 1125–1132. http://dx.doi.org/10.5897/AJB10.908

Fernandez-Piñas, F., Mateo, P., & Bonilla, I. (1995). Cadmium toxicity in *Nostoc*UAM208: Protection by calcium. *New Phytologist*, *131*(3), 403–407. http://dx.doi.org/10.1111/j.1469-8137.1995.tb03077.x

Gaballa, A., & Helmann, J. D. (2003). *Bacillus subtilis* CPx-Type ATPases: Characterization of Cd, Zn, Co and Cu efflux systems. *BioMetals*, *16*(4), 497–505. http://dx.doi.org/10.1023/A:1023425321617

Gibbons, S. M., Feris, K., McGuirl, M. A., Morales, S. E., Hynninen, A., Ramsey, P. W., et al. (2011). Use of microcalorimetry to determine the costs and benefits to *Pseudomonas*

putida strain KT2440 of harboring cadmium efflux genes. *Applied and Environmental Microbiology*, *77*(1), 108–113. http://dx.doi.org/10.1128/AEM.01187-10

Gipps, J. F., & Coller, B. A. W. (1982). Effect of some nutrient cations on uptake of cadmium by *Chlorella pyrenoidosa*. *Marine and Freshwater Research*, *33*(6), 979–987. http://dx.doi.org/10.1071/MF9820979

Goswami, S., Syiem, M. B., & Pakshirajan, K. (2015). Cadmium removal by *Anabaena doliolum* Ind1 isolated from a coal mining area in Meghalaya India: Associated structural and physiological alterations. *Environmental Engineering Research*, *20*(1), 41–50. http://dx.doi.org/10.4491/eer.2014.059

Goswami, S., Diengdoh, O. L., Syiem, M. B., Pakshirajan, K., & Kiran, M. G. (2015). Zn(II) and Cu(II) removal by *Nostoc muscorum*: A cyanobacterium isolated from a coal mining pit in Chiehruphi, Meghalaya, India. *Canadian Journal of Microbiology*, *61*(3), 209–215. http://dx.doi.org/10.1139/cjm-2014-0599

Gupta, V. K., & Rastogi, A. (2008). Biosorption of lead(II) from aqueous solutions by non-living algal biomass Oedogonium sp. and *Nostoc*sp. – A comparative study. *Colloids and Surfaces B: Biointerfaces*, *64*(2), 170–178. http://dx.doi.org/10.1016/j.colsurfb.2008.01.019

Hayakaway, N., Tomiokay, R., & Takenaka, C. (2011). Effects of calcium on cadmium uptake and transport in the tree species *Gamblea innovans*. *Soil Science and Plant Nutrition*, *57*(5), 691–695. http://dx.doi.org/10.1080/00380768.2011.608196

Hudek, L., & Ackland, M. L. (2017). Selective metal ion homeostasis in cyanobacteria. *Prospects and Challenges in Algal Biotechnology*, 219–232.

Huertas, M. J., López-Maury, L., Giner-Lamia, J., Sánchez-Riego, A. M., & Florencio, F. J. (2014). Metals in cyanobacteria: Analysis of the copper, nickel, cobalt and arsenic homeostasis mechanisms. *Life (Basel)*, *4*, 865–886. http://dx.doi.org/10.3390/life4040865

Inouye, S., Franceschini, T., & Inouye, M. (1983). Structural similarities between the development-specific protein S from a gram-negative bacterium *Myxococcus xanthus*, and Calmodulin. *Proceedings of the National Academy of Sciences of the United States of America*, *80*(22), 6829–6833.

Jacobson, K. B., & Turner, J. E. (1980). The interaction of cadmium and certain other metal ions with proteins and nucleic acids. *Toxicology*, *16*(1), 1–37. http://dx.doi.org/10.1016/0300-483X(80)90107-9

Kanamura, K., Kashiwagi, S., & Mizuno, T. (1993). The cyanobacterium *Synechococcus* sp. PCC7942, possess two distinct genes encoding cation transporting P-type ATPases. *FEBS Letter*, *330*, 99–104.

Kaneko, T., Sato, S., Kotani, H., Tanaka, A., Asamizu, E., Nakamura, Y., Miyajima, N., et al. (1996). Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. *DNA Research*, *3*, 109–136.

Kim, Y., Yang, Y., & Lee, Y. (2002). Pb and Cd uptake in rice roots. *Physiologia Plantarum*, *116*(3), 368–372. http://dx.doi.org/10.1034/j.1399-3054.2002.1160312.x

Lavoie, M., Campbell, P. G. C., & Fortin, C. (2014). Predicting cadmium accumulation and toxicity in a green alga in the presence of varying essential element concentrations using a biotic ligand model. *Environmental Science and Technology*, *48*(2), 1222–1229. http://dx.doi.org/10.1021/es402630z

Liorti, A., Crease, T., & Heyland, A. (2017). Interactive effects of copper and calcium in *Daphnia pulex*. *Journal of Limnology*, *76*, 281–291. http://dx.doi.org/10.4081/jlimnol.2016.1498

Lowry, O. H., Roserbough, N. J., Farr, A. L., & Randall, R. J. (1951). The Folin by Oliver. *Journal of Biological Chemistry*, *193*, 265–275. http://dx.doi.org/10.1016/0304-3894(92)87011-4

Lu, L., Tian, S., Zhang, M., Zhang, J., Yang, X., & Jiang, H. (2010). The role of Ca pathway in Cd uptake and translocation by the hyperaccumulator *Sedum alfredii*. *Journal of Hazardous Materials*, *183*(1–3), 22–28. http://dx.doi.org/10.1016/j.jhazmat.2010.06.036

Mackinney, G. (1941). Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry*, *140*, 315–322.

Marchetti, C. (2012). Role of calcium channels in heavy metal toxicity. *ISRN Toxicology*, http://dx.doi.org/10.1155/2013/184360

Mateo, P., Fernandez-Pinas, F., & Bonilla, I. (1994). O_2-induced inactivation of nitrogenase as a mechanism for the toxic action of Cd^{2+} on *Nostoc*UAM208. *New Phytologist*, *126*(2), 267–272. http://dx.doi.org/10.1111/j.1469-8137.1994.tb03945.x

Meeks, J. C. (2007). Physiological adaptations in nitrogen-fixing *Nostoc*–plant symbiotic associations. In K. Pawlowski (Ed.), *Prokaryotic symbionts in plants. Microbiology monographs* (Vol. 8). Berlin, Heidelberg: Springer.

Mishra, S. K., Shrivastav, A., Maurya, R. R., Patidar, S. K., Haldar, S., & Mishra, S. (2012). Effect of light quality on the C-phycoerythrin production in marine cyanobacteria *Pseudanabaena* sp. isolated from Gujarat coast India. *Protein Expression Purification*, *81*(1), 5–10.

Morgan, R. C. (1967). The carotenoids of Queensland fruits – Carotenes of the watermelon (*Citrullus vulqaris*). *Journal of Food Science*, *32*(3), 275–278. http://dx.doi.org/10.1111/j.1365-2621.1967.tb01311.x

Nies, D. H. (1999). Microbial heavy-metal resistance. *Applied Microbiology and Biotechnology*, *51*(6), 730–750, http://www.ncbi.nlm.nih.gov/pubmed/10422221.

Nongbri, B. B., & Syiem, M. B. (2012). Diversity analysis and molecular typing of cyanobacteria isolated from various ecological niches in the state of Meghalaya North-East India. *Environmental Engineering Research*, *17*(S1), 21–26. http://dx.doi.org/10.4491/eer.2012.17.S1.S21

Nongrum, N. A., & Syiem, M. B. (2012). Effects of copper ion (Cu^{2+}) on the physiological and biochemical activities of the cyanobacterium *Nostoc*ANTH. *Environmental Engineering Research*, *17*(S1), S63–S67.

Outten, C. E., & O'Halloran, T. V. (2001). Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. *Science*, *292*(5526), 2488–2492. http://dx.doi.org/10.1126/science.1060331

Pandey, P. K., Singh, B. B., Mishra, R., & Bisen, P. S. (1996). Ca^{2+} uptake and its regulation in the cyanobacterium *Nostoc*MAC. *Current Microbiology*, *32*, 332–335. http://dx.doi.org/10.1007/s002849900059

Pitta, T. P., Sherwood, E. E., Kobel, A. M., & Berg, H. C. (1997). Calcium is required for swimming by the non-flagellated cyanobacterium *Synechococcus* strain WH8113. *Journal of Bacteriology*, *179*(8), 2524–2528.

Rippka, R., Dereulles, J., Waterbury, J. B., Herdman, M., & Stanier, R. Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology*, *111*, 1–61. http://dx.doi.org/10.1099/00221287-111-1-1

Robinson, S. J., Deroo, C. S., & Yocum, C. F. (1982). Photosynthetic electron transfer in preparations of the cyanobacterium *Spirulina platensis*. *Plant Physiology*, *70*(1), 154–161. http://dx.doi.org/10.1104/pp.70.1.154

Sampaio, M. J. A. M., Rowell, P., & Stewart, W. D. P. (1979). Purification and some properties of glutamine synthetase from the nitrogen-fixing cyanobacteria *Anabaena cylindrica* and a *Nostoc* sp. *Journal of General Microbiology*, *111*(1), 181–191. http://dx.doi.org/10.1099/00221287-111-1-181

Shcolnick, S., & Keren, N. (2006). Metal homeostasis in cyanobacteria and chloroplasts balancing benefits and risks to the photosynthetic apparatus. *Plant Physiology*, *141*, 805–810. http://dx.doi.org/10.1104/pp.106.079251

Singh, J. S., Kumar, A., Rai, A. N., & Singh, D. P. (2016). Cyanobacteria: A precious bio-resource in agriculture. *Ecosystem and Environmental Sustainability*, *7*, 529. http://dx.doi.org/10.3389/fmicb.2016.00529

Stewart, W. D., Fitzgerald, G. P., & Burris, R. H. (1967). *In situ* studies on N_2 fixation using the acetylene reduction technique. *Proceedings of the National Academy of Sciences*, *58*(5), 2071–2078. http://dx.doi.org/10.1073/pnas.58.5.2071

Sulaymon, A. H., Mohammed, A. A., & Al-musawi, T. J. (2013). Competitive biosorption of lead, cadmium, copper and arsenic ions using algae. *Environment Science and Pollution Research*, *20*(5), 3011–3023. http://dx.doi.org/10.1007/s11356-012-1208-2

Torrecilla, I., Leganés, F., Bonilla, I., & Fernández-Piñas, F. (2004). A calcium signal is involved in heterocyst differentiation in the cyanobacterium *Anabaena* sp. PCC7120. *Microbiology*, *150*(11), 3731–3739. http://dx.doi.org/10.1099/mic.0.27403-0

Warjri, S. M., & Syiem, M. B. (2018). Analysis of biosorption parameters, equilibrium isotherms and thermodynamic studies of chromium (VI) uptake by a *Nostoc*sp. isolated from a coal mining site in Meghalaya, India. *Mine, Water and the Environment*, *37*, 713. http://dx.doi.org/10.1007/s10230-018-0523-3

Watkins, N. J., Knight, M. R., Trewavas, A. J., & Campbell, A. K. (1995). Free calcium transients in chemotactic and non-chemotactic strains of *Escherichia coli* determined by using recombinant aequorin. *The Biochemical Journal*, *306*(3), 865–869.

Werthén, M., & Lundgren, T. (2001). Intracellular Ca^{2+} mobilization and kinase activity during acylated homoserine lactone-dependent quorum sensing in *Serratia liquefaciens*. *Journal of Biological Chemistry*, *276*(9), 6468–6472. http://dx.doi.org/10.1074/jbc.M009223200

Wolk, C. P. (1965). Control of sporulation in a blue-green alga. *Developmental Biology*, *12*(1), 15–35, http://www.ncbi.nlm.nih.gov/pubmed/5833109.

Zeng, X., Tang, J., Liu, X., & Jiang, P. (2012). Response of *P. aeruginosa* E1 gene expression to cadmium stress. *Current Microbiology*, *65*(6), 799–804. http://dx.doi.org/10.1007/s00284-012-0224-2

Zorrig, W., Shahzad, Z., Abdelly, C., & Berthomieu, P. (2012). Calcium enhances cadmium tolerance and decreases cadmium accumulation in lettuce (*Lactuca sativa*). *African Journal of Biotechnology*, *11*(34), 8441–8448. http://dx.doi.org/10.4314/ajb.v11i34