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AMELIORATING POTENTIAL OF Ca^{2+} ON Cd^{2+} INDUCED TOXICITY ON CARBON ASSIMILATION IN THE CYANOBACTERIUM *NOSTOC MUSCORUM* Meg 1

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ABSTRACT: The cyanobacterium *Nostoc muscorum* Meg 1 was used to study the protective role of Ca^{2+} on Cd^{2+} toxicity. The isolate was treated with 0.5 ppm Cd^{2+} in presence of 0.5, 1, 1.5, 2, 3, 5 and 10 ppm Ca^{2+}. Protective role of Ca^{2+} on the cellular contents was evident from Bright Field Microscopy. Various photosynthetic pigments, chlorophyll *a*, phycocyanin, allophycocyanin and carotenoids were inhibited by 91.7%–81.2% respectively in presence of 0.5 ppm Cd^{2+}. However, there was significant improvement (7.9–67%) in these pigment contents when Ca^{2+} was simultaneously present in the medium. Presence of Ca^{2+} in the medium also modulated Cd^{2+} uptake. In absence of Ca^{2+} there was 93% Cd^{2+} uptake by the cyanobacterial cells within 24 h of which 4.5% was internally accumulated. The uptake of Cd^{2+} was reduced to 74.8% and 50.7% in presence of 5 and 10 ppm Ca^{2+} within the same period of time. Internal accumulation was also reduced to 4.3% and 2.1% under these conditions. PSII activity that was inhibited by 78.1% in presence of Cd^{2+}, was inhibited only by 60.2% and 51.6% in presence of 5 and 10 ppm Ca^{2+}. Inhibition on respiration rate was down to 48.6% and 40% in presence of Ca^{2+} in comparison to 77.5% in Cd^{2+}. Total photosynthetic production expressed as carbohydrate content was reduced by 76.7% in presence of 0.5 ppm Cd^{2+}, presence of 5 and 10 ppm Ca^{2+} released the inhibition by 10.5% and 17% in the cyanobacterium. These results provided evidence of ameliorating effect of Ca^{2+} on Cd^{2+} induced toxicity on the carbon fixation in cyanobacterium *Nostoc muscorum* Meg 1.

KEYWORDS: *Nostoc muscorum* Meg 1, cadmium uptake and toxicity, calcium mediated protection, photosynthetic PSII activity, carbon assimilation

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1. INTRODUCTION

Since industrial revolution, environmental pollution has become one of the serious concerns of recent times [1]. Organic and inorganic pollutants are increasing in the environment on daily basis. Even if many organic pollutants can be degraded; heavy metal pollutants constantly remain in the environment. Among heavy metals, many such as copper (Cu), zinc (Zn), iron (Fe), cobalt (Co), nickel (Ni), and molybdenum (Mo) are crucial for biological processes, however others like arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) are non-essential for living organisms and are toxic even at low concentrations. Among the non-essential metal ions, prevalence of Cd^{2+} in the environment is ubiquitous and due to its toxic and non-biodegradable properties with a half-life of 20 years, this metal ion is a threat to the every components of the food web starting from primary producers to human beings [2–4]. Cd^{2+} and its derivatives present in the earth are soluble in water and thus can enter food web easily. Cd^{2+} gets released into the environment due to natural processes like volcanic eruption, grazing as well as from different anthropogenic activities such as mining of coal and lime, smelting and refining of ores, disposed from batteries, paints and pigments, alloys, cement and phosphate fertilizers [5]. Cd^{2+} is listed as one of the 126 priority contaminants and as human carcinogen by the International Agency for Research on Cancer [6]. As reported by Nriagu and Pacyna in 1988 [7], 30,000 tons of Cd^{2+} is released into the environments annually. This attains significance as accumulation of Cd^{2+} in the human beings can cause osteoporosis and fractures, anemia, eosinophilia, kidney damage, apoptosis, diabetes-mellitus, oncogene activation and Itai-Itai diseases [8–10]. Thus, metal removal is becoming a focus of environmental clean-up. Various physical and chemical processes tried for environmental metal remediation have been moderately efficient at best and in recent times, bioremediation using plants and microbes is gaining popularity. Many microbes such as algae, fungi, bacteria can grow in metal contaminated soil and water and can absorb and accumulate various metal ions including Cd^{2+}, that is the metal of interest in our study [11]. Thus, metal ions get introduced into the food web by trophic transfer and ultimately get deposited in human. Many researchers have reported metal binding on the microbial cell surfaces [12, 13]. As microbes have short generation time they can increasingly present higher amount of cell surface for metal binding within short period of time thereby effecting their removal from the surrounding [14, 15]. Amongst microorganisms, cyanobacteria show great potential as economically

viable bioremediators due to their ability to fix both atmospheric carbon and nitrogen. They have been shown to flourish in metal stress conditions because of their high resilience and adaptability to changing environmental conditions [16, 17]. These organisms can concentrate Cd^{2+} among other metal ions on themselves from the surrounding by both energy independent sorption process or energy dependent internal accumulation [18]. However, chronic exposure to high concentration of Cd^{2+} is detrimental to the organism as this leads to generation of reactive oxygen species (ROS) within the cells that alters cell morphology, physiology and biochemistry [17, 19]. As defensive strategy, cyanobacteria display different metal resistant strategies such as metal precipitation, transformation, intracellular sequestration in polyphosphate bodies, and binding metal ions to phytochelatins, metallothionein, glutathione and other thiol containing compounds [20–22]. Additionally, cyanobacteria efficiently regulate metal ion concentrations inside the cells by influx and efflux of some common cations Na^{+}, K^{+}, Mg^{2+} and Ca^{2+} into or out of the cells maintaining ionic gradient [23–25]. In cyanobacteria, Ca^{2+} plays essential roles in heterocyst differentiation, nitrogen fixation, PSII activity and for phosphate uptake [26–29]. Hence, in cyanobacteria there exist different Ca^{2+} transporters [4]. Apart from being part of ion homeostasis mechanism, Ca^{2+} being similar in charge (i.e. +2 for both Ca^{2+} and Cd^{2+}) and of similar ionic radii shares Cd^{2+} transporters in cyanobacteria [30] and therefore presence of Ca^{2+} could reduce the transport of Cd^{2+} into the cells [31]. There are reports in *Synechocystis* sp. of occurrence of P-type Cd^{2+} ATPases (e.g. *cadA1* and *cadA2*) that transport Cd^{2+} and P-type Ca^{2+} ATPases (e.g. *pacL1*, *pacL2* and *pacL3*) that transport both Ca^{2+} and Cd^{2+} into the cyanobacterium [32]. Thus, presence of Ca^{2+} in the growth medium potentially could compete with Cd^{2+} for transport through Cd^{2+} and Ca^{2+} transporters which in turn may reduce Cd^{2+} accumulation. Reduced Cd^{2+} accumulation would therefore produce lessened toxicity in the exposed cells [33]. In the present study, we have investigated the impact of different Ca^{2+} concentrations on Cd^{2+} removal by the cyanobacterium *Nostoc muscorum* Meg 1. Internal accumulation of Cd^{2+} in absence and presence of Ca^{2+} was also analyzed. Effect of Cd^{2+} exposure alone and along with Ca^{2+} on various components of photosynthesis was evaluated in order to assess the ameliorating effect of Ca^{2+} on Cd^{2+} mediated toxicity on the carbon fixation of the cyanobacterium.

2. MATERIALS AND METHODS

2.1. Growth and maintenance of the cyanobacterium *Nostoc muscorum* Meg 1

The cyanobacterium *Nostoc muscorum* Meg 1 was earlier isolated from a contaminated rice field in Sohra, Meghalaya, India, purified and identified using 16S rRNA sequencing with a GenBank Accession No. KM596855 [15]. The organism was grown and maintained in BG-11_0 medium in aseptic conditions inside a culture room and the initial inoculum size, pH, temperature and light at

a photon fluence rate were maintained 3 µg/mL, pH 7.5, 25±2°C and 50 µmol/m^2s, respectively. For all experiments, ten days old mid log phase cultures were taken [14].

2.2. Chemicals and reagents used

All the chemicals used in this study were of analytical grade procured from Sigma-Aldrich, India, Sisco Research Laboratory, HiMedia Laboratories Pvt. Ltd. The prepared reagents were stored at 4 °C in the refrigerator and used when required.

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

$3CdSO_4.8H_2O$ and $CaCl_2.2H_2O$ were used as the source of Cd^{2+} and Ca^{2+} for all the experiments carried out. Stock solution of 100 ppm of Cd^{2+} and Ca^{2+} were prepared. Experimental Cd^{2+} and Ca^{2+} solutions were diluted with $BG\text{-}11_0$ medium. Cd^{2+} removal study was done after 24 h of exposure in Cd^{2+} supplemented medium in absence / presence of Ca^{2+}. To study the effects on various components of photosynthesis an incubation period of seven days was maintained in order to allow sufficient time for the ions to produce their effects.

2.4. Chlorophyll *a* estimation

Three mL of cyanobacterial culture was centrifuged; the supernatant discarded and to the pellet 3 mL methanol was added. The solution was kept in the refrigerator at 4 °C overnight for extraction of chlorophyll *a*. At the end of the incubation period, the solution was centrifuged and the absorbance of the supernatant was read at 663 nm using UV-Vis spectrophotometer (Smart Spec Plus; Bio-Rad, USA) [34]. Chlorophyll *a* concentration was calculated by using following formula:

$$[\text{Chlorophyll } a] = \text{Absorbance 663 nm} \times 12.63\ \mu g/mL \quad (1)$$

2.5. Estimation of phycobiliproteins

Phycobiliproteins estimation was done by taking 5 mL cyanobacterial culture, centrifuged at 2500 rpm for 3 min and to the pellet 5 mL of phosphate buffer saline, PBS (pH 7.0) was added. The culture was ultrasonicated and centrifuged at 13000 rpm for 45 min at 4°C. The absorbance of the supernatant was read at 615 and 652 nm by taking PBS as blank. The amounts of phycocyanin (PC) and allophycocyanin (APC) were calculated based on the formulae developed by Bennett and Bogorad (1973)[35].

$$[PC] = \frac{OD615 - (0.474 \times OD652)}{5.34}\ mg/mL \quad (2)$$

$$[APC] = \frac{OD652 - (0.4208 \times OD615)}{5.09}\ mg/mL \quad (3)$$

2.6. Carotenoids estimation

Carotenoids concentration was estimated according to the method described by Morgan (1967)[36]. Three mL cyanobacterial culture was centrifuged and the pellet was re-suspended in 95% N, N-

dimethyl formamide and incubated in dark for 5 min. After the incubation period, the solution was centrifuged at 3000 rpm for 5 min and the absorbance was read at 461 and 664 nm.

$$[\text{Carotenoids}] = [OD_{461} - (0.046 \times OD_{664})] \times 4 \ \mu g/mL \quad (4)$$

2.7. PSII activity and rate of respiration

A clark-type oxygen electrode (Rank Brothers, England) was used to measure PSII activity and rate of respiration as described by Robinson et al. (1982)[37]. Cyanobacterial cultures (3 mL) were added to the sample chamber and allowed to stabilize for 3 min with continuous stirring. PSII activity was initiated providing light using 100 W Tungsten filaments bulb. The electrode was polarized and O_2 evolution was monitored for a period of 3 min. In order to measure respiration, the sample chamber was wrapped with aluminum foil creating the dark condition. O_2 consumption was monitored for the next 3 min. The rate of oxygen evolution / or consumption was expressed as nmol O_2 evolved or consumed/min/µg Chl *a*.

2.8. Carbohydrate estimation

Carbohydrate content of the cyanobacterial cells was measured according to Roe (1995)[38]. Five mL culture was centrifuged at 2500 rpm for 3 min and the pellet was re-suspended in 5 mL milliQ water. The solution was sonicated using Sonic Vibra cell sonicator (USA) fitted with a microprobe to disrupt the cyanobacterial cells. The supernatant collected after centrifugation was used for carbohydrate estimation. To 1 mL of the supernatant, 4 mL of anthrone reagent (0.2% in concentrated H_2SO_4) was added and mixed well. The tubes were incubated in boiling water bath for 10 min. After cooling, the solutions were centrifuged at 2500 rpm for 5 min and the absorbance of the resulting supernatant was read at 630 nm. A calibration curve was prepared using glucose solution (concentration range of 10–100 µg/mL) as the standard for the determining carbohydrate content of the cyanobacterial cells.

2.9. Cd^{2+} removal and its distribution in the cell in absence and in presence of Ca^{2+}

Cd^{2+} removal by the cyanobacterium was determined using Graphite Furnace Atomic Absorption Spectrophotometry (GF-AAS, Analytik Jene AG Vario 6) [39]. Twenty mL cultures were treated with 0.5 ppm Cd^{2+} in absence or in presence of Ca^{2+} for 24 h, centrifuged at 2500 rpm for 3 min and the amount of metal present in the supernatant was analyzed using GF-AAS. Percent Cd^{2+} removal was calculated using the following equation.

$$\%\ Cd^{2+}\ \text{removal} = \frac{(C_I - C_F)}{C_I} \times 100 \quad (5)$$

Where, C_I is the Cd^{2+} concentration initially supplied in the medium; C_F is the remaining Cd^{2+} concentration in the supernatant.

After the percent removal study was done, 20 mL BG-11_0 medium was added to the pellet, vortexed

and centrifuged at 2500 rpm for 3 min. The supernatant was analyzed for precipitated Cd^{2+} on the cell surface. Afterwards, 0.1 N of 20 mL HCl solution was added to the pellet and left for 5 min after vortexing to desorb surface bound metal ions into the supernatant. The desorbed Cd^{2+} ions were determined in the supernatant collected after centrifugation. After this step, the residual pellet was again re-suspended in 20 mL medium, ruptured by ultrasonication for 3 min and the resulting solution was analyzed using GF-AAS for the determination of internalized Cd^{2+}.

2.10. Bright Field Microscopy

For Bright Field Microscopic study, 1 mL cyanobacterial sample each was taken from different experimental sets in an Eppendorf tube and centrifuged at 3000 rpm for 3 min. The pellets were washed thrice by adding 1 mL of phosphate buffer saline, pH 7.0. Following this, the samples were mounted on glass slides, covered and viewed in 100X magnification under Fluorescence Microscope using EMP TL-BF filter (Leica Microsystems, SFL 4000).

3. RESULTS AND DISCUSSION

3.1. Effect on growth

Chlorophyll *a* is one of the important pigments of photosynthesis and estimation of chlorophyll *a* is a standard measure of growth in cyanobacteria. Chlorophyll *a* concentration in control culture was found to be 11.1 µg/mL after seven day. This value was taken as 100%. On addition of 0.5 ppm Cd^{2+} in the culture medium, chlorophyll *a* concentration was drastically reduced by 91.7%. However, simultaneous supplementation of 0.5 to 10 ppm Ca^{2+} in the medium containing 0.5 ppm Cd^{2+} showed reduction in Cd^{2+} induced toxicity on chlorophyll *a* (91.3%, 88.5%, 87.7%, 87.3%, 86.2%, 83.8% and 70.3%, respectively) (Fig. 1). Maximum protective effect on the chlorophyll *a* content was seen in the cultures in presence of 10 ppm Ca^{2+}.

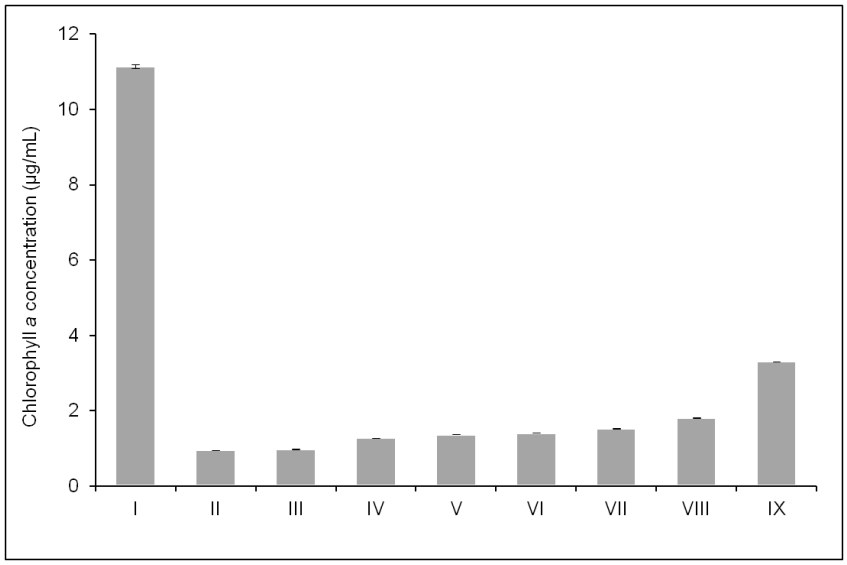

Fig. 1: Growth measurement in terms of chlorophyll *a* concentration in the cyanobacterium *Nostoc*

muscorum Meg 1 after seven day treatment. I: Control cultures; II: Cd^{2+} (0.5 ppm) treated; III: Cd^{2+} (0.5 ppm) + Ca^{2+} (0.5 ppm); IV: Cd^{2+} (0.5 ppm) + Ca^{2+} (1 ppm); V: Cd^{2+} (0.5) + Ca^{2+} (1.5 ppm); VI: Cd^{2+} (0.5) + Ca^{2+} (2 ppm); VII: Cd^{2+} (0.5 ppm) + Ca^{2+} (3 ppm); VIII: Cd^{2+} (0.5 ppm) + Ca^{2+} (5 ppm) and IX: Cd^{2+} (0.5 ppm) + Ca^{2+} (10 ppm). Taking control cultures as 100%. All values are expressed as mean ± SD. Samples were taken in triplicate (n = 3).

Henceforth, three different Ca^{2+} (1, 5 and 10 ppm) concentrations were chosen for the rest of the experiments. At these Ca^{2+} (1, 5 and 10 ppm) concentrations, growth of the cyanobacterium in terms of chlorophyll *a* was improved by 3.2%, 7.9% and 21.4%, respectively compared to 0.5 ppm Cd^{2+} treated culture.

3.2. Effect on photosynthetic accessory pigment contents: phycocyanin, allophycocyanin and carotenoids

Phycocyanin is one of the important accessory pigments present in cyanobacteria that assist the photosynthetic process by collecting light at 615 nm. In addition, phycocyanin plays a role as natural antioxidant which regulates and maintains oxidative stresses under conditions of high intensity of light, salinity, temperature, metals, etc. In the 0.5 ppm Cd^{2+} treated cells, phycocyanin content was reduced by 92.1% after seven-day exposure. The adverse effect was lowered to 70.1%, 54.3% and 24.3%, respectively (Fig. 2a) upon inclusion of 1, 5 and 10 ppm Ca^{2+} in the Cd^{2+} supplemented medium emphasizing that addition Ca^{2+} in the above mentioned concentrations could lessen the Cd^{2+} induced toxicity on the phycocyanin content by 22% – 67.8%. Allophycocyanin absorbs light at 650 and 660 nm maximally and increases the range of absorption of PAR light for photosynthesis. Allophycocyanin content in the culture medium supplemented with 0.5 ppm Cd^{2+} was reduced by 93.7% at the end of seven days. However, reduction seen upon inclusion of 1, 5 and 10 ppm Ca^{2+} in the culture medium was much less (54.9%, 32% and 27.8%), thus indicating the protective role of Ca^{2+} towards adverse effect brought about by Cd^{2+}. Carotenoids in cyanobacteria absorb light energy in the range between 461 and 664 nm and it performs the additional role of protecting chlorophyll pigments from photo-damage. Upon 0.5 ppm Cd^{2+} treatment, carotenoids content of the organism was lessened by 81.2% in seven days. When Ca^{2+} (1, 5 and 10 ppm) were added in the medium containing 0.5 ppm Cd^{2+}; the effect of Cd^{2+} was lowered to 69.4%, 65.2% and 59.1%, respectively (Fig. 2c) suggesting that addition of 1, 5 and 10 ppm Ca^{2+} lowered the effect of Cd^{2+} by 11.8%–22.1%. Among the pigments, allophycocyanin was most sensitive to Cd2+ whereas carotenoids were most tolerant. Highest improvement was seen in case of phycocyanin content when Ca^{2+} was introduced in the medium containing 0.5 ppm Cd^{2+}.

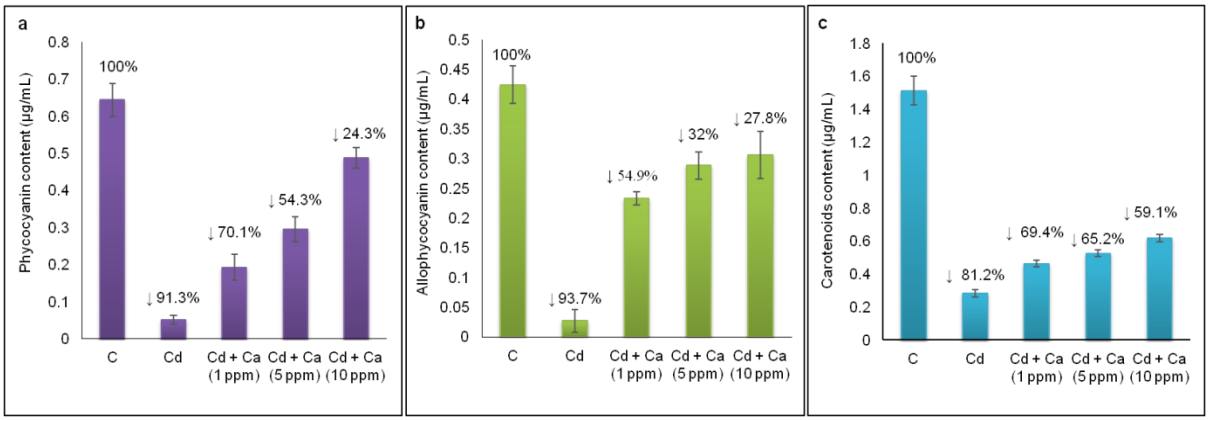

Fig. 2: Measurement of photosynthetic accessory pigments of the cyanobacterium *Nostoc muscorum* Meg 1. Period of treatment: seven days. **a:** phycocyanin; **b:** allophycocyanin and **c:** carotenoids. C: control culture; Cd: culture treated with 0.5 ppm Cd^{2+}; Cd + Ca: culture treated with 0.5 ppm Cd^{2+} along with Ca^{2+} (1 or 5 or 10 ppm). Taking control cultures as100%. All the values are expressed as mean ± SD. Samples were taken in triplicate (n = 3).

3.3. Effect on PSII activity and rate of respiration

PSII activity in terms of rate of O_2 evolution by the organism under continuous light was reduced by 78.1% after seven days of exposure to 0.5 ppm Cd^{2+}. However, severity of inhibition recorded in PSII activity in the cells treated with 0.5 ppm Cd^{2+} in presence of 1, 5 and 10 ppm Ca^{2+} was noticeably less by 68%, 60.2% and 51.6% (Table 1). The trend was similar for respiration. Respiration rate was significantly declined by 77.5% in Cd^{2+} treated cultures. However, inclusion of 1, 5 and 10 ppm Ca^{2+} during Cd^{2+} treatment brought down the percent inhibition to 64.1%, 48.6% and 40%, respectively.

Table 1: Photosynthetic PSII activity and respiration rate of the cyanobacterium *Nostoc muscorum* Meg 1 treated with 0.5 ppm Cd^{2+} in absence and in presence of 1, 5 and 10 ppm Ca^{2+}. Taking control cultures as 100%. C: control culture; Cd: culture treated with 0.5 ppm Cd^{2+} and Cd + Ca: culture treated with 0.5 ppm Cd^{2+} supplemented with Ca^{2+} (1 or 5 or 10 ppm).

	PSII activity (O_2 evolution)		Respiratory activity (O_2 consumption)	
	nmol O_2 evolved/min/µg Chl *a*	% ↑ / % ↓	nmol O_2 consumed/min/µg Chl *a*	% ↑ / % ↓
Control	443.6 ± 19.8	100	341.4 ± 18.5	100
Cd	97.1 ± 13.9	↓ 78.1	76.9 ± 12.7	↓ 77.5
Cd + Ca (1 ppm)	142.1 ± 14.3	↓ 68.0	122.7 ± 13.4	↓ 64.1
Cd + Ca (5 ppm)	176.4 ± 15.4	↓ 60.2	175.5 ± 15.6	↓ 48.6
Cd + Ca (10 ppm)	214.8 ± 17.1	↓ 51.6	208.4 ± 17.7	↓ 40.0

3.4. Effect on carbohydrate content

The biosynthesis of carbohydrate was inhibited by 76.7% in presence of 0.5 ppm Cd^{2+} however; the percent inhibition by Cd^{2+} was reduced to 69.2%, 66.2% and 59.7% in presence of 1, 5 and 10 ppm Ca^{2+} in the experimental medium. Thus, Ca^{2+} could lower the Cd^{2+} mediated toxicity in the carbohydrate synthesis by 7.5 - 17% (Fig. 3)

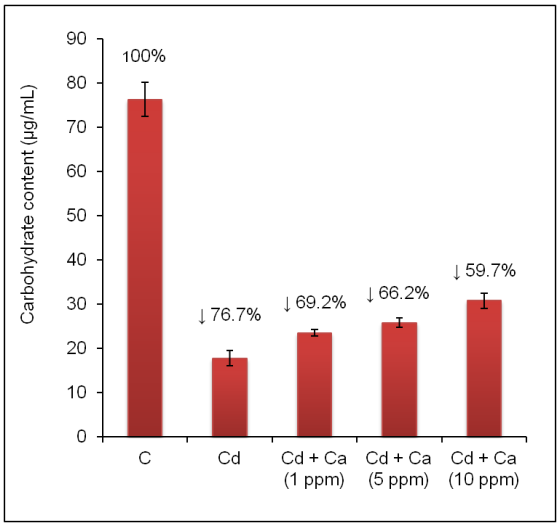

Fig. 3: Measurement of carbohydrate content of the cyanobacterium treated with 0.5 ppm Cd^{2+} in absence and in presence of 1, 5 and 10 ppm Ca^{2+} at the end of seven day treatment. C: control culture; Cd: culture treated with 0.5 ppm Cd^{2+} and Cd + Ca: cultures treated with 0.5 ppm Cd^{2+} along with Ca^{2+} (1 or 5 or 10 ppm). Taking control cultures as 100%. All the values are expressed as mean ± SD. Samples were taken in triplicate (n = 3).

3.5. Cd^{2+} removal and its cellular distribution in absence and in presence of Ca^{2+}

Metal ions internally accumulated are responsible for most of the toxic effects seen in the organism. Thus, percent Cd^{2+} uptake by the cyanobacterium from the medium was studied in detail. The organism removed 93% of the total Cd^{2+} supplemented in the medium (0.5 ppm) within 24 h (Fig. 4a). Further analysis showed that of the removed amount, 91.5% was adsorbed on the cell surface while 4% was precipitated and 4.5% (0.021 ppm) was internally accumulated (Fig. 4b). However, presence of 1, 5 and 10 ppm Ca^{2+} in the medium reduced Cd^{2+} removal to 83.8%, 74.8% and 50.7% at the end of 24 h (Fig. 4a) of which 91%, 90% and 91.8%, respectively were adsorbed on the cell surface. Of the removed Cd^{2+}, 4.7% (0.02 ppm), 4.3% (0.016 ppm) and 2.1% (0.005 ppm) was internalized (Fig. 4c; d; e). The increasingly reduced uptake of Cd^{2+} in presence of increasing concentration of Ca^{2+} pointed towards competition between the ions for the same binding sites as well as for ion transporters for entry into the cells.

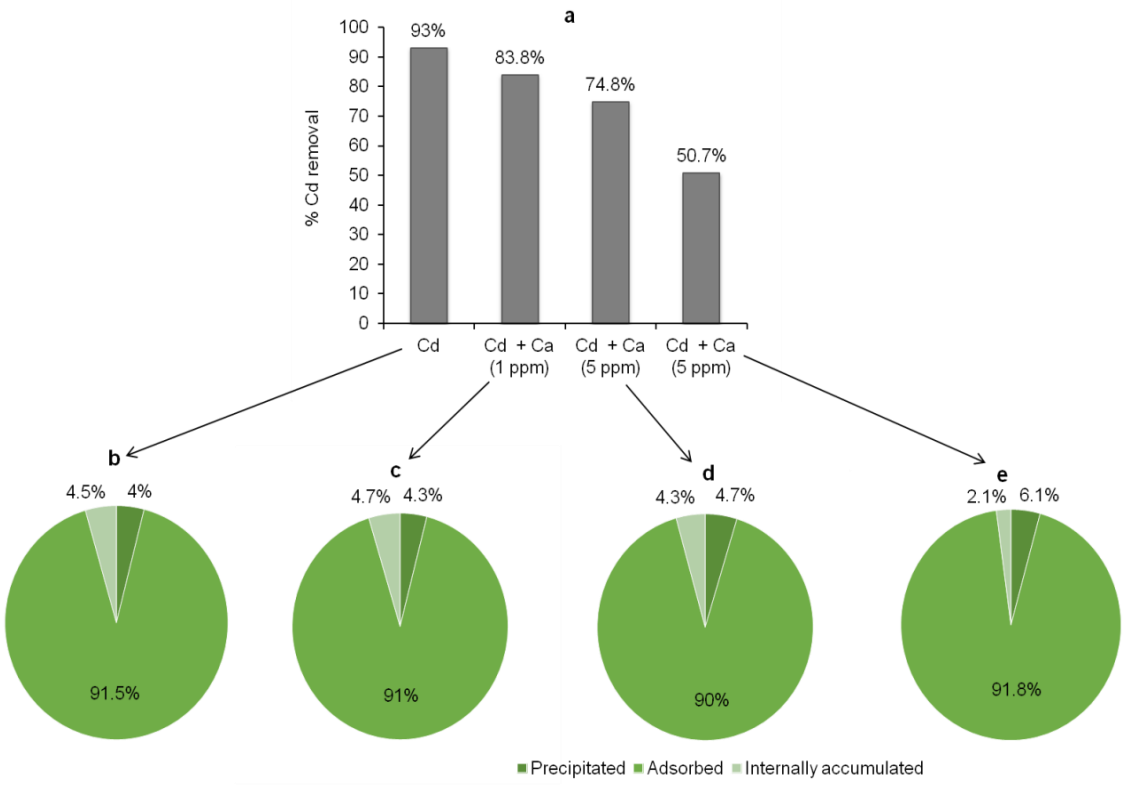

Fig. 4: Cd^{2+} removal and its cellular distribution in absence and in presence of Ca^{2+} (1 or 5 or 10 ppm) within 24 h. **a:** Total Cd^{2+} removal; **b:** cellular distribution in presence of 0.5 ppm Cd^{2+}; **c:** cellular distribution of Cd^{2+} in presence of 1 ppm Ca^{2+}; **d:** cellular distribution of Cd^{2+} in presence of 5 ppm Ca^{2+} and **e:** cellular distribution of Cd^{2+} in presence of 10 ppm Ca^{2+}. C: control culture; Cd: culture treated with 0.5 ppm Cd^{2+} and Cd + Ca: culture treated with 0.5 ppm Cd^{2+} supplemented with Ca^{2+} (1 or 5 or 10 ppm).

3.6. Morphological changes observed under Bright Field Microscope

Severe alterations in the morphology of the cells treated in 0.5 ppm Cd^{2+} for seven days were seen as filament breakage, disintegration of cells from the filaments, discoloration to blue color and distinctive cell damages (Fig. 5b). However, supplementation of Ca^{2+} (1, 5 and 10 ppm) showed progressive protection against Cd^{2+} induced damages in the cyanobacterial culture (Fig. 5c; d; e).

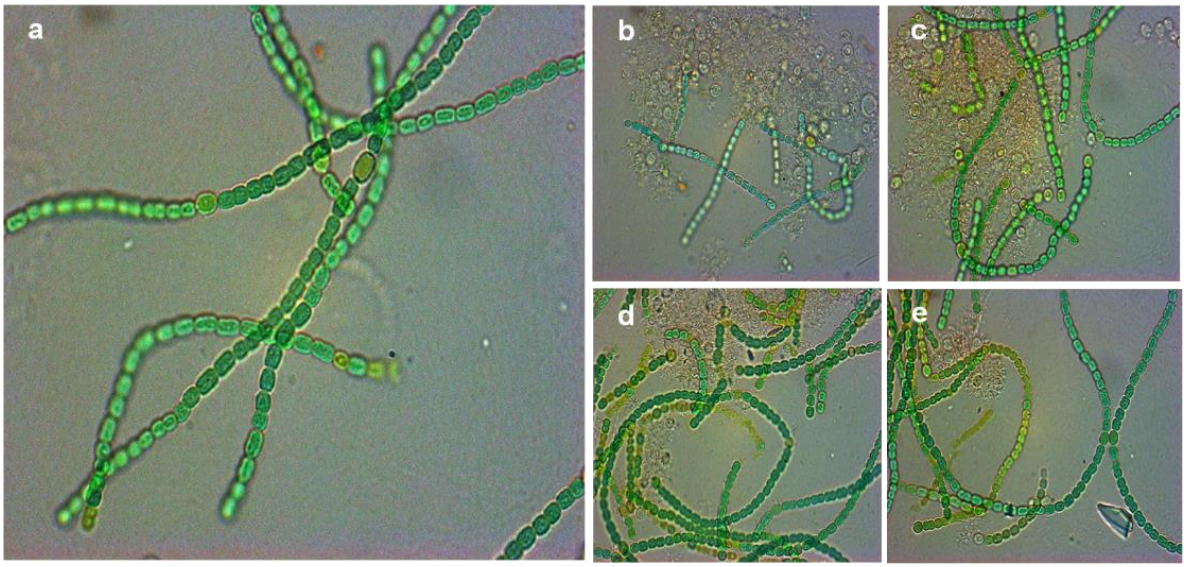

Fig. 5: Bright Field Microscopic images viewed in 100X magnification – **a:** control cells; **b:** cells treated with 0.5 ppm Cd^{2+}; **c**: cells treated with 0.5 ppm Cd^{2+} in presence of 1 ppm Ca^{2+}; **d**: cells treated with 0.5 ppm Cd^{2+} along with 5 ppm Ca^{2+}; **e:** cells treated with 0.5 ppm Cd^{2+} supplemented with 10 ppm Ca^{2+}.

Although both Cd^{2+} and Ca^{2+} are divalent cations, Ca^{2+} is essential in cyanobacteria for heterocyst differentiation, nitrogen fixation, PSII activity and phosphate uptake [26–29]. In contrast, presence of Cd^{2+} produced toxic effects on various carbon and nitrogen fixing components in the organism [17, 43]. Babich and Stotzky (1978)[41] and Fernandez□Piñas et al. (1995)[33] had reported that the toxicity of Cd^{2+} in plants and microorganisms could be reduced/eliminated in presence of various divalent cations such as Zn^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Ca^{2+} and Na^{+}. Fernandez□Piñas et al. (1995)[33] had also shown that in cyanobacterium *Nostoc* UAM208, supplementation of Ca^{2+} reduced the toxic effects of Cd^{2+} on growth, photosynthesis, N_2-fixation and reduce Cd^{2+} bioaccumulation. Although this is known for some time now, we decided to explore in detail effect of presence of Ca^{2+} on the Cd^{2+} induced changes in cyanobacteria isolated from a coal mining area in Meghalaya. This is because both coal and lime mining in the state release Cd^{2+} and Ca^{2+} in the surrounding environment and many cyanobacteria that are found to proliferate in these mining areas might have been taking advantage of regulatory influence of Ca^{2+} on the Cd^{2+} induced toxicity. Our results revealed that the presence Ca^{2+} in the culture medium containing Cd^{2+} countered the toxic effect of Cd^{2+} on growth, photosynthetic pigments (chlorophyll *a*, phycocyanin, allophycocyanin and carotenoids), PSII activity and rate of respiration and in production of carbohydrate. A deeper look into uptake of Cd^{2+}

in presence of Ca^{2+} (1, 5, 10 ppm) revealed that Cd^{2+} removal by the organism was reduced by 9.7–42.3% compared to cells that removed 93% of the supplemented Cd^{2+} from the medium containing only Cd^{2+} (0.5 ppm). Further, internal accumulation of Cd^{2+} was also reduced in presence of Ca^{2+} from 4.7% (~ 0.020 ppm) to 2.1% (0.005 ppm) within 24 h. Gipps and Coller (1982)[42]; Kim et al. (2002)[43] and Lu et al. (2010)[44] explained the possible reason of less internalization of Cd^{2+} in presence of Ca^{2+} as Ca^{2+} and Cd^{2+} ions compete for same transporters to move into the cells due to their similar ionic radii and charge. Apart from that concentration also plays a decisive role in uptake across any membrane. Therefore, increase in Ca^{2+} concentrations in the culture medium may employ both Ca^{2+} and Cd^{2+} transporters to transport more Ca^{2+} into the cells in a concentration dependent manner [45, 46]. In our study, presence of 1, 5 and 10 ppm Ca^{2+} in Cd^{2+} supplemented culture medium improved growth in terms of chlorophyll *a* by (0.4 – 21.4%), phycocyanin by (22 – 67.8%), allophycocyanin by (38.8 – 65.9%), carotenoids (11.8 – 22.1%), PSII activity (10.1 – 26.5%), rate of respiration by (13.4 – 37.5%) and carbohydrate production by (7.5 – 17%) compared to Cd^{2+} treated cells. Morphological alterations were also minimized in presence of Ca^{2+} (Fig. 5c; d; e). These findings point towards the fact that presence of Ca^{2+} did provide protection against Cd^{2+} induced toxicity in the cyanobacterium. From these results it can be concluded that the cyanobacteria growing in the coal mining area must be experiencing less Cd^{2+} toxicity due to the presence of Ca^{2+} in the same surrounding. The same logic applies also to other heavy metal contaminants whose toxicity may have been reduced due to presence of Ca^{2+} in the vicinity.

4. CONCLUSION

Presence of Ca2+ in the medium containing Cd2+ reduced the overall metal induced toxicity in the cyanobacterium Nostoc muscorum Meg 1 providing evidence towards the ameliorating effect of Ca2+ in Cd2+ induced toxicity. Ca2+ might replace Cd2+ at their site of action in the PSII thereby reducing Cd2+ toxicity on photosynthesis and hence on carbon assimilation. This may be the cause of the organism being able to grow in coal and lime mining areas where the presence of Ca2+ reduces the toxic effects produced by various heavy metal ions including Cd2+. Hence, Ca2+ supplementation in rice fields in the coal mining areas may be beneficial to these microbes that act as a natural biofertilizer in rice cultivation.

6. CONFLICT OF INTEREST

Authors declare no conflict of interest.

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