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Copper-Induced Morphological, Physiological and Biochemical Responses in the Cyanobacterium *Nostoc muscorum* Meg 1

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ABSTRACT

The cyanobacterium *Nostoc muscorum* Meg 1 was exposed to Cu^{2+} for seven days to assess its Cu^{2+} removal potential and the changes brought about in its morphology, physiology and biochemistry on Cu^{2+} exposure. Cu^{2+} binding was established by the EDX study. Identification of various functional groups on the cell surface involved in Cu^{2+} binding was done by FTIR analysis. AAS study showed that the organism was able to remove 96.3% Cu^{2+} from the medium supplemented with 3 ppm Cu^{2+}. Cellular distribution analysis indicated internalization of 6.1% Cu^{2+}. Another study showed that the maximum Cu^{2+} removal was a surface phenomenon that did not require energy. The IC_{50} value for Cu^{2+} was determined to be 9 ppm as growth was compromised by 50.5%. We used a sub-lethal dose of 3 ppm for all the experiments which are 3-fold higher than the WHO recommended value for Cu^{2+} (1 ppm) in drinking water. At this concentration, various photosynthetic pigments were reduced by 35.3% (chlorophyll *a*), 31.3% (phycocyanin), 17% (allophycocyanin), 21.3% (phycoerythrin) and 16% (carotenoids). Photosynthetic PSII activity and respiration rate were also compromised by 45% and 46.2%. Heterocyst frequency, nitrogenase and glutamine synthetase activities were declined by 18.5%, 14.8% and 16.2%. Bright field and SEM images showed distinct morphological changes, where there was filament breakage, disintegration and degradation of individual cells as well as cell elongation, distortion and shriveling. The cyanobacterium generated 436.4% more ROS compared to the control cells.

INTRODUCTION

The issues of water pollution are going to be critical in the upcoming decades due to global water scarcity. The rapid increase of mining activities, petroleum refining, metal finishing, smelting and electroplating, production of paints and pigments, etc. are releasing heavy metals into the environment (Akbari et al. 2015). Some heavy metals are essential to human beings, plants and microbes, but can be toxic in high concentration and on chronic exposure. Many heavy metals both essential and non-essential, e.g. Cu, Fe, Zn, Mn, Cr, Co, Cd, Pb, As, Hg, etc. Cd, Pb, As and Hg prevail in the environment and being carcinogenic, mutagenic and non-degradable are of serious health concern via their incorporation in the food web (Yan & Pan 2002, Jin et al. 2003, Qaiser et al. 2007, Reddy et al. 2012b, Bilal et al. 2013, Yu et al. 2014). In the context of the organism under the study, Cu^{2+} plays an essential role as a structural component of plastocyanin (a component of electron transport chain in cyanobacteria) and also an essential cofactor of enzyme superoxide dismutase (Yruela et al. 2000, Vermaas et al. 2001). According to the World Health Organization (WHO), the United States of Environmental Protection Agency (USEPA), Indian Council of Medical Research (ICMR) and Indian Standard Institution (ISI), the permissible limit of Cu^{2+} in water is between 0.05 and 1.5 ppm, and has many health risks associated with the exposure to excess Cu^{2+} in humans (James & Cook 1983, Veglio & Beolchini 1997, Dikshith 2009) (Table 1).

As reported by Surosz & Palinska (2004) and Nongrum & Syiem (2012), Cu^{2+} in high concentrations adversely affect the photosynthesis and rate of respiration, inhibition of cell division and cell death in primary producers of the food web such as plants, algae and bacteria including cyanobacteria. In tropical areas, one of the most conducive places for cyanobacterial growth is waterlogged rice fields with optimum light, temperature and nutrient supply. Cyanobacteria are highly beneficial to crops as they have short generation time that allows increase in cell biomass, thereby aiding to increase provision of fixed nitrogen and maintenance of soil fertility, soil health, and texture in way of releasing extracellular polysaccharides that bind soil particles (Castenholz 2001, Nisha et al. 2007, Ahad et al. 2015). As a consequence, these organisms are being used as biofertiliser in rice fields in many Asian countries such as China, India, Thailand, Vietnam and Myanmar (Kaushik 2014). Apart from that many cyanobacteria are also found

Table 1: Permissible limits of Cu^{2+} in water and its harmful effects on human health.

Metal ion	WHO (ppm)	USEPA (ppm)	ICMR (ppm)	ISI (ppm)	Health risk
Cu^{2+}	1	1.3	1.5	0.05	Gastrointestinal disorder, irritation of nose, mouth, eyes, headache

growing in metal contaminated environments, including crop fields in these areas (Shukla et al. 2012). In these situations, they can act as bioremediators of contaminants, especially heavy metal ions by virtue of sequestering the metal ions on to their cell surfaces due to the presence of negatively charged hydroxyl, carboxyl, carbonyl, etc. groups those are available to bind with positively charged metal ions (Chojnacka et al. 2005). As a result they are able to remove metal ions from the vicinity of the crop plants (Yang et al. 2015).

As pointed out earlier, many heavy metal ions are essential to organisms. However, at higher concentration they become detrimental. In the context of the present study, there are reports available that show that exposure to excess amount of Cu^{2+} in cyanobacteria is harmful, as it generates reactive oxygen species (ROS) leading to breakdown of membrane lipids and proteins (Murphy & Tiaz 1997, Chen et al. 2000, Wang et al. 2004). Subsequently, GSH and other metallo-protein functions are also compromised (Waldron & Robinson 2009, Gregoire & Poulain 2014, Imlay 2014). To combat such exposure, cyanobacteria are known to adopt protective and regulatory mechanisms (Nongrum & Syiem 2012, Goswami et al. 2015). One such protective measure is the production of extracellular polymeric substances that can sequester metal ions outside the cell surface (Khataee et al. 2010). Cu^{2+} is transported into the cells via *cop1/cop2*

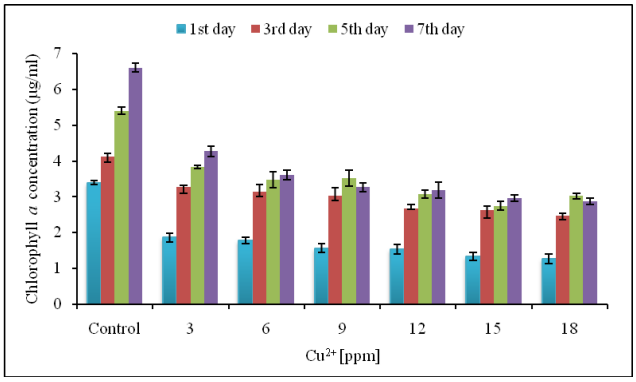

Fig. 1: Determination of IC_{50} in the cyanobacterium *Nostoc muscorum* Meg 1 cells in presence of 3-18 ppm Cu^{2+} within seven days of treatment. The inhibition was measured in terms of chlorophyll *a* content. All values are expressed in mean ± SD and the samples were taken in triplicates (n=3).

and *cta A* transporters in *Synechocystis* sp. (Phung et al. 1994, Kaneko et al. 1996). However, there are other cellular mechanisms that maintain homeostasis by exporting metals using P-type ATPase. The efflux of Cu^{2+} was done using transporter *pacS* in the *Synechocystis* sp. 7942 (Kanamura 1993). Inside the cells, the ion concentrations are regulated by binding into metallothioneins and phytochelatins and sequestration in polyphosphate bodies (Clarke 1987, Zhou & Goldsborough 1994, Keasling & Hupf 1996, Baptista & Vasconcelos 2006, Nies 2003, Cobette 2000). Other than these cellular strategies, cells produce antioxidants such as superoxide dismutase (SOD), catalase (KatG) and peroxidase (Prx) converts superoxide anion to hydrogen peroxide by SOD and subsequently into water (Narainsamy 2013).

The organism used in the present study (the cyanobacterium *Nostoc muscorum* Meg 1) was isolated from a rice field in Sohra, Meghalaya, India adjacent to the coal mining site contaminated with various heavy metal ions such as Cu, Cd, Fe, Zn, Mn, Cr, Ni, etc. due to the flow of coal mining effluents along with rainwater to the fields. Our previous study indicated that the cyanobacterium *Nostoc muscorum* Meg 1 is tolerant to 0.5 ppm Cd^{2+} (Ahad et al. 2017). In the present study we aimed at investigating the details of exposure to high Cu^{2+} concentration in this cyanobacterium as well as Cu^{2+} uptake and its distribution in the cells, energy requirement in Cu^{2+} removal and alterations in the physiological, biochemical and morphological characters.

MATERIALS AND METHODS

The cyanobacterium *Nostoc muscorum* Meg 1: The cyanobacterium *Nostoc muscorum* Meg 1 isolated from a contaminated rice field in Sohra, Meghalaya, India and identified using 16S rRNA sequencing (Ahad et al. 2017), was grown and maintained in BG-11_0 medium inside a culture room (pH 8, temperature 25±2 °C and photon fluence rate 50 µmol/m^2s, respectively).

Cu^{2+} treatment: $CuSO_4.5H_2O$ was used as the source of Cu^{2+} for all the experiments carried out. A stock solution of 100 ppm Cu^{2+} solution was prepared and diluted with BG-11_0 medium to make 1, 2, 3, 6, 9, 12, 15 and 18 ppm Cu^{2+}.

Chlorophyll *a* estimation: The chlorophyll *a* extracted in methanol and its absorption was measured at 663 nm in UV-Vis spectrophotometer (MacKinney 1941).

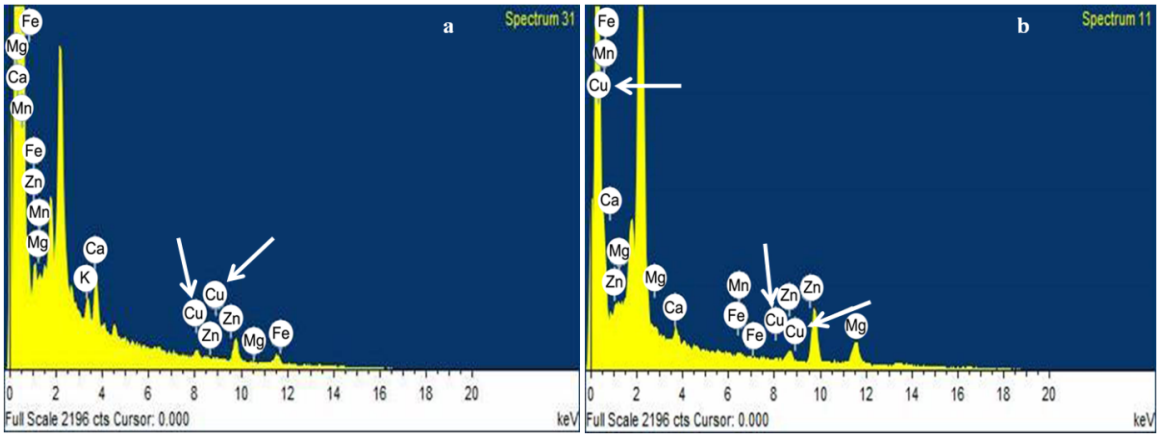

Fig. 2: EDX spectra of control and Cu^{2+} (3 ppm) treated *Nostoc muscorum* Meg 1 cells after seven days of exposure. EDX images - a: control cells and b: Cu^{2+} treated cells. Arrows indicate Cu^{2+} spectra.

SEM-EDX analysis: SEM-EDX analysis was performed using INCA Penta FETX3 in combination with SEM, JEOL-JSM-6360; JEOL, Tokyo, Japan according the method described in Ahad et al. (2017).

FTIR spectroscopic analysis: FTIR spectroscopic analysis was used to determine various cell surface functional groups involved in Cu^{2+} binding as detailed by Diengdoh et al. (2017).

Cu^{2+}removal and its distribution in the cell: Cu^{2+} removal and its cellular distribution were analysed using GF-AAS (Nongrum & Syiem 2012). Percent Cu^{2+} removal was calculated by the Eq.1.

$$\%\ Cu^{2+}\ \text{removal} = \frac{C_I - C_F}{C_I} \times 100 \qquad ...(1)$$

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

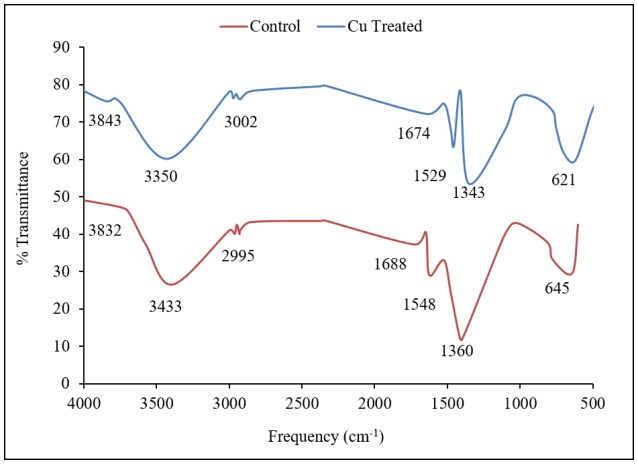

Fig. 3: FTIR spectra of control and Cu^{2+} (3ppm) treated cells of *Nostoc muscorum* Meg 1 at the end of seven days.

Provision of energy for Cu^{2+} accumulation: Provision of energy for Cu^{2+} accumulation was studied as described by Goswami et al. (2015).

Bright field microscopy: Bright field microscopic observation was made under fluorescence microscope (Leica Microsystems, SFL 4000).

Reactive oxygen species (ROS) measurement: ROS generated was determined by using a fluorometric indicator 2, 7 dihydrodichloro-fluoresce in diacetate (H_2DCF-DA) as described by Gomes et al. (2005). ROS generated was expressed in relative fluorescence unit (RFU).

Protein estimation: Protein was estimated according to the protocol of Lowry et al. (1951).

Phycobiliproteins estimation: Various phycobiliprotein [phycocyanin (PC), allophycocyanin (APC) and phycoerythrin (PE)] content was determined by the method developed by Bennett & Bogorad (1973).

Carotenoids estimation: Carotenoids were measured according to Morgan (1967).

PSII activity and rate of respiration: PSII activity and rate of respiration were measured using a Clark-type oxygen electrode as described by Robinson et al. (1982).

Heterocyst frequency and nitrogenase activity: Heterocyst frequency of the cyanobacterial cells was calculated by counting 1000 cells under a light microscope (Wolk 1965). Nitrogenase activity was measured as described by Stewart et al. (1967) using acetylene reduction method.

Glutamine synthetase activity: Glutamine synthetase activity was measured by the method developed by Sampaio et al. (1979).

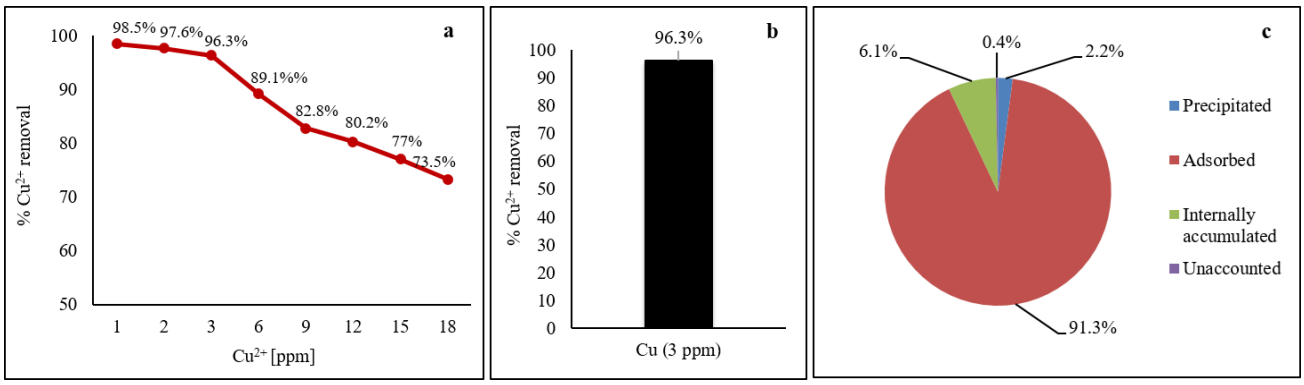

Fig. 4: Percent Cu^{2+} removal and its cellular distribution in the cyanobacterium *Nostoc muscorum* Meg 1 cells within seven days. a: percent Cu^{2+} removal in different Cu^{2+} (1- 18 ppm) concentrations; b: % Cu^{2+} removal in the 3 ppm Cu^{2+} exposure and c: cellular distribution of Cu^{2+} in the cyanobacterium.

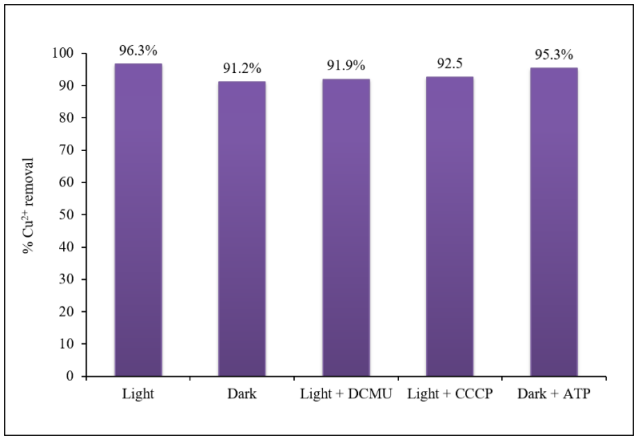

Fig. 5: Energy provision for Cu^{2+} uptake in different conditions. Light; Dark; Light + DCMU; Light + CCCP and Dark + ATP. Cu^{2+} concentration: 3 ppm; Duration: seven days.

RESULTS

***IC_{50}* and growth of the cyanobacterium *Nostoc muscorum* Meg 1:** The cyanobacterium *Nostoc muscorum* Meg 1 cultures were exposed to 3-18 ppm of Cu^{2+} for seven days (Fig. 1). The reduction (50.5%) in growth in terms of chlorophyll *a* concentration indicated 9 ppm Cu^{2+} concentration to be the IC_{50}. Incubation for seven days at a sub-lethal dose of 3 ppm Cu^{2+} that was chosen for the subsequent study showed a significant reduction in chlorophyll *a* content (35.3%).

EDX study: EDX analysis in the cyanobacterium *Nostoc muscorum* Meg 1 in absence or presence of 3 ppm Cu^{2+} is shown in Fig. 2. A clear peak of Cu^{2+} binding on the cell surface of the treated cells is visible in Fig. 2b. The small peaks of Cu^{2+} that appeared in both the EDX spectra were due to the presence of $CuSO_4$ as one of the component of $BG\text{-}11_0$ medium (Fig. 2a,b).

FTIR fingerprints: FTIR analysis of control and Cu^{2+} (3 ppm) treated *Nostoc muscorum* Meg 1 cells were performed at the end of seven-day treatment (Fig. 3). A change in fingerprint frequencies of different functional groups in the FTIR spectra indicated binding of Cu^{2+} ions on the cell surface. A shift is seen in the region from 3832 cm^{-1} → 3,843 cm^{-1} established the involvement of O-H group. A shift towards the lower frequency from 3433 cm^{-1} → 3450 cm^{-1} and from 2995 cm^{-1} → 3002 cm^{-1} indicated the possible involvement of OH stretching of alcoholic and phenol groups. A shift in frequency from 1688 cm^{-1} → 1674 cm^{-1} indicated the participation of C=O stretch of α, β-unsaturated aldehyde and ketone groups. The shift from 1548 cm^{-1} → 1529 cm^{-1} and 1360 cm^{-1} → 1343 cm^{-1} established the involvement of NO of nitro groups. Finally, a change in frequency from 645 cm^{-1} → 621 cm^{-1} reflected the participation of NH (2° amines, NH_2 and N-H wagging) group in Cu^{2+} binding.

Cu^{2+} removal by the *Nostoc muscorum* Meg 1 and its cellular distribution: The amount of Cu^{2+} biosorbed by the cyanobacterium *Nostoc muscorum* Meg 1 after seven days of exposure under optimum conditions was found to be 98.5-73.5% when 1-18 ppm of Cu^{2+} was supplemented in the medium (Fig. 4a). As seen in the figure, percent Cu^{2+} removal was found to decrease when the concentration of Cu^{2+} increased from 1 to 18 ppm. This experiment indicated the removal capability of the organism in different concentrations.

Batch experiments showed that *Nostoc muscorum* Meg 1 could remove 96.3% of Cu^{2+} when the culture was treated with 3 ppm Cu^{2+} for seven days (Fig. 4b). Of the total removed metal, 91.3% of Cu^{2+} was found to be adsorbed on the cell surface indicating Cu^{2+} removal primarily to be a surface phenomenon (Fig. 4c). The same figure shows that

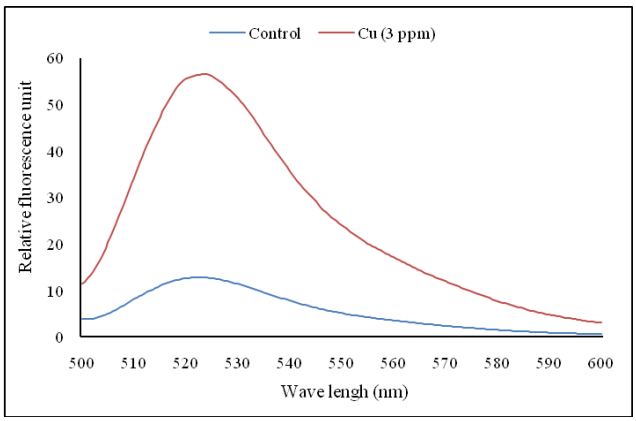

Fig. 6: Measurement of ROS generation in control and Cu^{2+} (3 ppm) treated cells in the cyanobacterium *Nostoc muscorum* Meg 1 at the end of seven days.

6.1% Cu^{2+} was internally accumulated and 2.2% was precipitated on the cell surface of the cyanobacterium.

Energy provision and role energy in Cu^{2+} uptake: The cyanobacterium *Nostoc muscorum* Meg 1 was capable of removing 96.3% of Cu^{2+} from the medium supplemented with 3 ppm Cu^{2+} after seven days in presence of continuous light. A similar experiment conducted in dark showed 91.2% removal. In presence of DCMU (the photosynthetic electron transport blocker), the organism was able to remove 91.9% Cu^{2+}. Another similar experiment in presence of CCCP (an uncoupler of electron transport chain and generation of proton motive force for ATP production) resulted in 92.5% of Cu^{2+} removal. These three experiments together established beyond doubt that light driven photosynthetic ATP production did not contribute significantly towards overall metal removal. The addition of ATP in dark resulted in only a small increase of ~ 4.1% in Cu^{2+} removal from the experiment conducted in only dark indicated that ATP probably was required for energy dependent intracellular metal accumulation (6.1%) that was seen within seven days (Fig. 5). This experiment provided evidence that the Cu^{2+} removal by the organism was mainly a sorption phenomenon.

Net reactive oxygen (ROS) generation: Net ROS generated in absence and presence of 3 ppm Cu^{2+} by the cyanobacterium *Nostoc muscorum* Meg 1 within seven days is presented in Fig. 6. The relative ROS generated in the organism in presence of 3 ppm Cu^{2+} was 436.4% compared to control cells which was ~ 4.4-fold higher than the control

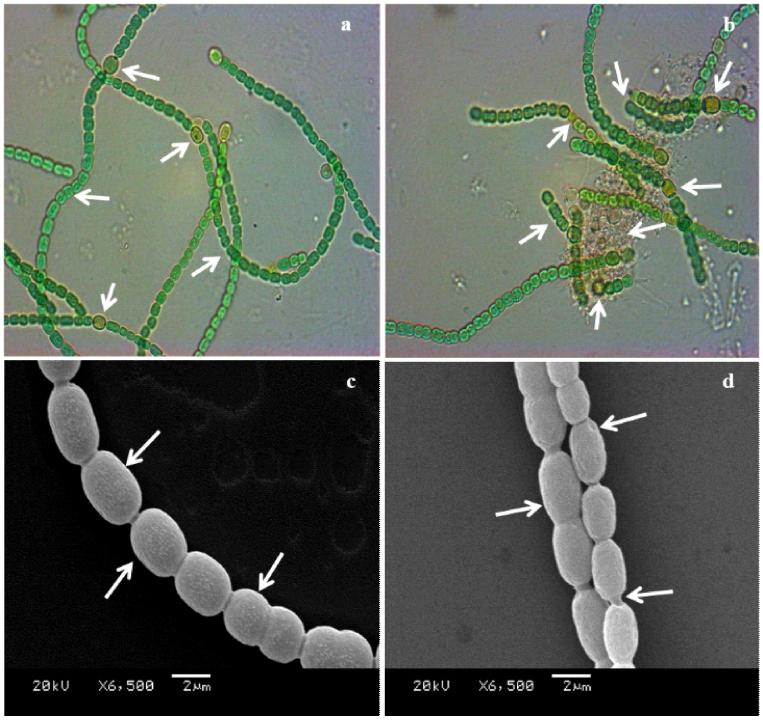

Fig. 7: Bright field and scanning electron microscopic images of control and Cu^{2+} (3 ppm) treated cells at the end of seven day exposure. Bright field images - a: control cells and b: Cu^{2+} treated cells. Scanning electron micrographs - c: control cells and d: Cu^{2+} treated cells. Arrows indicate heterocyst in the filaments of control cells (Fig. 6a) and heterocyst cells and broken filaments in Fig. 6b. Arrows indicate round and healthy cells in control culture (Fig. 6c) and elongated, distorted, shriveled cells in Cu^{2+} treated cells (Fig. 6d).

Table 2: Photosynthetic PSII activity and rate of respiration of control and Cu^{2+} (3 ppm) treated *Nostoc muscorum* Meg 1 cells within seven day treatment.

	PSII activity		Rate of respiration	
	nmol O_2 evolved/µg Chl*a*/h	↑ / ↓ %	nmol O_2 consumed/µg Chl*a*/h	↑ / ↓ %
Control	574.3 ± 10.9	100%	377.4 ± 12.1	100%
Cu^{2+} treated	258.3 ± 8.2	↓ 45%	175.6 ± 7.4	↓ 46.2

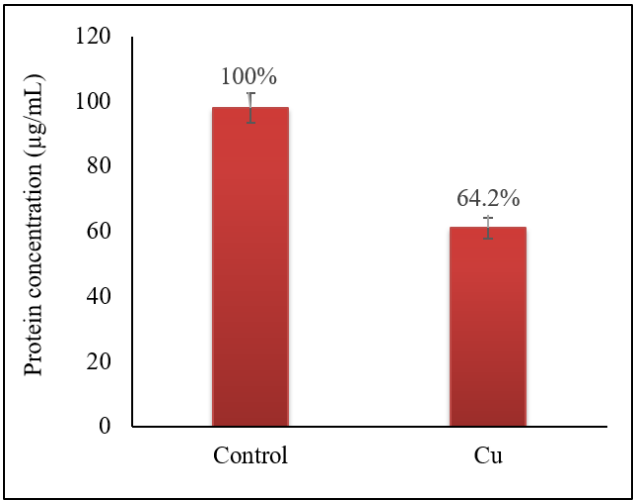

Fig. 8: Measurement of total protein content upon 3 ppm Cu^{2+} exposure with respect to control culture. Duration of treatment: seven days.

value indicating that Cu^{2+} mediated its adverse effects via ROS production.

Morphological alterations under Cu^{2+} exposure: Bright field microscopic observations under fluorescence microscope (Leica Microsystems, SF 4000) of control and Cu^{2+} treated cells are presented in Fig. 7a&b. Cu^{2+} at 3 ppm concentration was moderately toxic to the cyanobacterium at the end of seven days exposure. The control cultures were seen as long filaments with round and healthy cells with intact heterocysts in the bright field microscopic images (Fig. 7a). In contrast, many broken filaments and degraded cells were visible in cultures treated with 3 ppm Cu^{2+} (Fig. 7b).

More detailed changes in the morphology of the organism were visible under SEM (Fig. 7c&d) that corroborates the observation seen under bright field microscopy. The cells were elongated, distorted and distinctly shriveled upon Cu^{2+} treatment.

Protein content: The total protein content in the cyanobacterium was decreased by 35.8% when exposed to 3 ppm of Cu^{2+} at the end of seven day treatment indicating adverse effect of Cu^{2+} upon chronic exposure (Fig. 8).

Alterations in the photosynthetic accessory pigments: Photosynthetic accessory pigments phycocyanin, allophycocyanin, phycoerythrin and carotenoids were compromised by 31.3%, 17%, 21.3% and 16% respectively at the end of seven days treatment in 3 ppm Cu^{2+} compared to control cells (Fig. 9). Among the various accessory pigments, phycocyanin was the most sensitive and carotenoids was the most stable and tolerant to Cu^{2+}exposure.

Photosynthetic PSII activity and rate of respiration: PSII activity upon Cu^{2+} treatment was reduced by 45%, whereas the rate of respiration was down by 46.2% at the end of seven days when compared to control cells (Table 2). Lowered PSII activity could also be attributed to the compromised status of various photosynthetic pigments that play a crucial role in harvesting light energy at different wavelengths for the photosynthetic machinery.

Percent heterocyst frequency, nitrogenase and glutamine synthetase activities: Percent heterocyst frequency in the cyanobacterium was compromised by 18.5% in the presence of 3 ppm Cu^{2+} within seven days (Fig. 10a). Within the same period, activities of the nitrogenase and glutamine synthetase enzymes were reduced by 14.8% and 16.2% respectively (Fig. 10b&c). These results showed the negative effect of Cu^{2+} exposure on the nitrogen metabolism machinery of the organism.

DISCUSSION

The state of Meghalaya falls under Eastern Himalayan region and the topography of the state is hilly. The State is enriched in natural resources such as coal, limestone and uranium. Mining of coal and limestone activities is highly unscientific in approach. The effluents of these mining activities readily drain into various streams and rivers as well as into low lying rice fields. The mining effluents are rich in various heavy metal ions, including Cu, Zn, Fe, Cr, Cd, etc. (Ahad et al. 2017, Diengdoh et al. 2017). Analysis of water samples from different locations including rice fields around coal mines showed presence of Cu^{2+} to be higher than the normal limit (>1.5 ppm) in drinking water recommended by USEPA (James & Cook 1983, Veglio & Beolchini 1997,

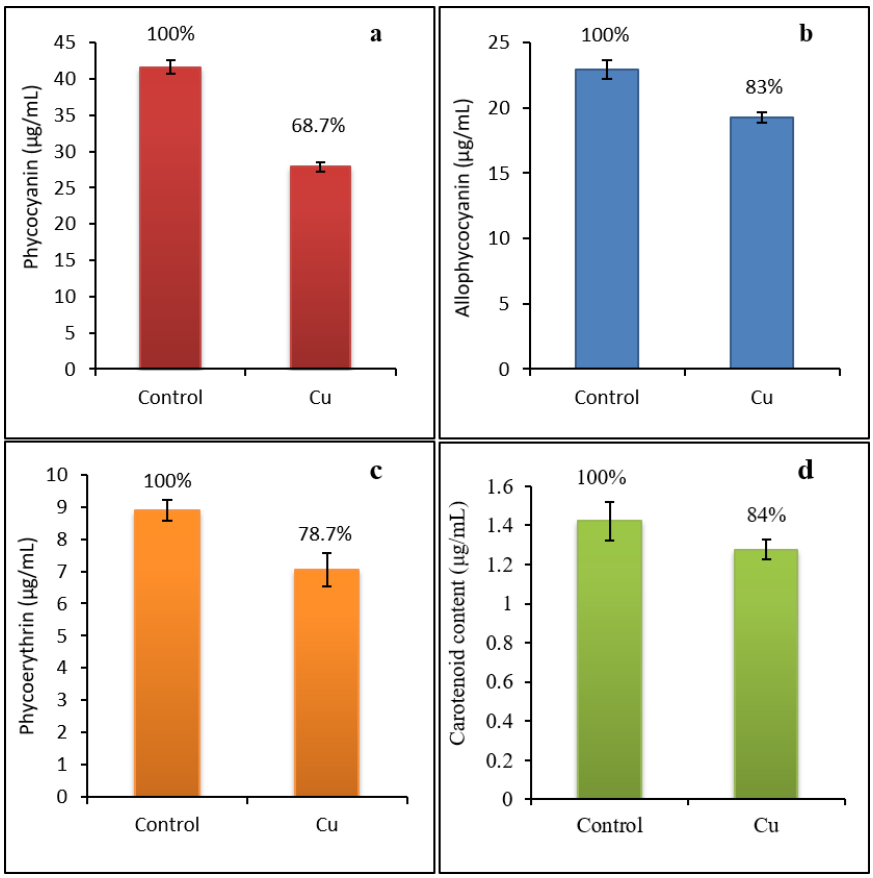

Fig. 9: Photosynthetic accessory pigment contents in presence of 3 ppm Cu^{2+} in the *Nostoc muscorum* Meg 1 cells at the end of seven days. a: Phycocyanin; b: allophycocyanin; c: phycoerythrin and d: carotenoids content.

Dikshith 2009). The organism was capable of removing a very high percentage of Cu^{2+} (96.3%) within seven days from 3 ppm Cu^{2+} supplemented medium. Within the same period of time it could accumulate 6.1% (0.177 ppm) of Cu^{2+} internally, probably using ATP derived from photosynthetic and respiratory electron chain activities. There are reports of Cu^{2+} entering cyanobacterial cells via *cop1/cop2* and *ctaA* P-type ATPase transporters (Phung et al. 1994, Kaneko et al. 1996) as well as there are reports of Cu^{2+} efflux via *pacS* transporter (Kanamura 1993). However, increased concentration of Cu^{2+} in the vicinity might have created imbalance in the influx-efflux system leading to higher intracellular accumulation of Cu^{2+} with the result of expression of toxicity in the organism in all measured parameters/major characters (Fig. 11).

Although the small percentage of Cu^{2+} internally accumulated required energy, the major portion of removed Cu^{2+} was biosorbed onto its cell surface that did not require any involvement of energy. EDX and FTIR studies comprehensively established surface binding of the metal ions to the organism's various cell surface functional groups. Even though Cu^{2+} is an essential element for the organism, chronic exposure to high Cu^{2+} concentration adversely affected its morphology, physiology and biochemical characteristics. Various photosynthetic pigments, total protein content, nitrogen metabolism, PSII activity and respiration were compromised at least by 15-40%. High ROS production observed in our study upon Cu^{2+} exposure indicated that probably these adverse effects may have been mediated via different reactive oxygen species that are known to create havoc in cellular environments (Chen et al. 2000, Wang et al. 2004, Goswami et al. 2015a). In addition, interactions of Cu^{2+} ions with the free thiols and other -SH groups of various enzymes' active sites might have also led to limited cell division, thus compromising growth and other parameters of the organism as pointed out by earlier researchers (Surosz & Palinska 2004, Waldron & Robinson 2009, Nongrum & Syiem 2012, Gregoire & Poulain 2014, Imlay 2014).

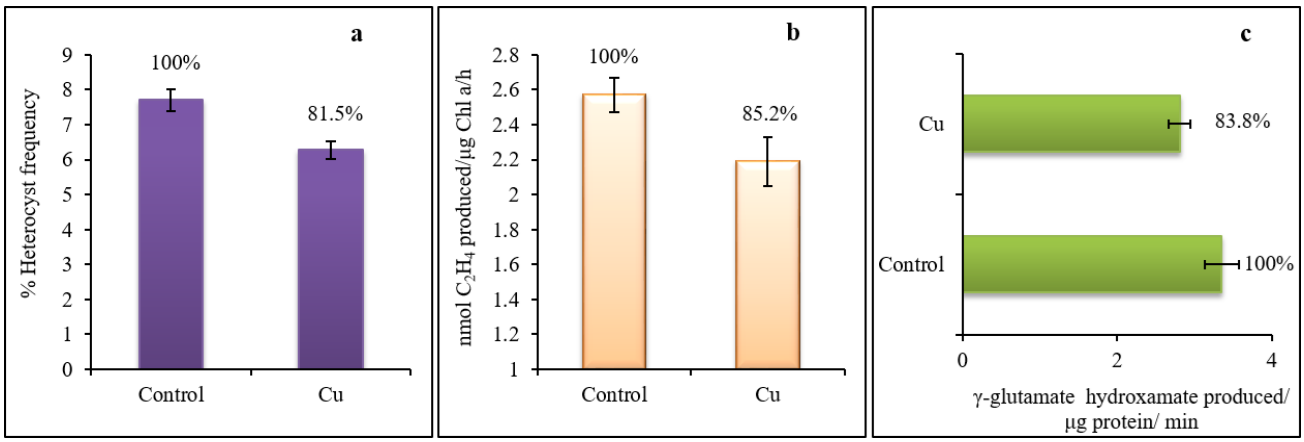

Fig. 10: a- Percent heterocyst frequency; b- nitrogenase and c- glutamine synthetase activitiesof the cyanobacterium in absence and presence of 3 ppm Cu^{2+} at the end of seven days.

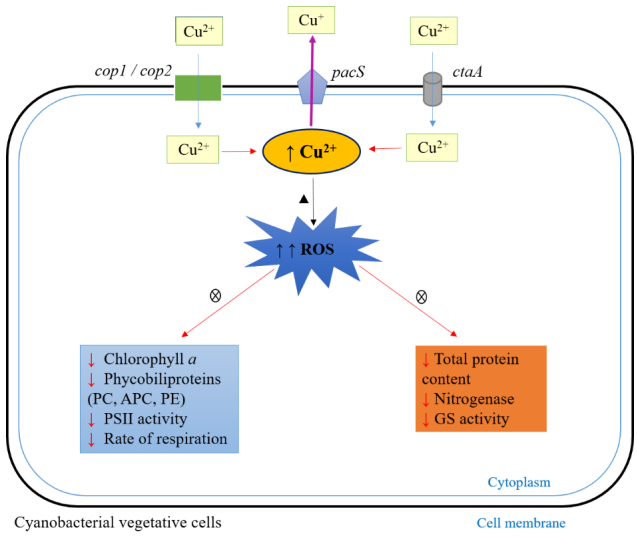

Fig. 11: Pictorial representation of Cu^{2+} entry into the cells and excess Cu^{2+} in the cells leading to the generation of high ROS in the cyanobacterium *Nostoc muscorum* Meg 1. Excess ROS production inhibits cellular physiological and biochemical characteristics. The above figure is a comprehensive representation of the involvement of various transporters for the entry of Cu^{2+} into the cells using Cu^{2+} transporters *cop1 / cop2* and *ctaA* in cyanobacteria (Phung et al. 1994, Kaneko et al. 1996, Tottey et al. 2005) and Cu^{2+} efflux by P-type ATPase *pacS* in cyanobacteria which helps the cells to maintain the Cu^{2+} concentration inside the cell (Kanamura et al. 1993). ▲ represents activation/ increase and ⊗ represents inhibition/ decrease.

CONCLUSION

Thus, our study provides evidence that although Cu^{2+} is essential for the organism as it is a crucial constituent of plastocyanin and cytochrome b_6f of photosynthetic electron transport chain, chronic exposure to high Cu^{2+} concentration is deleterious to the organism, although not fatal. Cyanobacteria are known biofertiliser in rice fields, where they aid to soil fertility via addition of nitrogen and carbon on their turnover. Additionally, the exo-polysaccharide secreted by many cyanobacteria helps in maintaining soil character and texture. Being minute and ubiquitous these significant contributions of cyanobacteria go unnoticed. However, persistent presence of various contaminants, including heavy metal ions in rice fields due to various anthropogenic activities are compromising the cyanobacterial population thereby harming soil fertility and productivity. This study has shown that even an essential element at higher concentration could lead to substantial harmful effects on the beneficial organisms.

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