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A dose-dependent study of carbon metabolism under Pb stress in the cyanobacterium *Nostoc muscorum* Meg 1

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Abstract

Lead (Pb) is one of the most abundant heavy metals and its toxic effects cause environmental and health problems. Pb occurs naturally in the environment. However, most lead concentrations that are found in the environment are a result of human activities including burning fossil fuels, mining and manufacturing. In the present study, we checked into the effects of Pb on growth, pigments, PSII activity, carbohydrate and biomass production in the cyanobacterium *Nostoc muscorum* Meg 1 using different concentrations ranging from 10 ppm to 80 ppm. Pb at lower concentration (10-40 ppm) enhanced the growth by increasing photosynthetic pigments such as chlorophyll *a*, phycobiliproteins and carotenoids; by increasing concentration of D1 protein that hosts the PSII complex of photosynthetic electron transport chain and by positively modulating the concentration of RuBisCO, the key enzyme for CO_2 fixation. Conversely, a higher concentration of Pb (60-80 ppm) adversely affected all parameters studied bringing down the organism's growth and biomass production. Augmentation of various components of carbon fixation at a lower concentration in this organism indicates that Pb at lower doses was not considered toxic but it could lead to eutrophication of lakes, rivers and water reservoirs rendering them unusable for human and animal consumption.

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Introduction

Water and soil contamination is a major problem in the world. Recent studies showed various water bodies being contaminated with fluoride, toxic metals, pesticides, fertilizers, dyes, hydrocarbons, and pathogens, (Jin *et al.*, 2003; Qaiser *et al.*, 2007; Akbari *et al.*, 2015). Most of these pollutants are toxic and non-biotransferable in nature (Reddy *et al.*, 2012; Bilal *et al.*, 2013). Metals being one of the prevalent toxic pollutants may have the potentiality of bioaccumulation in the higher trophic levels (Wen-Xiong, 2002). Metal toxicity is never related to metal concentration in all cases; it depends on bioavailability, its solubility and ease of entry into the cells (Hesler, 1974, Singh *et al.*, 1997; Heng *et al.*, 2004). A number of metals are essential for biological processes (Cu, Fe, Zn, Mn, Mo, Ni, Cr, Co, etc.) since they play vital roles as cofactors of enzymes or proteins and their presence in optimum concentrations are always biologically required. However, if their concentration crosses the threshold limit, they hamper physiological and biochemical processes by removing or competing with other cofactors from the binding sites or binding to unrelated proteins and enzymes (Surosz and Palinska, 2004; Goswami *et al.*, 2015; Diengdoh *et al.*, 2017; Ahad and Syiem, 2018). Amongst non-essential metals, Cd, As, Hg, Be, Pb, are a few to name and they producing toxicity in organisms even when present in trace amount (Yu *et al.*, 2014). Lead (Pb) is one of the prevalent, toxic and non-biodegradable pollutants found in the earth surface (Wuana *et al.*, 2010).

It is released into the environment by mining activities, agricultural runoffs, domestic effluents and industrial wastages, from lead compounds, lead alloys, pipes, batteries, paints and pigments, dyes and ceramic glazes (Gupta and Rastogi, 2008; Naja and Volesky, 2010). Pb is classified as a priority pollutant by the US Environment Protection Agency (USEPA 2006). In human, Pb is known to cause blood and brain disorders (ATSDR 2007). It is also recognized as a potent carcinogen (Silbergeld *et al.*, 2000). USEPA and WHO drinking water standard for Pb have been set at 50 µg L^{-1} and 10 µg L^{-1}, respectively (James and Cook, 1983; Veglio and Beolchini, 1997; Dikshith, 2009). Pb concentration at an average in soil is 15mg Kg^{-1} and in water <0.45 – 14mg L^{-1} (Smedley *et al.*, 2002). The accumulation of Pb in the environment affects tremendously the aquatic and terrestrial organisms. Amongst the organisms, the primary producers such as algae and cyanobacterial have been reported to be mostly affected (Kelly, 1988; Vymazal, 1990). Cyanobacteria are prokaryotes and can synthesize chlorophyll *a* and hence able to perform photosynthesis (Heng *et al.*, 2004). They are the most primitive oxygenic photosynthetic organisms found in all environments of this planet (Whitton, 2012). Almost all the cyanobacteria possess atmospheric N_2 fixing ability thereby contributing ~ 50% of global nitrogen fixation (Elbert *et al.*, 2012). These groups of organism occupied a crucial position amongst the microorganisms as they are self-sufficient in respect of managing their nutritional needs on their own by fixing atmospheric carbon and nitrogen. They are primary producers of most ecosystems and significantly contribute oxygen to the atmosphere due to their photosynthetic activity (Hader *et al.*, 2007). Presence of cyanobacteria in soil enhances soil quality and fertility by way of adding C, N and O (Prasanna *et al.*, 2014).

Arunakumara *et al.*, (2007) reported that presence of 8 ppm Pb in the growth medium led to destruction and/or disorganization of thylakoid membranes in the cyanobacterium *Synechocystis* sp. PCC 6803 and as a consequence the photosynthetic activity of this organism was severely altered. Inhibition of photosynthetic electron transport would be expected to affect O_2-evolution first and then C-fixation (Stratton *et al.*, 1979). Pb at low concentrations could inhibit the photosynthetic electron transport chain primarily by inhibiting the PSII activity although at higher concentrations, Pb interferes with electron transport at multiple sites (Wong and Govindjee, 1976). Kojima *et al.*, (1987) theorised that Pb induced inhibition in PSI activity in *Anacystis nidulans* might be due to its high affinity for thiol groups of proteins, disrupting the biological activities of plastocyanin and iron sulfur centres. Various other reports on Pb-induced toxicity in photosynthetic efficiency in cyanobacteria were attributed to inhibition of electron

transport mainly at the PSII reaction centre slowing down electron flow from PSII to PSI; inactivation of large number of RuBisCO enzyme molecules (Chaloub *et al.*, 2005); altered biosynthetic turnover of chlorophyll *a* (Poskuta *et al.*, 1996), and to the compromised integrity of photosynthetic apparatus (Pinchasov *et al.*, 2006) and C-fixation.

In our present investigation, the cyanobacterium *Nostoc muscorum* Meg 1 was treated with Pb (10 - 80 ppm) till seven days. The effects of Pb on the carbon anabolism were studied in detail measuring the concentration of photo-absorbing pigments (chlorophyll *a*, carotenoids, phycocyanin, allophycocyanin and phycoerythrin), activity of oxygen evolving complex (OEC) and total carbohydrate production. Expression of D1 protein and RuBisCO enzyme content was also determined. Finally, influence of chronic exposure to Pb on overall biomass production was compared between the control and the treated cells.

Materials and methods

Growth conditions

Nostoc muscorum Meg 1 (Ahad *et al.*, 2017), a cyanobacterial species that has been isolated from Sohra, Meghalaya, India was grown in BG-11_0 culture medium [Macronutrients: K_2HPO_4 (40 g/L), $MgSO_4{\cdot}7H_2O$ (75 g/L), $CaCl_2{\cdot}2H_2O$ (36 g/L), citric acid (6 g/L), ferric ammonium citrate (6 g/L), Na_2CO_3 (20 g/L), EDTA (1 g/L); Micronutrients: H_3BO_3 (2.86 g/L), $MnCl_2{\cdot}2H_2O$ (1.81 g/L), $ZnSO_4{\cdot}7H_2O$ (0.22 g/L), $Na_2MoO_4{\cdot}2H_2O$ (0.39 g/L), $CuSO_4{\cdot}5H_2O$ (0.079 g/L), $Co(NO_3)_2{\cdot}6H_2O$ (0.0494 g/L)]. The cyanobacterium was grown at pH 7.5 in the culture room at a temperature of 25±2°C under continuous light (Photosynthetically active radiation) of photon fluence rate of 50 µmol m^{-2} s^{-1} (Rippka *et al.*, 1979).

Metal treatment

For all of the experiments conducted, lead nitrate (PbN_2O_6) was used as the source of Pb. A stock solution of Pb of 200 ppm strength was prepared in doubled distilled water and diluted to different concentrations of Pb (10-80 ppm) as per the requirement.

Chlorophyll a estimation

Increase in chlorophyll *a* is taken as a standard measure of the growth of cyanobacteria. It was estimated according to the method described by Mackinney (1941) in the test organism under Pb treatment. Three mL of the cyanobacterial cell culture was centrifuged at 2500 rpm for 3 min. The supernatant was discarded and the pellet was resuspended in 3 mL methanol. The solution was mixed properly, kept overnight in the refrigerator at 4° C to extract chlorophyll *a*. It was then centrifuged again at 2500 rpm for 3 min and the absorbance of the supernatant was read spectrophotometrically at 663 nm using spectrophotometer (Smart Spec Plus, BioRad, USA). Chlorophyll *a* concentration was calculated by using the formula:

Chlorophyll *a* (µg/mL) = $OD_{663} \times 12.63$

Estimation of phycobiliproteins

Phycobiliproteins, i.e, phycocyanin (PC), allophycocyanin (APC), phycoerythrin (PE) were estimated according to the method of Bennett and Bogorad (1973). Five mL of the cell culture was centrifuged at 2500 rpm for 3 min. The supernatant was discarded and the pellet was washed and resuspended in phosphate buffer saline (PBS), pH 7.5. The solution was sonicated using Sonicator (Sonics Vibra cell sonicator, USA) for 3 min and centrifuged at 13000 rpm for 45 min at 4° C. The absorbance of the supernatant was read at 615, 562 and 652 nm respectively. PC, APC and PE concentration were then calculated by using the following formulae:

$$PC = \frac{OD615 - 0.475(OD652)}{5.34}\ \mu g/mL$$

$$APC = \frac{OD652 - 0.208(OD615)}{5.09}\ \mu g/mL$$

$$PE = \frac{OD562 - 2.41(PC) - 0.849(APC)}{9.62}\ \mu g/mL$$

Estimation of carotenoids

Carotenoids content was estimated according to Morgan (1967). Three mL of cell culture was centrifuged at 2500 rpm and the supernatant was discarded. The pellet was resuspended in 3 mL of 95% N, N-dimethylformamide and the mixture was sonicated. It was then kept in dark for 5 min and later centrifuged at 3000 rpm for 5 min.

The absorbance was read at 461 and 664nm, respectively. Concentrations were calculated following the formulae:

Coloured carotenoids = $(OD_{461} - (0.046) \times OD_{664}) \times 4$ μg/mL

Total carotenoids = $OD_{664} \times 1192$ μg/mL

Oxygen evolving complex (OEC) activity

For OEC activity, a Clark-type oxygen electrode installed in a 3 mL Plexi glass container with a magnetic stirrer (Rank Brothers, England) was used (Robinson *et al.*, 1982). Three mL of culture was taken into the plexi glass container and stirred keeping under a light source 10 cm away. The culture was allowed to equilibrate for 3 min with continuous stirring and the rate of oxygen evolution was obtained. Following, oxygen consumption was measured in dark for 3 min by shielding the plexi glass container using aluminium foil. The rate of oxygen evolution and consumption were expressed as nmol O_2 evolved or consumed/ μg chlorophyll *a*/ h.

Estimation of protein

The protein concentration was estimated by the method of Bradford (1979). Three mL of cyanobacterial cell culture was washed with 50 mm Tris buffer and resuspended in the same buffer. The mixture was sonicated and 100 μL of cell extract was taken for protein estimation. Three mL of Bradford reagent was added to the cell extract and incubated for 5 min in dark. The absorbance was then read at 595 nm. A calibration curve was made using Bovine serum albumin (10 – 200 μg/mL) as a standard.

Estimation of carbohydrate

Carbohydrate content was estimated according to the method described by Roe (1995). Three mL of cell culture was washed with distilled water and one mL of the cell extract was taken. Four mL of anthrone reagent (0.2% in H_2SO_4) was added to the cell extract. The solution was mixed properly and incubated in boiling water bath for 10 min. It was then centrifuge at 3000 rpm for 5 min and the absorbance of the supernatant was read at 630 nm using UV-Vis spectrophotometer. The carbohydrate content was then calculated from the calibration curve where glucose was used as a standard solution with a concentration ranging from 10–100 μg/mL.

Biomass estimation

Biomass concentration was calculated from three mL of culture after drying the cell at 40° C in an oven. It was expressed as mg/mL.

Western Blot

Concentrations of D1 and RuBisCO proteins were measured by Western blot analysis. The proteins that have been extracted from the control and the treated cells were separated using SDS-PAGE (BioRad). It was then transferred onto a PVDF membrane using Trans-Blot Turbo™ transfer system (BioRad) for 7 min. 5% skim milk in TTBS (pH 7.5) was used for blocking the membrane and the membrane was then washed three times with TBS buffer followed by TTBS for 5 min each. The membrane was incubated at 4° C overnight with respective primary antibody and the excess antibodies were removed by washing with TBS and TTBS buffer. The membrane was incubated for 2 h with anti-rabbit HRP conjugated secondary antibody and excess antibodies were again washed off. The membrane was then incubated in dark for 3 min with enhanced chemiluminescence (ECL) substrate and viewed under ChemiDoc™ MP imaging system (BioRad).

Statistical analysis

All the values are calculated as mean ± SD (N = 3). To compare the statistical difference (*p*) between the control and the treated cells ANOVA was performed using statistical software GraphPad Prism version 8.

Results

Effect of Pb on photosynthetic pigments

The effect of different concentrations of Pb (10–80 ppm) on the chlorophyll *a*, phycobiliproteins and carotenoid pigments are shown in the Fig. 1. The respective values of control cultures were taken as 100 % and the values obtained for treated cultures were compared against the control value(s). There was an increase in chlorophyll *a* concentration in the presence of 10, 20 and 40 ppm of Pb by 15, 20 and

30% after one day. This increase continued after seven days, the increase was 32, 39 and 45%, respectively. However, a decrease of chlorophyll *a* content by 4% and 7% in the first day and 16% and 43% at the end of seven day were seen in the presence of 60 and 80 ppm Pb, respectively (Fig. 1a).

The photosynthetic accessory pigment phycocyanin showed a similar trend of increasing at lower doses of Pb (10-40 ppm) by 4, 10 and 20% after one day and 24, 43 and 61% after seven days. At higher doses, a decrease in the concentration of phycocyanin was seen both on first day and on seven day by 1, 4% for 60 and 80 ppm and 18% and 35% in presence of 60 and 80 ppm (Fig. 1b). However, allophycocyanin (APC) and phycoerythrin (PE) were more sensitive among the photo-pigments and in all concentrations of Pb, these two pigments were inhibited between 13–72% (Fig. 1c; d) at the end of first and seventh day compared to control cells. From the Fig. 1e; f, both colored and total carotenoids showed a similar pattern like chlorophyll *a*; colored and total carotenoids content increased in 10-40 ppm of Pb and decreased at 60 and 80 ppm.

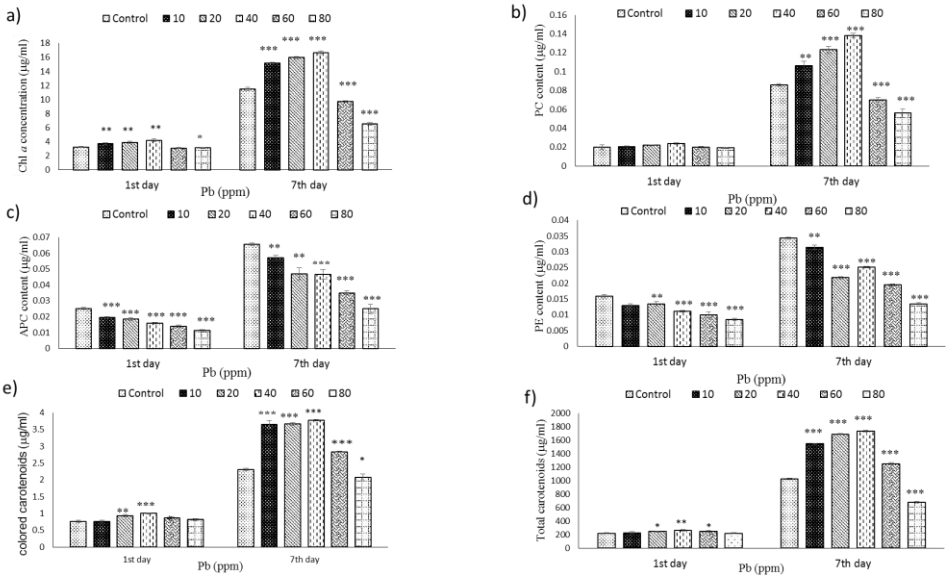

Fig. 1. Effects of Pb on the photosynthetic pigments after first and seven days of exposure. (a) Chlorophyll *a*; (b) phycocyanin; (c) allophycocyanin; (d) phycoerythrin; (e) colored carotenoids and (f) total carotenoids. All the values are expressed as Mean ± SD (N = 3) and asterisks (*p < 0.05, ** p <0.01 and *** p < 0.001) above the histogram bars specify significance in differences between control and Pb treated cells.

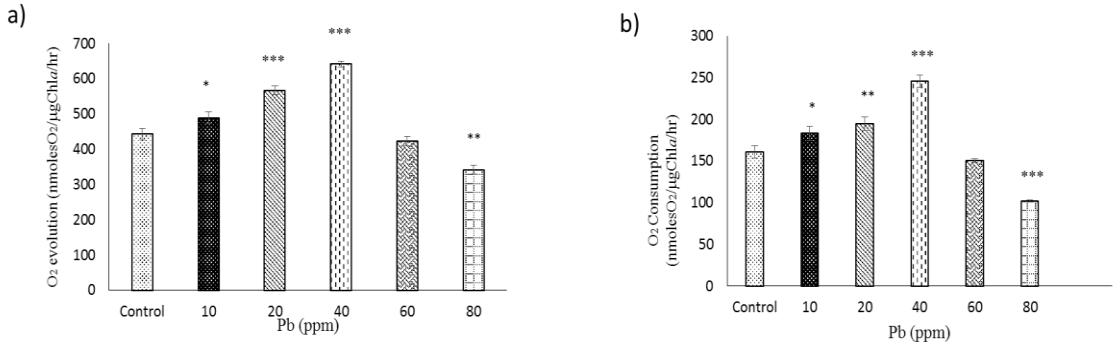

Fig. 2. Effects of Pb on PSII activity and rate of respiration on exposure to Pb (10 – 80 ppm) for seven days. (a) PSII activity and (b) rate of respiration. All the values are expressed as Mean ± SD (N = 3) and asterisks (* p < 0.05, ** p < 0.01 and *** p < 0.001) above the histogram bars specify significance in differences between control and Pb treated cells.

Effect of Pb on oxygen evolving complex (OEC) activity

The effect of 10-80 ppm of Pb in the PSII activity and the rate of respiration are shown in Fig. 2a; b. There was an increased in PSII activity and rate of respiration on exposure to 10, 20 and 40 ppm by 11 – 53% but at 60 and 80 ppm, a reduction of 4–37% at the end of seven days was recorded (Fig. 2a; b).

Impact of Pb on carbohydrate concentration

End product of carbon fixation is carbohydrate. At the end of seven day exposure of the cyanobacterium to Pb, the total carbohydrate content was increased in presence of 10, 20 and 40 ppm by 12, 18 and 30%, respectively. However, at 60 and 80 ppm Pb, the carbohydrate content was reduced by 15% and 28% (Fig. 3).

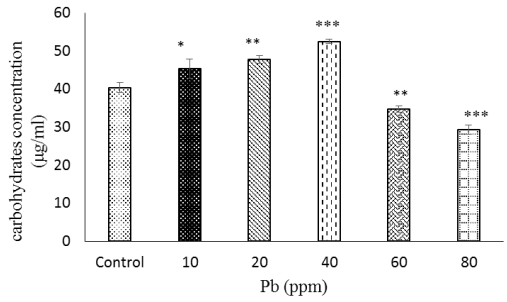

Fig. 3. Carbohydrate concentration in the cyanobacterium *Nostoc muscorum* Meg 1 in presence of Pb (10-80 ppm) at the end of seven days. All the values are in Mean ± SD (N = 3) and asterisks (* p < 0.05, ** p < 0.01 and *** p < 0.001) above the histogram bars specify significance in differences between control and Pb treated cells.

Influence of Pb on protein concentration and dry biomass

At the end of seven days, both protein and dry biomass contents were found to be augmented in 10-40 ppm of Pb and decreased at higher Pb exposure (Fig. 4a; b). In the presence of 10, 20, 40 and 60 ppm of Pb, protein concentration was increased by 40, 60 and 111%. A point to note that an increase was also seen at 60 ppm of Pb. Both protein and biomass production was also seem to increase in 10, 20 and 40 ppm of Pb by 16, 35 and 54%. The biomass content was also at the level of control in 60 ppm Pb exposure. Both protein and biomass production at 80 ppm of Pb comparatively reduced by 14% and 21%, respectively.

Western blot analysis of D1 protein and RuBisCO enzyme

Western blot analysis of D1 protein showed an increase of 1.4 to 4.5 fold in culture exposed to 10–40 ppm Pb whereas in presence of 60 ppm and 80 ppm, it registered a decrease compared to 40 ppm but still was higher by 51% and 6%, respectively compared to the control (Fig. 5a).

A similar trend was noticed for RuBisCO (Ribulose-1, 5 Bisphosphate carboxylase/ oxygenase) which is the crucial enzyme of Calvin cycle for carbon fixation. Its content in presence of 10, 20 and 40 ppm of Pb was increased by 31, 104 and 126% whereas on exposure to 60 and 80 ppm of Pb, the expression of RuBisCO enzyme was decreased by 30 and 43%, respectively (Fig. 5b).

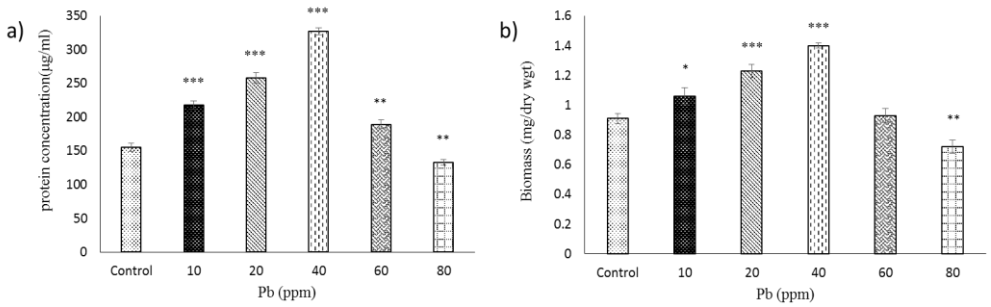

Fig. 4. Biomass production measurement in the *Nostoc muscorum* Meg 1 in terms of (a) protein concentration and (b) dry biomass at the end of seven days in control and treated cultures. All the values are expressed as Mean ± SD (N = 3) and asterisks (* p < 0.05, ** p < 0.01 and *** p < 0.001) above the histogram bars specify significance in differences between control and Pb treated cells.

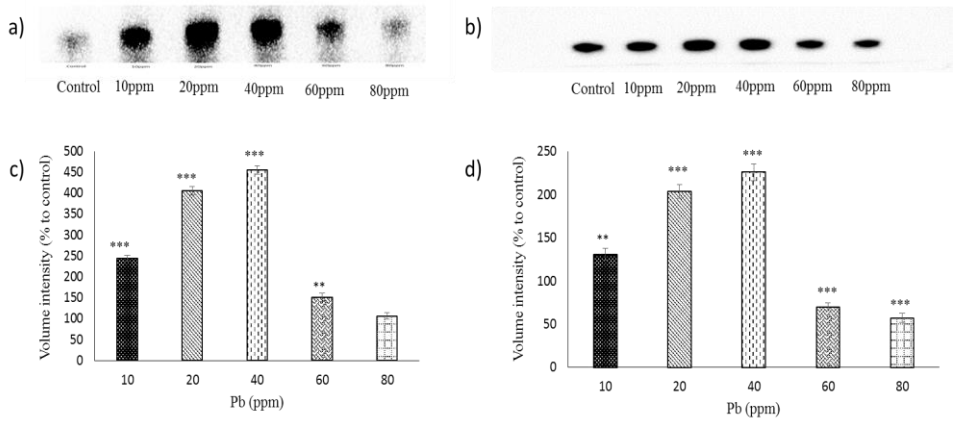

Fig. 5. Images of Western blot of (a) D1 protein and (b) RuBisCo enzyme. Western blot analysis of (c) D1 protein and (d) RuBisCO enzyme of the cyanobacterium *Nostoc muscorum* Meg 1 at different Pb (10 – 80 ppm) concentrations where control is taking as 100%. All the values are expressed as Mean ± SD (N = 3) and asterisks (* p <0.05, ** p< 0.01 and *** p< 0.001) above the histogram bars specify significance in the differences between control and Pb treated cells.

Discussion

Pb is present in almost all environments and is released mostly from industrial activities contaminating air, soil and aquatic environments (Rossi, 2008; Mesquita *et al*., 2016). Presence of Pb in the environment, particularly in aquatic ecosystems affects various forms of microorganisms including algae, cyanobacteria as well as plants (Kelly, 1988, Vymazal, 1990; Carpentier *et al*., 2001). There are reports that in cyanobacteria, Pb can induce toxicity by binding to the thylakoid membranes thereby damaging these membranes and impede photosynthetic activities (Ayya Raju, 2016). Another, possible reason of Pb mediated toxicity could be the interaction of Pb ions with polyphosphates in the cells that result in loss of phosphate nutrient through the precipitation of insoluble Pb phosphate (Vymazal, 1995). It had been reported that Pb exposure in the *Saccharomyces cerevisiae* inhibits assimilation of nutrients (Chen and Wang, 2007) and metabolic activities (Van der Heggen *et al*., 2010); decreases the intracellular level of reduced glutathione (Perez *et al*., 2013); induces oxidative stress (Bussche and Soares, 2011; Sousa and Soares, 2014); DNA damage (Cui and Tang, 2000) and loss of proliferation capacity (Soares *et al*., 2003) followed by cell death (Bussche and Soares, 2011). It has been documented that accumulation of metal ions affects many physiological and biochemical functions in plants, algae and cyanobacteria and in these photoautotrophic organisms, CO_2-fixing machinery are mostly disrupted (Singh *et al*., 1997; Danilov and Ekelund 2001; Heng *et al*., 2004). Moreover, Pb mimics metal cofactors for many enzymes and therefore it can replace these cofactors disrupting various biological activities (Arif *et al*., 2015).

Adir (2005) showed that cyanobacteria revealed that Pb essentially compromises crucial functions of many enzymes, increases ROS contents in the cells, reduces antioxidant proteins and enzyme contents and reduce ATP pool. In response, cyanobacteria attempt to counter these threats employing various corrective measures. Some of the mechanisms are: binding metal ions to the cell surface or to the extra polysaccharides (EPS) preventing intracellular accumulation of the metal, binding internalized Pb in metallothioneins and phytochelatins and by sequestrating them in polyphosphate bodies (Ayansina and Olubukola, 2017). Cyanobacteria also adopt energy dependent influx-efflux mechanism which regulates and maintains internal ionic balance inside the cells (Agrawal *et al*., 2015; Ahad *et al*. 2019). In our study, Pb at higher concentrations (above 40 ppm) was seen to be exerting toxic effects on the organism. The synthesis of primary photosynthetic pigment chlorophyll *a* was reduced by 4 and 7% within 24 h of exposure to 60 and 80 ppm

Pb, respectively. A week long exposure to the same concentrations inhibited chlorophyll *a* concentration by 16 and 43%. This is highly significant ($p<0.001$) emphasizing the toxic nature of Pb. Additionally, exposure to metals in photosynthetic organisms is known to substitute central Mg atom of the chlorophyll pigment leading to compromised harvesting of light and consequently damaging the photosynthetic efficiency (Krupa and Baszynski, 1995). The similar results of compromised photosynthetic ability obtained in the present study in cells exposed to higher Pb (60 and 80 ppm) concentrations may further reiterate the above finding. Comparable effects were also noticed on phycocyanin as well as on colored and total carotenoids. Both increased in the lower concentrations (10-40 ppm) and decreased in higher concentrations of Pb (60-80 ppm) were found to be significant. Bryant (1994) reported that carotenoids play a significant role in preventing DNA and lipid membranes from oxidative damages. In the present study, carotenoids production was found to be enhanced by 58-64% in lower concentrations of Pb (10-40 ppm) indicating that the organism had initiated the biosynthesis of carotenoids as a defense mechanism against the oxidative stress generated due to presence of Pb ensuring survival of the organism (p value between 0.05 and 0.001). However, inhibition in allophycocyanin and phycoerythrin in presence of all Pb tested concentrations signified susceptibility of these two pigments to presence of any Pb in its surrounding (p value between 0.01 and 0.001) (Fig 1c; d). Thus the negatively altered phycobiliproteins concentrations at higher Pb exposure further affected the light absorption capacity in the organism. A similar observation was also reported by Santos *et al.*, (2012).

Heng *et al.*, (2004) reported that Pb is capable of binding to thylakoid membranes disturbing their vital functions. One of the major functions which occur in the thylakoid membrane is the OEC activity. OEC activity was higher (11 - 45%) in presence of 10-40 ppm of Pb while the same activity was decreased by 4 - 30% in the cultures treated with 60 and 80 ppm of Pb compared to control cultures (p value between 0.05 and 0.001). The harmful effects of Pb on the photosynthetic performance of cyanobacteria have been attributed to inhibition of the photosynthetic electron transport mainly at the PSII reaction center of OEC (Chaloub *et al.*, 2005). Similar observation was that exposure to excessive amount of essential and non-essential metals impairs synthesis of photosynthetic pigments, transport of electrons in reaction centers and synthesis of ATP was made by Osmond, (1981). In cyanobacteria respiratory electron transport chain is also hosted on the thylakoid membrane. The compromised status of thylakoid membrane under Pb exposure was reflected in the reduced respiratory activity (6 - 37%) recorded at higher Pb exposure. However, the activities of both photosynthetic and respiratory chains were enhanced between 11-53% under the influence of Pb at a concentration of 10-40 ppm (p value between 0.05 and 0.001).

Western blot study of D1 protein after one day of Pb (10-80 ppm) treatment revealed that the production of D1 protein of PSII reaction centre had increased tremendously by 6-355% within 7 days. This corroborates with the findings of other researchers who pointed out that D1 protein is the catalytic site where the water oxidation takes place and thus provides the majority of the amino acid ligands for the Mn_4CaO_5 cluster (Umena *et al.*, 2011). The redox active residues of the D1 protein are susceptible to the oxidative damage and in order to counter the damage, cells trigger the repair process that favourably remove damage D1 protein and replace with new D1 protein to sustain active PSII complexes in the cell (Nagarajan and Burnap, 2014). Even in this study we found that this response was immediate and the cyanobacterium *Nostoc muscorum* Meg 1 countered Pb exposure with enhanced production rate of D1 protein (p value between 0.01 and 0.001) (Fig. 4a).

Although D1 protein registered a steep increase under all Pb concentrations tested, Western blot analysis of RuBisCO – the primary enzyme of Calvin cycle showed substantial increase in its protein content by 31–126% only under exposure to lower concentrations (10-40 ppm) of Pb.

However, there was 30–43% reduction in its content in the cells exposed to 60 – 80 ppm Pb (*p* value between 0.01 and 0.001). This observation was similar to the other parameters studied (other than D1 protein). Singh *et al.*, (2016) had observed that heavy metal exposure decreases CO_2 assimilation in two different ways: one is by diminishing RuBisCO activity and the other is by reacting with the crucial thiol group of the enzyme. Our study quantifying the amount of RuBisCO by western blot adds another reason for decreased CO_2 assimilation, i.e. the metal induced reduction in the actual amount of the enzyme.

The end result of CO_2-fixation is carbohydrate production and the synthesis of carbohydrate is a cumulative effort of various factors including pigment concentrations that captures light energy; activity of electron transport chain that generates ATP and NADPH and CO_2 assimilation via Calvin cycle. Our results showed that at lower concentrations (10-40 ppm) of Pb exposure, there was increase in carbohydrate content by 12– 30%, however carbohydrate content was significantly compromised by 15–28% at higher concentrations of Pb (60-80 ppm) (*p* value between 0.05 and 0.001). Additionally, the adverse effect of Pb exposure on soluble protein content and total biomass content was visible only at an exposure of 80 ppm (decrease in soluble protein was 14% while it was 21% in case of total biomass) but both these parameters showed augmentation under the exposure of 10-60 ppm of Pb (*p* value between 0.05 and 0.001). An interesting observation in this study was the elevated levels of all parameters under exposure to lower doses of Pb (10–40 ppm).

This could be attributed to the well-known phenomenon known as hermetic effect where organisms respond to a lower level of threat in its vicinity by increasing all its metabolic functions resulting in enhanced biomass production in order to neutralise the perceived threat. Similar observations had been reported under presence of low doses of Cd and Zn in cyanobacteria (Goswami *et al.*, 2015; Diengdoh *et al.*, 2017).

The cyanobacterium *Nostoc muscorum* Meg 1 seems to possess finest defense mechanism against metal threats. Augmentation of all parameters of carbon fixation under exposure to lower concentrations of Pb actually points to the fact that low level of Pb exposure could promote proliferation of microbes especially cyanobacteria in contaminated water bodies that could lead to eutrophication thereby potentially render these water bodies useless for human and animals consumption.

Thus although low levels of heavy metals such as Pb in the water bodies that escapes our attention as harmless could actually affect these water system from a different angle.

Conclusion

The lower doses of Pb (10-40 ppm) influenced positively on the physiology and biochemistry of the cyanobacterium *Nostoc muscorum* Meg 1, although at higher doses (60 and 80 ppm), the effects were detrimental. The cyanobacterium attained higher growth and biomass production compared to control cells under low doses of Pb treatment indicating that although at low dose concentrations, Pb was not considered to be toxic, however, it could lead to eutrophication of lakes, rivers and water reservoirs rendering them unusable for human and animal consumption.

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Declaration of interest

Authors have no competing interest for authorship and funding.

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