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SCREENING OF CYANOBACTERIAL STRAINS FOR SELECTION OF MODERATELY SENSITIVE STRAIN: A TOOL FOR TOXICITY ASSAY

FAROOQUI A[1*], SHAMIM A[2] AND MAHFOOZ S[2] AND SIDDIQUI MH[1]

1: Department of Bioengineering, Integral University, Lucknow

2: Department of Bioscience, Integral University, Lucknow

***Corresponding Author: E Mail: alvinafarooqui@yahoo.co.in; farooqui.alvina@gmail.com**

ABSTRACT

The aim of the present study was to screen five cyanobacterial strains namely *Nostoc muscorum, Phormidium, Plectonema. boryanum, Chroococcus* sp. and *Scytonema*sp. against five metals mercury (Hg), nickel (Ni), cadmium (Cd), copper (Cu) and aluminum(Al). The order of toxicity based on survival results for all the strains was found to be Hg> Ni> Cd> Cu> Al. The survival of different cyanobacterial strains towards metal stress led to the finding that response to stress is subjected to interspecific variations and therefore different organisms respond differently and specifically to the environmental stress conditions. *N. muscorum* was found to be the most sensitive of all the strains so it was used for further growth pattern standardization. Amongst the selected metals Hg was found to be the most toxic at all the selected concentrations. Cu gave contrasting results at different treatment doses. Cd and Ni showed a certain degree of adaptation at low doses whereas Al adapted well at all the chosen concentrations. The present results suggest the unequivocally differential response exhibited by the five environmentally prevalent metals in various cyanobacterial strains establishing Cyanobacteria as an efficient toxicity tool.

Keywords: Cyanobacteria, Screening, Toxicity, adaptation, Metal

INTRODUCTION

Due to unrestricted anthropogenic activitiessuch as industrialization and urbanization, there has been a tremendous rise in the levels of pollution **[1, 2]**. Environmental pollutants originating from these anthropogenic sources have been known

to degrade the ecological integrity of the aquatic environment. Amongst the aquatic pollutants, a great deal of research has been devoted to metals **[3, 4]**. Metals are integrated components of the ecosystem. Though some metals are essential as micronutrients, uptake of higher concentrations of metal is found to be toxic for plants and many aquatic organisms including algae and cyanobacteria.

Cyanobacteria are a group of ubiquitous, photosynthetic prokaryotes which perform two key biological processes such as oxygenic photosynthesis and nitrogen fixation in same filaments. They are ecologically important group of eubacteria that evolved to compete in a wide range of habitats and are well adapted to environmental stress including exposure to UV, high solar radiation and temperatures, scarce and abundant nutrients. Adaptation in such harsh environment has favored the dominance of cyanobacteria in many aquatic habitats, from freshwater to marine ecosystems. Since aquatic organisms possess tremendous ability to concentrate metals in their tissues or cells, these toxicants may accumulate to levels which may pose a threat to their proper growth and metabolism **[5]** by inactivating the photosynthetic machinery, enzymatic pathways or by altering the nutrient transport and availability **[6]**.

Mercury (Hg) and Cadmium (Cd), have unknown function as nutrients and seem to be toxic to plants, algae and micro-organisms **[7]**. Mercury (Hg), one of the non-essential heavy metals for plants, is frequently reported to be released into the biosphere including air, waters and soils **[8]**. Due to its transition properties, mercury uptake by plants is quite fast, thus it accumulates at high level, and consequently results in toxicity or even death of plants **[9, 10]**.Cadmium (Cd) has been recognized as one of the most toxic contaminants and its concentration ranges from 0.1 mg L^{-1} in open ocean water to several mgL^{-1} in coastal areas with industrial establishments **[11]**. Cd is principally used in Ni–Cd batteries, pigment formulations, coverings and stabilizers for synthetics and plastics **[11]**. Although Cu in the form of Cu^{2+} is an essential microelement but when present in high concentration it accumulates in microalgae and interferes with numerous physiological processes and has also been shown to disturb chlorophyll synthesis **[12, 13]** and many metabolic processes like nucleic acid and protein synthesis. Al is not regarded as an essential nutrient, but low concentrations can sometimes increase plant growth or induce other desirable effects **[14]**. There are also number of reports dealing with Al toxicity to higher plants, **[15]**, algae **[16]**

and cyanobacteria **[17]**. Accumulating evidence shows that Al toxicity affects light absorption **[18]**, photosynthetic electron transport **[19]**, gas exchange **[20, 21]**, photoprotective systems **[22, 23]**, pigments **[20]**, ultrastructure **[22, 24]**, carbohydrates **[25]** and photosynthetic enzymes **[18]** in plant leaves.

Understanding the fundamental physicochemical mechanisms of metal bio-uptake by cyanobacteria in natural systems is a step towards identifying under what conditions cyanobacterial growth is favoured and to ascertain the mechanisms by which the excess and type of metal is detoxified making cyanobacteria an excellent tool for toxicity assay. Thus our study deals with the screening of various cyanobacterial strains under metal stress for selection of moderately sensitive strain to be used as an excellent tool for metal toxicity assay.

MATERIAL AND METHODS

Experimental Organisms

Cyanobacteria *Nostoc muscorum(N.muscorum), Plectonema boryanum (P. boryyanum)*, and *Scytonema*sp. were obtained from Department of Botany, University of Allahabad and Department of Biological Sciences, Allahabad Agricultural Institute, Deemed University, Allahabad, respectively. *Chroococcus* sp. and *Phormidium* sp. were isolated from rice paddy fields of different cities of UP. India. The strains were purified and Identification was confirmed **[26]** for microscopic parameters and maintained in the cyanobacterial culture room, Department of Biotengineering, Integral University, Lucknow.

Growth Conditions

The desired strains of cyanobacteria were maintained in the culture room at 27±20C under 75 µmol $m^{-2}s^{-1}$ photon flux density with a photoperiod of 14:10 h. For the routine lab work BG-11 and Chu-10 medium were used with or without extra supplementation of combined nitrogen depending upon the heterocystous and non-heterocystous cyanobacteria **[27]**.

Isolation and Purification

The cyanobacterial strains were collected by gentle scraping using sterile blades and needles. The specimens were stored in screw cap bottles. A pinch of the sample was homogenized and added to 150 ml Erlenmayer flasks containing 50 ml of sterile medium with or without combined nitrogen and incubated at room temperature under fluorescent light. Clonal and axenic population were obtained by serial dilution.

Incubation and Maintenance of Cultures

The Stock Culture

For maintenance of laboratory culture, 2-3 mL of a 3 weeks old cyanobacterial stock culture was used as inoculum in 50 mL of autoclaved BG 11 medium in 150 mL Erlenmeyer flasks. These samples were maintained at 27 ± 2°C under 75 µmol m-2s-1 photon flux density (PFD) by cool fluorescence lamps with a photoperiod of 14:10 h. The stock cultures were maintained for 20-30 days.

Agar Slants

Cyanobacterial strains were inoculated into sterilized tubes containing agar prepared in the above medium. After 15 days of incubation in light and in darkness, cultures were examined microscopically and absence of bacteria was confirmed. Such bacteria free clones were selected and maintained on different agar slants.

The Batch Culture

Aliquots of 50 mL from the stationary phase stock cultures were used to inoculate 500 mL of autoclaved BG11 medium in 1.0 liter round bottom flasks. These samples were cultivated at 27 ± 2°C under 75 µmol m-2s-1 photon flux density (PFD) by cool fluorescence lamps with a photoperiod of 14:10 h.

Metal Treatment

Stock solution of different metal was prepared in sterilized BG-11 and CHU-10 medium and was further sterilized by passing through Millipore membrane filter (0.22 µm). From the stock solution various required concentrations 2, 4, 8 and 16 µM of metal were prepared in the BG-11 and CHU-10 medium. Different salts of various metals used were cupric chloride ($CuCl_3$), Cadmium chloride ($CdCl_2.H_2O$), Nickel chloride ($NiCl_2.6H_2O$), Mercuric chloride ($HgCl_2$), Aluminium chloride ($AlCl_3.6H_2O$).

Percent Survival Under the Metal Stress

Percent survival of different cyanobacterial strains was determined by spreading the aliquots onto agar plates containing different concentrations (2, 4, 8 and 16 µM) of Al, Cu, Cd, Ni and Hg separately. Plates were kept in dark for 24 h then incubated in a temperature controlled culture room at 27 ± 20C illuminated with white fluorescent light. After 15 days of incubation, the Petri plates were taken out and the colonies were counted with the help of binocular microscope. The percent survival was calculated with reference to the untreated samples referred to as control.

Growth

Growth experiments were performed in liquid medium and protein content was determined after regular intervals **[28]**.

RESULTS

Survival

The survival of test cyanobacteria such as *N. muscorum, Phormidium*sp., *P. boryanum, Chroococcus* sp. and *Scytonema* sp. against metals (Al, Cu, Cd, Ni and Hg) at various concentrations (2, 4, 8, and 16 µM) was studied following the plate colony count method. The results related to the percent survival of these cyanobacteria under various metals clearly demonstrated that the survival responses were concentration of metal dependent. Figure [1] shows the survival of all the test cyanobacteria against various concentrations of Al. It was observed that *N. muscorum* was the most sensitive strain as compared to *Phormidium* sp., *P. boryanum, Chroococcus* sp. And *Scytonema* sp. whereas *Scytonema* sp. appeared to be the most resistant. Al at low concentration reduced the colony of *N. muscorum*, *Phormidium* sp., *P. boryanum* and *Chroococcus* sp. by 4, 2, 2 and 2% whereas no loss was observed in *Scytonema* sp. Further decrease in the survival was noticed on increasing the concentration indicating the dose dependant effect on the test cyanobacteria. Al at 16 µM concentration exhibited 75%, 80%, 82%, 86% and 90% survival of *N. muscorum*, *Phormidium* sp., *P. boryanum*, *Chroococcus* sp. and *Scytonema* sp., respectively.

As compared to Al, Cu was found to be more toxic to all the test cyanobacteria. The low concentration (2 µM) of Cu reduced the survival by 8, 6, 4, 3 and 1% in *N. muscorum*, *Phormidium* sp., *P. boryanum, Chroococcus* sp. and *Scytonema* sp., respectively. However, the effect became more prominent on increasing the concentration and at 16 µM of Cu 70, 62, 58, 53 and 46 % decrease was observed in *N. muscorum*, *Phormidium* sp., *P. boryanum, Chroococcus* sp. and *Scytonema* sp., respectively **(Figure 2)**

The effect of Cd on the survival of *N. muscorum*, *Phormidium*sp., *P. boryanum, Chroococcus* sp. and *Scytonema* sp. was similar to the effect of Cu. Interestingly Cd at low concentration was more toxic as compared to Cu. The effect on the survival was dose of metal dependant **(Figure 3)**.

Among all the five metals Ni and Hg had pronounced effect on the survival of the test cyanobacteria **(Figure 4 and 5)**. Ni at 2 µM concentration reduced the survival of *N. muscorum*, *Phormidium* sp., *P. boryanum*, *Chroococcus* sp. and *Scytonema*sp. by 25, 20, 18, 14, 12% whereas, Hg at the same concentration caused 60, 45, 42, 38 and 35% reduction in the same organisms. Further both the metal showed dose dependent decrease in the survival of the test cyanobacteria.

Growth

Growth pattern of *N.muscorum* was monitored at regular intervals for 10 days in

liquid medium by estimating the protein content after the supplementation of 2, 4, 8 and 16 µM concentration of metals such as Al, Cu, Cd, Ni and Hg. *N. muscorum* was found to be least sensitive to Al and most to Hg at all the concentrations.

N. muscorum showed inhibitory growth response against all the test metals **(Figure 6 to 10)**. Figure 6 shows the effect of various doses (2, 4, 8 and 16 µM) of Al on the growth pattern of *N. muscorum*. Al at 2 and 4 µM did not cause marked effect even after 10 days of exposure as shown in the growth curve. Further, the effect became pronounced after 8 µM exposure. Decline phase did not occur even at 16 µM after 10 days of the treatment.

Low dose of Cu (2 and 4 µM) and untreated control exhibited a lag phase of 2 days. The lag phase continued for six days when culture was treated with 8 µMof Cu while the high dose (16 µM) of Cu did not show any growth even up to 10 days. The decline in growth from 9% to 5% was noticed after six days of incubation on raising the concentration of Cu from 2 to 16 µM. The inhibitory action of Cu was more pronounced after 6 days of treatment, depicting a concentration and time dependent inhibition of growth **(Figure7)**. The growth inhibitory trend of Cu and Cd was very much similar but interestingly Cd was more inhibitory at low concentrations while Cu at high concentrations **(Figure 8)**.

When the cells were treated with a dose of 2 µM of Ni lag phase occurred for 2 days then log phase continued upto 10 days **(Figure 9)**. At 4 µM decline in growth commenced only after 2 days. At 8 and 16 µM there was a sharp decline phase after 2 days. Till 6 days no significant decline was noticed. The decline phase became prominent after 6^{th} day and continued upto 10 days.

There was no lag phase in the growth pattern of cells treated with all the selected concentrations of mercury **(Figure 10)**. Even at the lowest dose of 2 µM there was a sharp decline phase just after 2 days. At 4 and 8 µM complete death occurred only after 4 days. At the highest dose ie. 16 µM complete death occurred just after 2 days of exposure**.**

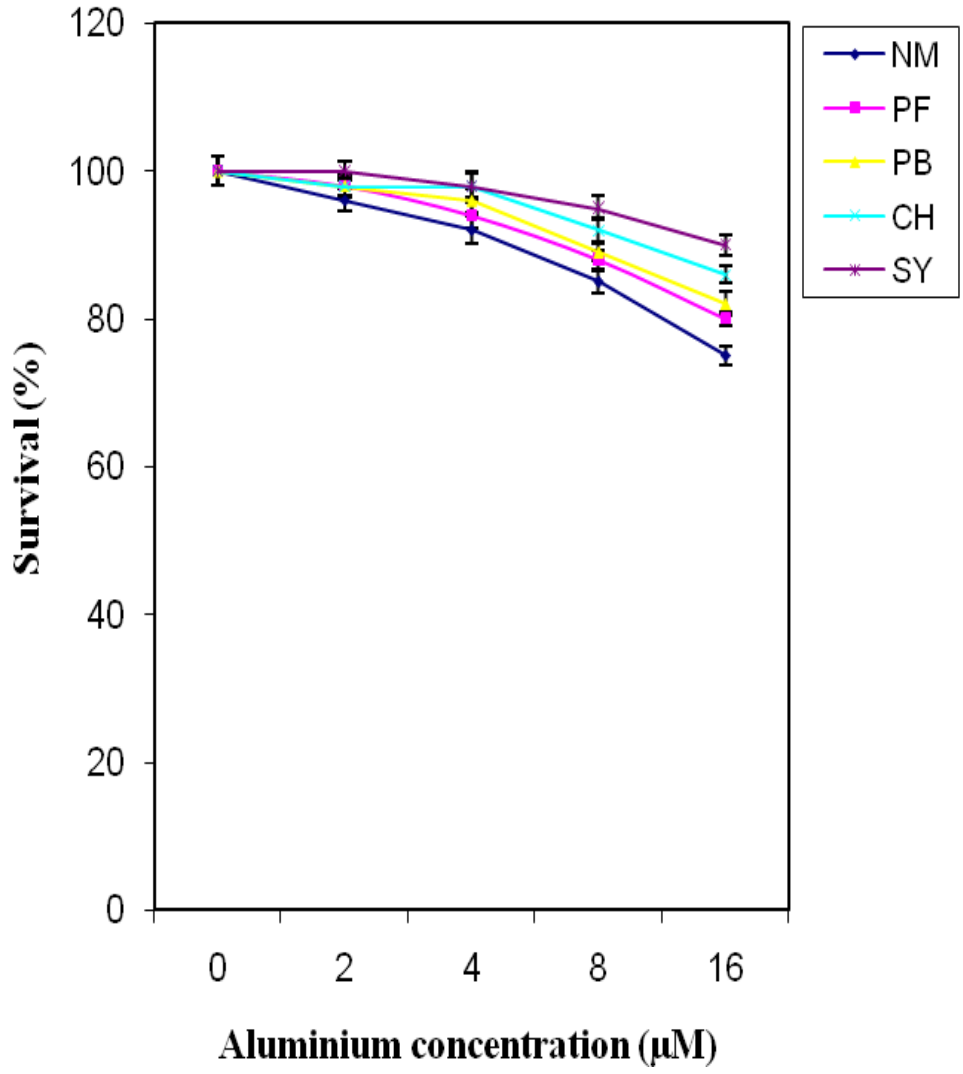

Figure 1: Effect of different concentrations of Al on survival of *N. muscorum, Phormidium* sp., *P. boryanum, Chroococcus* sp. and *Scytonema* sp. Values are means ± SE with n=3

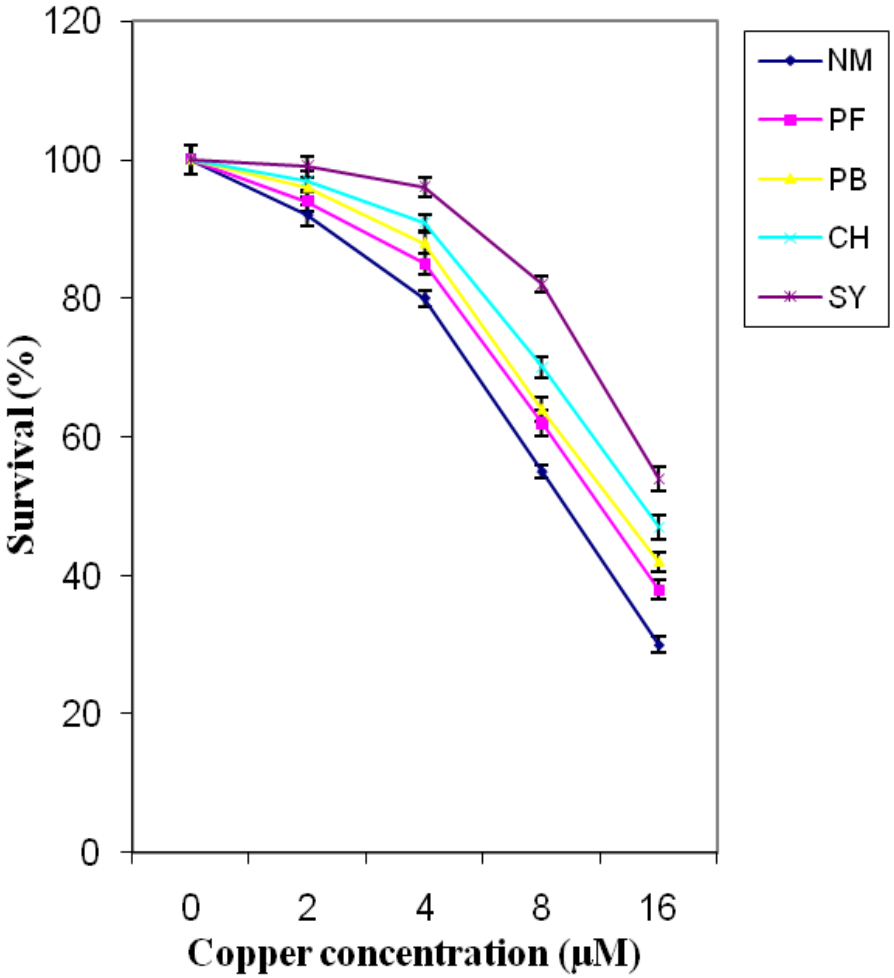

Figure 2: Effect of different concentrations of Cu on survival of *N. muscorum*, *Phormidium* sp., *P. boryanum*, *Chroococcus*sp. and*Scytonema* sp. Values are means ± SE with n=3

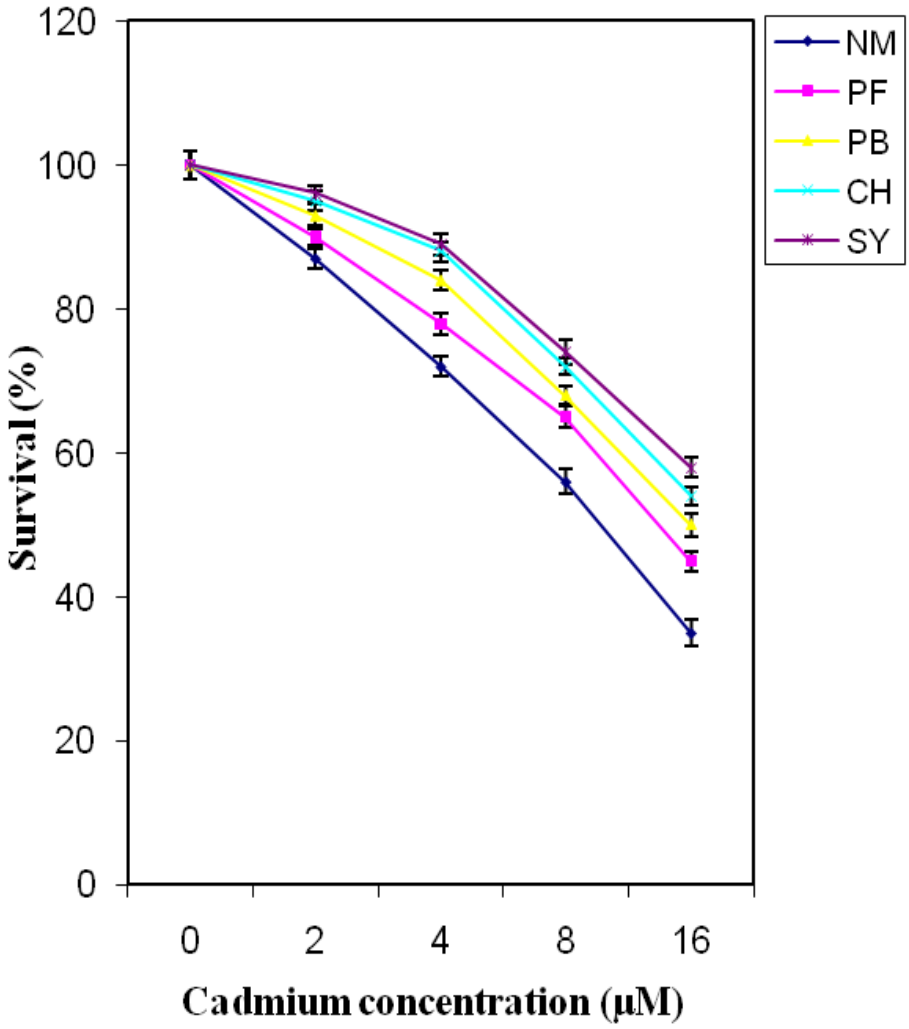

Figure 3: Effect of different concentrations of Cd on survival of *N. muscorum*, *Phormidium* sp. *P. boryanum*, *Chroococcus* sp. and *Scytonema* sp. Values are means ± SE with n=3.

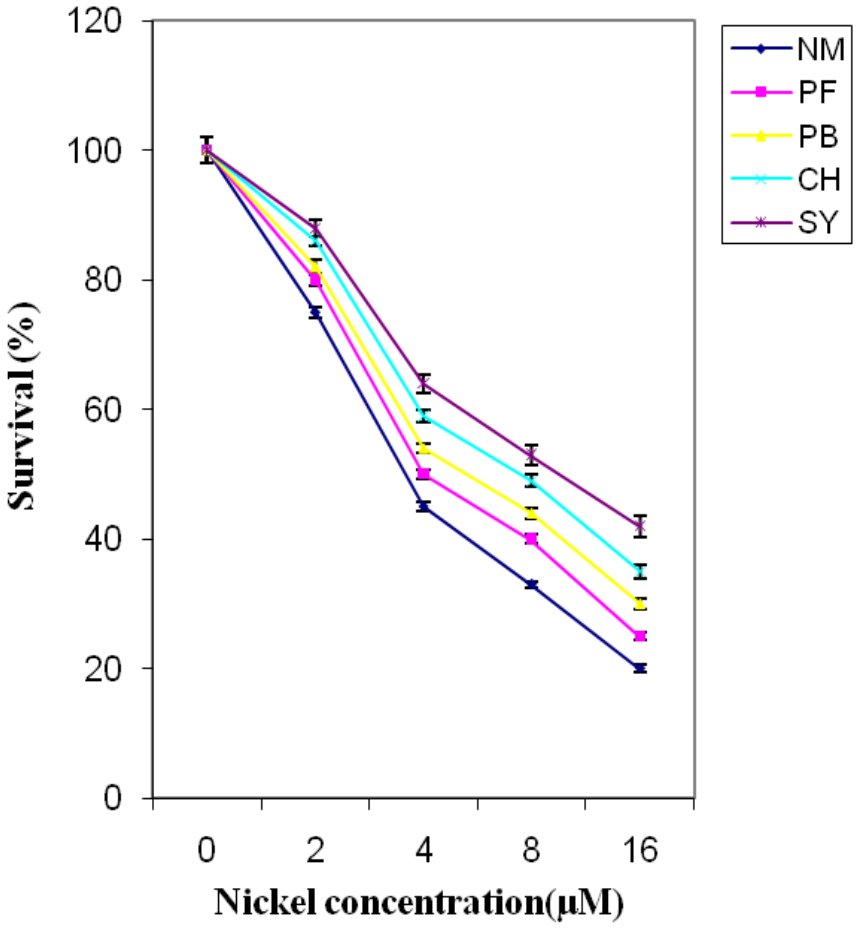

Figure 4: Effect of different concentrations of Ni on survival of *N. muscorum, Phormidium* sp., *P. boryanum, Chroococcus* sp. and *Scytonema* sp. Values are means± SE with n=3

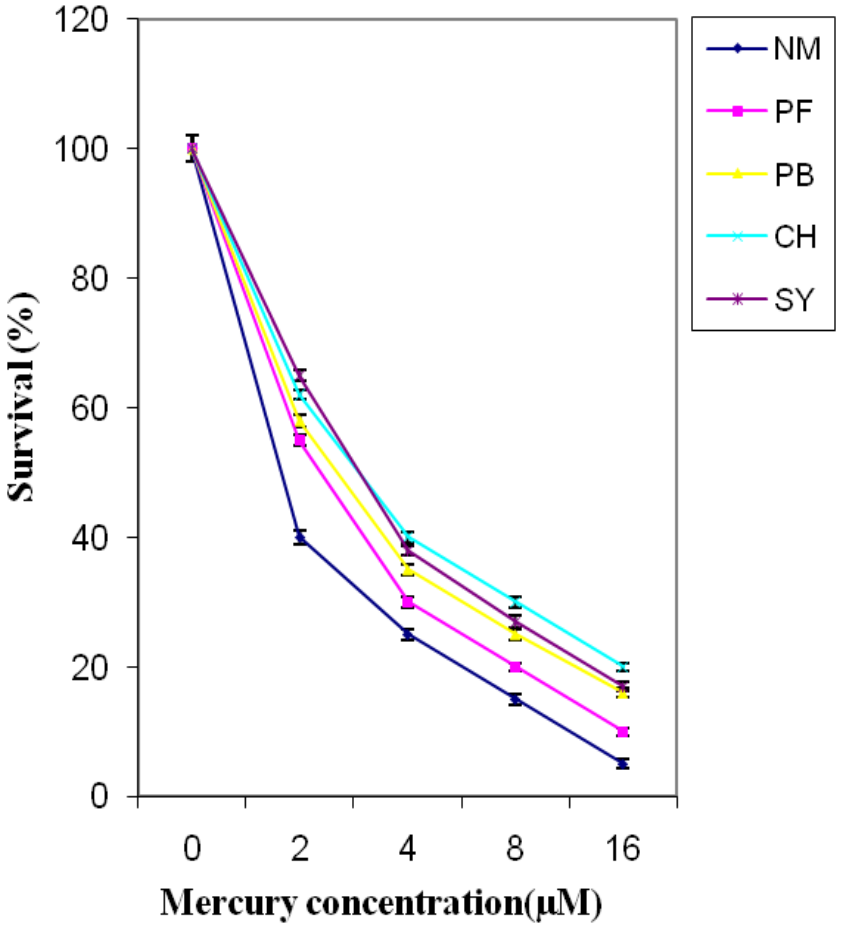

Figure 5: Effect of different concentrations of Hg on survival of *N. muscorum*, *Phormidium* sp., *P. boryanum*, *Chroococcus* sp. And *Scytonema* sp. Values are means± SE with n=3

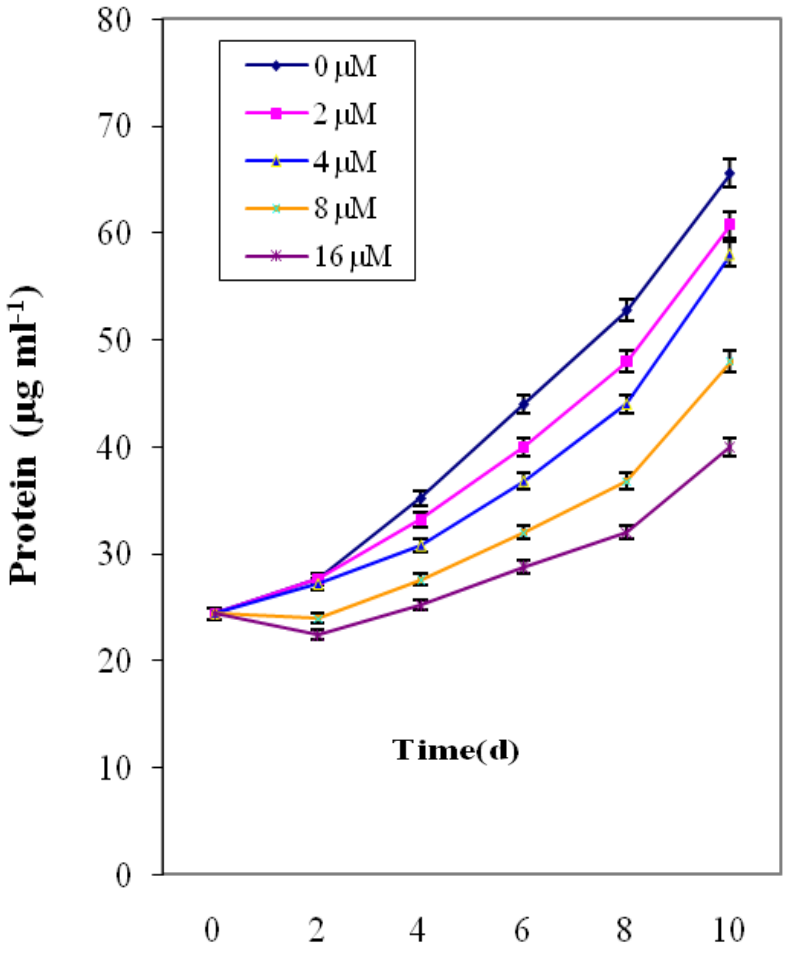

Figure 6: Effect of different concentrations of Al on growth of *N. muscorum*.Values are means ± SE with n=3

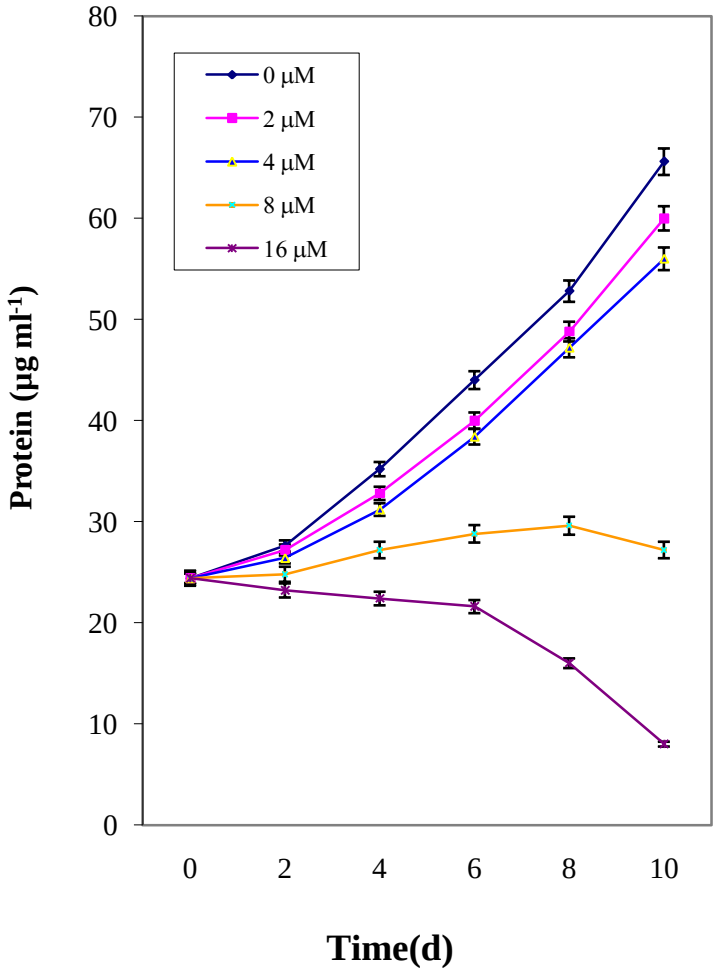

Figure 7: Effect of different concentrations of Cu on growth characteristic of *N. muscorum*. Values are means ± SE with n=3

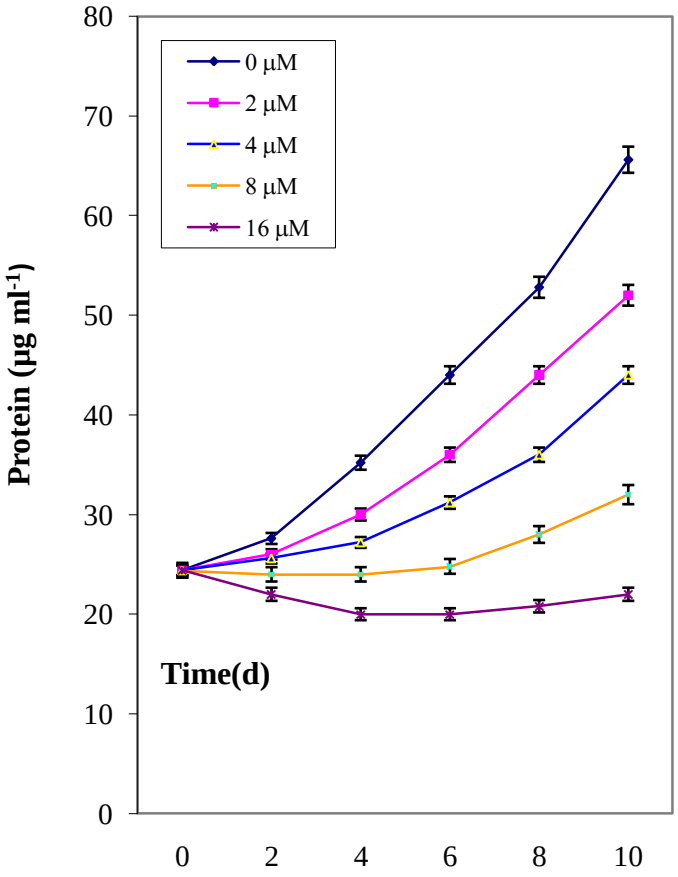

Figure 8: Effect of different concentrations of Cd on growth characteristic of *N. muscorum*. Values are means ± SE with n=3

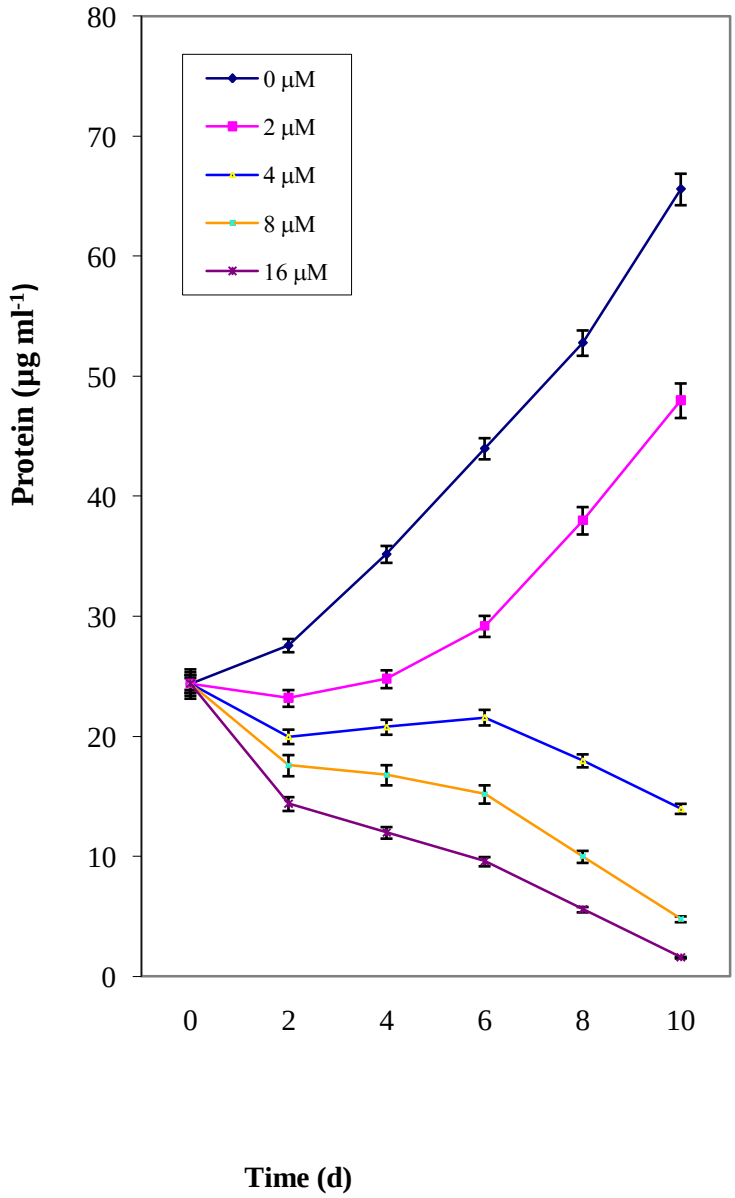

Figure 9: Effect of different concentration of Ni on growth characteristics of *N. muscorum*. Values are means ± SE with n=3

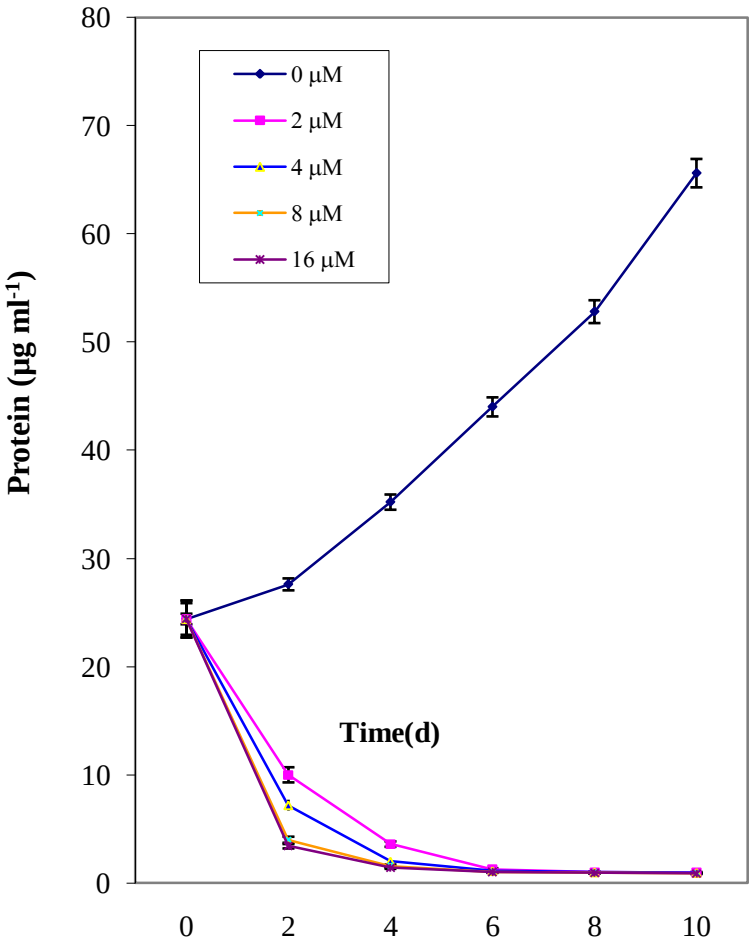

Figure 10: Effect of different concentration of Hg on growth characteristics of *N. muscorum*.Values are means ± SE with n=3

DISCUSSION

Survival and Growth

Though some heavy metals are needed by living organisms for various metabolic processes, the physiological and/or metabolic requirements of such metals as Cd, Cr, Pb, Ni, Ag and Hg are not properly understood. The results of present study show metal induced

changes in survival and growth of*N. muscorum*. Our study reveals reduction in survival of *N. muscorum, Phormidium sp., P. boryanum, Chroococcus sp. and Scytonema sp.* **(Figure 1- 5)** and growth in *N. muscorum* exposed to metals **(Figure 6-10)**.These results are in agreement with the findings of **[29]**. The decrease in growth have been described for various cyanobacterial strains and green algae exposed to abiotic stresses including heavy metals **[30, 31]**.

The decreasing trends in survival and growth following metal exposure might be due to the arrest of the physiological and biochemical processes in cyanobacteria. Among the five metals (Al, Cu, Cd, Ni and Hg) Hg was the most toxic. Even at low dose there was pronounced effect on growth and survival. Hg is a strong phytotoxic metal that causes inhibition of plant growth as well as produces long term effects on soil fertility. It affects membrane structural integrity **[32]**, mineral nutrient uptake **[33, 8]**, photosynthesis and transpiration **[34]**. The high level of toxicity of mercury was also reported by **[35]** who observed effects of different concentrations of Cd, Cu, Co, Hg, Li, Mn, Mo, Ni and Pb on growth, chlorophyll, DNA and protein contents of *Chlorella vulgaris.*

Cd causes inhibition of normal cell division, damage to various metabolic processes, Chlorophyll synthesis, photosynthetic activity and ultrastructure of membrane [36], finally arresting the overall growth of the stressed cyanobacteria. Studies of the effects of Cd, Cu, Zn, Pb and Fe on the green alga *Scenedesmusquadricauda* revealed that the toxicity for all the above parameters including the growth was increased in a dose-dependent manner **[37]**, which is in good agreement with the present results of growth.

The study carried out by **[38]** revealed a reduction in the survival and growth of *Anabaena doliolum* with increasing concentration of Cu and Cd thereby confirming the toxicity of these metals. During the past decades, significant progress has been made in our understanding of the mechanisms of Al toxicity and tolerance in plants **[15]**, algae **[16]** including blue green algae **[17]**.

In the present study the least inhibition in growth of *N.muscorum* under all the test concentrations of Al is also supported well by the survival results clearly demonstrating the high tolerance of the cyanobacterium to Al. This finding is in consonance with the reports of **[39]**, which showed that low concentration of Al in the form of $AlCl_3$ were non-inhibitory to *N. linckia* at pH 7.5

CONCLUSION

The understanding of the response of cyanobacteria can be used as a simple model for plants to various stress and is of utmost relevance, as deciphering their adaptive mechanisms will surely contribute not only to understand the plant response but also to design and construct plants resistant to such an environmental limitation.This work aims to redress the problem of metal toxicity to plants by exploiting the differential responses of several cyanobacterial strains to different metals. It can be inferred from these preliminary metal screening results against the *N. muscorum* that as usual Hg, Cu, Ni and Cd are toxic while Al is relatively less toxic showing that *N. muscorum* tolerated Al exceedingly well.

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