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# SCREENING OF CYANOBACTERIAL STRAINS FOR TOXICITY ASSAY OF SILVER AND SILVER NANOPARTICLES

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## ABSTRACT

The growing incorporation of nanomaterials and excessive heavy metal discharge into the environment calls for the better understanding of their toxicological effects on the ecosystem. For analyzing their toxic effects we have chosen cyanobacteria as our model system. Cyanobacteria is known to accumulate heavy metals and act as a sink for many aquatic contaminants. The present study aims towards screening of four cyanobacterial strains namely *Synechococcus sp*., *Scytonema sp*., *Nostoc muscorum* and *Plectonema boryanum*, against silver stress. Our results indicated that out of four strains, two strains namely *Nostoc muscorum* and *Plectonema boryanum* were found to be less sensitive while *Scytonema sp*. exhibited highest sensitivity towards Ag stress. Our results state that the growth inhibition of cyanobacterial strains is in the order of *Scytonema sp*> *Synechococcus sp* >*Nostoc muscorum*> *Plectonema boryanum* which was estimated in terms of photosynthetic pigment (Chl a). Effect of Ag stress on carotenoids (Car) and total protein was also studied. Based on the above results, the less sensitive strains namely *Nostoc muscorum* and *Plectonema boryanum* were further studied against silver nanoparticles stress. All experiments were repeated three times to ascertain the reproducibility of the results. Values are represented as mean ± SE. Thus, the understanding of the responses incurred in cyanobacteria during heavy metal and metal nanoparticle stress can be very well used to evaluate their toxic responses in the environment.

**KEYWORDS**: **Cyanobacteria, heavy metals, silver, silver nanoparticles**

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# INTRODUCTION

A broad range of pollutants including heavy metals and metallic nanoparticles are rapidly discharged into the environment for the sake of human development.[1] Heavy metals in the aqueous environment are lethal for biotic life in water ecosystem as well as for humans using these resources. Apart from this, nanoparticles affect different forms of life and induce the generation of free radicals, which results in deleterious effects on cellular functions of the organism. An enormous increase in the level of these pollutants is posing a serious threat to the environment. These contaminants make their way into the living cells and generate physiological stress that generate reactive oxygen species (ROS).These ROS are responsible for inhibition of antioxidative enzymes, cellular redox imbalance, ionic imbalance, DNA damage and protein oxidation.[2] Among these metals, silver has been considered as one of the most toxic heavy metals, surpassed only by mercury and thus has been assigned to the highest toxicity class. Silver and silver salts can make their way into the ecosystem and eventually enter the food chain. The toxicity of silver is hypothesized to be due to the disruption of membrane transport processes that disturb osmoregulation and finally lead to cell death.[3] Chronic exposure of silver may cause various diseases thus, there is an immediate need to analyze the effect of silver toxicity on the environment. The last decade has been distinguished by the dramatic growth in production and use of manufactured nanoparticles (NPs). The release of such nanoparticles into the ecosystem can pose serious environmental threats which can lead to major consequences in the future. The consequent discharge of engineered nanoparticles to the environment during and after their manufacture and usage is a major concern leading towards the risk of its exposure to both environment as well as human.[4] Nanotechnology coupled with antibacterial properties of AgNP has a rising application in biomedical field as well as in the production of commercial products. Silver nanoparticles have emerged up with diverse medical applications ranging from silver based dressings, silver coated medicinal devices, such as nano gels, nana lotions, etc. Effects of AgNP and potential implication for human and environment health have been described in several papers.[5] The lethal silver concentration of silver nanoparticles for humans is about 3 times higher than that of silver ions.[6] Silver nanoparticles (AgNPs) may be introduced into aquatic ecosystems because of their widespread use as antimicrobial agents.[7] However, few studies have investigated the impacts of AgNPs on aquatic ecosystem in an environmentally relevant context. Ag is one of the most toxic metal found in natural waters systems and considering that situation together with the lack of information on behavior of AgNP in the environment, there is a need to assess the toxicity of AgNP to aquatic species and which will have a contribution on building the knowledge concerning their nontoxic production and application. Cyanobacteria are the only group of prokaryotes that can perform oxygenic photosynthesis and are globally distributed in almost every ecological niche from fresh and salt water to extreme environments including highly contaminated areas.[8] Due to their wide distribution they are exposed to various kinds of stresses whether tropospheric or aquatic. They have diverse in built mechanisms such as their ability to excrete heavy metal ligands or extracellular polymeric substances (EPS) that have established them to resist high metal and metal nanoparticle concentrations.[9] Despite their high degree of stress tolerance, it has been shown that cyanobacteria are highly sensitive to heavy metal pollution and have been used in a variety of ways to understand environment related problems.[10] Therefore, these organism can act as a promising detector system for heavy metal and metal nanoparticle toxicity analysis.[11] Thus, our study deals with the screening of various cyanobacterial strains under heavy metal and metallic nanoparticle stress for selection of moderately sensitive strains to be used as an ideal tool for heavy metal and metal nanoparticle toxicity analysis. This study might prove to be a preliminary step towards assessing the harmful effects of silver and silver nanoparticle into the environment and also reveal favoring mechanisms in cyanobacteria that make it such an ideal model system for evaluation of toxicity.

# MATERIALS AND METHODS

### *Experimental organisms*

Four strains of cyanobacteria namely *Nostoc muscorum (N.muscorum), Plectonema boryanum (P. boryanum*), *Synechococcus sp*. and *Scytonema sp*. were chosen for the present study. Cyanobacteria *N. muscorum* and *P. boryanum* were obtained from Department of Botany, University of Allahabad and Department of Biological Sciences, Allahabad Agricultural Institute, Deemed University, Allahabad. While *Synechococcus sp*. and *Scytonema sp*. were obtained from Indian Agricultural Research Institute (IARI), New Delhi. The strains were maintained at cyanobacterial culture room, Department of Bioengineering, Integral University, Lucknow.

### *Growth conditions*

The desired strains of cyanobacteria were maintained in the culture room at 27 ± 2 °C. For regular lab work, cultures were grown in BG 11medium (pH 7.0)[12] with or without extra supplementation of combined nitrogen depending upon the heterocystous and non-heterocystous cyanobacteria under the light intensity of 2400 Lux and 14:10 h light and dark photoperiod.

### *Metal Treatment*

Stock solution of silver metal was prepared in deionized water (MilliQ, Millipore) and was further sterilized by passing through Millipore membrane filter (0.22 µm). From the stock solution various required concentrations 2, 4, 8, 16, 32, 64 and 128µM of Ag were prepared in the BG-11 medium. Silver nitrate ($AgNO_3$) was used as a source of silver.

### *Metal Nanoparticle Treatment*

Silver nanoparticle (<100nm) was purchased from Sigma–Aldrich. The stock suspensions/solutions of the tested chemical was prepared in deionized water (MilliQ, Millipore). The stock suspensions of metal nanoparticles were sonicated for 30 min, stored in the dark at +4 °C and used for testing within 2 months. Dilution of required concentrations was done same as in case of silver.

***Growth measurement***
Growth experiments were performed in liquid medium at regular interval up to 10 days in terms of chlorophyll a.

***Photosynthetic pigment extraction and estimation***
***Organic solvent soluble pigments***
***Chlorophyll a and Carotenoids***
For extraction of Chl-a and Carotenoids, equal volume of each organism was centrifuged and pellet was suspended in a desired volume of 80% (acetone: water, v/v). After overnight incubation at $4^0$C, suspension was centrifuged and supernatant was used for measuring Chl-a and carotenoids. The absorbance of pigment extracts was read spectrophotometrically for Chl-a at 665 nm and carotenoid at 480 nm. The specific coefficients were used for the calculation of Chl-a and carotenoids concentrations in cultures.[10] Quantitative estimation of these pigments in terms of g/l was done using formulae given below.

$$C = D/d\ \alpha \qquad (1)$$

Where α = absorption coefficient (value of α for Chl-a is 82.04 and for carotenoids is 200).
D = Optical density.
d = inside path length of spectrophotometer in (cm)
C = Concentration of pigment in $gL^{-1}$

***Estimation of protein***
Protein was measured by the method developed[13] and modifiedby[14]. For an analysis of protein, 0.5 ml of thoroughly washed cyanobacterial cell suspension was digested with 0.5ml of 1 N NaOH in a boiling water bath for 10 min. After sufficient cooling, 2.5 ml of reagent B was added and reaction mixture was incubated for 15 min at room temperature followed by addition of 0.5 ml of Folin-Ciocalteu's reagent. The intensity of the resulting blue color after 15 min was determined spectrophotometrically at 650 nm. The amount of protein was estimated from a standard curve prepared by using BSA as a source of protein.

***Statistical Analysis***
All experiments were performed using exponentially growing cultures and repeated three times to ascertain the reproducibility of the result. Values are represented as mean ± SE (n = 3).

## RESULTS

When *P.boryanum* was treated with a dose of 2 µM of Ag, log phase continued up to 96 h after that decline was observed whereas, at 4 µM log phase continued only up to 24 h and then decline in growth was observed even after 48h. At higher concentrations (8-28 µM) decline in growth commenced from the same day and continued up to 120 h (Figure.1).Whereas in case of *N.muscorum* (Figure. 2), no significant deviation from control culture was observed at 2µM and log phase continued up to 96 h. At 4 µM of silver decline in growth was observed after 48 h showing its sensitivity towards Ag stress. However at higher concentrations (16-128 µM) constant decline was observed after 24 h of treatment.The growth characteristics of *Synechococcus sp.* reveals slight effect at lower dose (2µM), whereas at 4 & 8 µM log phase continued up to 48 h and after that sharp decline was observed up to 120 h. At higher concentration (16-128µM), there was a rapid decrease in growth from the same day which continued up to 120 h and eventually led to death phase. This shows higher sensitivity of *Synechococcus sp*. towards Ag stress (Figure. 3). The growth characteristics of *Scytonema sp*. reveal that even from the lowest concentration (2 µM), a sharp decline in growth was observed after 24 h. On exposure to higher doses of Ag, a drastic decline in growth of cells was observed at the same day. This shows that *Scytonema sp*. exhibits extreme sensitivity towards Ag stress (Figure. 4).From the above results, we can conclude the sensitivity order which is as follows: *Scytonema sp.>Synechoccous sp.>Nostoc muscorum>Plectonema boryanum*. Thus, the above results were taken into consideration for selecting the cyanobacterial strains for silver nanoparticle stress. Thus, less sensitive strains *Nostoc muscorum* and *Plectonema boryanum* were chosen as representative models to study silver nanoparticle stress accordingly. When the cells of *P.boryanum* and *N. muscorum* were treated from 2-16 µM of AgNP, log phase continued up to 120 h. showing no significant variations in growth pattern. In case of *P.boryanum* (Figure. 5) rapid decline was observed at the higher concentrations (64 &128 µM) at the same day whereas in *N. muscorum* (Figure. 6) decline was observed from 32-128µM that continued up to 120 h.

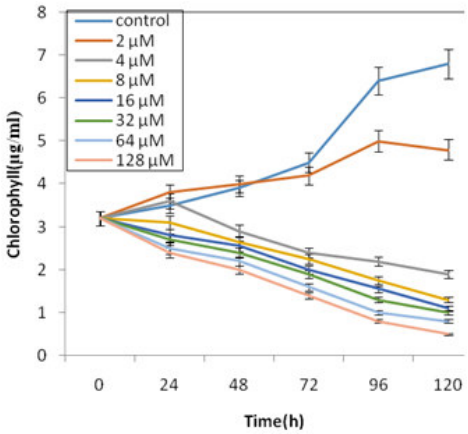


**Figure 1**
***Effect of different concentration of Ag on growth of P.boryanum.Values are means ± SE.***

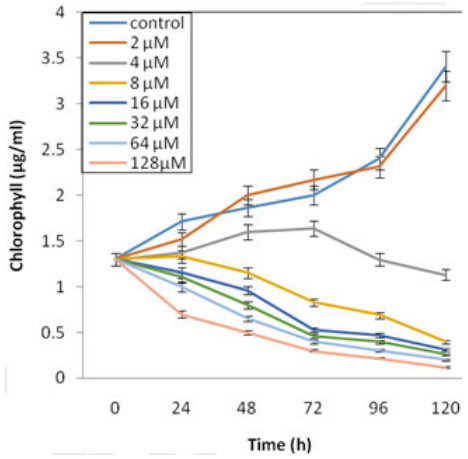


**Figure 2**
***Effect of different concentration of Ag on growth of N.muscorum. Values are means ± SE.***

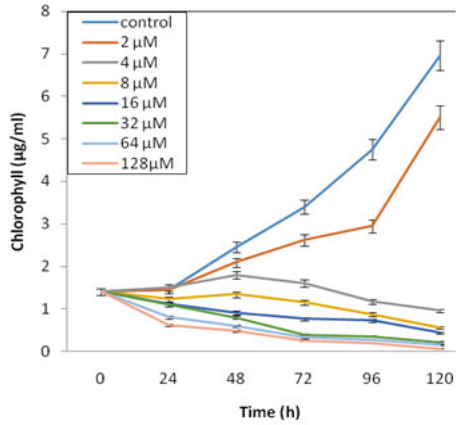


**Figure 3**
***Effect of different concentration of Ag on growth of Synechcoccus sp.Values are means ± SE.***

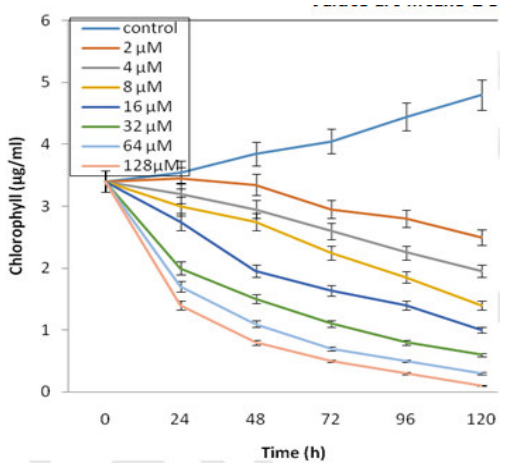


**Figure 4**
***Effect of different concentration of Ag on growth of Scytonema sp.Values are means ± SE.***

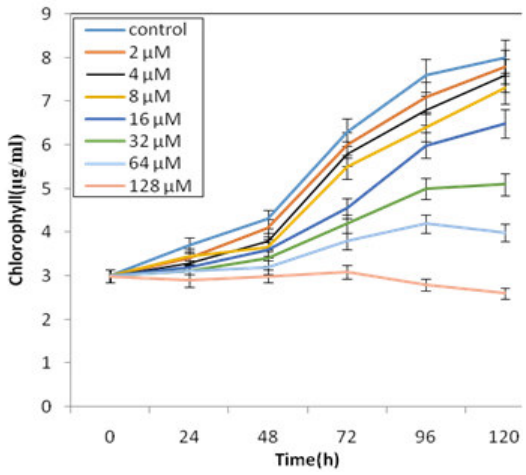


**Figure 5**
***Effect of different concentration of AgNP on growth of P.boryanum.Values are means ± SE.***

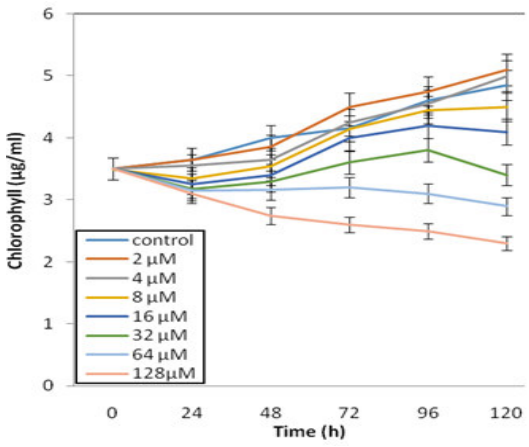


**Figure 6**
***Effect of different concentration of AgNP on growth of N.muscorum. Values are means ± SE***

To investigate the damaging effect of Ag on different cyanobacterial strains (*Scytonema sp., Synechococcus sp.,P.boryanum and N.muscurom*) after 96 h of treatment on photosynthetic pigments, the chlorophyll a (Chl a) carotenoids (Car) and total protein were estimated. Results presented in the tables (1, 2, 3 & 4) demonstrate that the photosynthetic pigments (Chl a & Car) of *Scytonema sp., Synechococcus sp., P.boryanum and N.muscurom* were severely affected with increasing concentration of Ag. When the *P.*

*boryanum* and *N. muscurom* was treated with increasing concentrations of Ag, the chl a followed a dose dependent decrease from 2-128 µM except the Car content which showed slight increase at 2 µM, showing its antioxidant potential in case of *N. muscurom*. In case of *P.boryanum* (Table 1), the protein content was found to increase at lower doses (2-8µM) of Ag and then a decline of 41- 62 % was seen from 16-128 µM, whereas in case of *N.muscurom*, protein content increased up to 8 µM and then a decline of 32-58% from 32-128 µM was observed (Table 2).Similar pattern of effect was perceived in case of the *Synechococcus sp*(Table 3) and *Scytonema sp.* (Table 4). In both the strains, dose dependent decline in chl a, car and total protein was observed. Among these four strains, *Scytonema sp*. and *Synechcoccus sp.* exhibited extreme sensitivity whereas, *P. boryanum* and *N.muscorum* exhibited moderate sensitivity towards Ag stress, therefore these strains were chosen to study the effect of AgNP. When *N.muscorum* (Table 6) was treated with increasing concentrations of AgNP, the chl a content was less severely affected as compared to *P. boryanum* (Table 5). In both the strains, there was a dose dependent decrease in case of chl a except at 2 µM and car except at 2 & 4 µM, showing its antioxidant activity at low concentrations. In case of *P. boryanum*, total protein increased in a dose dependent manner from 2-128 µM as compared to the control culture whereas in case of *N. muscorum* total protein increased only at lower concentrations (2 & 4 µM).

**Table 1**
***Effect of silver on photosynthetic pigments and Total Protein of P.boryanum***

| Ag (µM) | Chl *a* (µg mL$^{-1}$) | Car (µg mL$^{-1}$) | Total protein (µg mL$^{-1}$) |
|---|---|---|---|
| 0 | 7.35 ± 0.15 | 1.26 ± 0.06 | 39.45 ± 0.55 |
| 2 | 2.35 ± 0.15 (68) | 1.14 ± 0.04(9) | 44.05± 0.25(+11) |
| 4 | 1.85 ± 0.15 (74) | 0.74 ± 0.003(41) | 39.35± 0.71(0.2) |
| 8 | 1.3 ± 0.1 (82) | 0.68 ± 0.005(46) | 30.88 ±0.9(21) |
| 16 | 1.1 ± 0.09 (85) | 0.56 ± 0.005(55) | 2.34 ±0.75(41) |
| 32 | 0.8±0.03(89) | 0.48±0.002(61) | 21.2± 0.2(46) |
| 64 | 0.6±0.1(91) | 0.4±0.003(68) | 18.1±0.32(53) |
| 128 | 0.32±0.06(95) | 0.36±0.04(71) | 14.6±0.63(62) |

***The values represent Means ± SE. Values in parenthesis are [%] decrease or increase (+)***

**Table 2**
***Effect of silver on photosynthetic pigments and Total Protein of N.muscurom***

| Ag (µM) | Chl *a* (µg mL$^{-1}$) | Car (µg mL$^{-1}$) | Total protein (µg mL$^{-1}$) |
|---|---|---|---|
| 0 | 2.4 ± 0.03 | 1.35 ± 0.05 | 73.57 ± 0.3 |
| 2 | 2.31 ± 0.1 (+6) | 1.38 ± 0.05(+1) | 81.15±0.5 |
| 4 | 1.3 ± 0.03 (66) | 0.625 ± 0.025(53) | 72.75±0.4 |
| 8 | 0.69 ± 0.015 (88) | 0.33 ± 0.01(75) | 57.25 ±0.3 |
| 16 | 0.47 ± 0.03 (90) | 0..235 ± 0.005(82) | 49.2±0.3 |
| 32 | 0.4±0.03(83) | 0.2±0.002(85) | 45.2± 0.2(38) |
| 64 | 0.3±0.1(87) | 0.13±0.003(90) | 38.1±0.32(48) |
| 128 | 0.22±0.06(90) | 0.9±0.04(33) | 30.6±0.63(58) |

*The values represent Means ± SE. Values in parenthesis are [%] decrease or increase (+)*

**Table 3**
***Effect of silver on photosynthetic pigments and Total Protein of Synechococcus sp.***

| Ag (µM) | Chl *a* (µg mL$^{-1}$) | Car (µg mL$^{-1}$) | Total protein (µg mL$^{-1}$) |
|---|---|---|---|
| 0 | 4.75 ± 0.15 | 3.15 ± 0.05 | 67.95 ± 0.15 |
| 2 | 2.9 ± 0.1 (38) | 2.95 ± 0.05(63) | 60.82± 0.72 (10) |
| 4 | 1.1 ± 0.04 (76) | 0.7 ± 0.02(48) | 52.95± 0.75 (22) |
| 8 | 0.8 ± 0.02 (83) | 0.47 ± 0.005(85) | 31.08 ±0.75 (54) |
| 16 | 0.6 ± 0.04 (87) | 0.26 ± 0.005(91) | 24.04± 0.25 (64) |
| 32 | 0.35±0.03(92) | 0.18±0.002(94) | 22.2± 0.2(80) |
| 64 | 0.27±0.1(94) | 0.11±0.003(96) | 20.1±0.32(74) |
| 128 | 0.2±0.06(95) | 0.7±0.04(77) | 17.6±0.63(70) |

*The values represent Means ± SE. Values in parenthesis are [%] decrease or increase (+)*

**Table 4**
***Effect of silver on photosynthetic pigments and Total Protein of Scytonema sp.***

| Ag (µM) | Chl *a* (µg mL$^{-1}$) | Car (µg mL$^{-1}$) | Total protein (µg mL$^{-1}$) |
|---|---|---|---|
| 0 | 4.4 ± 0.2 | 1.5 ± 0.1 | 72.7 ± 0.5 |
| 2 | 2.8 ± 0.01 (47) | 1.35 ± 0.05(10) | 53.5± 0.9 (26) |
| 4 | 1.9 ± 0.15 (56) | 1.07 ± 0.035(28) | 49.7± 0.1 (31) |
| 8 | 1.3 ± 0.1 (70) | 0.65 ± 0.045(56) | 34.8 ±0.7 (52) |
| 16 | 0.9 ± 0.1 (79) | 0.42 ± 0.01(72) | 29.95± 0.15 (58) |
| 32 | 0.3±0.03(93) | 0.3±0.002(80) | 20.2± 0.2(72) |
| 64 | 0.18±0.1(95) | 0.14±0.003(90) | 16.3±0.32(77) |
| 128 | 0.14±0.06(96) | 0.6±0.04(60) | 12.6±0.63(82) |

***The values represent Means ± SE (n=3). Values in parenthesis are [%] decrease or increase (+)***

**Table 5**
***Effect of silver nanoparticle on photosynthetic pigments and Total Protein of. P.boryanum***

| Ag NP (µM) | Chl *a* (µg mL$^{-1}$) | Car (µg mL$^{-1}$) | Total protein (µg mL$^{-1}$) |
|---|---|---|---|
| 0 | 8.05 ± 0.15 | 2.35 ± 0.05 | 237.45 ± 0.6 |
| 2 | 7.05 ± 0.15 (12) | 2.46 ± 0.04(+4) | 294.8± 0.9 (+24) |
| 4 | 7.4 ± 0.1 (8) | 3.1 ± 0.06(+31) | 288.7± 0.6 (+21) |
| 8 | 7.15 ± 0.05 (11) | 2.15 ± 0.05(8) | 263.8 ±0.4 (+11) |
| 16 | 6.6 ± 0.1 (18) | 1.95 ± 0.5(17) | 258.35± 0.5 (+9) |
| 32 | 6.0±0.17(25) | 1.6±0.23(32) | 250±0.21(+5) |
| 64 | 5.2±0.33(35) | 1.23±0.21(47) | 246.2±0.26(+3) |
| 128 | 4.7±0.44(41) | 0.9±0.32(61) | 239±0.12(+0.6) |

***The values represent Means ± SE (n=3). Values in parenthesis are [%] decrease or increase (+)***

**Table 6**
***Effect of silver nanoparticle on photosynthetic pigments and Total Protein of N. muscorum.***

| Ag (µM) | Chl *a* (µg mL$^{-1}$) | Car (µg mL$^{-1}$) | Total protein (µg mL$^{-1}$) |
|---|---|---|---|
| 0 | 4.6 ± 0.1 | 1.85 ± 0.1 | 108.9 ± 0.1 |
| 2 | 4.75 ± 0.2 (+5) | 2.02 ± 0.08(+9) | 128.5± 0.5 (+3) |
| 4 | 4.55 ± 0.2 (3) | 2.04 ± 0.06(+10) | 110.7± 0.5 (+1) |
| 8 | 4.4 ± 0.1 (4) | 1.78 ± 0.08(3) | 97.6 ±0.6 (10) |
| 16 | 4.2 ± 0.15 (8) | 1.71 ± 0.09(7) | 91.5± 0.6 (15) |
| 32 | 3.8±0.03(17) | 1.6±0.02(13) | 90.0± 0.2(17) |
| 64 | 3.1±0.1(32) | 1.3±0.03(29) | 83.4±0.32(23) |
| 128 | 2.5±0.06(45) | 0.9±0.04(51) | 79.2±0.33(27) |

***The values represent Means ± SE. Values in parenthesis are [%] decrease or increase (+)***

## DISCUSSIONS

Our study reveals effect on growth characteristics (Figure. 1- 4), photosynthetic pigments (Chl a and Car) and total protein (Table 1-4) of *N. muscorum*, *P. boryanum*, *Synechococcus sp*. and *Scytonema sp*. exposed to silver stress. Further, *N. muscorum* and *P. boryanum* were selected for the growth measurement (Figure. 5 & 6) and photosynthetic pigment (Table 5 & 6) content under silver nanoparticle stress. Growth inhibition of microalgae is related to the concentration of heavy metal ions bound to the algal cell surface, in some cases to intracellular heavy metal concentration and to the chemical nature of heavy metal ion. From the present results, it was observed that there was pronounced effect of silver stress on growth of *Synechococcus sp.* and *Scytonema sp*. and moderate effect on *N. muscorum* and *P. boryanum*. Alterations in morphology and ultrastructure have also been frequently reported by many researchers which might also be a reason of growth inhibition. Our data also show that *Scytonema sp* exhibited greatest sensitivity against silver stress followed by *Synechococcus sp*., *N. muscurom* and *P.boryanum*. The decreasing trend in growth following metal exposure might be due to the arrest of the physiological and biochemical processes in cyanobacteria. Another cyanobacterium *Chlorella vulgaris* also showed decrease in growth and chlorophyll content with an increasing concentration of Cr (IV) along the exposure time.[15] Deleterious effects of different silver forms at antibacterial concentrations on osteoblasts and osteoclasts in vitro have also been reported showing silver lethality at lower doses.[16] The data obtained in our results during AgNP treatment reveal that *P.boryanum* was less sensitive compared to *N.muscorum* towards silver nanoparticle stress .In another study with the freshwater alga *Chlamydomonas reinhardtii*, AgNPs appeared to be much more toxic than silver ions, indicating a specific NP effect.[17] Similarly, the abnormal behavioral changes in *Chattonella marina* exposed to AgNPs were reported previously.[18] The exact mechanism of Ag toxicity has still not been deciphered however, according to the Biotic Ligand Model (BLM), Ag is known to compete with $Na^+$ for the binding sites at the enzyme $Na^+$,$K^+$-ATPase, which plays an important role on the transport of $Na^+$ and $Cl^-$ from water to the extracellular fluid in aquatic invertebrates such as *D. magna*.[19,20] This will cause ion or regulatory failure and death of the organism and it has been the most accepted mechanism to explain Ag toxicity.[21] Some authors suggested that the high toxicity of AgNPs was due to a high local concentration of Ag ions released from the NPs upon contact with the algal cell. The toxic effects induced by AgNP exposure in the present study seem to result from a mixture of parameters including exposure period, stability of the preparation, and speciation of the released silver. Other studies also showed the toxicity of AgNPs caused a highly dramatic reduction in the viability of cyanobacterial cells at higher concentrations.[22] Inhibition of photosynthetic pigment biosynthesis is one of the

primary events in plants and algae during heavy metal stress. Photosynthetic pigments are the central part of the energy manifestation of virtually every photosynthetic organism and therefore, any significant alteration in their levels may alter overall growth status and metabolic activities. The results in our study indicated that photosynthetic pigments of *Scytonema sp.*and *Synechococcus sp*. were severely affected in a dose-dependent manner in response to silver exposure including chlorophyll a, total carotenoids and total protein (Table 1 & 2) as compared to *P.boryanum* and *N.muscurom* which showed a comparatively lesser impact (Table 3 & 4). Our study shows dose dependent decrease in chl a content in case of *Scytonema sp*. and *Synechococcus sp*. at all concentrations while increase of car content at low concentrations, might be due to its antioxidant potential. A stress-induced inhibition of cyanobacterial growth occurs due to damage of the cellular constituents or inactivation of vital processes, such as nutrient uptake, enzyme activities, and photosynthesis.[23] Several studies suggest that NPs are toxic to some species while other authors find no differences in toxicity between metal NPs and the corresponding dissolved ion. The obtained results indicated that algal cells were affected by higher AgNP concentrations. Our results (Figure. 5 & 6) show that the total chlorophyll content of the culture is a measure of the effect of NPs on algal growth, since the AgNP concentrations were found to cause a drastic drop of chlorophyll level. In our study, the photosynthetic pigment was found to more inhibited in *N. muscurom* than *P. boryanum* when exposed to silver nanoparticle stress. This inhibition might be explained by that these NPs may in turn inhibit cell division and enzyme activities.[24] Our results also show an increase of carotenoid content at lower doses. Some researchers found reduction in growth, photosynthetic pigments and carbohydrate of *Aulosira fertilissima*, *Anabaena variabilis* and *Nostoc muscorum* were accompanied with increase in their total protein under higher doses of endosulfan which support our results.[25] Similarly, some reports show significant increase in concentrations of Car in two species of *Colophospermum mopane* after UV-B exposure.[26]

## CONCLUSION

The aim of this work was to investigate the toxicity of Ag and AgNP in the environment by using cyanobacterial strains as model systems. The present investigation revealed that photoautotrophic growth of *P.boryanum* was least susceptible to Ag and AgNP toxicity. The growth, pigments, and total protein in *Scytonema s*p. was more severely inhibited by Ag than in any other cyanobacterial strain. Therefore, the use and exposure of silver in our environment must be monitor very carefully at regular intervals. The understanding of the responses incurred in cyanobacteria during heavy metal and metal nanoparticle stress can be very well used to evaluate their toxic responses in the environment.

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## CONFLICT OF INTEREST

Conflict of interest declared none.

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