

Impact of Salinity and Copper Stress on the Growth and Physiology of Cyanobacteria: A Comparative Study

Shivaranjan C.S.* and G. Abraham

Division of Microbiology, Indian Agricultural Research Institute (New Delhi), India.

(Corresponding author: Shivaranjan C.S.*)

(Received: 24 June 2023; Revised: 18 July 2023; Accepted: 20 August 2023; Published: 15 September 2023)

(Published by Research Trend)

ABSTRACT: In this present study, we delved into the intricate world of *Anabaena doliolum*, a nitrogen-fixing cyanobacterium, to investigate how it copes with the combined stressors of salinity and copper exposure. Our study revealed that both salinity and copper stress lead to a significant reduction in growth and key cellular components, including protein, chlorophyll, and phycocyanin. The stress induced by salinity and copper was further evidenced by a decrease in the average filament length and heterocyst frequency of *A. doliolum*. Salinity treatment was found to enhance the activity of the nitrogen assimilation enzyme, nitrate reductase, while glutamine synthetase and nitrogenase exhibited significant inhibition in their activities. Conversely, copper treatment led to a decrease in the activity of all three nitrogen assimilation enzymes. The deleterious effects on growth, cellular components, and nitrogen assimilation enzymes were exacerbated when *A. doliolum* was exposed to both salt and copper simultaneously. Both individual and combined exposure to salt and copper was observed to stimulate the activity of antioxidant enzymes, including superoxide dismutase, ascorbate peroxidase, and catalase, as well as the accumulation of proline in the cyanobacterium. The study encountered several challenges, including the necessity to evaluate the synergistic effects of salinity and copper on *A. doliolum*, as well as the complex interplay of multiple physiological variables under stress conditions. These findings have advanced our understanding of how nitrogen-fixing cyanobacteria respond to the concurrent influence of multiple stressors, with potential implications for the ecological and environmental management of rice fields and similar ecosystems.

Keywords: Cyanobacterium, Salinity stress, Copper stress, N assimilation enzymes, Antioxidant enzymes.

INTRODUCTION

By the year 2050, it is projected that the global human population will surpass 9.6 billion, a demographic shift that will significantly elevate the demand for agricultural output. To meet this escalating need, a twofold augmentation in grain production is imperative. Nevertheless, conventional agricultural practices have engendered deleterious consequences, manifesting as soil degradation, diminished crop yields, and contamination of the environment. These conventional methodologies encompass the deployment of synthetic fertilizers, pesticides, intensive tillage, and excessive irrigation (Ali, 2023). These issues have contributed to an escalating cost associated with food production, underscoring the pressing necessity for a transition towards sustainable agricultural approaches. Sustainable agriculture underscores the adoption of organic techniques aimed at curtailing production expenditures while concurrently conserving vital resources such as soil and water (Bantider *et al.*, 2022). One pivotal facet of sustainable agriculture involves the utilization of beneficial microorganisms to safeguard soil fertility, bolster crop yields, and enhance the resilience of agroecosystems (Gamage *et al.*, 2023).

Cyanobacteria, a highly diverse group of photosynthetic prokaryotes, played a crucial role in the transformation of Earth's initially anaerobic conditions, which emerged approximately 3.5 billion years ago, into aerobic environments. These hardy organisms, which thrive in both terrestrial and aquatic habitats (Shokravi and Bahavar 2021), offer significant benefits within the realm of agriculture. They enhance soil quality, stimulate plant growth, and thereby contribute substantially to effective crop management practices. By facilitating nutrient cycling, nitrogen fixation, improving phosphorus availability, enhancing soil water retention and movement, and aiding in environmental protection by mitigating pollution and preventing land degradation, cyanobacteria become invaluable assets to farming economies (Chittora *et al.*, 2020). Moreover, cyanobacteria exhibit remarkable adaptability and tolerance to various environmental conditions (Singh, 2018; Sharma *et al.*, 2021). Studies indicate that cyanobacteria can contribute a substantial amount of organic matter and nitrogen to the soil, approximately 20–30 kg N ha⁻¹ (Issa *et al.*, 2014). This makes them particularly advantageous for resource-constrained farmers who might struggle to afford costly synthetic fertilizers. Additionally, cyanobacterial strains

serve as excellent models for studying stress responses due to their close relationship with eukaryotic photosynthetic organisms and plants (Burnap, 2015). Despite their potential for use in agriculture, cyanobacteria encounter several difficulties as a result of numerous abiotic stress factors. Salinity, which reduces rice yields and soil fertility, is one of the main stressors impacting cyanobacteria, especially in paddy fields. Although cyanobacteria can tolerate some salt, excessive amounts of salt can have a deleterious effect on their development, photosynthesis, and enzyme activity (Yadav *et al.*, 2022). There hasn't been much success utilizing cyanobacteria to restore salty and sodic areas. Additionally, as industrial and agricultural activity has grown, heavy metals have been released into the environment, straining cyanobacteria even more. The use of copper-containing herbicides, contaminated irrigation water, and excessive chemical fertilizer application all lead to soil pollution with heavy metals like copper (Cu). They can adversely affect the growth and physiology of cyanobacteria, disrupting their metabolic processes (Hooda *et al.*, 2023).

The excessive use of pesticides and chemical fertilizers, which raise the salinity and heavy metal content in the soil and threaten the soil microflora, especially cyanobacteria, make the agroecosystem particularly sensitive to these stresses. These difficulties emphasize the requirement for environmentally friendly agricultural methods that reduce the detrimental effects of abiotic stresses and retain the critical function of cyanobacteria in preserving soil fertility and agricultural output. Therefore, it is crucial to comprehend how the stress response is modulated in cyanobacterium under salinity and copper stress.

MATERIAL AND METHODS

Experimental organism and growth conditions: In the current study, we utilized the diazotrophic cyanobacterium *Anabaena doliolum*, which is regularly cultured at the Centre for Conservation and Utilization of BGA, ICAR-Indian Agricultural Research Institute, New Delhi, India. This bacterium was consistently grown as a unialgal culture in a BG-11 medium devoid of supplementary nitrogen. The pH of the growth medium was maintained at 7.5, and it was exposed to a light intensity of $72 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 16 hours during the day, followed by an 8-hour dark period, all at a temperature of $28 \pm 2^\circ\text{C}$.

Salinity and copper treatment: The selected cyanobacteria *A. doliolum* was exposed to different concentrations of NaCl (50, 100, 150, and 200 mM) and Copper chloride (1, 3, 5, 7, and 10 μM). It was used to determine the LD₅₀ concentration.

Treatment details: For estimation of growth and physiological response of the cyanobacterium to salinity and copper, the following treatments were followed: T₁ = Control, T₂ = 150 mM NaCl, T₃ = 5 μM CuCl₂, T₄ = 150 mM NaCl + 5 μM CuCl₂.

Dry Weight Determination: Following thorough agitation, a measured volume of the cyanobacterial suspension (10 ml) was filtered utilizing Whatman No.

42 filter paper. Subsequently, the cultures were subjected to oven-drying at 60°C and maintained in a desiccator until a consistent weight was achieved. The differences in weights before and after drying were recorded as the dry weight (Sorokin, 1973).

Estimation of Protein Content: The protein content of the cyanobacterium was determined using the method outlined by Lowry *et al.* (1951).

Pigment Profile Analysis

(a) Chlorophyll a: The total chlorophyll concentration was determined using the cold extraction technique as described by MacKinney, 1941.

(b) Carotenoids: The carotenoid content of the cyanobacteria was assessed following the method outlined by MacKinney, 1941.

(c) Phycocyanin: The phycocyanin content of the cyanobacteria was quantified using the method followed by Bennett and Bogorad, 1973.

Total Carbohydrate: The total carbohydrate content of the cyanobacterial samples was calculated using the method followed by Spiro, 1966.

Estimation of average filament length: A small quantity of the cyanobacterial suspension was pipetted out and placed on a clean grease-free slide. Under a low-power microscope, the filaments were focussed. The number of cells (vegetative cells and heterocysts) in each filament in a field was counted. The average length of a filament in a sample was calculated by counting the number of cells in each filament and dividing by the total number of cells (Mishra, 2003).

Estimation of heterocyst frequency: The number of heterocysts per hundred vegetative cells is known as heterocyst frequency, and it was determined as follows (Mishra, 2003)

$$\text{Heterocyst frequency} = \frac{\text{Total number of heterocyst}}{\text{Total number of vegetative cells}} \times 100$$

Assay of enzymatic and non-enzymatic antioxidant enzymes

(a) Superoxide dismutase: It was determined by measuring the reduction in optical density of formazan caused by superoxide radical and nitro-blue tetrazolium dye (Dhindsa *et al.*, 1981).

(b) Ascorbate peroxidase: It was measured using an approach followed by Nakano and Asada, 1981.

(c) Catalase: Catalase enzyme activity was assayed by monitoring the decrease of H₂O₂ (Aebi, 1984).

(d) Proline content: The proline content in the cyanobacterial samples was quantified following the procedure outlined by Bates *et al.* (1973).

Statistical analysis: All the experiments were performed in triplicate using triplicate samples. The data recorded on various parameters were subjected to statistical analysis.

RESULTS AND DISCUSSION

A. Effect of salinity and copper on the growth and cellular constituents of the cyanobacterium Anabaena doliolum

Based on the growth experiments using various concentrations of NaCl and copper chloride, it was observed that the cyanobacterium was able to grow well

up to a NaCl concentration of 150 mM and copper chloride concentration of 5 μ M. Therefore, these two concentrations have been used for further experiments. The rice field cyanobacterium *Anabaena doliolum* was exposed to NaCl (150 mM) and copper chloride (5 μ M) individually and together. Growth was recorded as an increment in the dry weight over some time. The cyanobacterium exposed to NaCl showed a reduction in the dry weight by 16.85% whereas a 40.44% reduction was observed in response to copper chloride on the twelfth day of treatment (Fig.1). However, the cells exposed to salinity and copper together showed further reduction in the dry weight by 52.98%. Stress tolerance in general reflects the total of various simple and complex interactions that take place at the structural, physiological, and molecular levels in the organism. Reduction in the growth may be considered as a general response to salinity and copper treatment (Yang *et al.*, 2020). A decrease in the growth of the cyanobacterium

Anabaena doliolum due to salinity stress was reported earlier (Srivastava *et al.*, 2009; Singh *et al.*, 2022). Similarly, the effect of copper on the reduction in growth was reported by Bhargava *et al.* (2008); Yadav *et al.* (2022). However, the exposure to combined salinity and copper treatment has resulted in a further drastic reduction in growth. Kholssi *et al.* (2023) reported a severe reduction in the growth and productivity of a cyanobacterium due to combined stress conditions. Reduction in growth could also be attributed to the loss of cell water and turgor coupled with toxicity by the accumulated ions. Osmotic stress results from salt stress, leading many organisms, such as cyanobacteria, to either accumulate or synthesize compatible solutes or osmoprotectants in order to uphold a lowered water potential within the cell (Kirsch *et al.*, 2019).

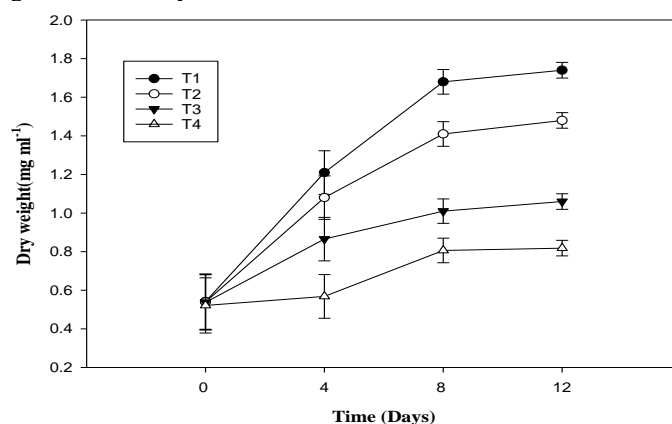


Fig. 1. Dry weight accumulation in the cyanobacterium *A. doliolum* in response to exposure to salinity and copper.

The protein content of the cyanobacterium significantly decreased following treatment with salt and copper (Fig. 2). Protein content was found to have decreased by 39.55% and 46.69% in response to salt and copper treatment, respectively. On the twelfth day following treatment, however, further protein content decrease (61.03%) was noted in response to combined exposure. There are conflicting studies about the effect of salt on the rise or fall in cellular protein concentration in

various species (Pathak *et al.*, 2022). According to Zhang *et al.* (2013), the protein content of the cyanobacterium *Microcystis aeruginosa* has decreased. According to Huertas *et al.* (2014), exposure to copper causes cyanobacteria to degrade proteins and alter how they function. The higher protein content loss brought on by simultaneous exposure to salt and copper, nevertheless, could be the result of an additive impact.

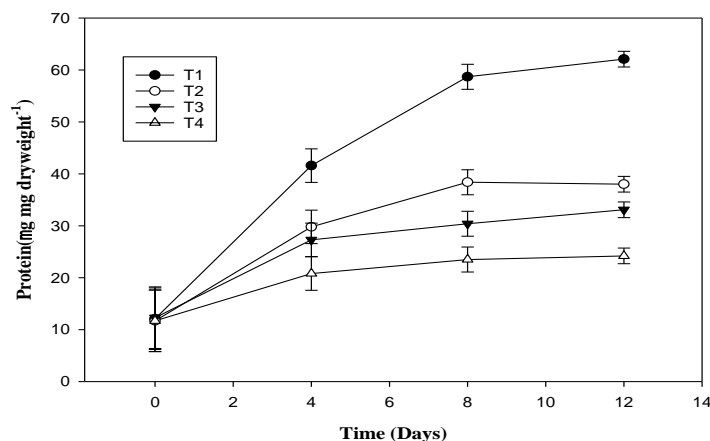


Fig. 2. Salinity and copper-induced changes in the protein content of the cyanobacterium *A. doliolum*.

The amount of chlorophyll in cyanobacteria may be used as a key indicator for determining productivity. With salinity and copper treatment, the cyanobacterium showed a decrease in chlorophyll concentration (Fig. 3). On the twelfth day of salinity incubation, a decrease in chlorophyll content of 51.72% was noted, whereas a decrease of 64.48% occurred in response to copper treatment. On the other hand, the amount of chlorophyll decreased by 80.34% when copper and salt were both present. In the same way, phycocyanin, one of the crucial accessory pigments for photosynthetic activity, significantly decreased in response to salinity and copper treatment (Fig. 4). In response to the stress brought on by exposure to salt and copper, it was discovered that the phycocyanin concentration decreased by 32.39 and 52.11%, respectively. Carotenoid, a second auxiliary photosynthetic pigment, significantly decreased in response to salt and copper treatment as well (Fig. 5). The observed increase in the carotenoid content was 36.95 and 39.94% due to exposure to salinity and copper, respectively. It appears that salinity and copper treatment lead to a severe decrease in the pigment profile of the cyanobacterium *A.doliolum*. Rai *et al.* (2021) reported that chlorophyll concentration changes with physiological conditions as well as environmental stressors. In addition, Kumar and Gaur (2014) noted that exposure to copper decreased

the chlorophyll content of the cyanobacterium *Phormidium bigranulatum*. Because of its location on the inner thylakoid membrane, phycocyanin is extremely sensitive to stressful situations (Chini Zittelli *et al.*, 2023). However, the test cyanobacterium *A. doliolum* showed a higher reduction in accessory pigments, regardless of the type, as a result of salinity and copper treatment. The pigments are vital in photosynthesis, and a drop in their concentration may result in decreased photosynthetic activity under stress circumstances in the test cyanobacterium, resulting in decreased growth. Bhargava *et al.* (2008) discovered that too much copper caused the cyanobacterium *Anabaena doliolum* to produce anoxygenic photosynthesis. On the other hand, we saw a rise in the cyanobacterium exposed to salinity's carotenoid concentration. However, the test cyanobacterium's carotenoid level was reduced by copper treatment, as well as by salt exposure combined with copper. According to Mao *et al.* (2020), cyanobacteria frequently build up carotenoids in response to oxidative stress. According to Lin and Wu (2014), increased carotenoid accumulation in cyanobacteria is associated with increased resistance to drought stress. Due to increasing copper levels, Kumar and Gaur (2014) found that the cyanobacterium's carotenoid concentration had decreased.

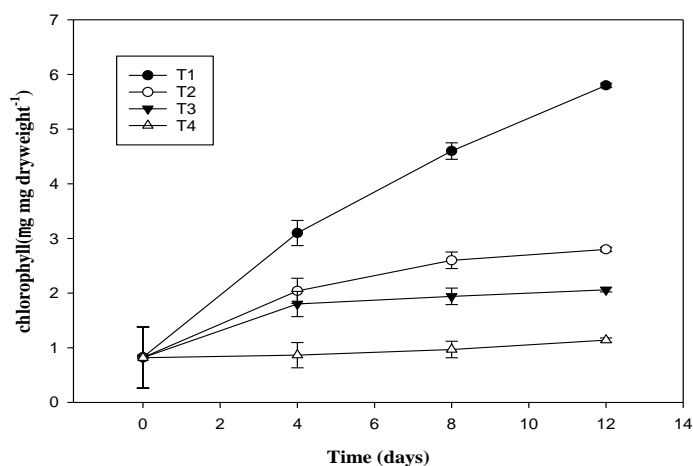


Fig. 3. Chlorophyll content of the cyanobacterium *A. doliolum* in response to salinity and copper treatment.

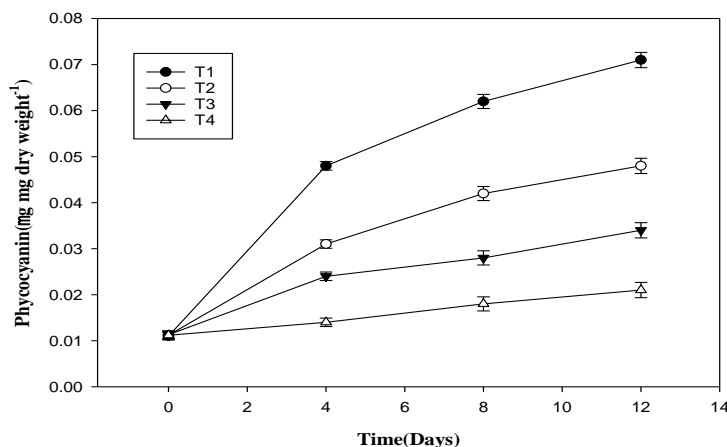


Fig. 4. Phycocyanin content of the cyanobacterium *A. doliolum* in response to salinity and copper treatment.

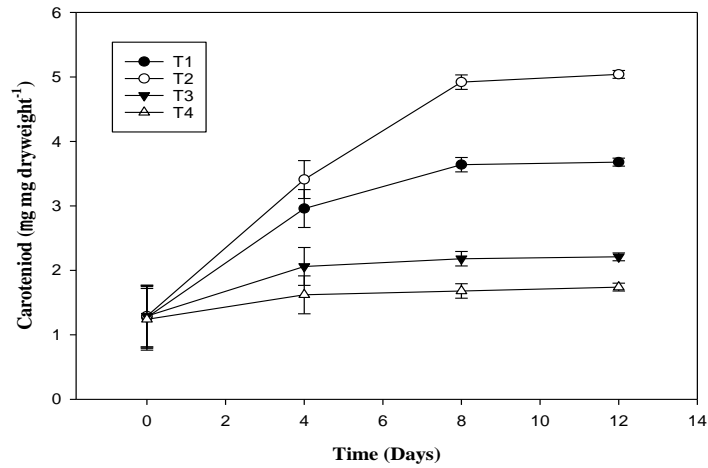


Fig. 5. Carotenoid content of the cyanobacterium *A. doliolum* in response to salinity and copper treatment.

B. Effect of salinity and copper exposure on the average filament length, heterocyst frequency and enzymes of nitrogen assimilation in the cyanobacterium A. doliolum

In response to salinity and copper exposure, we computed changes in the cyanobacterium *A. doliolum* average filament length (Fig. 6). The control (51.6) had the longest filament. However, with salinity treatment, a reduction in filament length was seen (40.1). In addition, the copper treatment resulted in a 30.1 reduction in filament length. The combined exposure to salt and copper led to the greatest decrease in average

filament length (20.6) that was seen. The salinity and copper exposure of the cyanobacterium *A. doliolum* resulted in a reduction in the heterocyst frequency (Fig. 7). The control cells had the highest frequency of heterocysts (12.1%), followed by the salt and copper treated cells (10.4%) and 6.8%) respectively. Salinity and copper treatment also resulted in a decrease in the heterocyst frequency and average filament length. The nitrogenase enzyme is located in the heterocyst. Zulkefli and Hwang (2020) observed a reduction in the heterocyst frequency and average filament length of cyanobacteria due to abiotic stress.

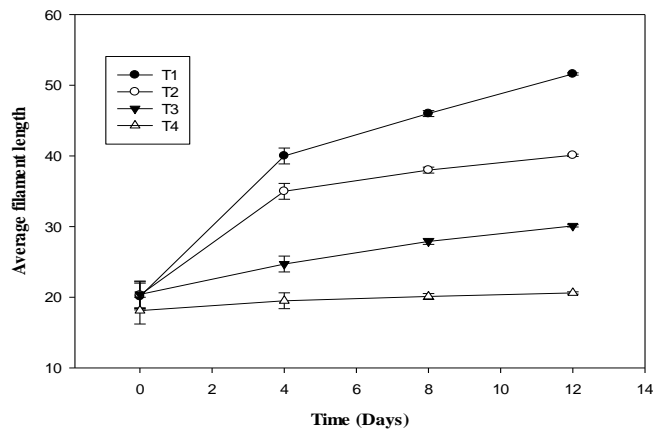


Fig. 6. Effect of salinity and copper on the average filament length of the cyanobacterium *A. doliolum*.

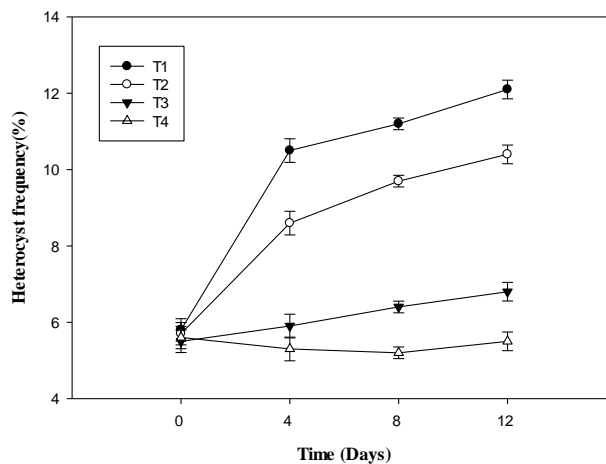


Fig. 7. Effect of salinity and copper on the heterocyst frequency of the cyanobacterium *A. doliolum*.

In the cyanobacterium *A. doliolum* subjected to salinity and copper, research on the nitrogen absorption enzymes was carried out (Table 1). Treatment with salinity increased *A. doliolum* nitrate reductase activity. In response to salinity treatment, a substantial increase in nitrate reductase activity was seen in the *A. doliolum*. However, exposure to copper boosted the activity of nitrate reductase by 88.10%. Combining exposure to salt and copper led to the greatest increase in nitrate reductase activity (82.78%). Assimilation of ammonia is mostly carried out by the enzyme glutamine synthetase. Salinity treatment resulted in a 12.81% reduction in glutamine synthetase activity in *A. doliolum*. When the cells were exposed to copper, there was an additional 25.64% decrease in the glutamine synthetase activity that was noted. The combined exposure to salt and copper resulted in a significant (38.09%) decrease in glutamine synthetase activity. The nitrogenase enzyme activity of the cyanobacterium *A. doliolum* exposed to salt and copper was assessed using the acetylene reduction assay (ARA). The control cells showed nitrogenase activity of 35.8 ± 0.252 $\mu\text{moles C}_2\text{H}_4 \text{ mg chl}^{-1} \text{ h}^{-1}$. Further, the nitrogenase activity declined to 29.7 ± 0.256 $\mu\text{moles C}_2\text{H}_4 \text{ mg chl}^{-1} \text{ h}^{-1}$ in the case of cells exposed to 150 mM salinity. Copper treated cells showed nitrogenase activity of 18.4 ± 0.254 $\mu\text{moles C}_2\text{H}_4 \text{ mg chl}^{-1} \text{ h}^{-1}$. However, the nitrogenase activity further declined to 10.6 ± 0.153 $\mu\text{moles C}_2\text{H}_4 \text{ mg chl}^{-1} \text{ h}^{-1}$ in response to combined exposure of salinity and copper.

The nitrate reductase enzyme plays a crucial role in the conversion of nitrate, a significant source of nitrogen in the environment, into ammonium. Previous research conducted by Kunui *et al.* (2020) has highlighted that

stress can lead to the accumulation of nitrate within cyanobacteria. Interestingly, this accumulation of nitrate appears to have a mitigating effect on its harmful consequences. In response to these stressful conditions, cyanobacteria tend to increase their production of nitrate reductase, which may serve as an adaptive mechanism to counteract the adverse effects of stress. However, it's worth noting that other nitrogen-related processes within cyanobacteria are affected differently under stress. Both salinity and copper stress have been found to reduce the activity of enzymes like glutamate synthetase and nitrogenase. Swapnil *et al.* (2015) reported that stress conditions led to a decrease in glutamine synthetase activity in the cyanobacterium *Anabaena* sp. Furthermore, the activity of nitrogenase, another crucial enzyme involved in nitrogen metabolism, was also adversely affected by stress exposure. It was observed that disruptions in electron transport could lead to the inhibition of nitrogenase activity (Dong *et al.*, 2021). This trend of stress-induced reduction in nitrogenase activity was further confirmed by Moisanter *et al.* (2002), who demonstrated that stress situations have a consistent negative impact on this enzyme in cyanobacteria. Bhargava *et al.* (2008) specifically noted that copper exposure could inhibit nitrogenase activity. Similarly, Yadav *et al.* (2016) found that exposure to high salt levels had a similar inhibitory effect on nitrogenase activity. Collectively, these research findings underscore the adverse effects of stressful conditions on nitrogen absorption enzymes within cyanobacteria, revealing a complex interplay of adaptations and limitations in response to environmental challenges.

Table 1: Effect of salinity and copper exposure on the N assimilation enzymes of the cyanobacterium *A. doliolum*.

Treatments	Nitrate Reductase ($\mu\text{mol mg protein}^{-1}$)	Glutamine Synthase ($\text{nmol mg protein}^{-1}$)	Nitrogenase ($\mu\text{mol C}_2\text{H}_4 \text{ mg chl}^{-1} \text{ h}^{-1}$)
T1	5.173±0.041	163.9±0.513	35.8±0.252
T2	11.43±0.231	142.6±0.458	29.7±0.256
T3	9.66±0.0153	121.8±0.0577	18.4±0.254
T4	9.45±0.0252	101.4±0.351	10.6±0.153

C. Effect of salinity and copper on the enzymatic and non-enzymatic antioxidant activity of the cyanobacterium A. doliolum

Antioxidants, both enzymatic and non-enzymatic, are crucial to the health of any organism under stress. We have also investigated the cyanobacterium *Anabaena* sp. subjected to salinity and copper's profile of antioxidant enzymes. Superoxide dismutase, ascorbate peroxidase, and catalase were all activated when the cyanobacterium was exposed to salt, copper, or all of these stimuli alone or in combination. The increase in the superoxide dismutase activity was 18.18% on the 4th day of NaCl treatment (150 mM) whereas it was found to enhance by 41.17% upon exposure to 5 μM copper chloride. The highest increase (58.28%) in the superoxide dismutase activity was observed when the organism was exposed to both NaCl and copper together (Fig. 8). Similarly, the increase in the ascorbate peroxidase activity was highest due to

combined exposure to NaCl and copper (82.52%). Salinity treatment resulted in an increase in the ascorbate peroxidase activity by 47.61% (Fig. 9) whereas the copper treatment enhanced the activity by 62.12%. Regarding the catalase activity, salinity treatment increased the activity significantly (Fig. 10). Copper chloride treatment also was found to enhance the activity whereas the maximum activity was observed in response to the combined application of NaCl and copper chloride. Superoxide dismutase, the primary antioxidant enzyme is involved in the conversion of the superoxide anion to H_2O_2 whereas the ascorbate peroxidase is involved in the reduction of H_2O_2 using ascorbate as the electron donor and is well known to be important in the detoxification of H_2O_2 . Whereas, catalase activity along with superoxide dismutase is the most effective strategy in preventing cellular damage (Kurutas, 2015). In stress conditions, several algae and cyanobacteria show enhancement in

antioxidant enzyme activities (Dwivedi and Ahmad 2023). Swapnil and Rai (2018) observed enhanced activity of antioxidant enzymes in the salt-stressed cyanobacterium *Anabaena fertilissima*. Therefore, an increase in enzymatic activity is correlated with the ability to tolerate salinity and copper. Intracellular accumulation of proline is related to the stress tolerance of the organism. The proline content of the cyanobacterium *A. doliolum* showed a differential response to salinity and copper treatment. A significant increase in the proline accumulation was observed in the cyanobacterium *A. doliolum* exposed to salinity, copper as well as combined exposure to salinity and copper (Fig. 11). The Increase in the proline content was 93.09% in response to the stress-induced by NaCl whereas it was enhanced to 35.52 % due to copper treatment. Combined exposure to copper and salinity on

the other hand enhanced the proline content by 55.26%. Lin and Wu (2014) reported that proline acts as an osmo-regulator and protects enzymes and proteins. Yadav *et al.* (2016) observed an increase in the accumulation of proline due to salinity stress conditions in the cyanobacterium *Anabaena* sp. Xiao *et al.* (2022) also observed enhanced accumulation of proline in response to heavy metal stress. Proline protects various cellular components/complexes induced by heavy metal or any other abiotic stress-induced damage. It also scavenges the reactive oxygen species produced in plants exposed to heavy metal stress. There are several reports that the synthesis of proline has a significant role in regulating NAD(P)⁺ to NAD(P)H ratio (Shabnam *et al.*, 2014). Increased intracellular proline content therefore shows the ability of the organism to tolerate the stress due to salinity and copper.

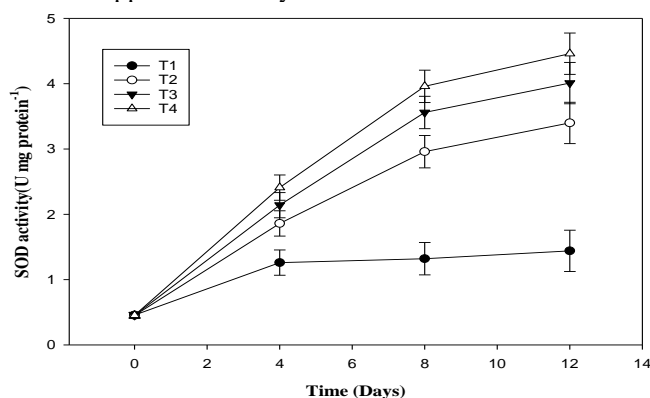


Fig. 8. Effect salinity and copper on the superoxide dismutase activity of the cyanobacterium *A. doliolum*.

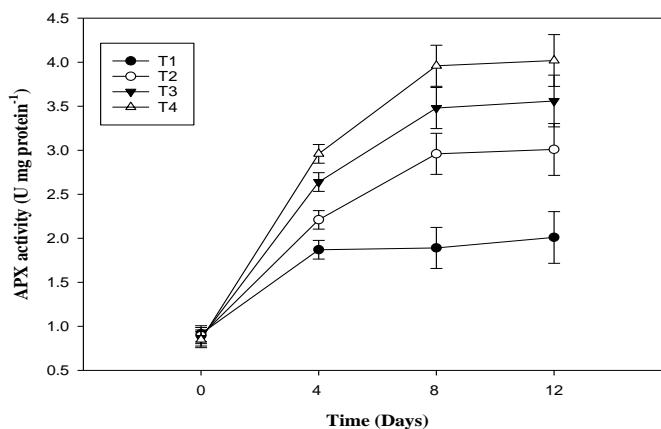


Fig. 9. Effect of salinity and copper on the ascorbate peroxidase activity of the cyanobacterium *A. doliolum*.

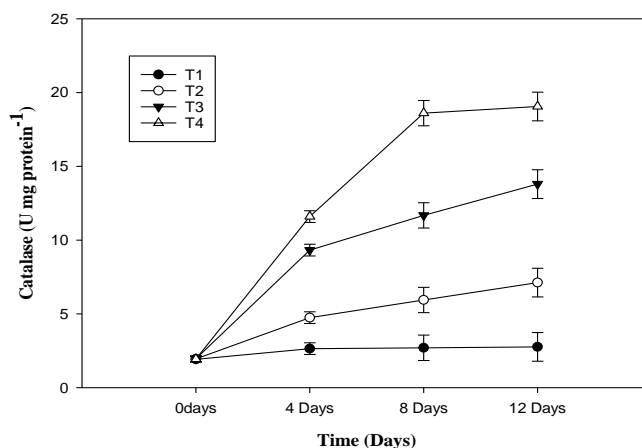


Fig. 10. Effect of salinity and copper on the catalase activity of the cyanobacterium *A. doliolum*.

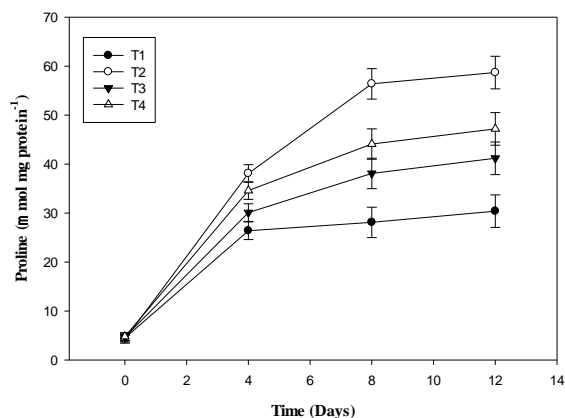


Fig. 11. Proline content of the cyanobacterium *A. doliolum* in response to salinity and copper treatment.

CONCLUSIONS

Cyanobacteria are significant bioinoculants in agriculture because of their specific ability to transform atmospheric nitrogen into ammonia using solar energy. Extreme temperatures, anaerobiosis, salt, drought, pesticides, and heavy metals are just a few of the environmental challenges that these microbes have evolved to survive. Abiotic stresses such as salinity and heavy metal pollution in rice fields, however, make it difficult to exploit them. In this work, the nitrogen-fixing cyanobacterium *Anabaena doliolum* is exposed to salt and copper, and their combined effects are investigated. The findings show that both stresses reduced growth as well as biological components including protein, chlorophyll, and phycocyanin. Furthermore, the *A. doliolum* average filament length and heterocyst frequency decreased as a result of the stress caused by salt and copper. Although salt treatment increased the activity of the enzyme responsible for assimilating nitrogen, nitrate reductase, the other two enzymes, glutamine synthetase and nitrogenase, demonstrated a decrease in activity. All three of the main nitrogen assimilation enzymes' activities were shown to be reduced by the copper treatment. The detoxification of free radicals depends heavily on antioxidant enzymes. Superoxide dismutase, ascorbate peroxidase, and catalase activities were increased when the cyanobacterium *A. doliolum* was individually and simultaneously exposed to salt and copper. Proline buildup by the cyanobacterium has had similar outcomes.

FUTURE SCOPE

The future scope of this research is multifaceted and holds promise across several domains. It offers avenues for advancing environmental stress management strategies, enabling the development of robust cyanobacterial strains for agricultural applications, and exploring the broader biotechnological potential of these microorganisms in areas such as bioremediation, biofuel production, and pharmaceuticals. Additionally, the study's insights into cyanobacterial adaptability have implications for ecosystem resilience, synthetic biology, and our understanding of climate change impacts on microbial communities.

Author contributions. Conceived and designed the analysis, Collected the data, Performed the analysis,

and wrote the paper: Shivarajan, C.S., and Dr. Gerard Abraham.

Acknowledgment: The authors acknowledge the Laboratory facilities supported at the Division of Microbiology, Indian Agricultural Research Institute, New Delhi, to conduct the experiments.

Conflict of Interest. None.

REFERENCES

- Aebi, H. (1984). Catalase in vitro. *In Methods in enzymology*, 105,121-126.
- Ali, I. M. (2023). The Harmful Effects of Pesticides on the Environment and Human Health: A Review. *Diyala Agricultural Sciences Journal*, 15(1), 114-126.
- Bantider, A., Haileslassie, A., Alamirew, T. and Zeleke, G. (2022). Soil and water conservation and sustainable development. In *Clean Water and Sanitation* (pp. 551-563). Cham: Springer International Publishing.
- Bates, L. S., Wadern, R. P. and Teare, I. D. (1973). Rapid estimation of free proline for water stress determination. *Plant Soil*, 39(1), 205-207.
- Bennett, A. and Bogorad, L. (1973). Complementary chromatic adaptation in a filamentous blue-green alga. *The Journal of Cell Biology*, 58(2), 419-435.
- Bhargava, P., Mishra, Y., Srivastava, A. K., Narayan, O. P. and Rai, L. C. (2008). Excess copper induces anoxygenic photosynthesis in *Anabaena doliolum*: a homology based proteomic assessment of its survival strategy. *Photosynthesis Research*, 96, 61-74.
- Burnap, R. L. (2015). Systems and photosystems: cellular limits of autotrophic productivity in cyanobacteria. *Frontiers in bioengineering and biotechnology*, 3, 1.
- Chini Zittelli, G., Lauceri, R., Faraloni, C., Silva Benavides, A. M. and Torzillo, G. (2023). Valuable pigments from microalgae: phycobiliproteins, primary carotenoids, and fucoxanthin. *Photochemical & Photobiological Sciences*, 1-57.
- Chittora, D., Meena, M., Barupal, T., Swapnil, P. and Sharma, K. (2020). Cyanobacteria as a source of biofertilizers for sustainable agriculture. *Biochemistry and biophysics reports*, 22, 100737.
- Dhindsa, R. S., Plumb-Dhindsa, P. A. M. E. L. A. and Thorpe, T. A. (1981). Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *Journal of Experimental Botany*, 32(1), 93-101.
- Dong, F., Lee, Y. S., Gaffney, E. M., Liou, W. and Minter, S. D. (2021). Engineering cyanobacterium with transmembrane electron transfer ability for

- bioelectrochemical nitrogen fixation. *ACS Catalysis*, 11(21), 13169-13179.
- Dwivedi, S. and Ahmad, I. Z. (2023). Evaluation of the effect of UV-B radiation on growth, photosynthetic pigment, and antioxidant enzymes of some cyanobacteria. *Environmental Research*, 218, 114943.
- Gamage, A., Gangahagedara, R., Gamage, J., Jayasinghe, N., Kodikara, N., Suraweera, P. and Merah, O. (2023). Role of organic farming for achieving sustainability in agriculture. *Farming System*, 1(1), 100005.
- Hooda, S., Malik, G., Saini, P., Grewall, A. and Pandey, V. C. (2023). Cyanobacteria as a potential bioasset for restoring degraded land. *Land Degradation & Development*, 34(12), 3435-3450.
- Huertás, M. J., López-Maury, L., Giner-Lamia, J., Sánchez-Riego, A. M. and Florencio, F. J. (2014). Metals in cyanobacteria: analysis of the copper, nickel, cobalt and arsenic homeostasis mechanisms. *Life*, 4(4), 865-886.
- Issa, A. A., Abd-Alla, M. H. and Ohyama, T. (2014). Nitrogen fixing cyanobacteria: future prospect. *Advances in biology and ecology of nitrogen fixation*, 2, 23-48.
- Kholssi, R., Lougraimzi, H. and Moreno-Garrido, I. (2023). Influence of salinity and temperature on the growth, productivity, photosynthetic activity and intracellular ROS of two marine microalgae and cyanobacteria. *Marine Environmental Research*, 186, 105932.
- Kirsch, F., Klähn, S. and Hagemann, M. (2019). Salt-regulated accumulation of the compatible solutes sucrose and glucosylglycerol in cyanobacteria and its biotechnological potential. *Frontiers in microbiology*, 10, 2139.
- Kumar, D. and Gaur, J. P. (2014). Growth and metal removal potential of a *Phormidium bigranulatum*-dominated mat following long-term exposure to elevated levels of copper. *Environmental Science and Pollution Research*, 21, 10279-10285.
- Kunui, K. K., Minj, R. A., Singh, S. S. and Vishwavidyalaya, G. G. (2020). Iron transportation and its impact on nitrate assimilation in the cyanobacterium *Anabaena sphaerica*: a promising bioaccumulator. *Plant Archives*, 20, 3701-3709.
- Kurutas, E. B. (2015). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition journal*, 15(1), 1-22.
- Lin, C. S. and Wu, J. T. (2014). Tolerance of soil algae and cyanobacteria to drought stress. *Journal of Phycology*, 50(1), 131-139.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin-phenol reagent. *Journal of Biological Chemistry*, 193, 265-275.
- Mackinney, G. (1941). Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry*, 140(2), 315-322.
- Mao, F., He, Y. and Gin, K. Y. H. (2020). Antioxidant responses in cyanobacterium *Microcystis aeruginosa* caused by two commonly used UV filters, benzophenone-1 and benzophenone-3, at environmentally relevant concentrations. *Journal of Hazardous Materials*, 396, 122587.
- Mishra, A. K. (2003). MSX-resistant mutants of *Anabaena* 7120 with derepressed heterocyst development and nitrogen fixation. *World Journal of Microbiology and Biotechnology*, 19, 675-680.
- Moisander, P. H., McClinton Iii, E. and Paerl, H. W. (2002). Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. *Microbial ecology*, 432-442.
- Nakano, Y. and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and cell physiology*, 22(5), 867-880.
- Pathak, J., Kumar, D., Singh, D. K., Ahmed, H., Kannaujiya, V. K. and Sinha, R. P. (2022). Ultraviolet radiation and salinity-induced physiological changes and scytonemin induction in cyanobacteria isolated from diverse habitats. *Biointerface Research in Applied Chemistry*, 12, 3590-3606.
- Rai, R., Singh, S., Rai, K. K., Raj, A., Sriwastaw, S. and Rai L. (2021). Regulation of antioxidant defense and glyoxalase systems in cyanobacteria. *Plant Physiology and Biochemistry*, 168, 353-372.
- Shabnam, N., Pardha-Saradhi, P. and Sharmila, P. (2014). Phenolics impart Au³⁺-stress tolerance to cowpea by generating nanoparticles. *PLoS One*, 9(1), e85242.
- Sharma, A., Rijal, R., Bhetwal, S., Das, S. and Malannavar, A. B. (2021). Cyanobacteria-A potential Gram-Negative Bacteria as an alternative for Fertilizers and Bioremediation. *Biological Forum – An International Journal*, 13(1), 590-601.
- Shokravi, S. and Bahavar, N. (2021). Growth and photosynthesis acclimated response of the cyanobacterium *Fischerella* sp. FS 18 exposed to extreme conditions: alkaline pH, limited irradiance, and carbon dioxide concentration. *Extremophiles*, 25(5-6), 493-500.
- Singh, H. (2018). Desiccation and radiation stress tolerance in cyanobacteria. *Journal of Basic Microbiology*, 58(10), 813-826.
- Singh, R. P., Yadav, P., Kujur, R., Pandey, K. D. and Gupta, R. K. (2022). Cyanobacteria and salinity stress tolerance. In *Cyanobacterial lifestyle and its applications in biotechnology* (pp. 253-280). Academic Press.
- Sorokin, C. (1973). Dry weight, packed cell volume and optical density. *Handbook of phycological methods: culture methods and growth measurements*, 321-343.
- Spiro, R. G. (1966). Analysis of sugars found in glycoproteins. In *Methods in enzymology*, 8, 3-26.
- Srivastava, A. K., Bhargava, P., Kumar, A., Rai, L. C. and Neilan, B. A. (2009). Molecular characterization and the effect of salinity on cyanobacterial diversity in the rice fields of Eastern Uttar Pradesh, India. *Saline Systems*, 5(1), 1-17.
- Swapnil, P. and Rai, A. K. (2018). Physiological responses to salt stress of salt-adapted and directly salt (NaCl and NaCl+ Na₂ SO₄ mixture)-stressed cyanobacterium *Anabaena fertilissima*. *Protoplasma*, 255, 963-976.
- Swapnil, P., Singh, M., Singh, S., Sharma, N. K. and Rai, A. K. (2015). Recombinant glycine betaine improves metabolic activities, ionic balance and salt tolerance in diazotrophic freshwater cyanobacteria. *Algal Research*, 11, 194-203.
- Xiao, X., Li, W., Jin, M., Zhang, L., Qin, L. and Geng, W. (2022). Responses and tolerance mechanisms of microalgae to heavy metal stress: A review. *Marine environmental research*, 105805.
- Yadav, P., Singh, R. P., Rana, S., Joshi, D., Kumar, D., Bhardwaj, N., Gupta, R. K. and Kumar, A. (2022). Mechanisms of stress tolerance in cyanobacteria under extreme conditions. *Stresses*, 2(4), 531-549.
- Yadav, R. K., Tripathi, K., Ramteke, P. W., Varghese, E. and Abraham, G. (2016). Salinity induced physiological and biochemical changes in the freshly separated cyanobionts of *Azolla microphylla* and *Azolla*

- caroliniana*. *Plant Physiology and Biochemistry*, 106, 39-45.
- Yang, Z., Li, J. L., Liu, L. N., Xie Q. and Sui N. (2020). Photosynthetic regulation under salt stress and salt-tolerance mechanism of sweet sorghum. *Frontiers in Plant Science*, 10, 1722.
- Zhang, X., Fan, L. and Roddick, F. A. (2013). Influence of the characteristics of soluble algal organic matter released from *Microcystis aeruginosa* on the fouling of a ceramic microfiltration membrane. *Journal of Membrane Science*, 425, 23-29.
- Zulkefli, N. S. and Hwang, S. J. (2020). Heterocyst development and diazotrophic growth of *Anabaena variabilis* under different nitrogen availability. *Life*, 10(11), 279.

How to cite this article: Shivanjan C.S. and G. Abraham (2023). Impact of Salinity and Copper Stress on the Growth and Physiology of Cyanobacteria: A Comparative Study. *Biological Forum – An International Journal*, 15(9): 104-113.