Role of Proline in Management of NaCl and pH Stress in Cyanobacteria

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Introduction

The role of nitrogen fixing cyanobacteria in enhancing soil fertility has been long known and is well documented[1-4]. Cyanobacteria contributes to overall soil health not only by its ability to perform biological nitrogen fixation but also because of its ability to produce polysaccharides and other bioactive compounds which has a growth stimulating effect on the vegetation/crop plants, as well as ensuring maintenance of soil quality and preventing erosion. Moreover it is also known that cyanobacteria increases the oxygen concentration of the environment in which they occur[5,6]. The general maintenance of soil fertility particularly in rice fields is attributed to the natural supply of assimilable nitrogen that is made available by the cyanobacteria through the process of biological nitrogen fixation[7]. Cyanobacteria generate cellular energy by harvesting light during the process of photosynthesis and utilizing this energy in the synthesis of carbon compounds required for their metabolic processes. Any alteration in the environment including change in pH and in salt concentration of the soil may have a direct negative impact upon photosynthesis and related metabolic processes of the cyanobacteria, including biological nitrogen fixation and overall performance of these organisms[8].

In nature microorganisms are constantly challenged by different factors and stresses including changes in temperature, moisture content (droughts and floods), salinity and so forth. Among these, salinity is known to be of major significance in limiting the growth and productivity of plants, eukaryotic microorganisms and bacteria[9]. Salinity posses to be a huge threat to agricultural productivity and approximately 7% of agricultural land are affected by high salt concentrations and the area is still increasing[10]. Owing to their considerable degree of salt tolerance, cyanobacteria have been applied in reclamation of saline and sodic lands with limited success[11,12]. Such a concept of biological soil reclamation using cyanobacteria was first advocated by Singh in 1961[4]. In trace amounts NaCl is known to act as a micronutrient to cyanobacteria and appears to be essential in certain metabolic processes[12-14]. However, elevated levels of the same may prove to be detrimental and result in inhibition of growth[15,16].

Cyanobacteria have evolved elaborate defense mechanisms in response to various environmental stresses that it encounters. Proline accumulation in response to stress is well documented in microbes, plants as well as marine invertebrates[17]. Proline is known to be an important indicator for stress tolerance and functions a stabilizer[18], a metal chelator[19], as an inhibitor of lipid peroxidation[20] and as a hydroxyl radical scavenger[21]. There are reports of proline being involved in ameliorating salinity stress as well as stress induced by drought situation and also its role a singlet oxygen scavenger in higher plants[22,23].

Since cyanobacteria play a prominent role in the maintenance of soil fertility and productivity, any loss in cyanobacterial biomass may result in noticeable consequences. Moreover, these organisms are now regarded as promising agents in bioremediation and in cleaning up the environment. Hence, it becomes imperative to study and investigate the effects of stresses, either singly or in combination on the growth and characters of some naturally occurring cyanobacteria. This work investigates the effect of salinity, changes in pH and a combined effect of both on the growth and proline accumulation in cyanobacteria.

Materials and methods

The effects of salt stress as well as pH fluctuations were assessed on some of the filamentous heterocystous cyanobacteria which were isolated from various locations in Meghalaya. These were then grown as axenic cultures in batches in nitrogen free $BG11_0$ medium (Rippka et al, 1979)[24] at 25±2°C and under a photon fluence rate of 50 μmol m^{-2} s^{-1}. The exponential cultures were used for all the experiments.

Initially, three organisms that were sampled for the present study include two belonging to the *Nostoc* species (N1 and N2) and one to *Anabaena* species (A1). Three sets of these were grown in different concentrations of NaCl (50,100,150.........500 mM) in 50ml conical flasks to find out which amongst them is most tolerant to salinity stress. This was done by estimating the chlorophyll *a* concentration every 48 hours in each flask[25] to compare their growth to a control culture. One *Nostoc* species (N1) that showed highest tolerance to salt stress was selected for further experiments. The soil and water bodies of Meghalaya distinctly show a wide variation in pH. Therefore, effect of pH and combined effect of pH and salt stress was also measured in this cyanobacterium. For this, pH range of 5, 6, 7, 8 and 9 was selected. Also, a combination of a constant salt concentration (500 mM) and variable pH was used to assess their combined effect on the organism. The constant salt concentration was based on the fact that beyond this any further increase in the salt concentration was lethal to the organism. The changes in proline content in the organism in response to the different stress conditions was estimated on the 8th day using the method described by Bates et al 1973[26] . Chlorophyll *a* concentration on the 8th day was also considered.

Results and Discussion

In case of all the three organisms the chlorophyll *a* content was found to decrease with increasing salt concentrations (Fig 1, 2 and 3). However, the unfavorable concentration of salt was different for the individual cyanobacterium, with N1exhibiting higher tolerance. The *Anabaena* sp. that was used for the present study appeared to be highly susceptible to salinity stress with maximal reduction in chlorophyll *a* content at 500 mM by the 8th day (Fig 3).

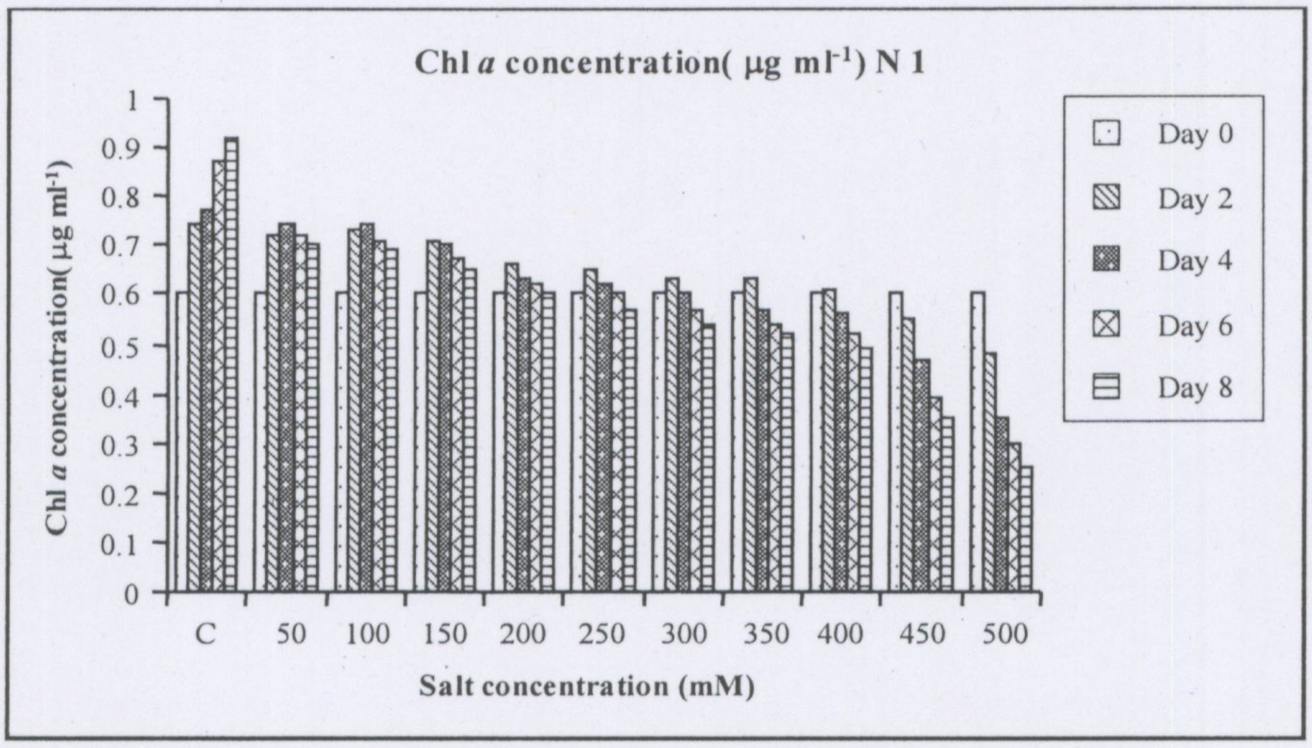

Fig 1: Effect of salt on growth of Nostoc sp.1. (C- Control condition)

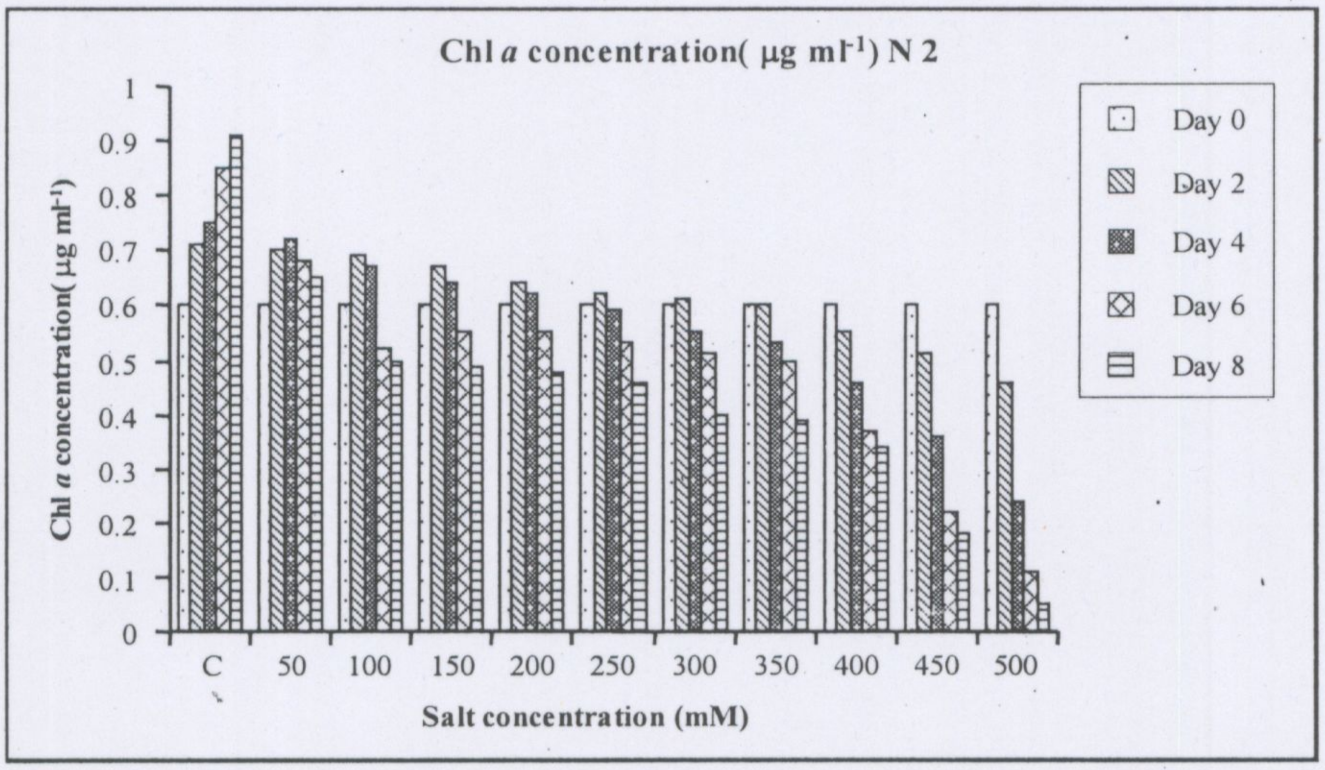

Fig 2: Effect of salt on growth Nostoc sp.2. (C- Control condition)

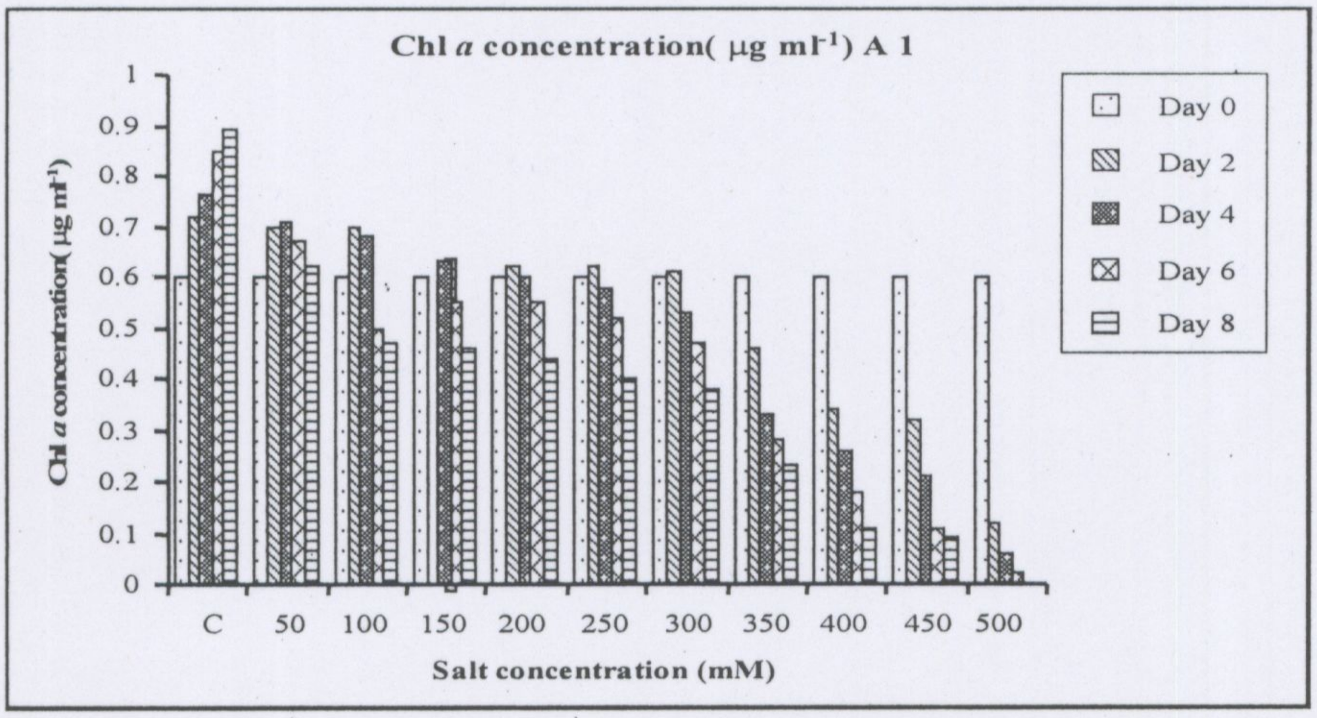

Fig 3: Effect of salt on growth Anabaena sp.1. (C- Control condition)

Studies with *N1*in different pH ranges revealed distinct changes in the growth pattern at pH 5 and 6. At higher pH of 8 and 9 there were no remarkable changes in growth. The cyanobacterium exhibited erratic growth at pH 5 and 6 and growth showed a decline in these pH with respect to time (Fig. 4). At pH 9, the exponential growth phase of the organism was shorter than the control (pH 7.6). However, the growth during this phase was comparable to the control. In combination with a constant salt concentration of 500mM, there was a steady decline in chlorophyll *a* concentration indicating low survival at pH 5 and 6. A similar behavior was also evident at the higher pH ranges of 8 and 9, although the effect was less drastic then that at lower pH (Fig. 5).

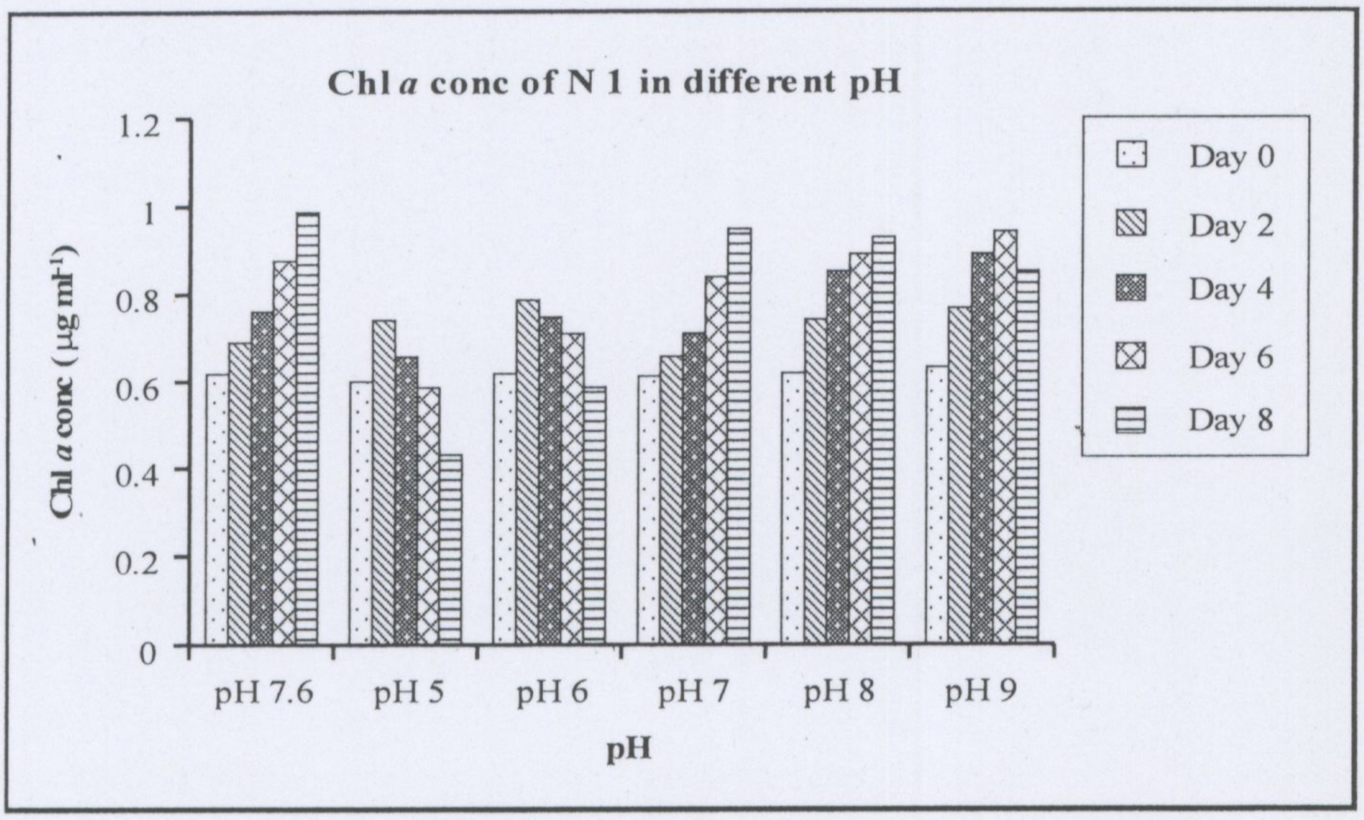

Fig 4: *Effect of pH on growth of Nostoc sp.1. (C- Control condition)*

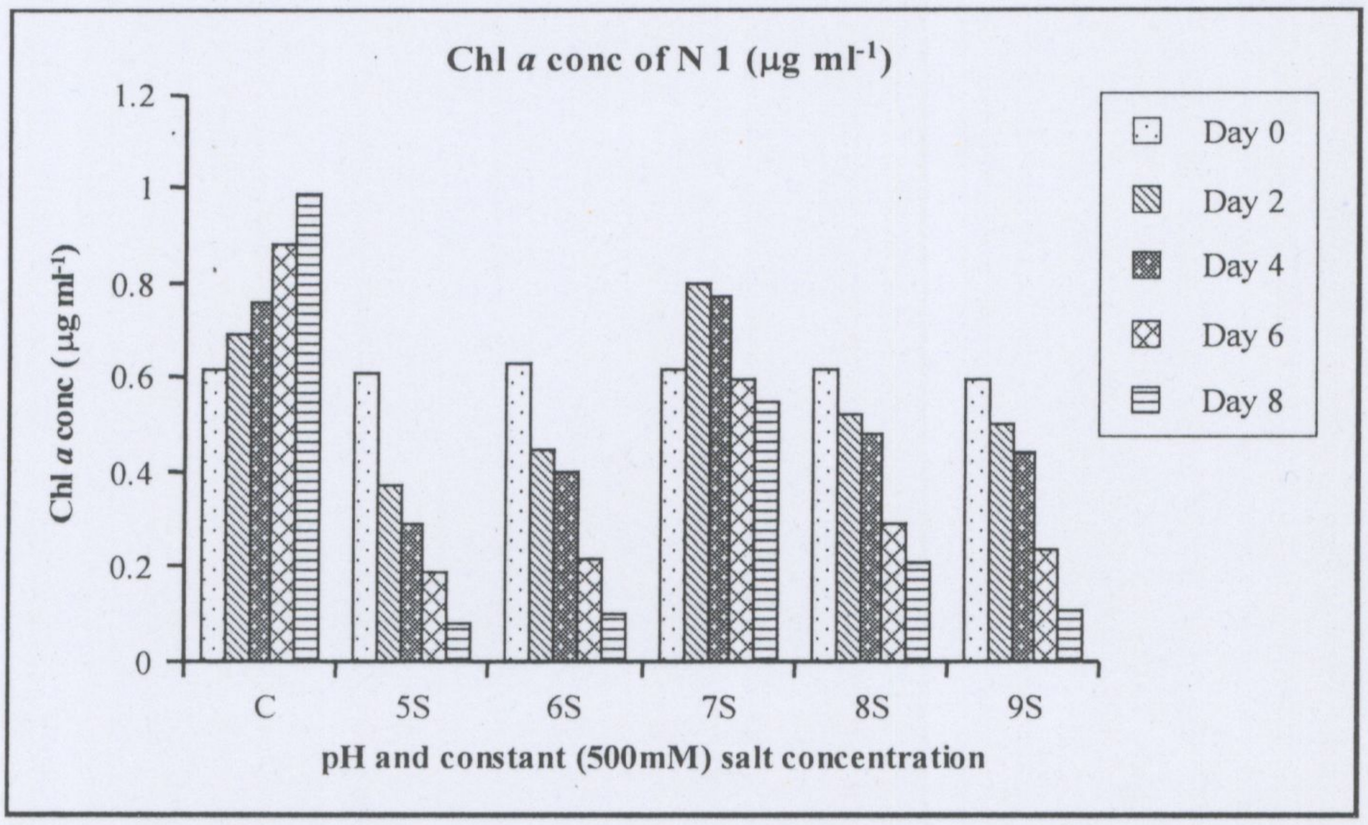

Fig 5: *Combined effect of pH and salt on growth of Nostoc sp.1. (C- Control condition; S-500 mM NaCl)*

Consistent with previous observations[8,27], it was found that with increase in stress, either as high salinity or unfavorable pH, there was an associated increase in proline content and a prominent decline in chlorophyll *a* concentration. High proline contents of 0.65 and 0.63μg g^{-1} were observed in pH range of 5 and 6 respectively followed by 0.63 and 0.61 μg g^{-1} for pH 9 and pH 8 as compared to 0.57 μg g^{-1} in unstressed condition in control culture (Fig 6). The proline content was found to be the highest in the case of the combined salt and pH stress (Fig 7). Proline was accumulated to an amount of 0.72 and 0.73 μg g^{-1} in pH 8 and 9 respectively which further increased in pH 5 and 6 to 0.92 and 0.76 μg g^{-1} respectively while in combination with salt.

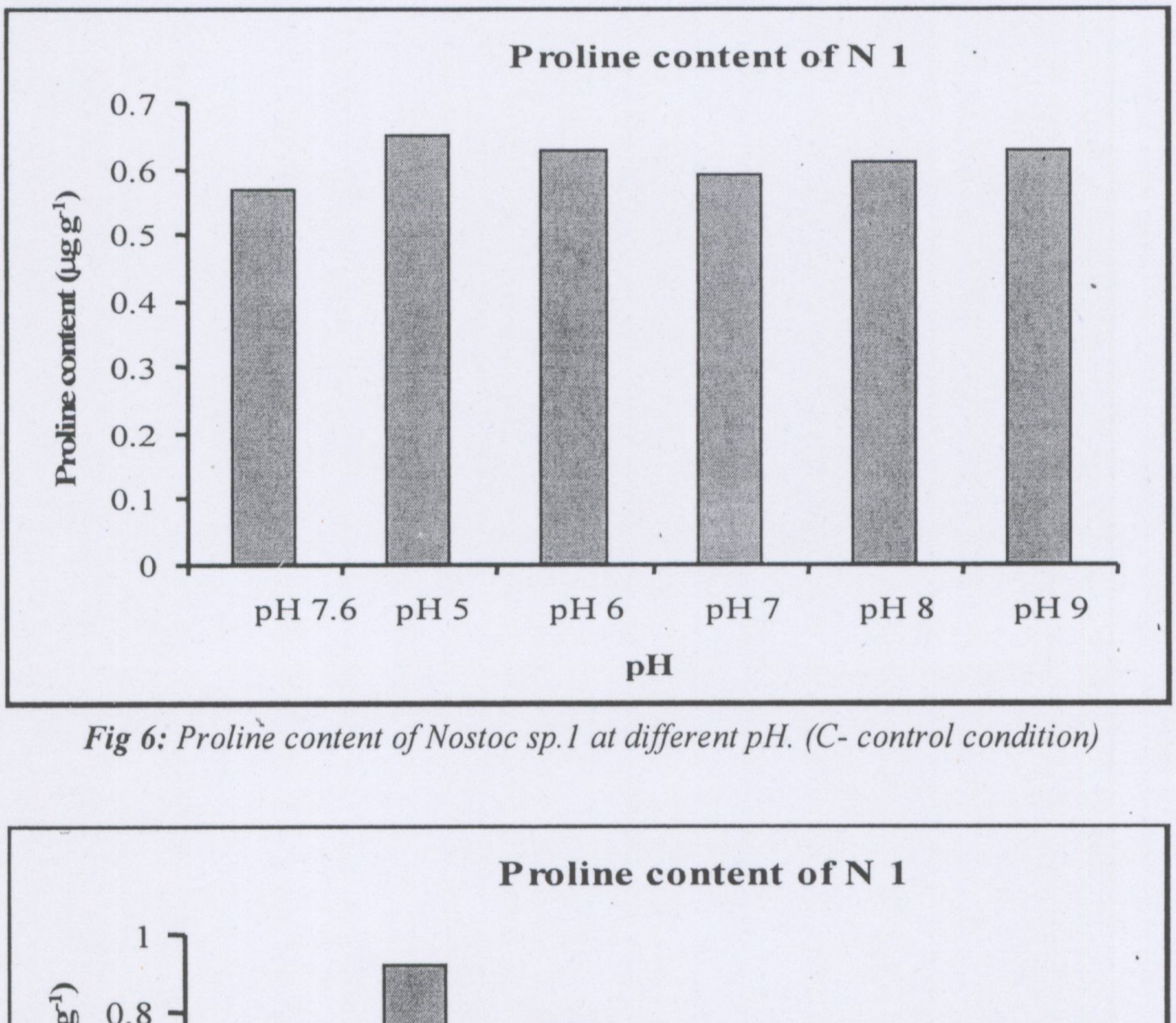

Fig 6: Proline content of Nostoc sp.1 at different pH. (C- control condition)

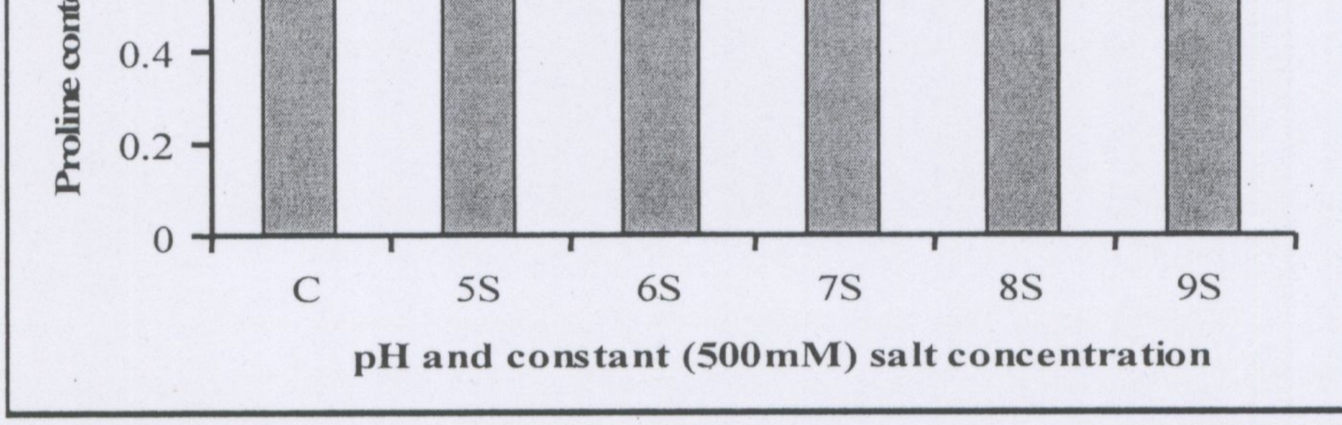

Fig 7: Proline content of Nostoc sp.1 under combined effect of salt and pH. (C- Control condition; S-500 mM NaCl)

There are extensive reports on stress induced proline accumulation in higher plants[28-32]. However, not much work has been done towards the assessment of the role of proline in cyanobacterial stress tolerance that may have a significant impact on their putative application in bioremediation as well as their use as biofertilizer in coastal areas. Wu et al (1998)[33] has reported increase in proline content under salt stress in *Chlorella*. It has been suggested that proline is involved in stabilizing folded protein structures[34]. Its role in stabilizing membranes by interacting with phospholipids[35] and being involved in osmotic adjustments[36] are also known. Stress induced by high salinity has a negative impact on the photosynthetic capacity and growth of cyanobacteria[37,38].

In the present study, it was observed that stress due to enhanced salinity and changes in pH in the immediate vicinity resulted in induction and accumulation in intracellular proline in the cyanobacterium. This finding is similar to earlier report on proline accumulation under stress conditions in the cyanobacterium *Westiellopsis prolifica*-Janet Strain-NCCU331 by Fatma et al., 2007. In these organisms, proline appears to be the stress-induced substance that helps in generating tolerance to adverse environmental stresses.

The potential of cyanobacteria as biofertilizer is immense. However, their susceptibility to environmental changes limits their optimum use. Carefully selected cyanobacterial strains with high nitrogen fixing capability that are resistant and/or tolerant to fluctuations in the surrounding environment could be ideal for future biofertilizer programme and also as bioremediators for reclaiming saline and sodic lands for agricultural usage. One way to identify such strains could be to look at their proline accumulating capacity under stressful environment. These strains with high proline synthesizing potential may prove to be an ideal candidate for bioremediation.

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References

1. Venkataraman G S, (1981). Blue-green algae: a possible remedy to nitrogen scarcity. *Curr. Sci.* 50:253-256.
2. De P K, (1939). The role of blue green algae in nitrogen fixation in rice fields, *Proc R Soc Lond, Ser B.* 127:121-139.
3. Watanabe A, Ito R & Konishi C, (1951). Effects of nitrogen fixing blue-green on the growth of rice plants. *Nature* (*Lond*). 168:748-749.
4. Singh R N, (1961). Role of blue green algae in the nitrogen economy of Indian agriculture, (Indian council of Agricultural research, New Delhi).
5. Nayak S, Prasanna R, (2007). Soil pH and its role in cyanobacterial abundance and diversity in rice field soils *J. Appl ecology and environmental research*, 5(2):103-113.
6. Mandal B, Vlek P L G, Mandal L N, (1998). Beneficial effect of blue green algae and Azolla excluding supplying nitrogen, on wetland rice fields: a review. *Biology and fertility of soil*, 27:329-342.
7. Matsuguchi T, (1979). Factors affecting heterotrophic nitrogen fixation in submerged rice soils. In: Nitrogen and rice. International Rice research Institute. Los Banos, Laguna, Philippines.
8. Chris A, Zeeshan Mohd, Abraham G, Prasad S M, (2006). Proline accumulation in *Cylindrospermum* sp. *Environment and Experimental Botany*. 57:154-159.
9. Inabha M, Sakamato A, Murata N, (2001). Functional expression in *E.coli* of low affinity and high affinity Na^+ (Li) $^+/H^+$ antiporters of Synechocystis. *J. Bacteriol*. 183:1376-1384.
10. Szabolcs I, (1994). In: Pessarkali, M. (Ed), Handbook of plant and Crop Stress. Mercel Dekker, New York. 3-11.
11. Singh R N, (1950). Reclamation of user lands in India through blue green algae. *Nature*. 165: 325-326.
12. Thomas J, Apte S K, (1984). Sodium requirement and metabolism in nitrogen fixing cyanobacteria. J.*Biosci*. 6:771-794.

13. Garcia-Gonzalez M, Sanchez-Maeso E, Quesada A, Fernandez E, (1987) Sodium requirement for photosynthesis and nitrate assimilation in am mutant of *Nostoc Muscorum. J Plant Physiol.* 127: 423-429.
14. Sanchez-Maeso E, Fernandez-pinas F, Garcia-Gonzalez M, Fernandez-Valiente E, (1987) Sodium requirement for photosynthesis and its relationship with dinitrogen fixation and external CO_2 concentration in cyanobacteria. *Plant Physiol.* 85: 585-587.
15. Apte S K, Reddy B R, Thomas J, (1987). Relationship between sodium influx and salt tolerance of nitrogen fixing cyanobacteria. *Appl. Environm. Microbiol.* 53: 1934-1939.
16. Jeanjean R, Matthijs H C P, Onana B, Havaux M, Joset F, (1993). Exposure of Cyanobacterium *Synechocystis* PCC 6803 to salt stress induces concerted changes in respiration and photosynthesis. *Plant Cell Physiol.* 43: 1073-1079.
17. Delauney A, Verma D P S, (1993). Proline biosynthesis and osmoregulation in plants. *Plant* J. 4:215-223.
18. Shah K, Dubey R S, (1998). Effect of Cadmium on Proline accumulation and Ribonuclease activity in rice seedlings: Role of Proline as a possible enzyme protectant. *Biol. Plant.* 40: 121-130.
19. Farago M E, Mullen W A, (1979). Plants which accumulate metals. Part IV. A possible copper-Proline complex from the roots of *Armeria maritima. Inorg Chim Acta.* 32:93-94.
20. Mehta S K, Gaur J P, (1999). Heavy metal induced proline accumulation and its role in ameliorating metal toxicity in *chlorella vulgaris. New Phytol.* 143:253-259.
21. Smirnoff N, Cumbes Q J, (1989). Hydroxyl radical scavenging activity of compatible solute. *Phytochemistry.* 28:1057-1060.
22. Kishore K P B, Hong Z, Miao G, Hu C-AA, Verma D P S, (1995). Over expression of Δ^1 – pyrroline – 5-carboxylate synthetase increase proline overproduction and confers osmotolerance in transgenic plants. *Plant Physiol.* 108:1387-1394.
23. Alia, Mohanty P, Matysik J, (2001). Effect of Proline on the production of singlet oxygen. *Amino Acid.* 21:195-200.
24. Rippka R, Dervelles J, Waterbury J B, Herdman M, Stanier R Y, (1979). Genetic assignment, strain histories and properties of pure culture of cyanobacteria. *J.Gen. Microbiol.* 111, 1-61.
25. Mackinney G, (1941). Absorption of light by chlorophyll solutions. *J.Biol.Chem.* 140: 315-322.
26. Bates L S, Waldren R P, Teare I D, (1973). Rapid determination of free proline for water stress studies. *Plant Soil.* 39:205-207.
27. Fatma T, Khan M A, Choudhary M, (2007). Impact of environmental pollution on cyanobacterial proline content. *J Appl Phycol.* 19:625-629.
28. Alia, Saradhi P P, (1991). Proline accumulation under heavy metal stress. *J Plant Physiol.* 138:554-558.
29. Bassi R, Sharma S S, (1993a). Changes in proline content accompanying the uptake of zinc and copper by *Lemna minor. Ann Bot.* 72:151-154.
30. Bassi R, Sharma S S, (1993b). Proline accumulation in wheat seedlings exposed to zinc and copper. *Phytochemistry.* 33:1339-1342.
31. Ashraf M, Harris P J C, (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant sci.* 166:3-16.
32. Mansour M N F, Salama K H A, Ali F Z M, Abou Hadid A F, (2005). Cell and plant responses to NaCl in *Zea mays* L. Cultivars differing in salt tolerance. *Gen. Appl. Plant Physiol.* 31: 29-41.
33. Wu J T, Hsieh M T, Kow L C, (1998). Role of Proline accumulation in response to toxic Copper in *Chlorella* sp. (Chlorophyceae) Cells. *J. Phycol.* 34: 13-117.
34. Low P S, (1985). Molecular basis of the biological compatibility of nature's osmolytes. In: Giller R ,Gilles-Baillen M (Eds) Transport processes, iono-and osmoregulation. *Springer*, Berlin. 469-477.
35. Rudolph A S, Crowe J H, Crow L N, (1986). Effects of three stabilizing agents Proline, Betaine, and Trihalose on membrane phospholipids. *Arch. Biochem. Biophys.* 245:134-143.
36. Watanabe S, Kojima K, Ide Y, Sasaki S, (2000). Effects of Saline and Osmotic Stress on Proline and Sugar Accumulation in *Populus euphratica* in vitro. *Plant Cell and Tissue Organ Cult.* 63: 199-206.
37. Vonshak A, Richmond A, (1981). Photosynthetic and respiratory activity in Anacystis nidulans adapted to osmotic stress. *Plant Physiol.* 68:504-505.
38. Schubert H, Fulda S, Hagemann N, (1993). Effects of adaptation to different salt concentrations on photosynthesis and pigmentation of the cyanobacteria *Synechocystis* sp. PCC 6083. *J. Plant Physiol.* 142: 291-295.