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Research Article

# MORPHOLOGICAL ANOMALIES IN *OSCILLATORIA WILLEI* DUE TO NaCl STRESS AND ITS ALLEVIATION BY GYPSUM

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**ABSTRACT**

Salinity is the presence of elevated levels of soluble salts in soil and water. It is an ever present threat to crop yield ant it severely limits agricultural productivity, because all crops are highly sensitive to elevated levels of NaCl. Therefore, more and more areas for agricultural use are disappearing. Hence, there is a pressing need to bring back such areas under cultivation and check their further spread and also to improve the fertility of saline soil. Algae including Cyanobacteria have attracted considerable attention in this respect. Though algae flourish well in the saline soil but, when salinity increases it has adverse effects on the algae. Therefore, in the present investigation an attempt has also been made to understand the impact of different concentrations of NaCl viz., 0.2, 0.4, 0.6 and 0.8M, besides control in one set and in the second set alga treated with different concentrations of NaCl in combination with gypsum (10mM) along with control over 30 days. The *Oscillatoria willei* exhibited multiple morphological anomalies like, light green cells at lower concentrations, while at higher concentrations the cells turned yellow and exhibited inward rolling of filaments with granulated protoplast, deformed cells with shrunken protoplast, elongated, zig-zag, ruptured cells and fragmentation of the filaments into hormogonia. It was speculated that some morphological anomalies might be due to unfavorable growth conditions created by salinity but inward rolling of filaments indicated an adaptation strategy of the alga to protect itself and survive successfully under saline stress. However, the addition of Gypsum to the culture medium protected cells against salt stress and alleviated the adverse effects of NaCl.



## INTRODUCTION

Among the various types of stress, saline stress is a challenging environmental stress for organisms to overcome. Salinity is the presence of elevated levels of soluble salts in soil and water. It is an ever present threat to crop yield especially in the countries where irrigation is an essential aid to agriculture. Abrol *et al.,* (1988) estimated that, the world as a whole is losing at least 3 hectares of fertile land every minute due to salinization/ sodification. It is increasing gradually in many parts of the world, particularly in arid and semiarid areas. According to Evelin *et al*. (2009) out of 1.5 billion hectares of cultivated land around the world, about 77 million hectares is affected by excess salt content.

According to Jadhav *et al*. (2010) nearly 40% of world's surface has salinity problem. However, salts are necessary components of soil and many salts are essential plant nutrients. But, when salts present in relatively high amounts the plant growth is adversely affected. Hence crop production has been seriously hampered by the soil salinity and this problem is becoming more serious every year. Therefore, it is needed to check their further spread and also to improve the fertility of saline soil which is utmost essential from the agricultural point of view. To counteract the adverse effects of saline stress many attempts have been undertaken by using chemical amendments. However, biological means of reclamation is another approach.

In this respect, Cyanobacteria and Algae have attracted considerable attention because they are inhabitants of biotopes characterized by changing salinities and can serve as model organisms for better understanding of salt acclimation in the more complex physiological processes of higher plants (Bohnert *et al.,* 1996 and 1998; Fogg,2001). The algae including cyanobacteria have tremendous capacity to increase the fertility of soil; they regulate the pH of soil, fix atmospheric nitrogen, increase moisture content of soil, suppress plant pathogens and promote plant growth. They offer an economically attractive and ecologically sound alternative to chemical fertilizers for reaching the ultimate goal of increased productivity, especially in rice cultivation All these features enhanced to employ them in the biotreatment and bioremediation of salinity problems**.** Though

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cyanobacteria thrive well in saline soil but higher concentrations adversely affect the cyanobacteria. Hence it is essential to understand successful development of algalization technology especially under salt affected soils. Hence, the present study also concentrated to asses weather addition of gypsum ($CaSO_4$. 2 $H_2O$) could alleviate the adverse effect of salinity on algae. The gypsum was selected because it is an excellent and inexpensive source of calcium, dissolves at high pH and does not contain elements or compounds that might interfere with reclamation. Gypsum is soluble in water (2.5 g/l) or approximately 200 times greater than lime ($CaCO_3$). This makes the calcium in gypsum more mobile than the calcium in lime and allows it to move more easily through the soil profile. The sulphate in gypsum is not likely to be a problem for crops, even though it is applied in excess of plants need. It improves plant growth through ameliorating deficiencies by providing plant nutrients (Liming and Warren, 2011).

Therefore, in the present investigation an attempt has also been made to understand the impact of different concentrations of NaCl and NaCl in combination with gypsum on the morphological characteristics of *Oscillatoria willei.*

## MATERIALS AND METHODS

The blue green alga *Oscillatoria willei* BDU 141621 was obtained from National Facility for Marine Cyanobacteria (NFMC) Tiruchirapalli. The ASN III medium (Rippka *et al.,* 1979) at pH 7.5 was best suited for the growth of the alga *O. willei.*

The present work was planned in such a way that, in one set, the cultures of *Oscillatoria willei was* treated with different concentrations of NaCl such as 0.2, 0.4, 0.6, 0.8 M besides control. In another set, alga was treated with different concentrations of NaCl in combination with gypsum (10mM) along with control. The experiments were carried out in 500 ml sterile Erlenmeyer flasks containing 300 ml of nutrient media. To this, 30 ml of exponentially growing *O. willei* was harvested, washed, centrifuged and inoculated separately into each of these flasks containing the media **b**esides control and kept for observation to 30 days. The samples were drawn periodically during growth ($10^{th}$, $20^{th}$ and $30^{th}$ day) from control and different concentrations of NaCl and NaCl along with gypsum and were subjected for the Photomicrographic observations for the analysis of morphological characteristics of *Oscillatoria willei.*

## RESULTS

Photomicrographic observations of blue green alga *Oscillatoria willei* exposed over 30 days to different concentrations of NaCl i.e., 0.2, 0.4, 0.6 and 0.8 M along with control exhibited distinct multiple morphological variations. The variations were not pronounced at lower concentrations but at higher concentrations distinct anomalies were seen (Figure-1 & 2).

The filaments of *Oscillatoria willei* at control were found to be healthy, normal and bluish green in color (Fig.-1 A). Whereas, at 0.2 M NaCl inward rolling of filaments was initiated with granulated protoplast (Fig.-1 B), it was interesting to note that at 0.4 M NaCl inward rolling was much conspicuous & the cells were with shrunken protoplast (Fig.-1 C&D), at 0.6 M NaCl cells became colorless, elongated, enlarged, zig zag and irregular (Fig-2 E & F). However at 0.8 M NaCl elongated ruptured cells and fragmentation of the filaments into hormogonia with disrupted and damaged cells were noticed (Fig-2 G &H).

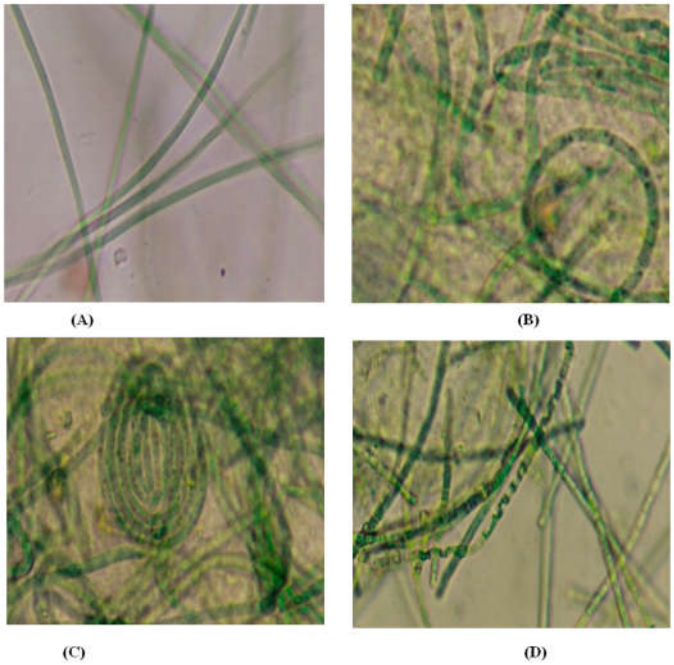


**Figure 1** Results on Photomicrographic observations of *Oscillatoria willei* exposed to different concentrations of NaCl revealed distinct morphological anomalies

A: *Oscillatoria willei*- Control - Filaments showing healthy and normal cells
B: *Oscillatoria willei* - 0.2M NaCl - Filaments showing initiation of inward rolling with granulated protoplast.
C: *Oscillatoria willei* - 0.4 M NaCl - Filaments showing increased inward rolling of with granulated protoplast.
**D**: *Oscillatoria willei*- 0.4 M NaCl – Filaments showing cells with shrunken protoplast

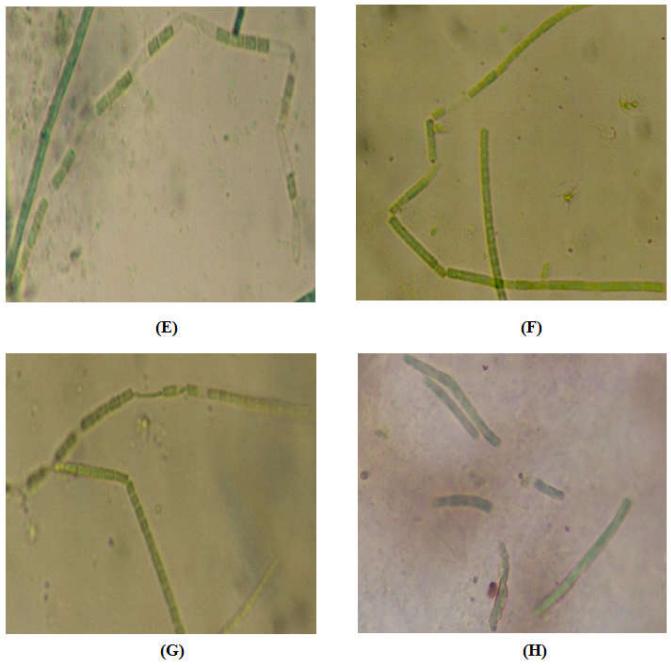


**Figure 2** Results on Photomicrographic observations of *Oscillatoria willei* exposed to different concentrations of NaCl revealed distinct morphological anomalies

E: *Oscillatoria willei*- 0.6 M NaCl - Filaments showing elongated, enlarged and colorless cells
F: *Oscillatoria willei*- 0.6 M NaCl - Filaments showing enlarged, irregular zig zag cells
G: *Oscillatoria willei*- 0.8 M NaCl - Filaments showing elongated, enlarged ruptured cells
H: *Oscillatoria willei* - 0.8M NaC l - Fragmentation of the filaments into hormogonia with disrupted and damaged cells.

In fact the alga exposed over 30 days to different concentrations of NaCl in combination with gypsum (10 mM), following changes were noticed. The addition of Gypsum to the culture medium exhibited, the filaments with reduced inward rolling and slight recovery in the cells and morphological anomalies gradually reversed by the addition of Gypsum (Fig- 3)

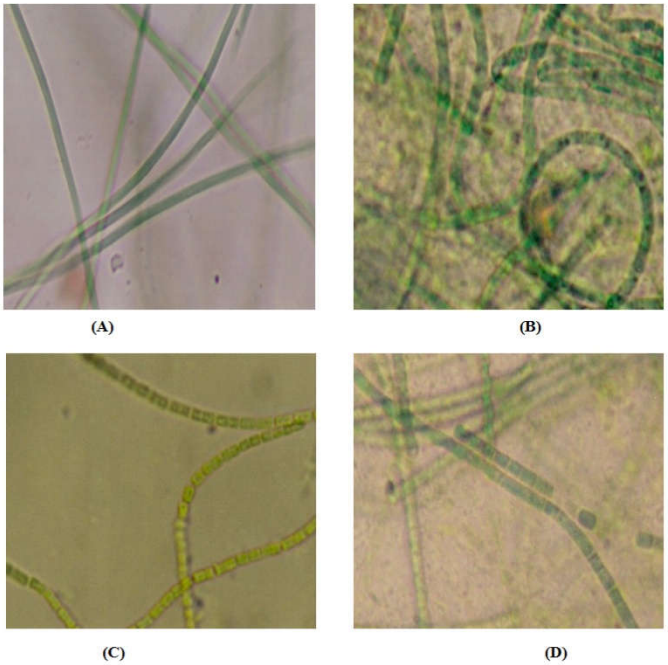


**Fig 3** Photomicrographic observations of *Oscillatoria willei* exposed to different concentrations of NaCl in combination with gypsum

A: *Oscillatoria willei*- Control - Filaments showing healthy and normal cells
B: *Oscillatoria willei* - 0.4 M NaCl + GP - Filaments with reduced inward rolling.
C & D: *Oscillatoria willei*- 0.6 & 0.8 M NaCl + GP – Filaments showed slight recovery in the cells

## DISCUSSION

The *Oscillatoria willei* exposed to different concentrations of NaCl over 10, 20 and 30 days of durations exhibited distinct morphological variations and anomalies.

Similarly Rai and Abraham (1993) noticed that, with increase in NaCl concentration (beyond 200 mM), the filaments of *Anabaena doliolum* were shorter with less heterocysts. In *A. halophytica* significant increase in the diameter of cells under increased NaCl stress (2.0 M) has been observed by Surasak *et al.* (2009). Further they reported that the shape of cells grown under stress conditions was longer than the cells grown under normal conditions. They also suggested that NaCl might arrest the formation of the shape of cells and cells under salt stress were highly heterogeneous with respect to their size and shape. Affenzeller *et al.,* (2009) indicated retraction of the chloroplast as well as plasmolysis the cells of *Micrasterias denticulata* under NaCl stress. According to Fodorpataki and Bartha (2004) shrunken chloroplast is due to a partial desiccation and a loss of turgor. The morphological disturbances may be due to differences in NaCl concentrations in the cells and in the extra cellular medium. Ferjani *et al*. (2003) have observed that under NaCl stress the cells were unable to complete cell division and lysed. They also indicated that the high concentrations of NaCl inhibit cell division without significantly affecting cell growth. Suggesting that NaCl might arrest the formation of the septum and the separation of daughter cells. It has been investigated that, the salt shock due to high concentrations of NaCl increased the cell size in halotolerant bacterium *Staphylococcus aureus* (Vijaranakul *et al.,* 1995 and 1997).

Our results also revealed that, addition of Gypsum efficiently reversed anomalies induced by NaCl stress. Our results are in accordance with the findings of ISSA (1996) who carried out an investigation on the role of calcium in the stress response of *Dunaliella bardawil* and observed readaptation of cells in the presence of 1, 5 and 10 mM of $Ca^{+2}$. Ferjani *et al.* (2003) stated that in *Synechocystis* sp. the structural aberrations and morphological abnormalities induced by NaCl stress were efficiently reversed by the addition of Glucosylglycerol to the growth medium.

## CONCLUSION

It was speculated that, the anomalies like elongated colorless cells, ruptured and irregular zig-zag cells, fragmentation etc. might be due to reduced metabolic activities of the cells and unfavorable growth conditions created by salinity.

Further, the variations like inward rolling of filaments indicated adaptation strategy of *Oscillatoria* to protect itself and survive successfully under saline stress.

The present study revealed that the application of gypsum could alleviate the adverse effects of salinity and increase the tolerance of alga to salinity.

## References

Abrol, I. P, Yadav, J. S. P. and Massoud, F. I., 1988. Salt-affected soils and their management. *FAO* Soils Bulletin, Soil Resources, Management and Conservation Service, FAO Land and Water Development Division, 39:131.

Affenzeller, M. J., Dareshouri, A., Andosch, A., Lutz, C., LutzMeindl, U., 2009. Salt stress-induced cell death in the unicellular green alga *Micrasterias denticulata*. *Journal of Experimental Botany,* 60: 939–954.

Ali Ferjani, Laszlo Mustardy, Ronan Sulpice, Kay Marin, Iwane Suzuki, Martin Hagemann and Norio Murata, 2003. Glucosylglycerol, a Compatible Solute, Sustains Cell Division under Salt Stress. *Plant Physiology,* 131: 1628–163.

Bohnert, H. J. and Jensen, R. G., 1996. Metabolic engineering for increased salt tolerance- the next step. *Aust. J. Plant Physiol.,* 23: 661-66.

Bohnert, H. J. and Sheveleva, E., 1998. Plant stress adaptations-making metabolism move. *Curr. Opin. Plant. Biol.*, 1: 267-274.

Evelin, H., Kapoor, R., Giri, B., 2009. Arbuscular mycorrhizal fungi in alleviation of salt stress. *J. Ann. Bot.,* 104(7): 1263-1280.

Fogg, G. E., 2001. Algal adaptation to stress - some general remarks, in Rai, L. C., Gaur, J. P. (Eds.), Algal adaptation to environmental stresses, Springer, Berlin, 1 – 19.

Fodorpataki, L. and Bartha, C. 2004. Salt stress tolerance of a freshwater green alga under different photon flux densities. Studia. Universitatis. Babes. Bolyat. Biologia. XLX, 2.

Issa, A. A., 1996. The Role of Calcium in the Stress Response of the Halotolerant Green Alga *Dunaliella bardawil* Phyton (Horn, Austria), 36: 295-302

Jadhav, G. G., Salunkhe, D. S., Nerkar, D. P., Bhadekar, R. K., 2010. Isolation and characterization of salt-tolerant nitrogen-fixing microorganisms from food. *J. Eur. Asia. Bio. Sci.*, 4: 33-40.

Liming Chen and Warren A. Dick, 2011. The Ohio State University. Gypsum as an Agricultural Amendment: General Use Guidelines.

Rai, A. K. and Abraham, G., 1993. Salinity tolerance and growth analysis of the cyanobacterium *Anabaena doliolum*. *Bulletin of Environmental contamination and Toxicology,* 51: 724-731.

Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., Stanier, R. Y., 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria, *Journal of General Microbiology,* 111: 1–61.

Surasak Laloknam, Aporn Bualuang, Bongkoj Boonburapong, Vandna Rai, 2009. Salt stresses induced glycine-betaine accumulation and Amino acid and fatty acid changes in cyanobacterium *Aphanothece halophytica. International Conference on the Role of Universities in Hands-On Education ajamangala University of Technology Lanna, Chiang-Mai, Thailand* 23-29.

Vijaranakul, U., Nadakavukaren, M. J., de Jonge, B. L. M., Wilkinson, B. J. and Jayaswal, R. K., 1995. Increased cell size and shortened peptidoglycan interpeptide bridge of NaCl- stressed *Staphylococcus aureus* and their reversal by glycine betaine. *Journal of Bacteriology,* 177: 5116 – 5121.

Vijaranakul, U., Nadakavukaren, M. J., Bayles, D. O., Wilkinson, B. J. and Jayaswal, R. K., 1997. Characterization of an NaCl-sensitive *Staphylococcus aureus* mutant and rescue of the NaCl-sensitive phenotype by glycine betaine but not by other compatible solutes. *Applied Environmental Microbiology,* 63:1889–1897.

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