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Salinity as a factor affecting the physiological and biochemical traits of *Scenedesmus quadricauda*

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ABSTRACT

A study was conducted to investigate the impact of salinity (NaCl) on the physiological and biochemical traits of an algal species. In order to determine the impact of NaCl, an algal species *Scenedesmus quadricauda* was exposed to different concentrations of NaCl ranging from 0.2-1.0mM alongwith control over a period of 15 days. It was found that the algal biomass yield was highest at 0.2mM NaCl concentration as compared to control and then it subsequently decreases with increase in NaCl concentration. Initial increase of NaCl concentration from 0.0-0.2 mM decreased the lipid accumulation from 6.75 to 6.12 % dcw. Total chlorophyll content of the algal species decreases as the salt concentration was increased when compared with control. It is interesting to note that carbohydrate content increased in all the concentrations of NaCl as compared to control for the culture studied. Alga exhibited initial decline in the total protein content at NaCl concentrations of 0.2 and 0.4mM and thereafter at NaCl concentration of ≥0.6mM the quantity of total protein content increases as compared to control. The results indicated that algal species showed diverse response to salinity stress.

Keywords: Biomass, Carbohydrate, Chlorophyll, Lipid, NaCl, *Scenedesmus*

INTRODUCTION

Plant cells are generally able to live within a certain range of enhanced salt concentrations or changing salinities, since most probably all life originated in the oceans, i.e. a highly saline environment. However, during evolution, the degree of salt resistance and salt tolerance became very divergent among the present day aquatic organisms. Algae (and cyanobacteria) have attracted considerable attention in this respect, since they are inhabitants of biotopes characterized by changing salinities and can serve as model organisms or a better understanding of salt acclimation in the more complex physiological processes of higher plants (Bohnert and Jensen 1996; Bohnert and Sheveleva 1998; Fogg 2001). Salinity is a serious agro-economical problem which leads to metabolic alterations and graded reduction in the plant growth in terms of all the growth parameters leading to severe crop losses. It is also considered an important ecological variable in the fresh water and marine environment. Salinity has been suggested as being a controlling factor for blooms of cyanobacteria in estuaries and is considered as one of the major constraints on species diversity and productivity of natural population of algae (Booth and Beardall 1991; Chen and Plant 1999). Particularly in estuarine water planktonic algae are often subjected to widely fluctuating salt concentrations (Guillard 1962; Moisander *et al.*,2002). Such changes in the salinity of water often affect the growth, metabolism and photosynthesis of phytoplanktons (Moisander *et al.*,2002; Lartigue *et al.*,2003). Salinity stress and unfavorable light conditions are main limiting factors of plant productivity both in aquatic and terrestrial, natural and anthropically modified environments (Fodorpataki and Bartha 2004). The biomass of algal species mainly comprises of protein, carbohydrate, and lipids (Spolaore *et al.*,2006). Mainly due to the high protein content some algal species like *Chlorella* sp. are widely used as health food for human beings and as animal nutritional supplements (Spolaore *et al.*,2006; Metting 1996; Guil-Guerrero *et al.*,2004) and finds its potential applications in food, cosmetic, and pharmaceutical industries (Singh *et al.*,2005). The total lipid contents of microalgae varied from 1 to 70% of the dry cell weight (Metting 1996). The lipid present in microalgae is mainly in the form of esters of glycerol and fatty acid, which are suitable for producing biodiesel (Chisti 2007; Chiu *et al.*,2009).

A number of factors are known to influence the lipid content of microalgae, such as nitrogen (Illman *et al.*,2000) and silicon (Lynn *et al.*,2000) deficiency, phosphate limitation (Reitan *et al.*,1994), high salinity (Rao *et al.*,2007). Light intensity (Kojima and Zhang 1999) and iron content of the medium also affect algal growth (Liu *et*

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al.,2008). The composition of intracellular lipid of microalgae was reported to change in response to environmental salinity. Increase of NaCl concentration from 0.4M to 4M increased saturated and monounsaturated fatty acids in *Dunaliella* cells isolated from an Antractic hypersaline lake (Xu and Berdall 1997), while polyunsaturated fatty acid decreased. The fatty acid composition of polar lipid in *Dunaliella salina* Teodoresco was affected significantly by the change in NaCl concentration (Peeler *et al.*,1989). The percentage of saturated fatty acid decreased as the concentration of NaCl increased, while the percentage of highly unsaturated fatty acid increased (Fujii *et al.*,2001). Salt might have a direct effect upon processes involved in electron transport and/or photophosphorylation and result in a decrease in the quantum efficiency of photosynthesis (Seeman and Critchley 1985). In this connection El-Sheekh and Omar (2002) indicated that ATP is severely affected by salt stress in *Chlorella vulgaris*, however NADPH was not affected. Other studies (Sharma and Hall 1991) showed that the light saturating rate of CO_2 uptake and maximum quantum yield decreased with increasing salt concentrations in barley and sorghum seedling leaves. Shen and Katoh (1991) working with chloroplasts from spinach localized the NaCl effect at photosystem II. However, there are several studies on the effect of salt stress on microorganisms, particularly in freshwater algae dealing with the inhibitory effect of NaCl on oxygen evolution, chlorophyll fluorescence, the photochemistry and function of photosystem II. (Joset *et al.*,1996; Murakami *et al.*,1997; Gonzales-Moreno *et al.*,1997; Lu and Vonshak 1999; Lu and Zhang 1999; Lu *et al.*,1999; Lu and Zhang 2000; Lu and Vonshak 2002).

The ability of cells to survive and flourish in saline environment under the influence of osmotic stress has received considerable attention. Cells develop many adaptive strategies in response to different abiotic stresses such as salinity, dehydration, cold and excessive osmotic pressure. Against these stresses, cells adapt themselves by undergoing different mechanisms including changes in morphological and developmental pattern as well as physiological and bio-chemical processes (Bohnert *et al.*,1995). In the present study impact of NaCl concentration on the physiological and biochemical attributes of an algal species *Scenedesmus quadricauda* has been evaluated.

MATERIALS AND METHODS

The alga used in the present study was isolated from the fresh water pond of village Ladwi, Haryana (India). Pure culture of *Scenedesmus quadricauda* was obtained by repeated streaking and plating at pH 7.0±1 using standard isolation and culturing techniques in BG-11 medium. The composition of medium is as (gl^{-1}): $NaNO_3$ 1.5; K_2HPO_4 0.04; $MgSO_4 \cdot 7H_2O$ 0.075; $CaCl_2 \cdot 2H_2O$ 0.036; Citric acid 0.006; Ferric ammonium citrate 0.006; EDTA (disodium salt) 0.001; Na_2CO_3 0.02; and 1ml of trace elements solution having composition (gl^{-1}): H_3BO_3 2.86; $MnCl_2 \cdot 4H_2O$ 1.81; $ZnSO_4 \cdot 7H_2O$ 0.222; $NaMoO_4 \cdot 2H_2O$ 0.39; $CuSO_4 \cdot 5H_2O$ 0.079; Co $(NO_3)_2 \cdot 6H_2O$ 0.0494. Cultures were maintained at a light intensity of 3000 lux using cool fluorescent tubes at 25±1°C in culture room. Further to study the impact of NaCl, the algal species was grown in BG-11 medium modified with varying salt concentrations (0.2mM to 1.0mM). Appropriate dosing was made from 1000mM NaCl stock solution. To study the effect of salinity on *Scenedesmus quadricauda* the experiments were carried out in 250ml Erlenmeyer flasks each containing 100 ml of BG-11 medium incubated at 25°C in an orbital shaker set to120 rpm in BOD incubator cum shaker for 15 days and control culture in BG-11 media was also run parallel. The medium and flasks were sterilized in an autoclave for 20 min at 121°C in order to prevent any contamination. One ml ten days fresh inoculums was used for all the experiments, keeping care that the cultures do not get too old and reach late stationary phase as depletion of nutrients and accumulation of waste products causes deterioration and damage to the cultures. The samples were drawn on 15th day and were subjected to analysis for various physiological and biochemical parameters. Chlorophyll content of the algae was estimated spectrophotometrically at 650 and 665nm by hot extraction method of Tandeau de Marsac and Houmard (1988). The chlorophyll content was calculated using the following formula and was expressed as mg/ml:

$$Chlorophyll\ (mg/ml) = 2.55 \times 10^{-2} OD_{650} + 0.4 \times 10^{-2} OD_{665}$$

Protein content was estimated at 660nm by the method of Lowry *et al.*,(1951). Carbohydrate was determined by anthrone reagent method (Dubois *et al.*,1956). Concentration of carbohydrate was determined using standard curve prepared by taking graded concentrations of glucose.

Total lipids were extracted by mixing methanol-chloroform (2:1.5 v/v) with the algal samples using slightly modified version of Bligh and Dyer's method (Bligh and Dyer 1959). Algal biomass was collected by centrifuging 50 ml of the algal culture at 5000 rpm for 10 min. Supernatant was discarded and the algal biomass was suspended in 2 ml methanol and 1.5 ml chloroform and incubated for 24 hrs at 25°C. After 24 hrs of incubation the mixture was agitated in a vortex for 2 min, 1.5 ml of chloroform was again added and the mixture was again agitated in a vortex for 1 min and the mixture was further agitated in a vortex again for 2 min after addition of 1.8 ml of distilled water. The mixture was separated in layers by centrifugation for 10 min at 2,000 rpm. The lower lipid layer was separated carefully using the eppendorf micropipette and transferred into a clean previously dried (104°C) and weighed 15 ml glass centrifuge tube. The chloroform phase was evaporated near to dryness in a water bath at 70°C and the residue was further dried at 104°C for 30 minutes. The weight of the glass centrifuge tube was again recorded and residual mass (lipid contents) was expressed as % dry cell weight (dcw). All the experiments were carried out at least in duplicate.

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RESULTS AND DISCUSSION

The results indicated that, the algal biomass yield was highest at 0.2mM NaCl concentration as compared to control and then it subsequently decreases with increase in NaCl concentration as shown in Table 1 and Fig. 1. The initial increase of NaCl concentration from 0.0-0.2 mM decreased the lipid accumulation from 6.75 to 6.12 % dcw. There was very little difference in lipid content i.e. 6.75 and 6.65 % dcw, when cells were grown in control culture and culture containing 0.8mM NaCl, respectively (Table 1 and Fig. 2). The increase in lipid content at higher NaCl concentration may be due to adaptation under stress conditions which help in accumulation of lipid content and these results are in accordance with the finding of Takagi and his coworkers (Takagi *et al.*,2006) in *Dunaliella* cells. Total chlorophyll contents decreases as the salt concentration is increased from 0.2 to 1.0mM when compared to control for the culture studied (Table 1 and Fig. 3).

Table 1: Values of various physiological and biochemical parameters of *Scenedesmus quadricauda* at different salt/ NaCl concentrations (mM) on 15th day of growth

Salt (NaCl) concentration in mM	Algal Biomass (g/l)	Lipid content (%dcw)	Total Chlorophyll (µg/ml)	Total Carbohydrate (mg/ml)	Total Protein (mg/ml)
Control (0.0)	1.4807	6.756757	20.0405	0.04884	0.018159
0.2	1.588438	6.199021	15.1025	0.05522	0.016343
0.4	1.547	6.299813	18.25025	0.06237	0.010169
0.6	1.481805	6.369637	17.83075	0.0539	0.018885
0.8	1.435395	6.645161	18.32575	0.05863	0.026149
1	1.355283	6.895369	16.125	0.06941	0.027965

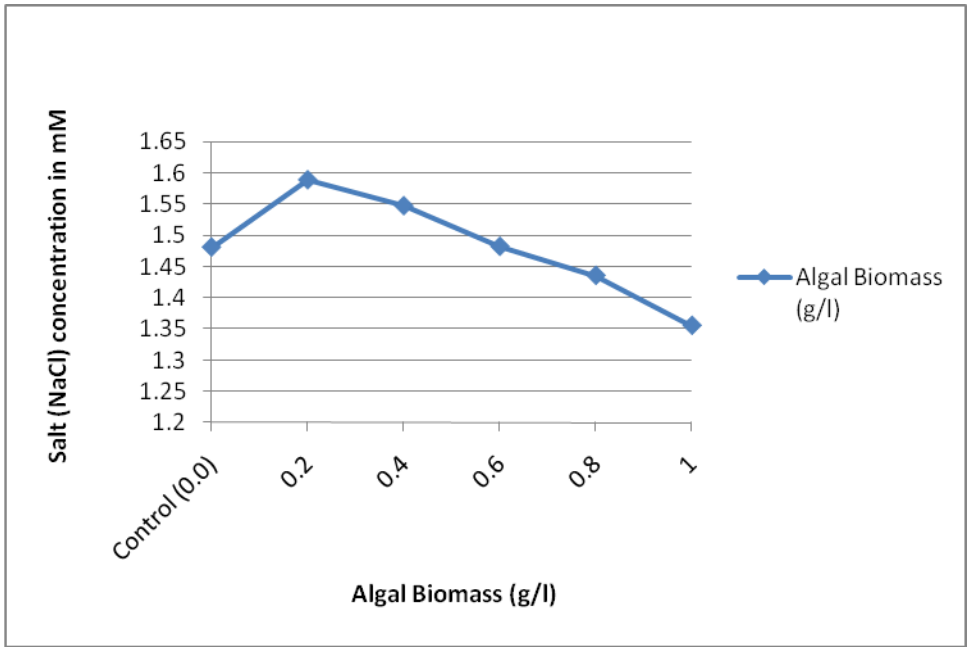

Fig. 1: Algal biomass yield (g/l) at different concentrations of salt (NaCl)

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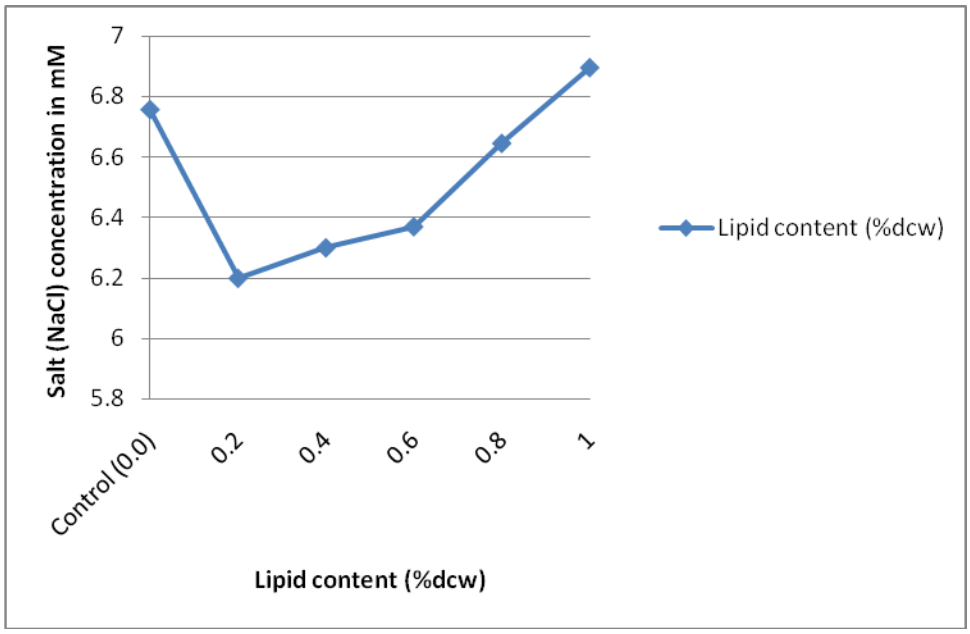

Fig. 2: Values of lipid content (%dcw) at different concentrations of salt (NaCl)

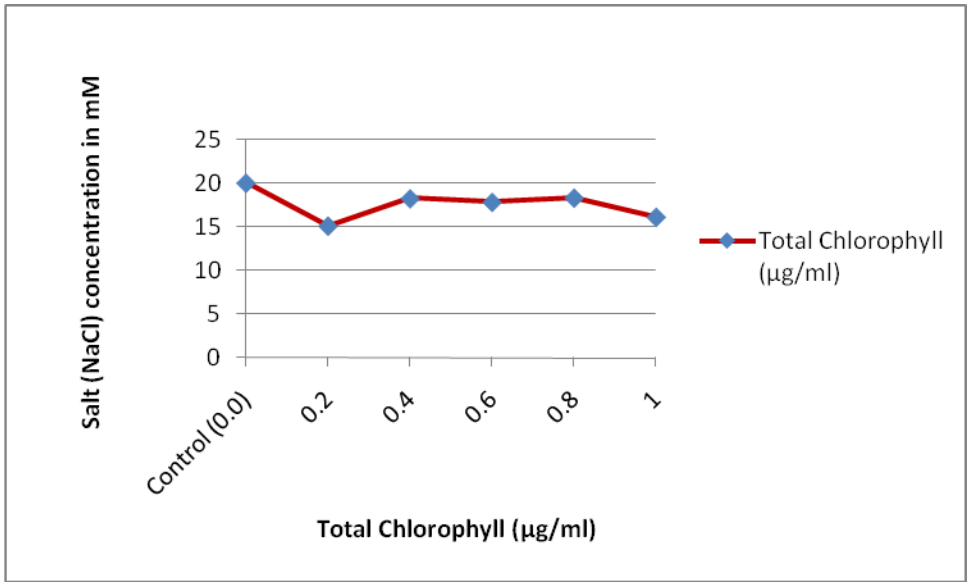

Fig. 3: Values of total chlorophyll (µg/ml) at different concentrations of salt (NaCl)

According to Moradi and Ismail (2007), reduced chlorophyll contents at higher salinities are due to decrease in photosynthetic rate because of salt osmotic and toxic ionic stress. Many previous studies reported that the cultivation with higher saline concentrations had lower chlorophyll and protein contents (Vonshak *et al.*,1996). It

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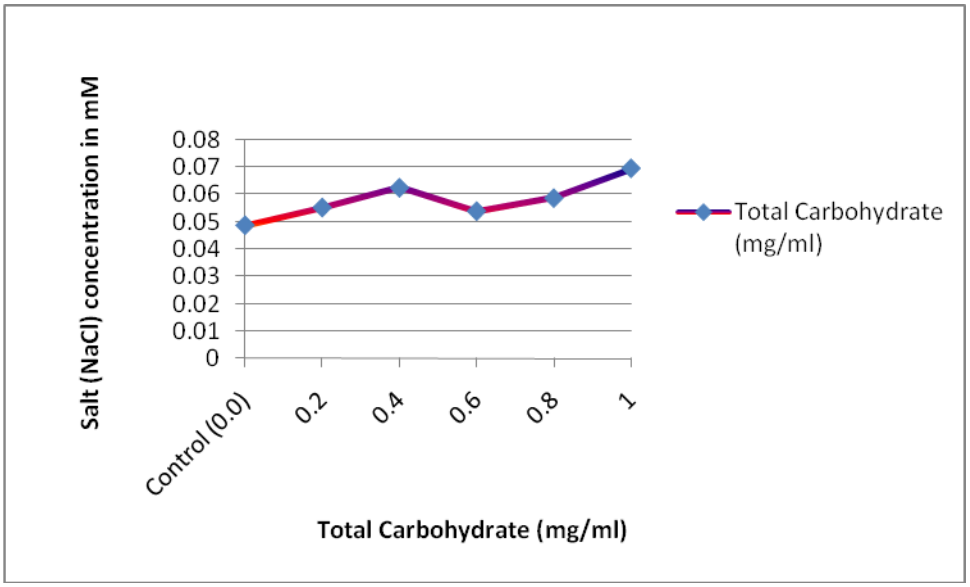
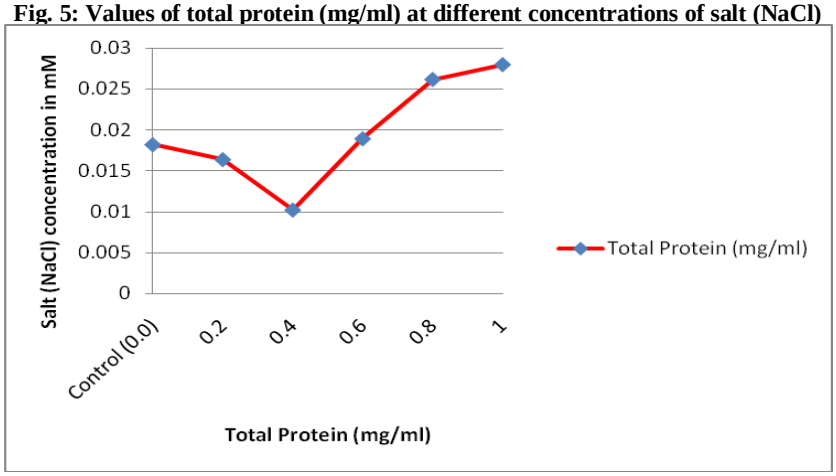
has also been reported that chlorophyll is the primary target to salt toxicity limiting net assimilation rate, resulting reduced photosynthesis and reduced growth (Rai 1990; Rai and Abraham 1993). Carbohydrate content increased in all the concentrations of NaCl as compared to control for the culture studied (Table 1 and Fig. 4).

Fig. 4: Values of total carbohydrate (mg/ml) at different concentrations of salt (NaCl)



Many previous studies reported that carbohydrates synthesis was stimulated by stress conditions (Warr *et al.*,1985; Tomaselli *et al.*,1987). Gill *et al.*,(2002) made an observation that soluble sugars play an important role in the osmotic regulation of cells during reproduction and stress conditions. Among different solutes accumulating in response to stress sugar may play a key role to maintain the osmotic regulation of cells. The increase in the sugar content may be an adaptive measure under saline conditions. Algae exhibited decline in the total protein content at the NaCl concentrations of 0.2 and 0.4mM and thereafter at NaCl concentration of ≥0.6mM the quantity of total protein content increases as compared to control (Table 1 and Fig. 5). Here results of total protein content are in accordance with the finding of Vonshak *et al.*,1996 and they reported that the cultivation with higher saline concentrations had lower chlorophyll and protein contents.

Fig. 5: Values of total protein (mg/ml) at different concentrations of salt (NaCl)



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CONCLUSION

The effect of various concentrations of NaCl on the isolated algal species of *Scenedesmus quadricauda* showed, increased biomass yield at 0.2mM NaCl concentration as compared to control and then it subsequently decreases with increase in NaCl concentration. Initial increase of NaCl concentration from 0.0-0.2 mM decreased the lipid accumulation from 6.75 to 6.12 % dcw. While, total chlorophyll and carbohydrate content increased in all the concentrations of NaCl as compared to control for the culture studied. Alga exhibited decline in the total protein content at the NaCl concentrations of 0.2 and 0.4mM and thereafter at NaCl concentration of ≥0.6mM the quantity of total protein content increased as compared to control. These beneficial properties indicated that, adaptation of the alga to salinity was characterized by the accumulation of chlorophyll, carbohydrates and protein.

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REFERENCES

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Canadian Biochem. Physiol. 37: 911 917.

Bohnert, H.J. Nelson, D.E. and Jensen, R.G. 1995. Adaptations to Environmental Stresses. The Plant Cell, 7: 1099-1111.

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

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

Booth W.A. and Beardall, J. 1991. Effect of salinity on inorganic carbon utilization and carbonic anhydrase activity in the halotolerant alga *Dunaliella salina* (Chlorophyta). Physiologia. 30: 220-225.

Chen, C.S. and Plant, A.L. 1999. Salt induced protein synthesis in Tomato roots. J. Exp. Bot. 50: 677-687.

Chisti, Y. 2007. Biodiesel from microalgae. Biotechnol. Adv. 25: 294–306.

Chiu, S.Y. Tsai, M.T. Kao, C.Y and Ong, S.C. 2009. The air-lift photobioreactors with flow patterning for high-density cultures of microalgae and carbon dioxide removal. Eng. Life Sci. 9: 254–260.

Dubois, M. Gilles, A.K. Hamilton, J.K. Rebes, P.A. and Smith, F. 1956. Colorimetric method for detemination of sugars and related substances. Anal. Chem. 28: 350-356.

EL-Sheekh, M.M. and Omar, H.H. 2002. Effect of high salt stress on growth and fatty acids content of the unicellular green alga *Chlorella vulgaris*. Az. J. Microbiol. 55: 181-191.

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.

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.

Fujii, S. Uenaka, M. Nakayama, S. Yamamoto, R. and Mantani, S. 2001. Effect of sodium chloride on the fatty acids composition in *Boekelovia hooglandii* (Ochramonadales, Chrysophyceae). Phycol. Res. 49: 73–77.

Gill, P.K., Sharma, A.D., Singh, P. and Bhullar, S.S. 2002. Osmotic stress induced changes in germination, growth and soluble sugar contents of *Sorghum bicolor* (L.) Moench seeds under various abiotic stresses. Plant Physiol. 128: 12 – 25.

Gonzalez-Moreno, S. Gomez-Berrera, J. Perales, H. and Monerono-Sanchez, R. 1997. Multiple effects of salinity on photosynthesis of the protist *Euglena gracilis*. Physiol. Plant. 101: 777-786.

Guil-Guerrero, J.L. Navarro-Juarez, R. Lopez-Martinez, J.C. and Campra-Madrid, P. 2004. Functional properties of the biomass of three microalgal species. J. Food Eng. 65: 511–517.

Guillard, R.R.L. 1962. Salt and osmotic balance. In: Lewin (ed.) Physiology and Biochemistry of Algae, pp 529-540. Academic Press, New York.

Illman, A.M. Scragg, A.H. and Shales, S.W. 2000. Increase in Chlorella strains calorific values when grown in low nitrogen medium. Enzyme Microb. Technol. 27: 631–635.

Joset, F. Jeanjean, R. and Hageman, N. 1996. Dynamics of the response of the cyanobacteria to salt stress: Deciphering the molecular events. Physiol. Plant. 96: 738-744.

Kojima, E. and Zhang, K. 1999. Growth and hydrocarbon production by microalga Botryococcus braunii in bubble column photobioreactor. J. Biosci. Bioeng. 87: 811–817.

Lartigue, J. Neill, A. Hayden, B.L. Pulfer, J. and Cebrian, J. 2003. The impact of salinity fluctuations on net oxygen production and inorganic nitrogen uptake by *Ulva lactuca* (Chlorophyceae). Aquat. Bot. 75: 339-350.

Liu, Z.Y. Wang, G.C. and Zhou, B.C. 2008. Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. Bioresour. Technol. 99: 4717–4722.

Lowry, O.H. Rosenbrough, N.J. Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin Phenol reagent. J. Biol. Chem. 193: 265-275.

Lu, C.M. and Torzillo, G. and Vonshak, A. 1999. Kinetic response of photosystem II photochemistry in the cyanobacterium *Spirulina platensis* to high salinity is characterized by two distinct phases. Austr. J. Plant Physiol. 26: 283-292.

Lu, C.M. and Vonshak, A. 1999. Characterization of PS II photochemistry in salt-adapted cells of the

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cyanobacterium *Spirulina platensi*. New Phytol. 141: 231-239.

Lu, C.M. and Vonshak, A. 2002. Effects of salinity stress on photosystem II function in cyanobacterial *Spirulina platensis* cells. Physiol. Plant. 114: 405-413.

Lu, C.M. and Zhang, J.H. 1999. Effects of salt stress on PS II function and photoinhibition in the cyanobacterium *Spirulina platensis* J. Plant Physiol. 155: 740-745.

Lu, C.M. and Zhang, J.H. 2000. Role of the light in the response of PS II photochemistry to salt stress the cyanobacterium *Spirulina platensi.* J. Exp. Bot. 51:911-917.

Lynn, S.G. Kilham, S.S. Kreeger, D.A. and Interlandi, S.J. 2000. Effect of nutrient availability on the biochemical and elemental stoichiometry in freshwater diatom *Stephanodiscus minutulus* bacillariophyceae. J. Phycol. 36: 510–522.

Metting, F.B. 1996. Biodiversity and application of microalgae. J. Ind. Microbiol. Biotechnol. 17: 477–489.

Moisnder, P.H. McClinton, E. and Paer, H.W. 2002. Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. Microb. Ecol. 43: 432-442.

Moradi, M. and Ismail, A.M. 2007. Responses of Photosynthesis, chlorophyll fluorescence and ROS – Scavenging systems to salt stress. During seedling and reproductive stages of Rice. Ann. Botany. 99: 1161-1173.

Murakami, A. Kim, S.J. and Fujita, Y. 1997. Changes in photosystem stoichiometry in response to environmental conditions for cell growth observed with the cyanophyte *Synechocystis* PCC 6714. Plant Cell Physiol. 38: 392-7.

Peeler, T.C. Stephenson, M.B. Einsphar, K.J. and Thompson, G.A.Jr. 1989. Lipid characterization of enriched plasma membrane fraction of Dunaliella salina grown in media of varying salinity. Plant Physiol. 89: 970–976.

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

Rai, A.K. 1990. Biochemical characteristics of photosynthetic response to various external salinities in halotolerant and fresh-water cyanobacteria. F.E.M.S. Microbiol. Lett. 69: 177-180.

Rao, A.R. Dayananda, C. Sarada, R. Shamala, T.R. and Ravishankar, G.A. 2007. Effect of salinity on growth of green alga Botryococcus braunii and its constituents. Bioresour. Technol. 98: 560-564.

Reitan, K.I. Rainuzzo, J.R. and Olsen, Y. 1994. Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. J. Phycol. 30: 972–979.

Seemann, J.R. and Critchley, C. 1985. Effect of salt stress on the growth, ion content, stomatal behavior and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. Planta. 164: 151-162.

Sharma, P.K. and Hall, D.O. 1991. Interaction of salt stress and photoinhibition on photosynthesis in barley and sorghum. J. Plant Physiol. 138: 614-619.

Shen, J.R. and Katoh, S. 1991. Inactivation and calcium-dependent reactivation of oxygen evolution in photosystem-II preparations treated at pH 3.0 or with high concentration of NaCl. Plant Cell Physiol. 32: 439-446.

Singh, S. Kate, B. N. and Banerjee, U. C. 2005. Bioactive compounds from cyanobacteria and microalgae: an overview. Crit. Rev. Biotechnol. 25: 73–95.

Spolaore, P. Joannis-Cassan, C. Duran, E. and Isambert, A. 2006. Commercial applications of microalgae. J. Biosci. Bioeng. 101: 87–96.

Takagi, M. Karseno and Yoshida, T. 2006. Effect of salt concentration on intracellular accumulation of lipids and Triacylglyceride in marine Microalgae *Dunaliella* cells. J. Biosci. Bioeng.101: 223-226.

Tandeau de Marsac, N. and Houmard, J. 1988. Complementary chromatic adaptation: physiological conditions and action spectra. Methods Enzymol. l67: 318-328.

Tomaselli, L. Torzillo, G. Giovanetti, L. Bocci, F. Tredici, M.R. Pusharaj, B. Pupuazzo, T. Balloni, T. and Meterassi, R. 1987. Recent research of *Spirulina* in Itali. Hydrobiol. 151: 79-82.

Vonshak, A.N. Bunang K. B. and Tanticharoen, M. 1996. Role of Right and Photosynthesis on the acclimation process of the cyanobacteria *Spirulina platensis* to salinity stress. J. Appl. Phycol. 8: 119-124.

Warr, S.R.C. Reed, R.H. Chudek, J.A. Foster, R. and Stewart W.D.P. 1985. Osmotic adjustment in *Spirulina platensis*, Planta. 163: 424 - 429.

Xu, X.Q. and Berdall, J. 1997. Effect of salinity on fatty acid composition of green microalgae from an Antarctic hypersaline lake. Phytochemistry. 45: 655–658.