# Growth and metabolic response of the filamentous cyanobacterium *Spirulina platensis* to salinity stress of sodium chloride

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**Abstract:** The filamentous cyanobacterium *Spirulina platensis* has been examined for salt tolerance. In this study, the influence of NaCl salinity on growth and some metabolites has been investigated. The cyanobacterium *Spirulina platensis* was grown at different salinities as 0.05, 0.10, 0.15, 0.20 and 0.25 M NaCl which were enriched with Zarrouk medium. Improved growth and most of the investigated metabolites were determined with the culture grown under salinity up to 0.15 M of NaCl compared to control. It was found that growth, soluble protein and glycine betaine were stimulated at lower concentrations of NaCl (0.05, 0.10 and 0.15 M) but were reduced at higher concentrations (0.20 and 0.25M). However, the growth rate was significantly reduced at higher concentrations of NaCl. Chlorophyll a and carotenoids were increased in NaCl concentration up to 0.15 M followed by a decrease at higher concentrations. On the other hand total phycobilins increased at the most lower concentration of NaCl (0.05 M), then it decreased with increasing NaCl concentration compared to control. It is interesting to note that insoluble and total protein decreased with increasing salinity, the same trend was also observed for conjugated and total amino acids. While, carbohydrates and proline content increased with the increase in NaCl concentrations. By investigating the effect of different levels of salinity on total antioxidant capacity; the results obtained revealed that, with the increase in concentration of NaCl up to 0.20 M, a progressively greater increase in the activity of the total antioxidants above the respective control was observed, while the minimum activity was detected at the most highest concentration of NaCl.

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**Key words:** Salinity, *Spirulina platensis*, growth, pigments, osmolytes, antioxidants.

## 1. Introduction

Salinity stress biology and plant responses to high salinity have been discussed over two decades (Flowers *et al*., 1977; Greenway and Munns, 1980; Ehret and Plant, 1999; Hasegawa *et al*., 2000; Zhu, 2002) and it has been over a decade since salinity tolerance in marine algae has been covered (Kirst, 1989; Parida and Das, 2005).

Salinity can cause significant accumulation of compatible solutes which acts as enzyme producers stabilizing the structure of macromolecules and organelles (Dahlich *et al*., 1983). Salinity stress may alter the metabolic pathways of stressed organism(s) leading to either enhancement or induction of biologically active compounds (Shalaby *et al*., 2010).

Algae often occur in extremely hostile environments, and this is a reflection of their remarkable ability to tolerate various kinds of stresses - natural as well as those resulting from human activities. They have to negotiate with low concentrations of essential nutrients (especially carbon, nitrogen, phosphorus and trace elements) in natural waters and, low availability of light and temperature on one hand. On the other, they have to survive and grow in habitats enriched with salts, toxic metals and pesticides, elevated temperature, UV -B radiation and light intensity. Algae are the principal primary producers of waterbodies - from a small rain puddle to the big oceans. Hence the tolerance of these organisms to diverse stresses assumes tremendous relevance from an ecological standpoint (Rai and Gaur, 2001).

*Spirulina*, is an edible blue-green microalga (cyanobacterium) characterized by high content of good quality protein as well as being rich in vitamins, minerals, and other components beneficial to health such as essential fatty acids and antioxidant pigments like carotenoids, chlorophyll, and phycocyanin, this cyanobacterium has received much attention as a most promising and nutritious food source (Dillion *et al*., 1995). It is generally a rich source of vitamins, essential amino acids, minerals, essential fatty acids (Mendes *et al*., 2003). Also, its diverse biological and pharmacological properties (Belay, 2002; Becker, 2003; Khan *et al*., 2005; Mani *et al*., 2008) have promoted *Arthrospira* (*Spirulina*) as being a functional food, and thus, consumption of this microorganism as nutritional therapeutic supplement gains popularity (Hutadilok-Towatana *et al*., 2010).

The aim of this work was focused to investigate the effect of different salinity levels on growth and synthesis of some important metabolites

which may vary with salt stressed culture conditions of the blue green filamentous alga *Spirulina platensis*.

## 2. Material and Methods

**Biological material and culture conditions:** The organism used in the present study was *Spirulina platensis*. The axenic culture of *Spirulina platensis* was grown in *Spirulina* medium (Zarrouk, 1966), this medium was found best suited for the growth of *Spirulina* in the laboratory. The organism was grown in Erlenmeyer pyrex-glass flasks (capacity 250 ml) each containing 50 ml culture medium (pH 9). The inoculated medium was adjusted to optical density above 0.1 unit in order to yield a linear growth curve with a lag phase. The cultures were grown in a culture chamber under controlled temperature and light (26˚C + 3˚C and 80 μ mol $m^{-2}$ $s^{-1}$). Cultures were conducted under a regime of 16 hour light/ 8 hour dark; swirled daily and lasted for 20 days.

**Salinity stress:** Salinity treatments were applied as 0.05, 0.10, 0.15, 0.20 and 0.25 M NaCl; with three replicates for each treatment besides control and kept for observation to 20 days, the results presented are mean values.

The samples were drawn periodically during growth ($5^{th}$, $10^{th}$, $15^{th}$ and $20^{th}$ day) from control and different concentrations of NaCl and were subjected for the analysis of some metabolites.

**Growth measurements:** The biomass or algal growth was measured by optical density at 560 nm. The growth rate (μ $day^{-1}$) was calculated from the results of optical density using the equations followed by Guillard (1979).

**Analytical procedures:** Chlorophyll a was estimated using the extinction coefficients given by Jeffrey and Humphrey (1975), Carotenenoids accumulation was estimated according to the method of Jensen (1978), Phycobilipigments were estimated from the extinction coefficients given by Bennett and Bogorad (1973). Protein was extracted according to Rausch (1981) and determined by the method described by Hartree (1972) with BSA as standard. Carbohydrate fractions were determined following the colorimetric phenol method described by Dubois *et al*. (1959) using glucose as standard. Proline concentration was determined using the acid ninhydrin method described by Bates *et al*. (1973) and Glycine betaine was estimated by the method of Barak and Tuma (1981). Free, conjugated and total amino acids were extracted by the method described by Speckmann *et al*. (1958) and were determined using an Eppendwarf (model LC 3000, Hamburg, Germany) amino acid analyzer. Determination of the total antioxidants capacity is performed by the reaction of antioxidants in the sample with a definite amount of exogenously provided hydrogen peroxide as described by Koracevic *et al*. (2001).

Significance of differences was tested at $P \leq 0.05$ using ANOVA. The data are means ± SE from determinants of at least three independent experiments.

## 3. Results

In the present study attempt has been taken to investigate the tolerance capacity to different five concentrations of NaCl; 0.05, 0.10, 0.15, 0.20 and 0.25 M NaCl by the cyanobacterium *S. platensis*. The experiments were designed to find out the change in the growth, pigments, protein, carbohydrates, proline, glycine betaine, amino acids content and antioxidant capacity of the test species when exposed to different concentration of NaCl in the culture media and incubated for twenty days.

It was found that the growth pattern showed a gradual increase from day 5 up to day 15 and thereafter steep decrease from day 15 to day 20 (Fig. 1). Compared to control; the growth increased with increase of NaCl concentration up to 0.15 M then decreased at 0.20 and 0.25 M. Results obtained for growth parameters of *S. platensis* (optical density and growth rate) represented in (Figs. 1and 2) revealed that growth was highest at initial NaCl concentration (0.05 M). The highest growth rate for all the five salinity levels and control were recorded at the $10^{th}$ day of culturing but with different values depending on the level of salinity. The more was salinity; the lower was growth rate at the most highest NaCl concentrations (0.20 and 0.25 M) during the experiment.

The results of chlorophyll a content in *S. platensis* under salt stress cultured for 20 days (Fig. 3) showed the same trend as in case of growth. *S. platensis* showed increase in chlorophyll a content with increase of NaCl concentration upto 0.15 M followed by decrease in highest test concentrations (0.20 and 0.25 M). The chlorophyll a content was highest at initial NaCl concentration (0.05 M) while the lowest content was attained at the most highest NaCl concentration.

Pigment fractions of *S. platensis* (Carotenoids and the water-soluble phycobilin individuals) under different salinity levels were estimated spectrophotometrically on the $10^{th}$ day of culturing (the day on which the culture attained maximum growth rate) were presented in Table 1. Among these pigments, carotenoids were increased gradually by increasing salinity up to 0.15 M NaCl, then it decreased at salinity 0.20 and 0.25 M; but still higher than that of control. The data obtained for total phycobilins increased at the most lower concentration of NaCl (0.05 M), then it decreased with increasing NaCl concentration compared to control. Also, within the phycobilin individuals; phycoerythrin was more sensitive to salinity stresses than the other two individuals. It is clear also that phycobilin individuals

are interconvertable i.e. the increase in one individual is at the expense of the other under the salinity stress.

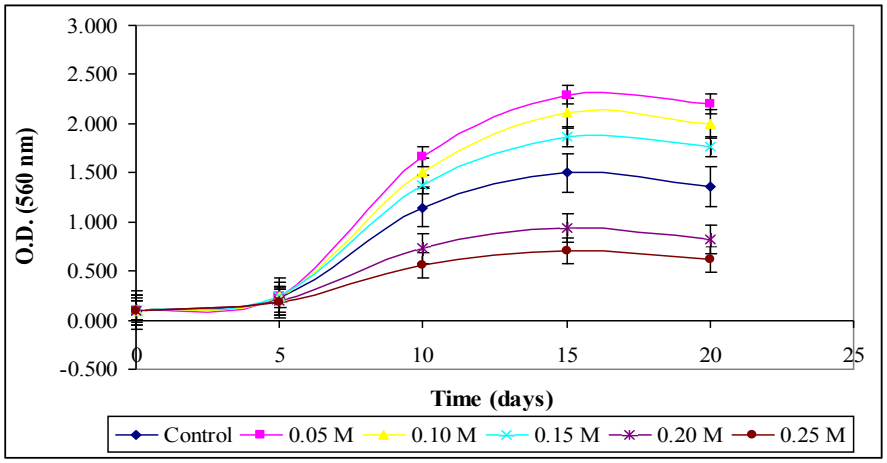


**Figure 1: Effect of different salinities on the growth of *Spirulina platensis* cultured for 20 days.**

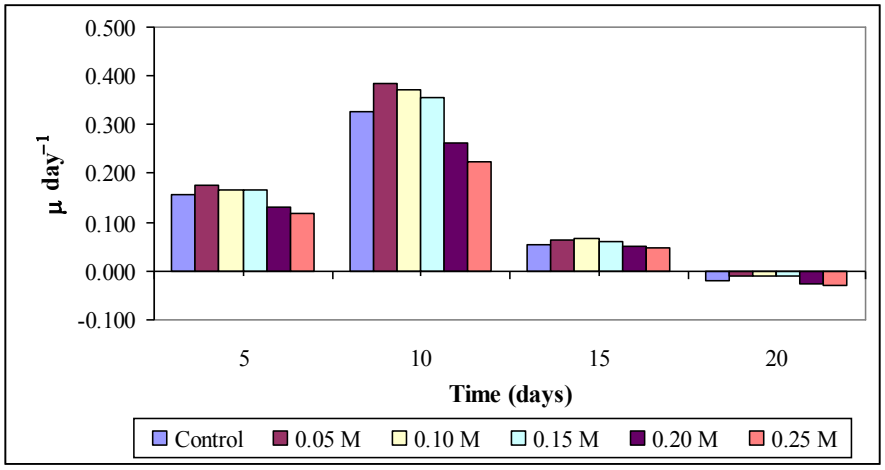


**Figure 2: Effect of different salinities on growth rate (μ day$^{-1}$) of *Spirulina platensis* cultured for 20 days.**

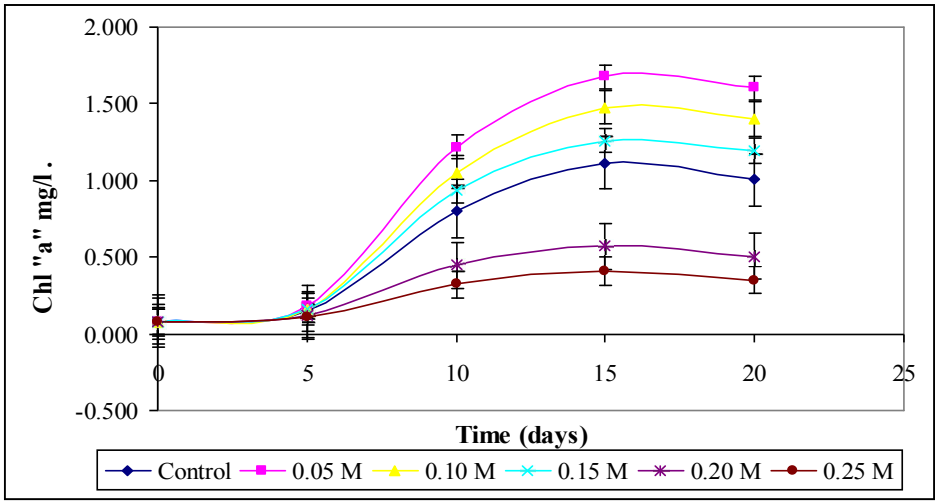


**Figure 3: Effect of different salinities on chlorophyll a (Chl "a") content of *Spirulina platensis* cultured for 20 days.**

**Table 1: Content of pigment fractions (mg/l) of *Spirulina platensis* cultured for 10 days under treatment with different salinities.**

| Treatment | Carotenoids | (PC) | (APC) | (PE) | Total phycobilins |
|---|---|---|---|---|---|
| **Control** | 0.113 ± 0.08 [a] | 0.04 ± 0.05 [a] | 0.04± 0.09 [a] | 0.07 ± 0.11 [a] | 0.15 |
| **0.05 M NaCl** | 0.124 ± 0.13 [b] | 0.05 ± 0.07 [b] | 0.05 ± 0.04 [b] | 0.06 ± 0.05 [b] | 0.16 |
| **0.10 M NaCl** | 0.162 ± 0.10 [c] | 0.05 ± 0.04 [b] | 0.04± 0.17 [a] | 0.06 ± 0.09 [b] | 0.15 |
| **0.15 M NaCl** | 0.171 ± 0.07 [d] | 0.02 ± 0.09 [c] | 0.06± 0.02 [c] | 0.04 ± 0.11 [c] | 0.12 |
| **0.20 M NaCl** | 0.140 ± 0.05 [e] | 0.02± 0.07 [c] | 0.05 ± 0.04 [b] | 0.04 ± 0.10 [c] | 0.11 |
| **0.25 M NaCl** | 0.131 ± 0.09 [f] | 0.01 ± 0.12 [d] | 0.04 ± 0.10 [a] | 0.03 ± 0.12 [d] | 0.08 |

**PC= Phycocyanin; APC= Allophycocyanin; PE= Phycoerythrin**
Different superscripts are significant

Table 2: Content of protein and carbohydrates fractions (mg/l) of *Spirulina platensis* cultured for 10 days under treatment with different salinities.

| Treatment | Protein | | | Carbohydrates | | |
|---|---|---|---|---|---|---|
| | Soluble | Insoluble | Total | Soluble | Insoluble | Total |
| **Control** | 29.33 ± 0.05 [a] | 40.00± 0.04 [a] | 69.33 | 17.31 ± 0.03 [a] | 27.91 ± 0.01 [a] | 45.22 |
| **0.05 M NaCl** | 31.10 ± 0.03 [b] | 33.11 ± 0.10 [b] | 64.21 | 21.12 ± 0.11 [b] | 31.25 ± 0.09 [b] | 52.37 |
| **0.10 M NaCl** | 34.65 ± 0.06 [c] | 23.21 ± 0.02 [c] | 57.86 | 23.43 ± 0.01 [c] | 35.20 ± 0.03 [c] | 58.63 |
| **0.15 M NaCl** | 35.22 ± 0.04 [c] | 18.77 ± 0.01 [d] | 53.99 | 30.12 ± 0.07 [d] | 39.29 ± 0.05 [d] | 69.41 |
| **0.20 M NaCl** | 29.55 ± 0.01 [a] | 16.26 ± 0.03 [e] | 45.81 | 34.26 ± 0.09 [e] | 42.58 ± 0.06 [e] | 76.84 |
| **0.25 M NaCl** | 21.82 ± 0.08 [d] | 14.29 ± 0.05 [f] | 36.11 | 39.66 ± 0.02 [f] | 44.05 ± 0.03 [f] | 83.71 |

Different superscripts are significant

**Table 3: Content of proline, glycine betaine and amino acid fractions of *Spirulina platensis* cultured for 10 days under treatment with different salinities.**

| Treatment | Proline (mg/l) | Glycine betaine (mg/l) | Amino acids (mg/g fresh weight) | | |
|---|---|---|---|---|---|
| | | | Free | Conjugated | Total |
| **Control** | 0.26 ± 0.02 [a] | 3.72 ± 0.04 [a] | 0.92 ± 0.03 [a] | 2.11 ± 0.06 [a] | 3.03 |
| **0.05 M NaCl** | 0.31 ± 0.09 [b] | 5.63 ± 0.01 [b] | 0.98 ± 0.02 [b] | 1.92 ± 0.08 [b] | 2.90 |
| **0.10 M NaCl** | 0.37± 0.06 [c] | 8.20 ± 0.02 [c] | 1.04 ± 0.05 [c] | 1.74 ± 0.10 [c] | 2.78 |
| **0.15 M NaCl** | 0.44 ± 0.10 [d] | 13.22 ± 0.01 [d] | 1.10 ± 0.09 [c] | 1.56 ± 0.01 [d] | 2.66 |
| **0.20 M NaCl** | 0.50 ± 0.11 [e] | 10.44 ± 0.04 [e] | 1.15 ± 0.06 [d] | 1.44 ± 0.04 [e] | 2.59 |
| **0.25 M NaCl** | 0.61 ± 0.09 [f] | 6.25 ± 0.05 [b] | 1.17 ± 0.03 [d] | 1.31 ± 0.08 [f] | 2.48 |

Different superscripts are significant

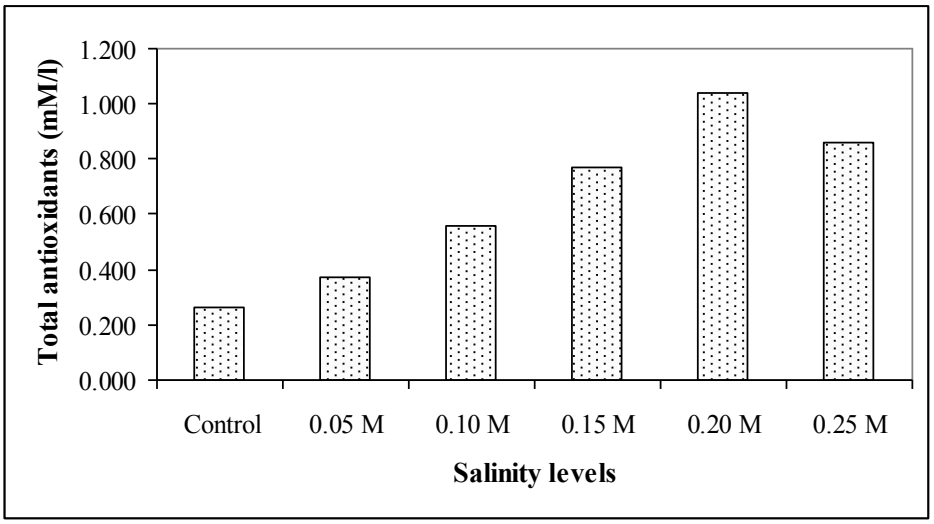


**Figure 4:** Effect of different salinities on total antioxidants of *Spirulina platensis* cultured for 10 days.

Table 2 showed the effect of the five different salinities on the content of protein and carbohydrates fractions of *S. platensis* cultured for 10 days. The results obtained cleared that soluble proteins increased by increasing the salinity reaching maximum at salinity o.15 M NaCl, but it decreased gradually at 0.20 and 0.25 M NaCl. The data cleared also that insoluble and total protein decreased with increasing salinity. The lowest protein content was observed in the salinized cultures treated with 0.25 M NaCl. Concerning the data obtained for the effect of different salinity levels on the content of carbohydrates fractions (soluble, insoluble and total), cleared a significant gradual increase with increasing salinity compared to control. The highest carbohydrates content were obtained at the most highest concentration of NaCl.

Data presented in Table 3 showed the results obtained for proline, glycine betaine and amino acid fractions of *S. platensis* cultured for 10 days under the effect of the five different concentrations of NaCl. A glimpse at these data it is clear that proline was more effective as a protective agent against salinity stress than glycine betaine. The first increased under all the investigated salinity levels, reaching maximum content at the most highest concentration of NaCl. While glycine betaine increased gradually by increasing salinity up to 0.15 M NaCl, then it decreased at salinities 0.20 and 0.25 M; but still higher than that than that of control. Regarding amino acids content, the results revealed that free amino acids increased by increasing salinity. While the content of conjugated and total amino acids decreased under all the studied salinities. It is clear therefore that under salinity stress the free amino acids increase at the expense of conjugated ones.

The result obtained from the antioxidants capacity of *S. platensis* at control and the five tested concentrations of NaCl were graphed in figure (4). The activity of total antioxidants increased gradually by increasing the NaCl concentrations but with different values. Maximum total antioxidant activity at salinized cultures reached at concentration 0.20 M NaCl, while the minimum activity was detected at the most highest concentration of NaCl. However the activity weather increased or decreased was higher than control under all the tested concentrations of NaCl.

**4. Discussion**

Salinity is one of the major environmental factors limiting plant growth and productivity (Allakhverdiev *et al*., 2000). Salinity is a physiological parameter to determine the ability of organisms to survive in their environment. The cyanobacterial response to salinity involves several physiological and biochemical process such as nucleic acid synthesis, carbohydrates and protein metabolism, photosynthesis and respiration (Priyadarshani *et al*., 2012).

The increase in salinity caused a decrease in growth; Priyadarshani *et al*. (2012) have indicated that the growth was reduced with increase in NaCl concentration in four test species of cyanobacteria (*Oscillatoria subbrevis, Phormidium tenue*, *Phormidium sp.*, *Lyngbya sp*.). When the concentration of NaCl was above 30.0 g $L^{-1}$, *Chlorella* could not tolerate the excessive salt concentration levels and therefore no algal growth was observed (Barghbani *et al*., 2012). Munns (2003); Singla and Garg (2005) stated that suppression of algal growth under saline conditions may be due to the increasing toxicity of sodium chloride associated with increasing salinity. The increase in salinity caused a decrease in growth rate of *Synechococcus* sp., under this conditions cells tend to have a larger size and greater production and accumulation of metabolites, though they are unable to grow fast (Rosales *et al*., 2004). The inhibition of growth under salt stress conditions was certainly due to alteration of algal metabolism which might be directed towards the production of substances which have a role in algal salt tolerance or defense mechanism. However, Ahmed *et al*. (1989) revealed that the growth of microalgae is retarded during salinity stress due to the accumulation of compatible solutes like proline and glycine to balance the external salt concentrations.

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 has also been reported that chlorophyll is the primary target to salt toxicity limiting net assimilation rate, resulting reduced photosynthesis and growth (Rai, 1990; Rai and Abraham, 1993). Ayachi *et al*. (2007) indicated that *Spirulina platensis* (*Arthrospira platensis*) showed an increase in chlorophyll amounts at 15 g $l^{-1}$ NaCl in 10 days old cultures but a decrease at high NaCl concentrations. Sudhir *et al*. (2005) reported that 0.8 M NaCl caused a remarkable decrease in photosystem II (PS II) mediated oxygen evolution activity of *S. platensis*.

Carotenoids were increased in all the concentrations of NaCl except at concentrations 0.20 and 0.25 M for all the cultures studied. Similar observations were made by Reddy *et al*. (2003) who indicated that carotenes content increased in o.1, 0.2 and 0.3 M and decreased in 0.4 M NaCl. The result of Vazquez *et al*. (1991) has also indicated that the *Botryococcus braunii* has adopted lower levels of salinity with an increased production of biomass, carbohydrate and carotenoid. Concerning the results

obtained for phycobilins revealed that they increased at the most lower concentration of NaCl (0.05 M), then it decreased with increasing NaCl concentration compared to control. Lu and Vonshak (2002) concluded that *Spirulina platensis* exposed to 0.8M NaCl for 12h, chlorophyll contents remain unchanged whereas phycocyanin contents significantly decrease by about 50% compared to the control. Also, optimum content of phycocyanin, allophycocyanin and phycoerythrine of *S. platensis* was attained at 0.14 M NaCl (Osman *et al*., 2012).

*Spirulina platensis* exhibited decline in the total protein contents in all the concentrations of NaCl studied. The present results are in agreement with the results of sheik *et al*. (2006). Hageman *et al*. (1990) found complete blockage of protein synthesis in cyanobacteria. Many previous studies reported that stress cells have lower protein synthesis capacity increasing lipid and carbohydrate metabolism (Warr *et al*., 1985; Tomaselli *et al*., 1987). Carbohydrate contents increased in all the concentrations of NaCl studied. 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.

During the study there was a drastic increase in the proline content in all the concentrations of NaCl in the cultures studied. Szekely (2004) made an observation that in higher plants proline is considered to play an important role in defense mechanism of stressed cells providing carbon, nitrogen and energy source after stress by degradation. According to Hong *et al*. (2000) increased resistance to oxidative stress is due to accumulation of proline and other metabolites. He also made an observation that proline increased salt tolerance of microorganisms. Therefore in microalgae and other plants proline acts as a free radical scavenging and increases salt tolerance of microorganisms. It is likely that proline accumulation may be one of the major mechanisms of salinity tolerance by the alga.

Glycine betaine was increased gradually by increasing salinity up to 0.15 M NaCl then decreased, this was in harmony with the results of Hiremath and Mathad (2010) who recorded that glycine betaine increased with increase in NaCl concentrations up to 0.3 M and thereafter declined. Hasegawa *et al*. (2000); Hoque *et al*. (2007) and Hasaneen *et al*. (2008) reported that compatible osmolytes such as glycine betaine, choline, soluble sugars and free amino acids especially proline are synthesized in response to salt stress. These osmotic adjustments protect sub-cellular structures and reduce oxidative damage caused by free radicals, produced in response to stress of high salinity.

Salt stress is now known to cause several physiological changes including oxidative stress (Lee *et al*., 2001, Panda and Upadhyag, 2003). In plant cells both enzymatic and non-enzymatic antioxidant defense systems exist, which help in detoxifying the active oxygen species (AOS) (Lee *et al*., 2001 and Malencic *et al*., 2003). Careful examination of the results obtained revealed that, with increase in concentration of NaCl, a progressively greater increase in the activity of the total antioxidants above the respective control. However, at the most higher concentrations of NaCl, the activity of total antioxidant slightly decreased but still higher than that of control. Our results lend a strong support to the results obtained by Chaparzadeh *et al*. (2004) and Jungklang (2005).

In conclusion: this study revealed that the effect of various degrees of salinity stress typically affects all the biochemical processes of *S. platensis* including growth, photosynthesis and metabolism. However, adaptation of this alga to salinity was characterized by the accumulation of osmolytes like carbohydrates, proline and glycine betaine.

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