Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © PHYCOSPECTRUM
ISSN: 2229 – 6905

Effect of pH, salinity and temperature on the growth of six species of marine phytoplankton

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ABSTRACT

In the present study, the influence of pH, salinity and temperature on the growth of three species of cyanobacteria, two species of diatoms and one species of planktonic green alga was investigated. The isolates preferred near neutral to alkaline pH. The growth was comparatively lower at pH 6.3. The high pH levels adversely affect the growth of isolates and significantly reduced at pH 10.6. All isolates were able to grow at all salinities except the diatom *Skeletonema costatum* which did not show any growth at 40 ppt. The best growth was obtained at 16 ppt and 25 ppt indicated that isolated species prefer alkaline condition for their optimum growth. At higher salinity of 40 ppt, the growth was drastically decreased. All isolates showed best growth at 20˚C and 30˚C. The growth has ceased at 40˚C and 50˚C. These factors are useful for establishing the appropriate culture conditions for optimizing the growth and also helpful to understand their response to varying environmental factors. Some of the species could be considered for the production of high value compounds and are employed in mariculture.

Key words: Marine phytoplankton, pH, salinity, temperature, chlorophyll-a.

INTRODUCTION

Ecophysiologica1 studies are necessary to determine environmental conditions which are optimal, favorable or merely tolerable for the growth of phytoplankton species (Sigaud and Aidar, 1993). The abiotic factors such as pH, light, salinity, temperature and nutrient responses are useful to establish the appropriate conditions for optimizing marine cyanobacteria and microalgae growth (Harrison *et al*., 1993; Rocha *et al*., 2003; Bano and Siddiqui, 2004; Raghavan *et al*., 2008). Chlorophyll-a content has been widely used to determine growth, harvesting time and nutrient addition time in the cultivation of phytoplankton (Gitelson *et al*., 1995). The seasonal variation of diatoms in response to the physico-chemical factors of sea water has been carried out by Sushanth and Rajashekhar (2012). Recently, Shruthi and Rajashekhar (2014) have stated the effect of pH and salinity on the growth of four species of estuarine cyanobacteria. Thajuddin *et al*. (2002) have stated the cyanobacterial diversity in the salt pans. The purpose of the present study was to investigate the effect of abiotic factors such as pH, salinity and temperature on the growth of six species of phytoplankton isolated from the West coast of India.

MATERIALS AND METHODS

Isolation and maintenance of cultures

The cyanobacteria and microalgae samples were collected by filtering 50 liters of surface sea water through plankton net (20 µm pore size). The sampling was also made by taking the scrapings from the rock surfaces and puddles. The cyanobacteria species were isolated by micropipette method, whereas diatoms and planktonic green alga were isolated by agar plate method (Gopinathan, 1996; Andersen, 2005). The cyanobacteria namely, *Chroococcus turgidus*, *Lyngbya confervoides* and *Nostoc commune* were cultured in f/2 media at 28±2˚C. The cultures of diatoms namely, *Chaetoceros calcitrans* and *Skeletonema costatum* and planktonic green alga *Nannochloropsis oceanica* were maintained in Walne's media at 20±2˚C. The cultures were incubated under illumination of 1000 lux with 8 : 16 h light and dark regime. The species were identified by microscopic observation using standard manuals and publications (Desikachary, 1959; Desikachary, 1989; Tomas, 1997). The planktonic green alga was identified by 18S rDNA sequence analysis (Si *et al*., 2011).

Experimental design

For the experiment, all the isolates were maintained in f/2 media. The salinity and pH of the stock culture media was 30±2 ppt and 7.4±0.5, respectively. The pH of the stock media was adjusted to 6.3, 7.5, 8.4, 9.5 and 10.2 with the help of 1 N NaOH and 1 N HCl. The salinity of the media was adjusted to 9, 16, 25, 32 and 40 ppt by diluting it with double-distilled water or by varying the amount of NaCl in the medium.

The known volume (0.5 ml) of well homogenized culture of each isolate was inoculated into each of the 100 ml conical flasks containing 30 ml of the medium. The cultures were incubated at room temperature under illumination of 1000 lux with 8 : 16 h light and dark regime. The temperature range for the growth was determined by incubating the cultures at 20, 30, 40 and 50˚C. The cultures were retrieved after the incubation period of 7 days by centrifugation except *Chroococcus turgidus* which was harvested after 10 days for the determination of growth in terms of chlorophyll-a content. For each culture, the experiments were repeated three times.

Estimation of chlorophyll-a

About 10 ml of 90 % acetone was added to the harvested cultures . The tubes were vigoursly shaken and homogenized so as to dissolve completely in the solvent. For complete extraction of the pigments, the tubes were kept in a refrigerator for 24 h. After the extraction period, the samples were centrifuged and the supernatant was collected. The supernatant was made upto 10 ml with 90 % acetone and absorbance was measured at 630, 647, 664 and 750 nm against 90 % acetone as blank. The amount of chlorophyll-a content was calculated using equation given by Jeffrey and Humphrey (1975).
Chl-a (µg/ml) = $(11.85 * OD_{664}) – (1.54 * OD_{647}) – (0.08 * OD_{630})$

RESULTS AND DISCUSSION

The effect of abiotic factors such as pH, salinity and temperature on the growth of isolated species was determined by quantifying their chlorophyll-a pigment. The growth of isolates at different pH conditions are shown in Figure 1 (a & b). The isolates preferred near neutral to alkaline pH. The cyanobacteria namely, *Chroococcus turgidus*, *Lyngbya confervoides* and *Nostoc commune* and the diatoms *Chaetoceros calcitrans* and *Skeletonema costatum* showed maximum growth at pH 7.5, whereas planktonic green alga *Nannochloropsis oceanica* showed maximum growth at pH 8.4. The pH value lower or higher than these values was associated with their decreased growth. All isolates also exhibited growth at pH 6.3 but it was very low. The high pH levels adversely affect the growth of isolates and significantly reduced at pH 10.6. The limitation of phytoplankton growth and photosynthesis at elevated pH levels was observed by Chen and Durbin (1994). At high or low pH, cells may have to spend energy for maintainance of an internal pH necessary for cell function (Raven and Lucas, 1985). In pH drift experiments, Goldman (1999) found that when once the pH of cultures increased above pH 8.45, 8.51 and 8.31 there was a decrease in the growth of *Stephanopyxis palmeriana*, *Ditylum brightwellii* and *Coscinodiscus* sp., respectively.

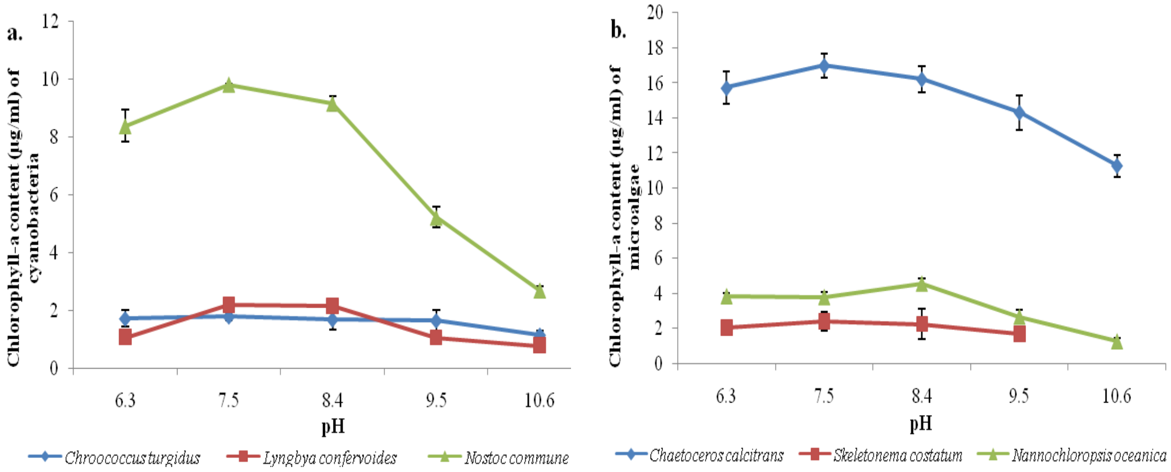

Figure 1 (a & b): Effect of pH on the growth (chlorophyll-a content in µg/ml) of cynaobacteria and microalgae species.

Earlier studies related to pH effect on the growth of cyanobacteria has revealed that pH between 7.4 and 8.0 is favorable for the optimum growth of cyanobacterial species (Rippka *et al.*, 1979; Bano and Siddiqui, 2004). The fact that all cyanobacteria were able to grow in acidic (pH 6.5) medium indicates that cyanobacteria can adapt to variable pH conditions as suggested by Buck and Smith (1995) and Burja *et al.* (2002). Thornton (2009) reported that photosynthetic efficiency of *Chaetoceros muelleri* decreases as the environment surrounding the cells becomes more acidic and also observed that growth rate of diatom was not affected by pH between 7.4 and 8.2, but growth rate at pH 6.8 was significantly lower.

The growth of isolated species at different salinities are shown in Figure 2 (a & b). All isolates were able to grow at all salinities except diatom *Skeletonema costatum* which did not show growth at 40 ppt. The cyanobacterium *Chroococcus turgidus*, diatom *Chaetoceros calcitrans* and planktonic green alga *Nannochloropsis oceanica* showed good growth at 16 ppt, whereas cyanobactia *Lyngbya confervoides* and *Nostoc commune* and the diatom *Skeletonema costatum* showed maximum growth at 25 ppt. The growth was decreased at higher salinities. The cyanobacteria species also exhibited best growth at lower salinity of 9 ppt. It has been reported that cyanobacteria can adapt to the variations in salinity but all cyanobacteria are not halotolerant (Blumwald and Tel-Or, 1982). Many freshwater species are reported to withstand higher salinities (Carr and Wyman, 1986). Similarly, many marine forms can survive at lower salinity, but for their optimum growth they express specific requirements for additional salts (Rippka *et al.*, 1979). The ability of cyanobacteria to grow with increased Na^{+} may be related to their ability to adjust respiration (Gabbay-Azaria *et al.*, 1992), regulate intake or efflux of Na^{+} (Molitor *et al.*, 1986), enhance cyclic electron transfer via photosystem I (Jeanjean *et al.*, 1998) and produce organic osmolytic compounds (Reed *et al.*, 1986).

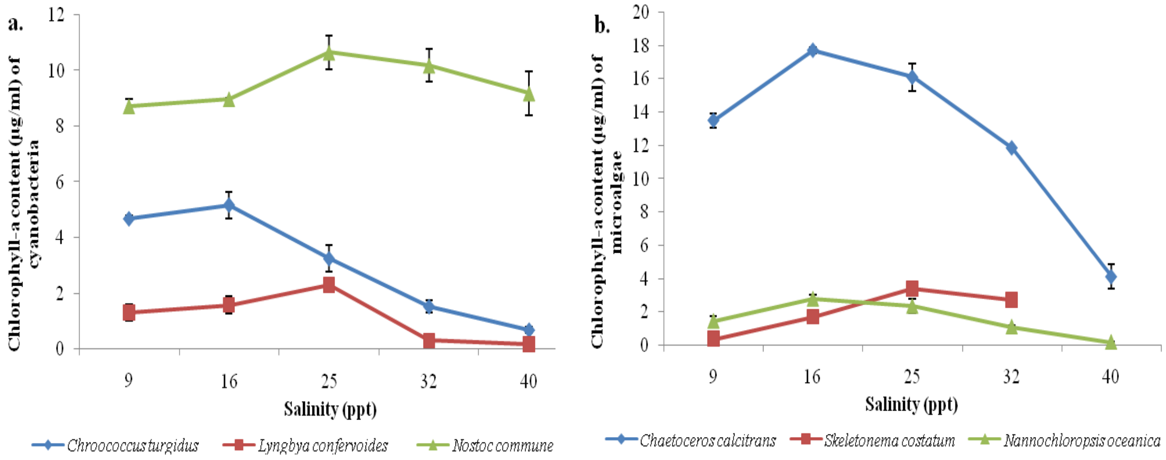

Figure 2 (a & b): Effect of salinity on the growth (chlorophyll-a content in µg/ml) of cynaobacteria and microalgae species.

Earlier studies have reported that the cultivation of phytoplankton with higher saline concentrations had lower chlorophyll contents (Vonshak *et al.*, 1996; Kirrolia *et al*., 2011). It has also been reported that chlorophyll is the primary target to salt toxicity limiting net assimilation rate, resulting in reduced photosynthesis and reduced growth (Rai and Abraham, 1993).

Saros and Fritz (2000) showed that diatom physiology can be affected directly or indirectly *via* interaction with other growth factors such as the ion composition in the saline system. The diatom species such as *Thalassionema eccentrica* and *Pseudo-nitzschia seriata* can able to survive at salinity up to 150 % (Nagasathya and Thajuddin, 2008). According to Raghavan *et al*. (2008), salinity of 25 % and 35 % had no significant effect on growth, maximum cell density, biomass and chlorophyll content of *Chaetoceros calcitrans* f. *pumilus* although a tendency of higher growth, biomass and lower cell density was observed at lower salinity. Hirata *et al*. (1981) observed that growth of marine *Chlorella saccharophila* linearly decreased with increasing salinities from 40 to 60 %. This suggests that *Chlorella* was affected by the overloading of the osmoregularoty mechanism therefore, affecting the growth, photosynthesis and respiration.

The effect of the temperature on the growth of the isolates is shown in the Figure 3 (a & b). The cyanobacteria *Chroococcus turgidus*, *Lyngbya confervoides* and *Nostoc commune* and planktonic green alga *Nannochloropsis oceanica* showed good growth at 20˚C, whereas diatoms *Chaetoceros calcitrans* and *Skeletonema costatum* exhibited high growth at 30˚C. *Chroococcus turgidus*, *Lyngbya confervoides*, *Skeletonema costatum* and *Nannochloropsis oceanica* were unable to grow at 40˚C and 50˚C. Only *Nostoc commune* and *Chaetoceros calcitrans* showed growth at higher temperature. Fogg and Thake (1987) stated that low growth rate of phytoplankton could be a result of the increase in respiration due to rise in temperature above the species optimum level.

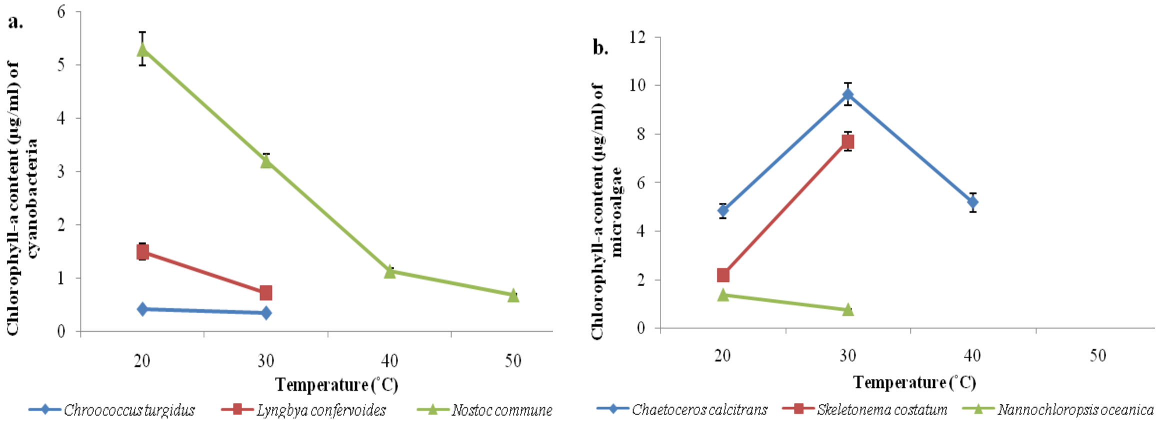

Figure 3 (a & b): Effect of temperature on the growth (chlorophyll-a content in µg/ml) of cynaobacteria and microalgae species.

Most of the phytoplankton species (tropical or subtropical) grew best at temperature ranging from 16 to 27˚C (Creswell, 2010). Kumar *et al*. (2011) found that *Spirulina platensis* has a wide range of temperature tolerance from 20 to 40˚C. Ukeles (1976) compared the growth response of several phytoplankton species with the control which cultured at 20.5˚C and observed that growth of *Monochrysis lutheri* was equal to control at temperature range of 14 to 25˚C, *Isochrysis galbana* at 14 to 22˚C, *Phaeodactylum tricornutum* at 8 to 24˚C, *Dunaliella euchlora* at 12 to 35˚C, *Tetraselmis* sp. at 12 to 32˚C, *Chlorella* sp. (isolate # 580) at 14 to 35˚C and *Chlorella* sp. (UHMC isolate) at 14 to 29˚C. According to Eppley (1972), temperature

ISSN: 2229 – 6905

which is optimum for growth of many temperate diatom species is 20 to 24˚C. Renaud *et al*. (2002) attributed the higher growth rate of *Chaetoceros* sp. to an increase in temperature from 25 to 30˚C.

CONCLUSION

Marine cyanobacteria and microalgae are considered to be a promising source of high value compounds for the pharmaceutical and food industry. They form a very important live feed component in aquaculture practices due to their high content of biochemical compounds. Thus it is essential to identify, isolate and cultivate monocultures under controlled laboratory conditions. The study on the effect of abiotic factors like pH, salinity and temperature on the growth of the isolates are useful for optimizing cell growth and is necessary to understand their response to varying environmental factors.

Acknowledgements: The authors are thankful to the Ministry of Earth Sciences, Government of India, New Delhi for the financial assistance and Dr. C. Krishnaiah, Co-ordinator, OASTC Department of Biosciences, Mangalore University for their help during the study period.

REFERENCES

Andersen, R. A. 2005. Algal Culturing Techniques. Elsevier Academic Press, Burlington. 578 pp.

Bano, A. and P. J. A. Siddiqui. 2004. Characterization of five marine cyanobacterial species with respect to their pH and salinity requirements. *Pak. J. Bot*. **36**: 133 - 143.

Blumwald, E. and E. Tel-Or. 1982. Osmoregulation and cell composition in salt-adaptation of *Nostoc muscorum*. *Arch. Microbiol*. **132**: 168 - 172.

Buck, D. P. and G. D. Smith. 1995. Evidence for a Na^+/ H^+ electrogenic antiporter in an alkaliphilic cyanobacterium *Synechocystis*. *FEMS Microbiol. Lett.* **128**: 315 - 320.

Burja, A. M., E. Abu-Mansour, B. Banaigs, C. Pyari, J. G. Burgess and P. C. Wright. 2002. Culture of marine cyanobacterium, *Lyngbya majuscula* (Oscillatoriaceae), for bioprocess intensified production of cyclic and linear lipopeptides. *J. Microbiol. Methods* **48**: 207 - 219.

Carr, N. G. and M. Wyman. 1986. Cyanobacteria: their biology in relation to the oceanic picoplankton. In: Platt, T. and W. K. W. Li (Eds), Photosynthetic Picoplankton. Canadian Bulletin of Fisheries and Aquatic Sciences 214, Department of Fisheries and Oceans, Ottawa. pp 159 - 204.

Chen, C. Y. and E. G. Durbin. 1994. Effects of pH on the growth and carbon uptake of marine phytoplankton. *Mar. Ecol. Prog. Ser.* **109**: 83 - 94.

Creswell, L. 2010. Phytoplankton culture for aquaculture feed. Southern Regional Aquaculture Center (SRAC) Pub. No. 5004, Department of Agriculture, US. 13 pp.

Desikachary, T. V. 1959. Cyanophyta. Indian Council of Agricultural Research, New Delhi. 686 pp.

Desikachary, T. V. 1989. Atlas of Diatoms. Madras Science Foundation, Madras. 809 pp.

Eppley, R. W. 1972. Temperature and phytoplankton growth in the sea. *Fish. Bull*. **70**: 1063 - 1085.

Fogg, G. E. and B. Thake. 1987. Algal Cultures and Phytoplankton Ecology. The University of Wisconsin Press, Madison, USA. 269 pp.

Gabbay-Azaria, R., M. Schonfeld, S. Tel-Or, R. Messinger and E. Tel-Or. 1992. Respiratory activity in the marine cyanobacterium *Spirulina subsalsa* and its role in salt tolerance. *Arch. Microbiol.* **157**:183 - 190.

Gitelson, A. A., S. Laorawat, G. P. Keydan and A. Vonshak. 1995. Optical properties of dense algal cultures outdoors and its application to remote estimation of biomass and pigment concentration in *Spirulina platensis*. *J. Phycol.* **31**: 828 - 834.

Goldman, J. C. 1999. Inorganic carbon availability and the growth of large marine diatoms. *Mar. Ecol. Prog. Ser.* **180**: 81 - 91.

Gopinathan, C. P. 1996. Live feed – microalgae culture. *C.M.F.R.I. Bull.* **48**: 113 - 118.

Harrison, P. J., P. A. Thompson, M. Guo and F. J. R. Taylor. 1993. Effects of light, temperature and salinity on the growth rate of harmful marine diatoms, *Chaetoceros convolutus* and *C. concavicornis* that kill netpen salmon. *J. Appl. Phycol*. **5**: 259 - 265.

Hirata, H., I. Andarias and S. Yamasaki. 1981. Effects of salinity and temperature on the growth of the marine phytoplankton *Chlorella saccharophila*. *Mem. Fac. Fish. Kagoshima Univ.* **30**: 257 - 262.

Jeanjean, R., S. Bedu, M. Havaux, H. C. P. Matthijs and F. Joset. 1998. Salt-induced photosystem I cyclic electron transfer restores growth on low inorganic carbon in a type 1 NAD(P)H dehydrogenase deficient mutant *Synechocystis* PCC6803. *FEMS Microbiol. Lett.* **167**: 131 - 137.

Jeffrey, S. W. and G. F. Humphrey. 1975. New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanzen* **167**: 191 - 194.

Kirrolia, A., N. R. Bishnoi and N. Singh. 2011. Salinity as a factor affecting the physiological and biochemical traits of *Scenedesmus quadricauda*. *J. Algal Biomass Utln*. **2**: 28 - 34.

Kumar, M., J. Kulshreshtha and G. P. Singh. 2011. Growth and biopigment accumulation of cyanobacterium *Spirulina platensis* at different light intensities and temperature. *Braz. J. Microbiol*. **42**: 1128 - 1135.

Molitor, V., W. Erber and G. A. Peschek. 1986. Increased levels of cytochrome oxidase and sodium-proton antiporter in the plasma membrane of *Anacystis nidulans* after growth in sodium-enriched media. *FEBS Lett.* **204**: 251 - 256.

Nagasathya, A. and N. Thajuddin. 2008. Diatom diversity in hypersaline environment. *J. Fish. Aquat. Sci*. **3**: 328 - 333.

Raghavan, G., C. K. Haridev and C. P. Gopinathan. 2008. Growth and proximate composition of the *Chaetoceros calcitrans* f. *pumilus* under different temperature, salinity and carbon dioxide levels. *Aquacult. Res.* **39**: 1053 - 1058.

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

Raven, J. A. and W. J. Lucas. 1985. Energy costs of carbon acquisition. In: Lucas, W. J. and J. A. Berry (Eds), Inorganic Carbon Uptake by Aquatic Phytosynthetic Organisms. American Society of Plant Physiologists, Rockville. pp 305 - 324.

Reed, R. H., L. J. Borowitzka, M. A. Mackay, J. A. Chudek, R. Foster, S. R. C. Warr, D. J. Moore and W. D. P. Stewart. 1986. Organic solute accumulation in osmotically stressed cyanobacteria. *FEMS Microbiol. Rev.* **39**: 51 - 56.

Renaud, S. M., L. V. Thinh, G. Lambridis and D. L. Parry. 2002. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. *Aquaculture* **211**: 195 - 214.

Rippka, R., J. Deruelles, J. B. Waterbury, M. Herdman and R.Y. Stainer. 1979. Generic assignments, strain histories and properties of pure cultures of cyanbacteria. *J. Gen. Microbiol.* **111**: 1 - 61.

Rocha, J. M., J. E. Garcia and M. H. Henriques. 2003. Growth aspects of the marine microalga *Nannochloropsis gaditana*. *Biomol. Eng.* **20**: 237 - 242.

Saros, J. E. and S. C. Fritz. 2000. Changes in the growth rates of saline lake diatoms in response to variation in salinity, brine type and nitrogen form. *J. Plankton Res*. **22**: 1071 - 1083.

Shruthi, M. S. and M. Rajashekhar. 2014. Effect of salinity and pH on the growth and biomass production in the four species of estuarine cyanobacteria. *J. Algal Biomass Utln.* **5**: 29 - 36.

Si, L., P. Kehou, Z. Baohua, M. Xiaolei, L. Xin and Y. Guanpin. 2011. Molecular identification of a species in genus *Nannochloropsis*. *J. Ocean Univ. China* **10**: 391 - 396.

Sigaud, T. C. S. and E. Aidar. 1993. Salinity and temperature effects on the growth and chlorophyll-a content of some planktonic algae. *Bol. Inst. Oceanogr.* **41**: 95 - 103.

Sushanth, V. R. and M. Rajashekhar. 2012. Seasonal variation in diatoms in response to physico-chemical characteristics of coastal waters of Uttara Kannada district, West coast of India. *Int. J. Environ. Sci.* **2**: 1543 - 1552.

Thajuddin, N., A. Nagasathya, R. Chelladevi and L. Saravanan. 2002. Biodiversity of cyanobacteria in different salt pans of Pudukottai district, Tamil Nadu. *Seaweed Res. Utiln.* **24**: 1 - 11.

Thornton, D. C. O. 2009. Effect of low pH on carbohydrate production by a marine planktonic diatom (*Chaetoceros muelleri*). *Res. Lett. Ecol.* **2009**: 1 - 4.

Tomas, C. 1997. Identifying Marine Phytoplankton. Academic Press, San Diego. 856 pp.

Ukeles, R. 1976. Cultivation of plants, unicellular plants. *In* Kinne, O. (Ed.), Marine Ecology III, Part 1. John Wiley and Sons, New York. pp 367 - 466.

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