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Investigating the impact of NaCl salinity on growth, β-carotene, and chlorophyll *a* in the content life of halophytes of algae *Chlorella* sp.

[1]Mojtaba Fathi and [2]Alireza Asem

[1] Department of Fishery, University of Afagh, Urmia, Iran; [2] Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao 266003, China. Corresponding author: M. Fathi, mojtabafathi2012@gmail.com

Abstract. Algae are used for many purposes as: food industry, animal feeding, soil enrichment and biodiesel production. *Chlorella* is widely cultivated from microalgae species as it is rich in nutrients and has its implementation as a healthy food use. In this study, the impact of NaCl salinity on growth, beta-carotene, and chlorophyll *a* of *Chlorella* sp. has been investigated. This study results in three salinities (10, 30, 50 g L^{-1}, respectively) have shown that 30 g L^{-1} concentration has the highest value of cell number in day 15th and there was no significant difference between salinities on day 15th ($p>0.05$). Beta-carotene result has shown 50 g L^{-1} salinity and the highest value of beta-carotene did not indicate any statistically significant differences between treatments ($p>0.05$). Chlorophyll *a* result has shown highest value in all treatments (it was in 50 g L^{-1}). However, there was no significant difference between salinities on day 5th ($p>0.05$).
Key Words: *Chlorella*, NaCl, beta-carotene, chlorophyll *a*, cell number.

Introduction. Microalgae are simple photosynthetic organisms, which are widespread in nature, playing fundamental roles as primary producers in marine, freshwater and sub-aerial terrestrial systems (Faria et al 2012). *Chlorella* is a kind of unicellular green algae living in freshwater and belongs to the phylum Chlorophyta (Barghbani et al 2012). *Chlorella vulgaris* is an essential phytoplankton with a high content of proteins and fatty acids, so it is used as live food for fisheries (Niu et al 2011). It contains highly-nutritious substances such as protein, carbohydrates, vitamins, minerals, fatty acids, dietary fibre, and nucleic acids (Horincar et al 2011). *Chlorella* sp. has a much higher utilization rate (10-20%) of light energy for the photosynthesis compared to other common plants (Zhang et al 2000). Its nutritional value has also great importance for food applications. According to Barghbani et al (2012) it contains 45% protein (w/w, dry basis), 20% fat, 20% carbohydrates, 5% fibre and 10% minerals and vitamins. B-1 and 3-glucan are the most important substances in *Chlorella* with a good capacity for scavenging radicals and reducing blood lipids. *Chlorella* has also indicated certain health benefits on hypercholesterolemia and tumor effects (Spolaore et al 2006). The production of environment-friendly fuel (biodiesel) which may be substituted for fossil fuels is another important application for the alga *Chlorella* (Chisti 2007; Lawal & Babakano 2011).

Material and Method

The sample preparing. Liquid stock of halophilic algae (*Chlorella* sp.) was prepared and purified at Artemia and Aquatic Animals Institute Phycolab - Urmia University.

Treatments. Purified *Chlorella* sp. sample was prepared in water with 30 g L^{-1} salinity. Then, the volume was increased to 3.3×10^{-6} cells/ml and cultured in three different salinities (10, 30 and 50 g L^{-1}) in three replicates in 500 ml volumes, respectively. At first, TMRL culture medium (1 ml per L of the culture medium) was used to improve the cultural conditions (Faramarzi et al 2010). Other conditions were fixed during the cultural

period as follows: pH = 7.5-8.0, light intensity: 3000-4000 μmol^{-2}s^{-1}, temperature: 25±1 °C. Direct airing method was used in order to create equal conditions for each culture medium.

Measurement of beta-carotene and chlorophyll a. Five (5) ml of each treatment was centrifuged at 4000 rpm for 10 minutes. The supernatant was evacuated. Due to the presence of salts, the sediment was centrifuged again. The resulted sediment was dissolute in 5 ml acetone 80%. The solution was centrifuged at 4000 rpm for 10 minutes. Then it was placed in spectrophotometer to measure the light absorbance at 412, 431, 460 and 480 nm. Beta-carotene and chlorophyll *a* were calculated according to Eijckelhoff & Dekker (1997).

Results

Cell (no. ml^{-1}). As indicated in Table 1 and Figure 1, the highest cell number among treatments was observed on the fifteenth day and there was no significant difference between salinities on day 15.

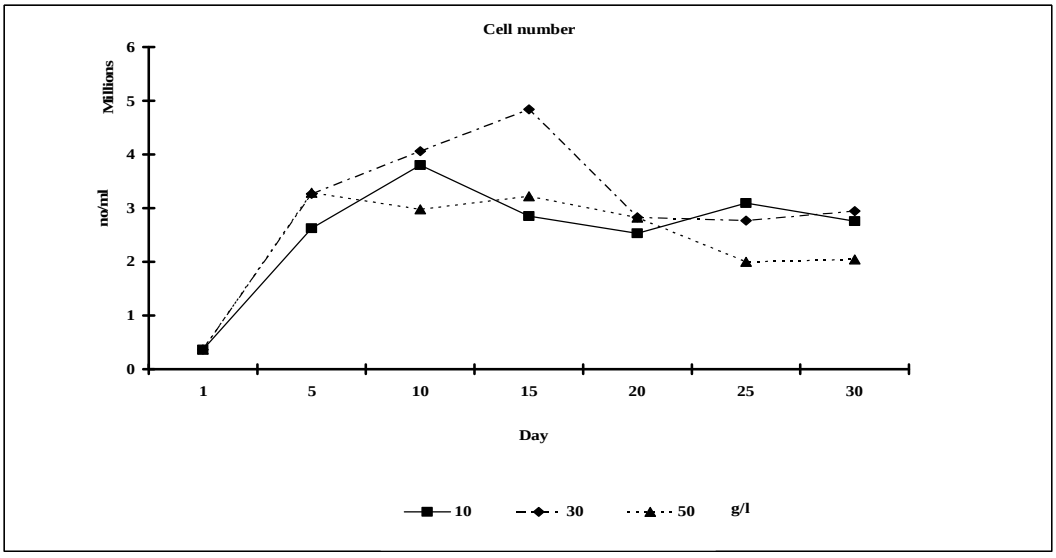

Figure 1. Mean of cell number (no mL^{-1}) in different salinity.

Beta-carotene (μg ml^{-1}). The highest values of beta-carotene was observed on day 10 in three salinities 10 g L^{-1}, 30 g L^{-1} and 50 g L^{-1}, respectively and did not indicate any statistically significant differences between treatments (Table 1 and Figure 2).

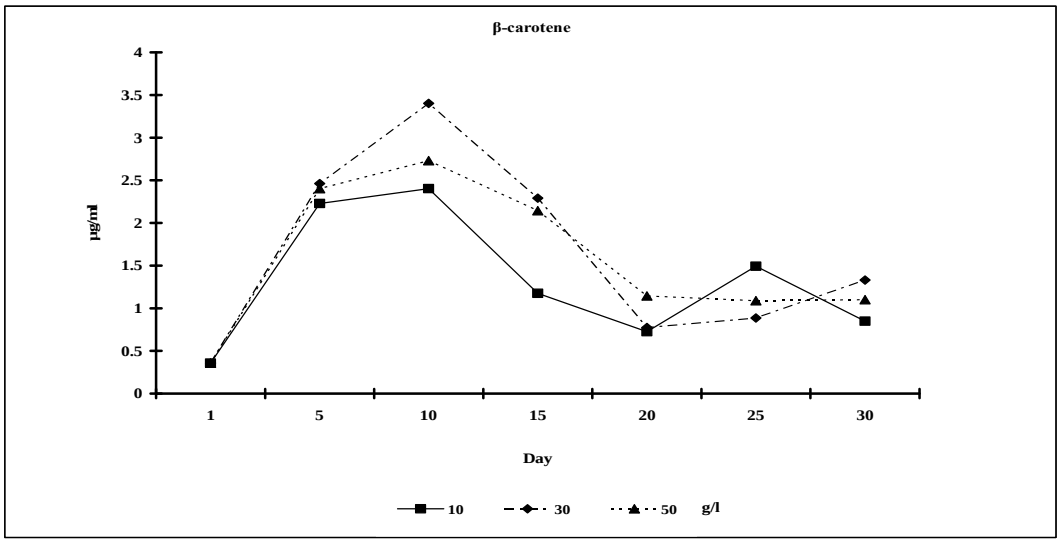

Figure 2. Mean of beta-carotene (μg mL^{-1}) in different salinity.

Chlorophyll a (µg ml^{-1}). Table 1 indicates that the highest value of chlorophyll an in all treatments were observed on day five and did not indicate any statistically significant differences between the treatments (Figure 3).

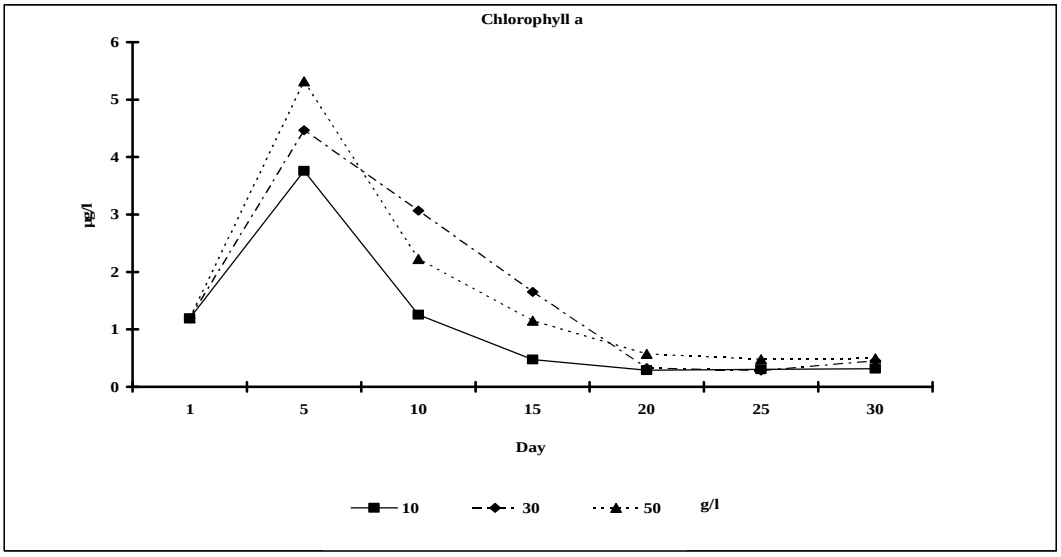

Figure 3. Mean of chlorophyll *a* (µg mL^{-1}) in different salinity.

Table 1

Mean (S.D.) of biological parameters of *Chlorella* sp. in different salinity (same letters in each column show non-significant difference, ANOVA, Tukey, p>0.05)

Salinity	*Day 1*	*Day 5*	*Day 10*	*Day 15*	*Day 20*	*Day 25*	*Day 30*
				Cell (no mL^{-1})			
10 g L^{-1}	3.6×10^5 (4.4×10^3) a	2.6×10^6 (7.5×10^5) a	3.8×10^6 (7.7×10^5) a	2.8×10^6 (2×10^5) a	2.5×10^6 (1×10^5) a	3×10^6 (4.7×10^5) a	2.7×10^6 (1.6×10^5) a
30 g L^{-1}	3.6×10^5 (4.4×103) a	3.2×10^6 (9.9×10^5) a	4×10^6 (3.6×10^5) a	4.8×10^6 (5.7×10^5)	2.8×10^6 (5.1×10^5) a	2.7×10^6 (6.6×10^4) a	2.9×10^6 (1.7×10^4) a
50 g L^{-1}	3.6×10^5 (4.4×10^3) a	3.2×10^6 (8.9×10^5) a	2.9×10^6 (4.4×10^3) a	3.2×10^6 (3.7×10^5) a	2.8×10^6 (3×10^4) a	2×10^6 (1.1×10^5) a	2×10^6 (2.3×10^5) a
				Beta-carotene (µg mL^{-1})			
10 g L^{-1}	0.36 (0.004) a	2.23 (0.556) a	2.40 (0.398) a	1.18 (0.069) a	0.72 (0.181) a	1.49 (0.242) a	0.85 (0.016) a
30 g L^{-1}	0.36 (0.004) a	2.46 (0.061) a	3.40 (0.267) a	2.29 (0.909) a	0.77 (0.057) a	0.89 (0.124) a	1.33 (0.229) a
50 g L^{-1}	0.36 (0.004) a	2.40 (0.293) a	2.73 (0.530) a	2.14 (0.655) a	1.14 (0.139) a	1.09 (0.440) a	1.10 (0.472) a
				Chlorophyll *a* (µg mL^{-1})			
10 g L^{-1}	1.19 (0.025) a	3.76 (0.638) a	1.26 (0.209) a	0.47 (0.042) a	0.29 (0.048) a	0.30 (0.056) a	0.31 (0.036) a
30 g L^{-1}	1.19 (0.025) a	4.46 (0.948) a	3.07 (0.691) a	1.65 (0.410) a	0.33 (0.057) a	0.29 (0.001) a	0.45 (0.077) a
50 g L^{-1}	1.19 (0.025) a	5.32 (1.612) a	2.22 (0.448) a	1.15 (0.234) a	0.57 (0.218) a	0.48 (0.215) a	0.49 (0.273) a

Discussion. In the present study, biomass result showed that 30 g L^{-1} salinity was more appropriate than 10 and 50 g L^{-1} salinities for cellular growth in *Chlorella* sp. (Table 1 and Figure 1). However, there were no significant differences between the treatments ($p>0.05$). Between all treatments, the highest number of cells was observed on day 15th with 30 g L^{-1} salinity, according to Jiang & Chen (1999); Sen et al (2005); Ranga Rao et al (2007); Wang et al (2011). Sankar & Ramasubramanian (2012) showed that each different growth environment had different effects on algae *Chlorella vulgaris*. 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) according to Table 1 and Figure 1. Kusumaningrum et al (2004) proved that *Dunaliella* sp. beta-carotene concentration fluctuation did not follow a regular pattern according to our result showed that the highest value of beta-carotene belonged to day 10 with 30 g L^{-1} salinity. Statistical comparison indicated that beta-carotene value in 30 g L^{-1} salinity had no significant differences on day 10 ($p>0.05$). Therefore we may conclude that day 10 to 30 g L^{-1} salinity is the most appropriate state for higher values of beta-carotene production in *Chlorella* sp. (Table 1 and Figure 2). However, Celekli & Donmez (2006); Fazeli et al (2006); Borowitzka & Siva (2007) proved the highest production of beta-carotene in high salinity, high temperature and high light intensity obtained. Although the higher values of cell number shown in *Chlorella* sp. in the 5th day to 30 g L^{-1}. Mohan et al (2009) reported that chlorophyll *a* had a downward trend in 30 days and this rule started in the first days to last days according to our result around chlorophyll *a* (Table 1 and Figure 3) and there was no significant difference between salinities on day 5th ($p>0.05$) may be this variation have been obtained due to fluctuations of lighting or salt interactions.

Conclusions. Our results showed that NaCl salinity had a great effect on factors such as beta-carotene, chlorophyll *a* and its growth, but increasing salinity, has a negative effect on the factors mentioned above. In fact, these results proved that the highest values of salinity have significant effect on the environmental factor and also salinity can play an important role in algae life.

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Authors:
Mojtaba Fathi, Department of Fishery, University of Afagh, Iran-West Azerbaijan-Urmia, St-Alborz-lands of omid - 88 Number, Urmia, Iran, e-mail: mojtabafathi2012@gmail.com
Alireza Asem, Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao 266003, China, e-mail: alireza_1218@yahoo.com

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