

The Effects of Different Salt Concentrations on the Biochemical Contents of *Gracilaria gracilis* Greville (Rhodophyta)

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Abstract

It has been known that, climate change causes changes in marine water salinity. Since salinity is one of the major factors on osmoregulation and ion concentration of algae, marine algal community will be effected by salinity changes. *Gracilaria gracilis* samples were collected from Izmir Bay on December 2014. After 2 days adjustment period in the laboratory conditions, the algae samples were divided into 4 groups and each group were cultured in different salinity concentrations (10‰, 25‰, 37‰ control group, 48‰) for 7 days. On Day 0, 2, 5 and 7, small pieces of samples collected from each group afterwards total protein, phycocyanin, phycoerythrin and chlorophyll a integrity and catalase activity were analyzed. Phycocyanin, phycoerythrin and chlorophyll a levels and catalase activity showed variations according to exposure time and salinity concentrations. Except for the catalase activity, all the parameters were decreased by the end of the 7th day at different salinities. The highest catalase activity was observed on the last day of the experiment in all groups which shows the salinity stress increasement according to exposure time. *Gracilaria gracilis* was not able to adapt both hiposalinity and hypersalinity conditions.

Keywords: *Gracilaria gracilis*, phycoerythrin, phycocyanin, catalase.

1. Introduction

One of the problems that occurs due to climate change is salinity change of the sea water. The Greenhouse effect increases the water temperature and evaporation rates. Depending on the increasing evaporation, the salinity concentration of seawater increases. Salinity is a limiting factor for macroalgae which inhabit intertidal zones and brakishwaters. Besides, salinity is an important factor on metabolism and growth rate of seaweeds and it affects distribution, growth, morphology and chemical composition of algae. Salinity stress might induce the amount of biological active components and changes the metabolic pathway of the organisms which are under stress.

Hyper/Hypo-osmolarity affects the external water potential and effects the ion distribution and turgor pressure adversely. High or low salinity exposure may inhibite cell division and prohibits the growth of the organism [1]. Besides, salinity stress causes inhibitions on photosynthetic activities [2].

Gracilaria has an economic importance for its high agar content. Also, *Gracilaria* species have antimicrobial activities and produce bioactive metabolites such as primer components [3] and contain important fatty acids

like arylc acid and eicosonoids which have important roles in oxydative pathways [4]. *Gracilaria* is a common alga of the intertidal zone of Izmir Bay.

In this study, we aim to determine the effects of salinity stress on the red alga *Gracilaria gracilis* Greville. The algae samples were exposed to different salt concentrations and total protein (TP), phycocyanin (PC), phycoerythrin (PE) levels and catalase activity were analysed. We believe that this study might contribute to determining the effects of salinity on macroalgae. As a result, we may predict how macroalgae will respond to salinity changes occured depending on global warming.

2. Materials and Methods

2.1. Sample collection and preparation

The *Gracilaria gracilis* samples were collected from Izmir Bay on December 2014. The water temperature (T), salinity, electrical conductivity (EC) and pH of sampling point were measured using a multi parameter probe (CyberScan 600, EUTECH Instruments). Probes were calibrated before the process.

The samples transferred to the laboratory were washed with artificial seawater and their epiphytes were cleaned using toothbrush. The samples were incubated in

artificial seawater in an aquarium for 2 days for acclimatization before the experiment. *G. gracilis* samples were divided into 4 groups (Figure 1). The artificial seawater was prepared using the sea salt (Red Sea coral pro salt) at the desired concentration. After filtration, 1/4 strength Provasoli enrichment medium [5] and other vitamins were added. The artificial seawater in culture aquariums was changed every three days.

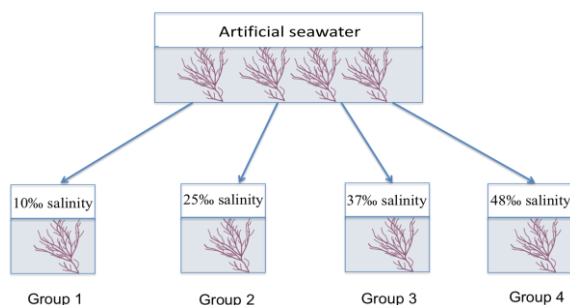


Figure 1. Experimental design of the aquariums.

2.2. Experimental design and analysis

After 2 days acclimatization period, total protein (TP), phycocyanin (PC), phycoerythrin (PE) and chlorophyll a (Chl a) content and catalase activity of the samples were analyzed and recorded as Day 0. All the samples were transferred into experimental aquariums which were differ only by their salinity levels. Group 1, 2, 3 and 4 had 10‰, 25‰, 37‰ (control group) and ‰ 48 salinity concentrations, respectively. The same analyses which were conducted on Day 0 were repeated on 2nd, 5th and 7th days.

TP content of the samples were estimated by Bradford method [6]. Bovine serum albumin standards ranging in concentration from 0.05-0.5 mg/ml were run simultaneously with the test samples.

PC and PE amounts were analyzed according to Beer and Eshel [7]. After samples were extracted with 0.1M phosphate buffer, the absorbance of the supernatants at

different wavelengths (455nm, 564nm, 592nm, 618nm, 645nm) were measured and quantities of PC and PE in the samples were determined.

Chl a was analyzed according to Meeks [8] and Catalase activity was analyzed according to Maksimovic and Zivanovic [9].

3. Results

Four different parameters (water temperature, pH, electrical conductivity, salinity) were considered during the measurement of water quality in the sampling point. The water temperature was 15°C and the pH was 7.9. Salinity was measured as 37.73‰ and the electrical conductivity was 53.82 mS/cm (Table 1).

After 2 days readjustment of the collected samples, they settled into 4 different aquariums with different salinity concentrations. Physical and chemical parameters of the artificial seawater in the culture aquariums were measured (Table 1).

Table 1. Physical and chemical parameters in the sampling point and the culture aquariums.

Physical and chemical parameters	The sampling point	G1* (10 ‰)	G2 (25 ‰)	G3 (37 ‰)	G4 (48 ‰)
Salinity (‰)	37.73	10.62	25.02	36.94	48.83
Temperature (°C)	15.0	19.0	19.0	18.7	18.8
pH	7.9	7.61	7.68	7.64	7.63
Electrical conductivity (mS/cm)	53.82	16.62	37.26	52.13	66.34

* G: Group.

The highest TP content was measured in Group 4 on Day 2 as 88.47±0.781 mg/g wet weight and the lowest value was on Day 7 as 2.35±0.384 mg/g wet weight in the same group. In Day 2 all other groups also showed the highest TP content among the each groups. On Day 5 the TP content was started to decrease and on Day 7 the lowest measurements were obtained for all the groups (Table 2; Figure 2).

Table 2. PE, PC, TP and Chl a and catalase analysis results of all groups.

Group	Day	PC (mg/g)	PE (mg/g)	TP (mg/g)	Catalase activity (U/mg)	Chl a (mg/g)
1 (10‰)	0	0.155 ± 0.013	1.635± 0.219	32.19 ± 13.160	0.427±0.241	0.421±0.22
	2	0.052 ± 0.022	0.734 ± 0.229	73.22 ± 3.853	0.438±0.002	0.341±0.06
	5	0.057 ± 0.025	0.679 ± 0.208	32.44 ± 1.669	0.565±0.110	0.180±0.02
	7	0.009 ± 0.004	0.385 ± 0.111	5.24 ± 1.555	3,064±0.122	0.172±0.01
2 (25‰)	0	0.155 ± 0.013	1.635± 0.219	32.19 ± 13.160	0.427±0.241	0.421±0.22
	2	0.110 ± 0.042	0.888 ± 0.340	79.95 ± 7.174	0.057±0.013	0.202±0.02
	5	0.096 ± 0.029	1.035 ± 0.263	65.04 ± 1.463	2.292±0.006	0.217±0.03
	7	0.005 ± 0.001	0.356 ± 0.177	5.84 ± 1.034	15.316±0.555	0.270±0.02
3 (37‰)	0	0.155 ± 0.013	1.635± 0.219	32.19 ± 13.160	0.427±0.241	0.421±0.22

	2	0.196 ± 0.006	2.048 ± 0.225	87.96 ± 3.339	0.521±0.124	0.144±0.002
	5	0.118 ± 0.010	1.238 ± 0.099	48.09 ± 7.643	0.667±0.021	0.192±0.02
	7	0.005 ± 0.000	0.421 ± 0.154	2.76 ± 0.543	1.662±0.256	0.275±0.02
	0	0.155 ± 0.013	1.635± 0.219	32.19 ± 13.160	0.427±0.241	0.421±0.22
4 (48‰)	2	0.125 ± 0.012	1.151 ± 0.143	88.47 ± 0.781	0.440±0.845	0.407±0.03
	5	0.100 ± 0.014	1.124 ± 0.174	72.29 ± 1.255	3.299±0.678	0.265±0.02
	7	0.014 ± 0.044	0.602 ± 0.044	2.35 ± 0.384	2.927±0.561	0.225±0.01

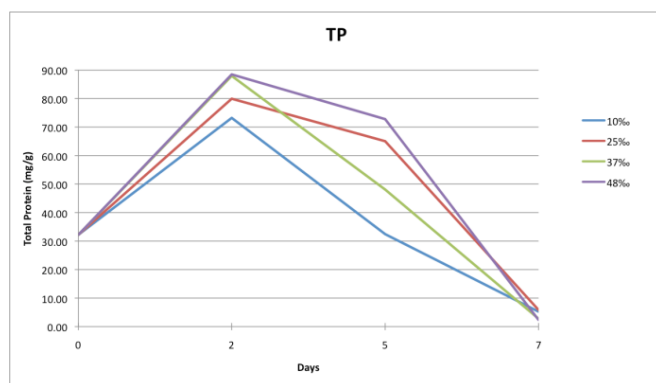


Figure 2. Total protein composition of *G. gracilis* exposed to different salinities.

PC and PE contents were decreased after Day 0 for all the groups except Group 3. The highest PC and PE amounts were measured in Group 3 on Day 2. Afterwards the PC and PE values decreased as seen in the other groups (Table 2). The PC amount decline in all groups in Day 2 whereas Group 3 showed a distinct increase. On Day 5 all the groups exhibited a decreasing pattern of PC content and continued on Day 7 to decline (Figure 3). The similar results for PE content of *G. gracilis* exposed to different salinity concentrations were obtained. The interesting result was obtained again in Group 3 on Day 2 with a considerable increase of PE content (Figure 4).

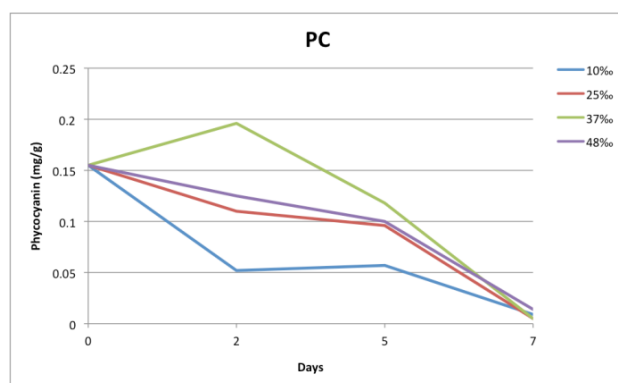


Figure 3. Phycocyanin composition of *G. gracilis* exposed to different salinities.

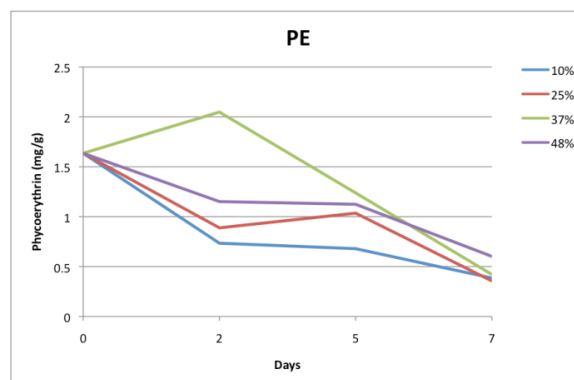


Figure 4. Phycoerythrin composition of *G. gracilis* exposed to different salinities.

G. gracilis showed decline in Chl a content at 25‰ and 37‰ and merely decline at 10‰ and 48‰ salinities in second experimental day. On Day 5, 25‰ and 37‰ salinities showed a small increase of Chl a content according to Day 2. On the other hand, 10‰ and 48‰ salinities showed a clear decrease in Chl a. On the last day of the experiment, Chl a content of the 25‰ and 37‰ salinities continue to increase while latter group's chl a content were decreasing (Table 2; Figure 5).

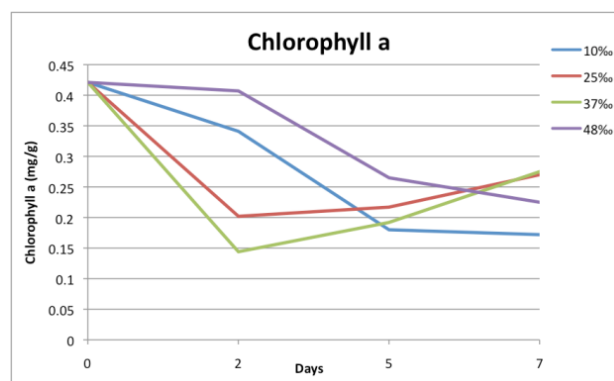


Figure 5. Chlorophyll a composition of *G. gracilis* exposed to different salinities.

Except for the group 4, the highest catalase levels were determined on the last day of the experiment within the other groups (Figure 6). The highest catalase level among all groups was obtained in Group 2 which has 35 times higher catalase activity than Day 0. First group which was exposed to 10‰ salt concentration did not showed a noticeable increase until the 7th day. It was the same for Group 2 which showed a marginal rise of

catalase on the last day. Third group did not show a remarkable response as the other groups. The last group which was the hypersalinated one gave a raised activity on the 5th day but it decreased on day 7.

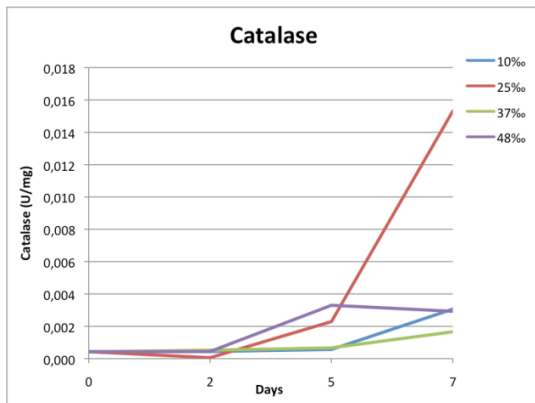


Figure 6. Catalase amounts of *G. gracilis* exposed to different salinities.

4. Discussion

Seaweeds contain three major photosynthetic pigments; carotenoids, chlorophylls and phycobilins. Phycobilins are important protein complexes anchored to thylakoid membranes. They consist of allophycocyanine, phycocyanin and phycoerythrin. Therefore we determined the effects of salinity on PE, PC and Chl a integrity which are important components of photosynthetic apparatus. Bhar et al. [10] mentioned about the way photosynthetic pigment variations with varied salinity gradients and causes alternations in chlorophyll content in mangrove plants. Their results also revealed that the mangroves from the high salinity regions have lower pigment concentrations while low salinity region plants have high pigment levels. They concluded that salinity causes chlorophyll degradation in photosynthetic organisms. Çetin [11] reported that salinity stress affected the amount of chlorophyll a in *Polysiphonia morrowii* species. In that study, hypo-salinity stress lead to an increase in chlorophyll a amount while hyper-salinity stress caused a decrease. The decrease in chlorophyll content at high salinity may result from increased chlorophyllase activity or changes in the lipid protein ratio of the pigment-protein complex [12]. In our experiment, the Chl a amounts of all the groups were analyzed and it was reported a decline on the seventh day (Figure 5). On the second day, Group 1 and 4 showed a slower decrease while group 2 and 3 displayed an apparent one. Group 3 could tolerate the decline in chlorophyll content after the second day because it was control group. Group 3 and group 2, the salinity concentration close to the control, had an increased graph on the 5th day and the increase in the graph continued on the seventh day. Group 1 and 4 displayed a permanent decline during all days. Eventually, it was obtained decreasing chlorophyll

results with the hyper-salinity stress which is consistent with Bhar et al. [10] and Çetin [11]'s studies. But contrary to these studies, a decrease in chlorophyll content was observed at hypo-salinity stress. It was thought that *G. gracilis* could not adapt to the changes in salinity.

As described before *Gracilaria* sp. grown well in the salinity ranges between 25‰ and 35‰ [13, 14, 15]. Thus in this study *G. gracilis* samples were grown in salinities ranging from 10, 25, 37 and 48 ‰. Israel, Martinez-Goss, and Friedlander [16] and Kim, Kraemer, Neefus, Chung, & Yarish [17], reported the accumulation of phycobiliproteins as a response to induced salinity stress although Macler [18] observed a decrease on PC and PE levels during hypo and hyper salinity conditions. Besides, Kumar et al. [15] did not obtain a significant increase of PE and PC levels in hypo-saline conditions as compared to hyper-saline ones. Our findings were congruent with Kumar et al. [15] and Macler [18]'s results while we have decreased PC and PE levels in all groups.

Lu and Vonshak [19] studied effects of salinity on *Spirulina platensis* and found PC and PE degradation according to increased salinity levels. On the fifth day of their experiment they observed a dramatic decrease of total protein amount and chlorophyll integrity. Afterwards they emphasized that the total protein decrease might be an evidence of chlorophyll break down. Our results were similar with the results of Lu and Vonshak [19], total protein variation is consistent with the chlorophyll integrity. Also PE and PC levels were decreased with TP levels. In the first group the PC and PE values were nearly 50% decreased from the beginning (Day 2). The decline continued on Day 5 and the lowest PC and PE results were observed within Group 1 on 7th day. The same results were obtained with Group 2 and 4 although Group 3 gave different results. Contrary to other groups it was obtained that the highest PC and PE amounts were in Group 3 on second day. On Day 5 the PC and PE levels started to decrease but they were still higher than Day 0. On Day 7 the PC and PE levels were similar to the other groups. Khan [20] and Gomes, Suzuki, Cunha, and Tullii [21] highlighted that saline stress slows down the pigment production. Besides they showed the photosynthetic apparatus degradation in transmission electron microscopy images and they suggested that this may have contributed to the reduction in the content of photosynthetic pigments.

If pigment accumulation is a response to salinity stress as introduced on previous studies [15, 17], our results supports this only at the beginning of the exposure. Later on *G. gracilis* couldn't able to adapt the different salinity levels and showed a decrease for PC, PE and Chl a levels. Our results were compatible with Macler [18], who observed a decrease in the level of

PC, PE and chlorophyll. Phycobilins are good antioxidant substances [22]. Increasing the amount of PC and PE may indicate that species exhibit adaptations to environmental stress.

When the catalase activity results are considered, the less stress was observed in the third group which have a similar salinity with the sampling area. Third group was followed by first group, which represents the hyposaline salinity within the groups. As it reported in the catalase activity results *G. gracilis* species was tolerate hiposaline conditions other than the hypersaline ones.

As reported in many studies, increased salinity levels in seawater would affect the seaweed communities. In this study, it was researched the response of a common red algae which has an economical importance in all over the world, against hypo and hyper salinity conditions. Our results showed that *G. gracilis* could not adapt salinity changes.

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