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Research Article

Variation in Photosynthetic Pigments, Antioxidant Enzymes and Osmolyte Accumulation in Seaweeds of Red Sea

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Abstract

The present investigation was carried out to evaluate the response of two groups of seaweeds of red sea to prevailing environmental conditions. Total four seaweeds were selected from two groups viz. (i) Red seaweeds (Rhodophyta): *Gracilaria salicornia* (Gs) and *Digenea simplex* (Ds); and (ii) Green seaweeds (Chlorophyta): *Ulva reticulata* (Ur) and *Chaetomorpha linum* (Cl). The performance of seaweeds was assessed in terms of photosynthetic pigments (Chl a, Chl b, total Chl content, Chl a: b ratio, phycocyanin and phycoerythrin), thiobarbituric acid reactive substances (TBARS), H_2O_2 content, accumulation of osmolytes (proline: Pro and glycine betaine: GB), activities of antioxidant enzymes (superoxide dismutase: SOD; peroxidase: POX; and catalase: CAT), and total protein and carbohydrates. The results show that green seaweeds contain higher level of all the photosynthetic pigments except carotenoids, phycocyanin and phycoerythrin which were higher in red seaweeds. Regarding activities of antioxidant enzymes, red seaweeds show higher activities of POX and CAT except SOD. Concentration of Pro, GB and total protein and carbohydrate were also higher in red seaweeds. Taken together, all the four studied seaweeds show an immense line of variation in their strategy of endurance under similar environmental conditions, but red seaweeds possess higher levels of antioxidant enzymes and osmolytes, thus better adapted to changing climatic conditions.

INTRODUCTION

Oceans contain about ninety percent of the world's biomass which constitutes approximately half of the total global biodiversity [1,2]. Seaweeds or marine algae are integral part of marine ecosystem and serve as good source of food and provide habitat for many animals. Several seaweeds are source of fertilizers, fodder [3,4], antioxidants, dietary fibres, vitamins, minerals, polyphenols, proteins, carbohydrates, agar, carrageenan and alginate [5-8]. Among 8,000 species of seaweeds along the world's coast [9], commercially important red, brown and green seaweeds are represented by 350, 90 and 25 species respectively [10]. Photosynthetic pigments are of vital importance in the life of any plant species, these pigments are classified into three major groups, chlorophylls (a, b, c), carotenoids (carotene and xantophylls) and phycobilins (phycoerythrin and phycocyanin). Chlorophylls are part of group of molecules which are required for photosynthesis. Carotenoids play essential role in passing light energy to chlorophyll and are very strong antioxidant. Likewise carotenoids, phycobilins capture light energy and pass it to chlorophylls.

Being submersed in water the requirement of light for the seaweeds is higher than other plant groups worldwide [11]. The seaweeds in the intertidal zones are constantly exposed to natural as well as anthropogenic sources which adversely affect marine environment through physical, chemical and biological processes and cause losses to seaweeds. These sources induce changes in turbidity, dissolved oxygen and nutrient composition of water and photosynthetic pigments of seaweeds. Moreover, the climatic conditions of Tabuk region (the study area) such as arid environment, negligible precipitation and no sources of fresh water also contribute to alterations in marine environment. These changes act as stressors and adversely affect pigment concentration of seaweeds leading to reduced growth and biomass production. Excessive generation of reactive oxygen (ROS) such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) is another damaging effect of wide range of environmental changes.

Excessive generation of ROS causes peroxidation of membrane lipids, denaturation of proteins, damage to nucleic acids, photosynthetic pigments, leakage of electrolytes and ultimately cell death [12,13], which reflects in the form of losses in biomass production. To cope with the detrimental effects of ROS, plants are equipped with a system of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT). SOD dismutates O_2^- radicals to H_2O_2, whereas CAT and POX are involved in converting H_2O_2 into water and oxygen. Moreover, excessive accumulation of osmolytes such as proline (Pro) and glycinebetaine (GB) is another strategy of plant's defense system to combat osmotic stress.

Seaweeds are precisely responsive to any climatic change and can be used as significant bioindicators for detecting various kinds of environmental changes [14,15]. Physiological stress is the prime impact of climate change, therefore, exploring the physiological response of seaweeds to climatic conditions would be of crucial importance in making environmental conservation policies in the area. Seaweeds of red sea have been identified as under-explored plant resources among the marine organisms and meager or insufficient information is available on the response of seaweeds to the present marine environment. Keeping the importance of seaweeds in view, the present work was undertaken to explore the variation in physiological attributes of red and green seaweeds of red sea coast in response to the prevailing climatic conditions.

MATERIALS AND METHODS

Sample collection

The samples were collected on 24th September, 2014 from Sharmaa sea coast, located 168 km west of Tabuk, and the northwestern province of Saudi Arabia. The collected samples were washed with sea water and surface adhered sand and epiphytes were removed, and samples were stored in 1 liter food grade plastic bottles. The samples were taken to the laboratory and were washed twice with double distilled water. The collected plant samples were divided in two groups (i) Red seaweeds (Rhodophyta); *Gracilaria salicornia* (Gs) and *Digenea simplex* (Ds) and (ii) Green seaweeds (Chlorophyta); *Ulva reticulata* (Ur) and *Chaetomorpha linum* (Cl). Collected algal material was used to analyze following attributes.

Analysis of physico-chemical properties of water

Water samples were collected at the depth of 50 cm and were stored in food grade transparent plastic bottles. The water samples were used for the analyses of pH, temperature, salinity, dissolved oxygen (DO) and concentration of nitrate (NO_3), nitrite (NO_2), ammonium (NH_4) and phpsphate (PO_4), which were estimated according to the protocol described by APHA and UNEP [16,17].

Estimation of photosynthetic pigments

Chlorophyll (Chl) and total carotenoids content were estimated using the method of Lichtenthaler and Buschmann [18]. The fresh tissue was ground with 100% acetone using a mortar and pestle. The optical density of the pigment solution was recorded at 662, 645 and 470 nm to determine Chl a, Chl b and total carotenoids content, respectively, using a spectrophotometer. Phycoerythrin and phycocyanin were estimated adopting the method of Beer and Eshel [19].

Determination of H_2O_2 content and TBARS

The hydrogen peroxide (H_2O_2) content was determined according to Velikova *et al*. [20]. Fresh samples (0.5 g) were homogenized with 5 mL of 0.1% (w/v) trichloacetic acid. The homogenate was centrifuged at 12,000 rpm for 15 min, and 0.5 mL of the supernatant was added to 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide. The absorbance of supernatant was recorded at 390 nm. The content of $H_2O_2$was calculated by comparison with a standard calibration curve, plotted using different concentrations of H_2O_2.

Lipid peroxidation was estimated by the content of thiobarbituric acid reactive substances (TBARS) as described by Cakmak and Horst [21]. TBARS were extracted from 0.5 g chopped material, ground with 5 mL of 0.1% (w/v) trichloroacetic acid (TCA). Following the centrifugation at 12,000gfor 5 min, an aliquot of 1 mL of the supernatant was added to 4 mL of 0.5% (w/v) TBA in 20% (w/v) TCA. Samples were incubated at 90˚C for 30 min. afterward, the reaction was stopped using an ice bath. Centrifugation was performed at 10,000 g for 5 min. and the absorbance of the supernatant was recorded at 532 nm with the help of a spectrophotometer and the values were corrected for non-specific turbidity by subtractingthe absorbance at 600 nm. TBARS content was expressed as nM g^{-1}FW

Determination of Pro and GB content

Proline (Pro) content was determined spectrophotometrically according to Bates *et al*. [22] 300 mg of samples were homogenized in sulphosalicylic acid, then 2 mL each of acid ninhydrin and glacial acetic acid was added. The samples were heated at 100˚C. The mixture was extracted with toluene and the free toluene was quantified spectrophotometrically at 528 nm using L-proline as a standard.

Glycine betaine (GB) content was estimated by the method of Grieve and Grattan [23]. Samples were weighed and oven-dried at80 ˚C, the dried leaves were finely ground with deionized waterat 100 ˚C for 60 min. GB concentration was determined at 365 nm, using aqueous extracts of dry-ground material afterreaction with KI_2–I_2.

Assay of antioxidant enzymes

Plant tissues were homogenized with three volumes (w/v) of anice-cold extraction buffer (50 mMTris–HCl, pH 7.8, 1 mM EDTA, 1 mM MgCl2and 1.5% (w/w) polyvinylpyrrolidone). The homogenate was centrifuged at 15,000gfor 20 min at 4˚C. The supernatant was used as the crude extract for the assay of enzyme activities.

Activity of SOD (E.C. 1.15.1.1) was determined according to Beauchamp and Fridovich [24] by following the photo-reduction of nitro blue tetrazolium (NBT). The reaction mixture contained; 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 75lM NBT, 2 µM riboflavin and 100 µL of the supernatant. Riboflavin was added as the last component and the reaction was initiated by placing the tubes under fluorescent

lamps. The reaction was terminated after 10 min by removing the reaction tubes from the light source. Non-illuminated and illuminated reaction without supernatant served as calibration standards. The absorbance of the solution was measured at 560 nm.

Activity of CAT (E.C. 1.11.1.6) was measured according to Cakmak and Marschner [25]. The decline in absorbance at 240 nm due to the decline of extinction of $H_2O_2$was recorded. The reaction mixture contained 25 mM sodium phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.1 mL enzyme extract.

Activity of POX (E.C. 1.11.1.7) was assayed by the method of Upadhyaya *et al.* [26]. The reaction mixture contained 2.5 mL of 50 mM potassium phosphate buffer (pH 6.1), 1 mL of 1% hydrogenperoxide, 1 mL of 1% guaiacol and 10–20lL of enzyme extract. The increase in absorbance at 420 nm was read.

Estimation of total protein content

Protein content was measured according to Bradford [27] using bovine serum albumin as standard. 100 mg of plant material was taken in a test tube containing 2 ml of 50 mM potassium-phosphate buffer at pH 7.0. Plant tissues were centrifuged at 7000-12000 rpm. The supernatant was centrifuged at 3000 rpm for 15 min at 4ºC. Samples were diluted 1:100 and read at 595 nm in a spectrophotometer.

Estimation of total carbohydrate

The total carbohydrate content of the samples were determined by the method of Hedge and Hofreiter [28], popularly known as Anthrone method. In this method the carbohydrate content was measured by hydrolyzing the polysaccharides into simple sugars by acid hydrolysis, and estimating the resultant monosaccharides.

STATISTICAL ANALYSIS

The mean and standard error were calculated and analyzed statistically using SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). The data is the mean of three replicates.

RESULTS

The results show a considerable variation within the algal groups as well as between the groups of Rhodophta and Chlorophyta.

Photosynthetic pigments

Regarding photosynthetic pigments a significant variation in Chl a, b and total Chl content was recorded among the seaweeds studied (Table 2). Comparison within the red seaweeds show that Gs gave higher Chl a, b, total Chl content and Chl a:b ratio while within green seaweeds Ur gave higher values for these parameters except Chl a:b ratio which was found higher in Cl. Overall, highest content of these pigments was recorded in Ur while lowest in Ds. The Ur gave 90.6%, 60.0%, 80.0% and 19.1% higher values for Chl a, b, total Chl and Chl a:b ratio respectively than Ds (Table 2). On the other hand a higher value for carotenoids was shown by Ds and Ur in red and green algal groups respectively. Taken as whole, red seaweed Ds gave highest values for carotenoids than Cl which gave the lowest value. An increase of 37.8% in carotenoids was recorded in Ds than Cl. Nonetheless, a different pattern of phycocyanin and phycoerythrin levels was recorded in these algal groups, Gs gave higher values for both phycocyanin and phycoerythrin, while in green algal group, Ur showed more phycoerythrin while Cl shows more phycocyanin content. However, among all the selected seaweeds red alga Gs gave highest values for phycocyanin as well phycoerythrin content. The Gs exhibited 132.4% and 116.1% higher concentration of phycocyanin and phycoerythrin than Ur and Cl respectively which gave lowest values (Table 2).

Table 1: Physico-chemical properties of water of collection site of red sea.

pH	Temperature (˚C)	Salinity (‰)	DO (mg/l)	NO_3 (µg/l)	NO_2 (µg/l)	NH_4 (µg/l)	PO_4 (µg/l)
8.28 ± 0.14	32 ± 1.05	46.17 ± 2.66	4.38 ± 0.39	33.65 ± 1. 37	16.29 ± 1.13	13.54 ± 1.87	7.41 ± 0.52

Table 2: Variation in photosynthetic pigments in red and green seaweeds.

Parameters	Species			
	Red seaweeds		Green seaweeds	
	Gs	Ds	Ur	Cl
Chlorophyll-a (mg/g FW)	0.82 ± 0.0071	0.64 ± 0.0064	1.22 ± 0.0100	1.16 ± 0.0106
Chlorophyll-b (mg/g FW)	0.29 ± 0.003	0.25 ± 0.011	0.40 ± 0.006	0.35 ± 0.004
Total chlorophyll (mg/g FW)	1.11 ± 0.010	0.89 ± 0.008	1.62 ± 0.014	1.51 ± 0.013
Chlorophyll a:b	2.83 ± 0.020	2.56 ± 0.035	3.05 ± 0.032	3.31 ± 0.052
Carotenoids (µg/g FW)	14.52 ± 0.126	15.39 ± 0.133	11.80 ± 0.102	11.17 ± 0.097
Phycocyanin (mg/g FW)	0.079 ± 0.001	0.066 ± 0.030	0.034 ± 0.002	0.057 ± 0.003
Phycoerythrin (mg/g FW)	1.88 ± 0.029	1.72 ± 0.021	0.93 ± 0.011	0.87 ± 0.009

Red seaweeds (*Gracilaria salicornia*: Gs; *Digenea simplex*: Ds), green seaweeds (*Ulva reticulata*: Ur; *Chaetomorpha linum*: Cl)

TBARS and H_2O_2 content

Response of seaweeds to oxidative stress was determined in terms of levels of TBARS and H_2O_2 content. In red algal group lower TBARS and H_2O_2 content were recorded in Ds, whereas, in green algal group, Ur gave the lower values for these attributes. Lowest TBARS and H_2O_2 content were recorded in Ds, which showed 31.3% and 44.8% lower levels respectively than Cl which showed highest accumulation of TBARS and H_2O_2 respectively (Figure 1,2).

Pro and GB content

Regarding Pro and GB content Ds gave higher values in red algal group, while in green algal group Ur gave higher values for these two osmolytes. Overall, Ds showed highest Pro as well as GB content than Cl which gave the lowest values. The Ds gave 19.4% and 36.3% higher Pro and GB content respectively than Cl (Figure 3,4).

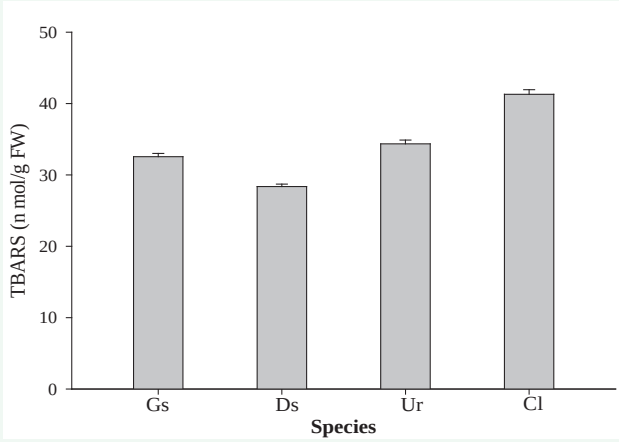

Figure 1 Variation in TBARS in red and green seaweeds. Red seaweeds (*Gracilaria salicornia*: Gs; *Digenea simplex*: Ds), green seaweeds (*Ulva reticulata*: Ur; *Chaetomorpha linum*:Cl).

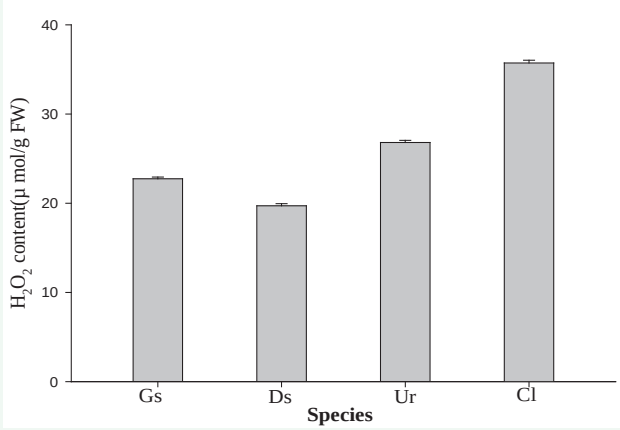

Figure 2 Variation in H_2O_2 content in red and green seaweeds. Red seaweeds (*Gracilaria salicornia*: Gs; *Digenea simplex*: Ds), green seaweeds (*Ulva reticulata*: Ur; *Chaetomorpha linum*:Cl).

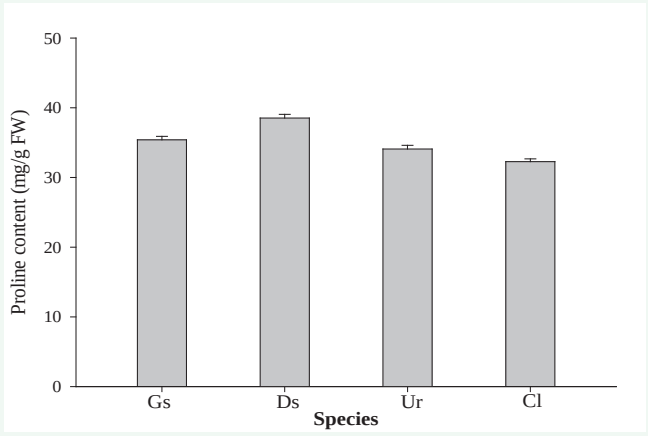

Figure 3 Variation in proline content in red and green seaweeds. Red seaweeds (*Gracilaria salicornia*: Gs; *Digenea simplex*: Ds), green seaweeds (*Ulva reticulata*: Ur; *Chaetomorpha linum*: Cl).

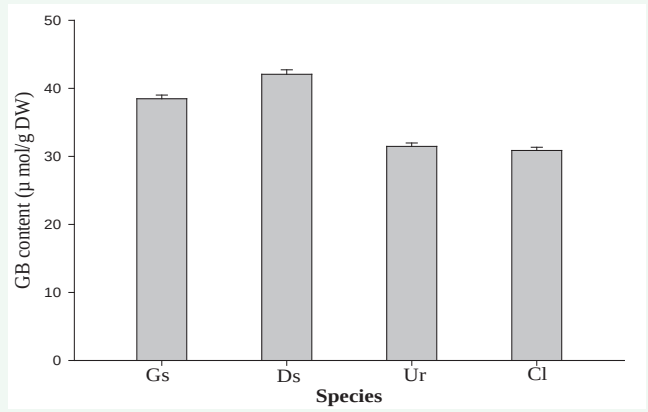

Figure 4 Variation in glycine betaine content in red and green seaweeds. Red seaweeds (*Gracilaria salicornia*: Gs; *Digenea simplex*: Ds), green seaweeds (*Ulva reticulata*: Ur; *Chaetomorpha linum*: Cl).

Antioxidant enzymes

A significant variation in the activities of antioxidant enzymes was recorded in both the algal groups (Table 3). As far as red seaweeds are concerned, Ds gave higher values for POX and CAT, while higher value of SOD was recorded in Gs. In green algal group, Ur gave higher values for all the antioxidant enzymes studied. While taken together, Gs proved best in terms of SOD and showed 24.4% more activity than Cl which gave least values. Regarding POX and CAT, Ur performed best and gave 60.4% and 17.5% higher activities respectively than Gs which gave lowest values for CAT (Table 3).

Total protein and carbohydrate

Regarding total protein content Ds and Ur gave higher value in red and green algal groups respectively (Table 3). On the other hand, Ds recorded the highest while Cl lowest protein content among all the species studied. The protein content of Ds was 125.5% more than Cl. Regarding total carbohydrates, Gs accumulated more carbohydrates in red algal group while Ur in green algal group. However, taken together Gs proved best

Table 3: Variation in SOD, POX, CAT, protein and carbohydrate in red and green seaweeds.

Parameters	Species			
	Red seaweeds		Green seaweeds	
	Gs	Ds	Ur	Cl
SOD (Units/g FW)	137.51 ± 1.191	122.49 ± 1.061	152.37 ± 0.792	146.28 ± 1.267
POX (Units/g FW)	18.27 ± 0.158	16.83 ± 0.146	11.39 ± 0.099	14.64 ± 0.127
CAT (μ mol H_2O_2 decomposed /g FW)	387.39 ± 3.355	366.20 ± 3.171	329.61 ± 2.855	351.08 ± 3.040
Total protein (%)	6.59 ± 0.057	7.60 ± 0.109	4.78 ± 0.041	3.37 ± 0.021
Total carbohydrates (%)	31.28 ± 0.271	29.49 ± 0.255	25.57 ± 0.221	22.34 ± 0.193

Red seaweeds (*Gracilaria salicornia*: Gs; *Digenea simplex*: Ds), green seaweeds (*Ulva reticulata*: Ur; *Chaetomorpha linum*:Cl)

and accumulated 40.0% more carbohydrate than Cl which gave lowest value (Table 3).

DISCUSSION

It is evident from the results that red and green algae show a significant variation in pigments, antioxidant enzymes as well osmolyte accumulation in response to prevailing environmental conditions. Moreover, the variation was not only between the two different groups but also between the species of each group.

Photosynthetic pigments are vital components for organic food production in plants, and cellular viability is associated with photosynthetic activity [29]. It is well known that a defined vertical distribution pattern of seaweeds allow them to expose during low tide and submersed at high tide. Thus seaweeds are continuously exposed to harmful effects of high light intensity, temperature, salinity, heavy metal stress, pollution, turbidity etc. Protection of photosynthetic apparatus against high light exposure is of considerable importance for the endurance of seaweeds [30, 31]. Seaweeds contain three main photosynthetic pigments i.e. chlorophylls, carotenoids and phycobilins. These pigments give protection against high light intensity and also assist in light absorption and energy transfer to the reaction centre. The results show that green seaweeds contain higher amount of Chl a, Chl b and total Chl, whereas, the concentration of carotenoids, phycocyanin and phycoerythrin were found higher in red seaweeds (Table 2). Our results corroborate the findings of Pereira *et al.* [32] who observed that red strain of *Gracilaria domingensis* possesses more phycoerythrin than the green strain, they also observed slight variation in Chl a and phycocyanin. Similar results were also reported by Plastino *et al.* [33] and Yokoya *et al.* [34]. Moreover, variation in the pigment concentration is a response to environmental variations that allows an organism to adapt under a particular habitat.

Increased temperature, heavy metal accumulation and ill-disposed light due to excessive exploitation of natural resources and uncontrolled anthropogenic activities, induces excessive generation of ROS which causes damage to biological membranes and adversely affect several plant physiological processes [35]. In the present study, we analyzed H_2O_2 content and TBARS as indicators of oxidative stress and lipid peroxidation. A significant variation in TBARS was recorded in the studied seaweeds. Highest TBARS was recorded in green alga Cl while lowest in Ds, which reflects variation in the degree of sensitivity among the species (Figure 1). Li *et al.* [36] in *Scenedesmus sp.* recorded that increased ROS levels were associated with cellular lipid accumulation under different environmental stress conditions. Higher damage to membrane lipids was also reported by Soto *et al.* [37] in green alga *Pseudokirchneriella subcapitata* exposed to copper and zinc. Yilancioglu [38] also reported increased lipid peroxidation in nitrogen depleted *Dunaliella salina* alga.

Seaweeds, along with oxidative stress, also face salinity induced osmotic stresses which adversely affect the plant life. Therefore, balance between generation and removal of ROS and reduction of osmotic stress is of crucial importance to cope with detrimental conditions of climatic changes. In order to execute the strategies against climate-induced stresses, plants are equipped with a system orchestrated by a set of antioxidant enzymes and osmolytes which have been proved to ameliorate the damaging effects of ROS and osmotic stress respectively. SOD is known as the cell's first line of defense against ROS that dismutase O_2^- to O_2 and H_2O_2 [39], whereas CAT and POX convert H_2O_2 into O_2 and H_2O. Therefore, higher level of H_2O_2 content is facilitated by either due to increased activity of SOD and/or depressed activities of CAT and POX. In the present study higher activities of SOD and lower activities of POX and CAT were recorded in green seaweeds which ultimately contributed to higher level of H_2O_2 content in green sea weeds, whereas, reverse is true for red seaweeds (Figure 2). Our results corresponded well with the findings of Zou *et al.* [40] who observed a significant increase in the activities of SOD, CAT and POX in seaweed *Sargassum fusiforme* under copper pollution. The results show that red seaweeds accumulated more Pro and GB content and protected the cells of red algae against osmotic and oxidative stress, more efficiently than green algae, which is also witnessed by lower levels of TBARS and H_2O_2 in red seaweeds. It has been observed that Pro and GB contributing to cellular osmotic adjustment, ROS detoxification, protection of membrane integrity and enzymes/protein stabilization [41]. Our results also strengthen the findings of Kebeish and Husain [42].

Proteins and carbohydrates are crucial for any living system. Therefore, accumulation of proteins and carbohydrates will be vital for the endurance of plants under stressful conditions of marine ecosystem. It has been well established that accumulated

proteins fulfill additional energy requirement in response to environmental stress and serve as antioxidant enzymes, defense responsive and photosynthesis-related proteins [43,44]. Proteins have been shown to play a significant role in the absorption of UV radiation. Increased anthropogenic activities cause nutrient enrichment of water bodies and induces turbitiy which reduces penetration of solar irradiance, thus the nutrients particularly nitrogen and lower solar irradiance induce higher accumulation of proteins [32]. The results show that red seaweeds accumulated more protein and carbohydrate and thus provided more protection to this group against abiotic stresses (Table 3). Relatively higher accumulation of protein in red seaweeds might have contributed to enhanced activities of antioxidant enzymes which ultimately resulted in lower levels of TBARS and H_2O_2 content (Figure 1,2).

CONCLUSION

On the basis of assessment of results it may be concluded that the two algal groups tested responded differently. Regarding pigment concentration, Chl a, b, total Chl content and Chl a: b ratio were higher in green seaweeds, whereas, carotenoids, phycocyanin and phycoerythrin were higher in red seaweeds. As far as protection against oxidative and osmotic stress is concerned, red seaweeds showed increased levels of POX, CAT, osmolytes, proteins and lower levels of TBARS and H_2O_2 content. Whereas, green seaweeds exhibited higher levels of SOD, Chl a, b, total Chl and Chl a: b ratio. This variation was not only between the two groups but also between the two species of each algal group. To put all in a nut shell, it can be postulated that red sea weeds posses a higher level of protection against changing climatic conditions than the green seaweeds through activating antioxidant enzymes and accumulating relatively more osmolytes and lower content of TBARS and H_2O_2.

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