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Comparative Response of Red and Green Algae to the Quality of Coastal Water of Red Sea, Haql, Saudi Arabia

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Abstract

Aquatic plants are always exposed to various types of stresses and the marine algae have been considered as useful bioindicators for detecting different kinds of environmental alterations. Present investigation was carried out to test the comparative response of red algae (*Gracilaria salicornia* and *Digenea simplex*) and green algae (*Enteromorpha compressa* and *Sargassum muticum*) to the contaminants present in their surrounding growth medium. The results of the study show that the studied macroalgae responded differently in terms of physiological and biochemical parameters. Green algae exhibited higher concentration of Chl a, b, total chlorophyll content and Chl a:b ratio and carbohydrates content. Whereas, red algae showed higher content of carotenoids, phycocyanin and phycoerythrin and protein content. Moreover, red algae *G. salicornia* and *D. simplex* showed lower level of hydrogen peroxide content and TBARS and higher values of proline and glycine betaine content and activities of antioxidant enzymes *viz.* superoxide dismutase, peroxidase, and catalase than the green algae. Taking all these together, it can be concluded that red algae possess strong defense system than the green algae. Among the studied species, red algae *G. salicornia* was found best in having a stronger defense system.

Keywords

Algae, Antioxidant Enzymes, Contaminants, Osmolytes, Photosynthetic Pigments

1. Introduction

Water is the prime determinant for the existence of life on any planet of the cosmos and water was the medium where life took its shape. The marine ecosys-

tem is considered a reservoir of plant and animal diversity and acts as a vital source of primary production. However, discharge of effluents from industries, sewage, agriculture runoff, and construction sites contains a variety of pollutants including toxic metals, detergents, grease, oil, pesticides, nutrients, suspended particles (Kassem, 1999 [1]), which leads to considerable modifications in the quality of coastal waters (Schulz *et al.*, 1994 [2]; Diez *et al.*, 2019 [3]; El-jaiek-Urzola *et al.*, 2019 [4]). Accumulation of nutrients especially N and P in the marine ecosystem supports algal expansion that results in algal blooms (Khan *et al.*, 2013 [5], 2018 [6]). The presence of these algae on the upper surface prevents the penetration of sunlight which affects marine life at lower surfaces. Moreover, death and decay of these plants require a huge quantity of oxygen which depletes oxygen level and such water exhibits higher values of biochemical oxygen demand (BOD) (Ferreira *et al.*, 2017 [7]; Vigiak *et al.*, 2019 [8]) that adversely affects the aquatic ecosystem. The excess and continuous presence of pollutants in the aquatic ecosystem makes their easy entry in the food chain and causes biomagnification which affects human health (Ahmed *et al.*, 2019 [9]).

Because of submergence in water, seaweeds require more light than other plant groups (Dennison *et al.*, 1993 [10]). In the intertidal zone, the seaweeds are constantly exposed to natural as well as anthropogenic sources which adversely affect marine environment and cause severe 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 the study area such as arid environment, low precipitation and no sources of fresh water also contribute to significant 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 species such as hydrogen peroxide (H_2O_2) is the primary response of plants to various environmental stresses. These ROS cause damage to macromolecules, photosynthetic pigments, leakage of electrolytes and ultimately cell death (Scandalios, 1993 [11]; Mittler, 2002 [12]). Plants cope with the detrimental effects of these ROS through their inbuilt system of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT). SOD converts superoxide ($O_2^{\bullet -}$) radicals to H_2O_2, whereas CAT and POX convert H_2O_2 into water and oxygen. Moreover, plants counter osmotic stress by synthesizing osmolytes such as proline (Pro) and glycinebetaine (GB).

Due to their precise responsiveness to changing climatic conditions, seaweeds can be used as significant bioindicators for detecting various kinds of environmental alterations (Harley *et al.*, 2006 [13]; Faveri *et al.*, 2010 [14]). 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. 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. Therefore, the present work was planned to explore the

quality of water and its impact on physiological and biochemical attributes of red and green seaweeds of the Haql coast of the Red Sea.

2. Materials and Methods

2.1. Water and Plant Sample Collection

Water samples were collected near the water surface at a distance of about 8 meters from the shoreline from three points of the Haql coast of the Red Sea, Saudi Arabia (Figure 1). Water quality was assessed using the mean values of three sampling data sets.

The plant samples were comprised of 1) Red algae (Rhodophyta); *Gracilaria salicornia* (*G. salicornia*), *Digenea simplex* (*D. simplex*) and 2) Green algae (Chlorophyta); *Enteromorpha compressa* (*E. compressa*); *Sargassum muticum* (*S. muticum*). These plant samples were collected from the same above-mentioned location from where water samples were collected. The collected plant samples were washed to remove surface adhered materials and stored in plastic bottles. Later, the algal samples were washed twice with double distilled water and used to analyze various attributes.

2.2. Analyses of Water Samples

Collected water samples were used for the estimation of following quality parameters: turbidity was measured using nephelometer, and pH using portable pH meter. Total dissolved solids (TDS) were estimated using a conductivity meter. Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were measured by the volumetric titration method (APHA, 1995) [15]. Total Kjeldahl nitrogen (TKN) was estimated by colorimetric method (EPA, 1978a) [16], ammonia

Figure 1. The study area, Haql, Saudi Arabia. (Source: Google Map: http://maps.google.com/maps?q=29.294+34.951+(Haql)&ll=29.294,34.951&spn=05.0,05.0&t=k&hl=en)

(NH_3) was estimated by Nessler method as described by Koch and McMeekin (1924) [17], nitrate (NO_3^-) was determined according to Rodger *et al.* (2017) [18] using cadmium reduction method. Fluoride was estimated by SPADNS method adapted from standard methods for the examination of water and wastewater (APHA, 1998) [19]. Total phosphorus (P) and total chlorine residual, and iron (Fe) were analyzed using standard methods as described in method 8048-Hach, 8167-Hach, and 8008-Hach, respectively (APHA, 1995) [15]. Oil and grease contents were quantified using gravimetric method 9070 (Blum and Taras, 1968) [20]. Total organic carbon (TOC) was assessed using EPA method 415.1 (EPA, 1999) [21], while phenols were estimated spectrophotometrically using EPA method 420.1 (EPA, 1978b) [22]. Concentration of following HMs was tested: Arsenic (As), Cadmium (Cd), Copper (Cu), Mercury (Hg), Lead (Pb), Selenium (Se), Barium (Ba), and Zinc (Zn). Concentration of various chemicals in water samples is mentioned in **Tables 1-3**.

2.3. Analyses of Algal Samples

2.3.1. Determination of Photosynthetic Pigments, Total Protein, and Carbohydrates Content

The method of Lichtenthaler and Buschmann (2001) [23] was used to estimate Chlorophyll (Chl) and total carotenoids content. The optical density of the pigment

Table 1. Physico-chemical characteristics of water of Red Sea along Haql coast.

Parameters				
Turbidity (NTU)	**pH**	**TDS (mg/L)**	**BOD (mg/L)**	**COD (mg/L)**
0.36 ± 0.027	8.49 ± 0.68	32471 ± 9.36	32 ± 1.49	3154 ± 8.52

Values are average ± SE of three independent replicates. TDS: total dissolved solids; BOD: biochemical oxygen demand; COD: chemical oxygen demand.

Table 2. Presence of oil and grease, phenols, total Kejldahl nitrogen (TKN), NH_3, NO_3^-, and P in the water of Red Sea along Haql coast.

Parameters (mg/L)	
Oil and grease	1.86 ± 0.0768
Total organic carbon	1.49 ± 0.0472
Phenols	0.002 ± 0.0710
TKN	0.81 ± 0.239
NH_3	10.65 ± 0.297
NO_3^-	2.10 ± 0.250
P	0.08 ± 0.0026
Fluoride	1.97 ± 0.049
Total chlorine residual	0.08 ± 0.0002
Iron	0.07 ± 0.019

Values are average ± SE of three independent replicates.

Table 3. Concentration of heavy metals in the water of Red Sea along Haql coast.

Heavy metals (mg/L)	
As	0.004 ± 0.0005
Cd	0.00013 ± 0.0032
Cu	0.00015 ± 0.0006
Hg	0.00093 ± 0.00029
Pb	0.009 ± 0.00039
Se	0.0003 ± 0.00042
Ba	0.0063 ± 0.00052
Zn	0.0002 ± 0.00042

Values are average ± SE of three independent replicates.

solution was recorded at 662, 645 and 470 nm to determine Chl a, Chl b and total carotenoids content, respectively. Phycoerythrin and phycocyanin were estimated adopting the method of Beer and Eshel (1985) [24].

Protein content was measured according to Bradford (1976) [25] using bovine serum albumin as standard. The total carbohydrates content in the algal samples was determined by the method of Hedge and Hofreiter (1962) [26].

2.3.2. Determination of Stress Markers

To determine the impact of water contamination on plants, H_2O_2 content and peroxidation of lipids in the plant samples were assessed as stress markers. Lipid peroxidation was assessed by measuring the content of thiobarbituric acid reactive substances (TBARS). H_2O_2 content was determined according to Velikova *et al.* (2000) [27]. The absorbance of the samples was read at 390 nm and H_2O_2 content was quantified by comparing with a standard curve and was expressed as $\mu mol \cdot g^{-1}$ FW. Content of TBARS was determined according to Cakmak and Horst (1991) [28]. The absorbance of the supernatant was measured at 532 nm and 600 nm. The values were corrected for non-specific turbidity by subtracting the absorbance and the values were expressed as $nmol \cdot g^{-1}$ FW.

2.3.3. Assay of Antioxidant Enzymes

Activity of SOD (E.C. 1.15.1.1) was determined according to Beauchamp and Fridovich (1971) [29] by following the photoreduction of nitro blue tetrazolium (NBT). Activity of POX (E.C. 1.11.1.7) was assayed by the method of Upadhyaya *et al.* (1985) [30]. Whereas, method of Cakmak and Marschner (1992) [31] was used to determine the activity of CAT (E.C. 1.11.1.6).

2.3.4. Statistical Analysis

The data were analyzed statistically using SPSS-20 statistical software (SPSS Inc., Chicago, IL, USA). Means of three independent replicates were presented ± SE. When F value was found to be significant at 5% level of probability, critical difference (CD) was calculated.

3. Results and Discussion

3.1. Photosynthetic Pigments, and Total Protein and Carbohydrates Content

A perusal of the data show that all the studied algal species showed a significant variation in photosynthetic pigments (Table 4). A comparison within the red algae shows that *G. salicornia* gave higher values for all the studied photosynthetic pigments (Chl a, b, total Chl, carotenoid, phycocyanin and phycoerythrin) except Chl a:b ratio which was higher in *D. simplex*. The studied green algae performed differently. *E. compressa* gave higher values for Chl a, b, and total Chl content, while lower values of Chl a:b ratio, carotenoid, phycocyanin and phycoerythrin were recorded as compared to the *S. muticum*. Overall, highest value of total Chl content was recorded in green alga *E. compressa*, whereas highest content of carotenoids, phycocyanin and phycoerythrin were found in red alga *G. salicornia* (Table 4). Photosynthetic pigments are of vital importance in plant life, these pigments are not only responsible for organic food production but also provide protection against high light intensity and also assist in light absorption and energy transfer to the reaction centre. As shown by the results green algae possess a higher concentration of Chl a, Chl b and total Chl, which resulted in enhanced accumulation of carbohydrates (Figure 2). However, the concentration of carotenoids, phycocyanin and phycoerythrin were found higher in red algae (Table 4). Our results corroborate the findings of Pereira *et al.* (2012) [32] and Nasir *et al.* (2015) [33] who observed that red strains of seaweeds possess more phycoerythrin than the green strain, they also observed variation in Chl a and phycocyanin. Similar results were also reported by Plastino *et al.* (2004) [34] and Yokoya *et al.* (2007) [35]. Seaweeds are continuously exposed to harmful effects of various climatic factors such as high light intensity, temperature, salinity,

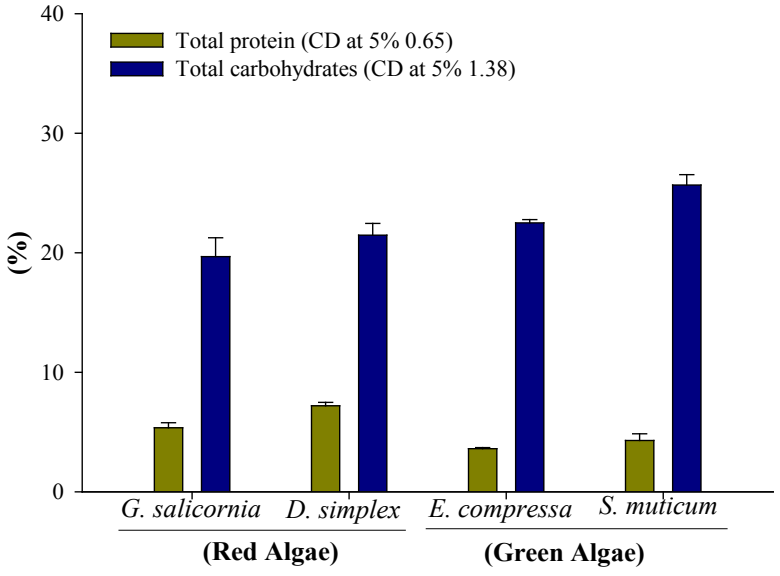

Figure 2. Concentration of total protein and carbohydrates in red and green algae. Average of three determinations is presented with CD at 5%, T bars indicating SE.

Table 4. Comparative concentration of photosynthetic pigments in red and green algae.

Parameters	Red algae		Green algae		
	G. salicornia	*D. simplex*	*E. compressa*	*S. muticum*	CD at 5%
Chlorophyll-a (mg/g FW)	0.91 ± 0.003	0.79 ± 0.004	2.18 ± 0.073	1.82 ± 0.087	0.035
Chlorophyll-b (mg/g FW)	0.45 ± 0.006	0.38 ± 0.018	0.41 ± 0.002	0.29 ± 0.011	0.029
Total chlorophyll (mg/g FW)	1.36 ± 0.021	1.17 ± 0.017	2.59 ± 0.035	2.11 ± 0.019	0.0062
Chlorophyll a:b	2.02 ± 0.018	2.08 ± 0.042	5.31 ± 0.061	6.28 ± 0.028	0.0019
Carotenoids (μg/g FW)	15.37 ± 1.47	14.08 ± 2.16	10.62 ± 1.52	11.54 ± 2.10	0.271
Phycocyanin (mg/g FW)	0.086 ± 0.002	0.072 ± 0.001	0.042 ± 0.002	0.051 ± 0.001	0.0004
Phycoerythrin (mg/g FW)	1.93 ± 0.036	1.65 ± 0.017	0.86 ± 0.012	0.91 ± 0.004	0.0057

heavy metal stress, pollution, turbidity etc. Protection of photosynthetic apparatus against high light exposure is of considerable importance for the survival of seaweeds and increased photosynthetic pigments in response to different light qualities have been reported by Borlongan *et al.* (2020) [36]. In addition, variation in these photosynthetic pigments in the studied plants was probably due to the quality of the water (**Tables 1-3**) because presence of heavy metals significantly reduces Chl content in seaweeds (Karimian *et al.*, 2020 [37]; Cheng *et al.*, 2016 [38]). Moreover, variation in the pigment concentration is a response to environmental alterations that allows an organism to adapt under a particular habitat. Higher values of the studied pigments in red algae show their better resistance capability to the changing marine environment.

Proteins and carbohydrates are integral part of any biological system. Carbohydrates provide structure and energy material to the cellular system, while proteins especially act as enzymes in various biochemical reactions. 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 induce turbidity which reduces penetration of solar irradiance, thus the nutrients particularly nitrogen and lower solar irradiance induce higher accumulation of proteins (Pereira *et al.*, 2012 [32]). The results show that red algae accumulated more protein and carbohydrate (**Figure 2**) and thus provided more protection to this group against abiotic stresses. The enhanced increase in protein content was probably due to presence of N in the water (**Table 2**) which has been shown to induce protein synthesis in microalga *Isochrysis galbana* (Zarrinmehr *et al.*, 2020 [39]).

3.2. Level of Stress Markers in Red and Green Algae

Exposure of plants to various natural stresses as well as to uncontrolled anthropogenic activities, induces excessive generation of ROS which causes damage to biological membranes and adversely affects several plant physiological processes (Khan *et al.*, 2020 [40]). In the present study, we analyzed H_2O_2 content and TBARS as stress markers of oxidative stress and lipid peroxidation. Marine

plants face various abiotic stresses such as salinity, osmotic stress which induce oxidative stress by excessive generation of ROS. It is evident from **Figure 3(A)** and **Figure 3(B)** that red algae exhibited lower levels of H_2O_2 and TBRAS than green algae. However, within the red algal group *G. salicornia* showed lower values of these stress markers than the red alga *D. simplex.* While, in the green algal group, *E. compressa* exhibited lower level of H_2O_2 and TBRAS. The increased level of H_2O_2 and TBARS was probably due to the presence of oil, grease, heavy metals and various other chemical compounds in the water (**Tables 1-3**). Our results corroborate the findings of Rezayian *et al.*, 2019 [41], and Cheng *et al.*, 2016 [38]. Therefore, it can be postulated that red algae possess a higher capacity of scavenging ROS as shown by decreased concentration of the stress markers. The lower concentration of H_2O_2 and TBRAS in red algae might be due to higher activities of antioxidant enzymes (SOD, POX, and CAT) that scavenged the ROS and resulted in decreased H_2O_2 level. Effect of lower level of H_2O_2 content was also reflected by the lower peroxidation of lipids as shown by decreased value of TBARS (**Figure 3(B)**).

3.3. Activities of Antioxidant Enzymes and Proline (Pro) and Glycine Betaine (GB) Content in Red and Green Algae

To cope with detrimental effects of oxidative stress plants are fitted with antioxidant system. The antioxidant enzyme SOD is known as the first line of defense against ROS that dismutase $O_2^{\bullet-}$ to O_2 and H_2O_2 (Hassan and Scandalios, 1990 [42]) whereas, CAT and POX convert H_2O_2 into O_2 and H_2O. Increased activities of SOD, POX, and CAT were observed in red algae (**Figure 4(A)** and **Figure 4(B)**). Therefore, lower level of H_2O_2 in red algae was due to increased activities of these antioxidant enzymes. Whereas in green algae, SOD activity was higher while POX and CAT were lower (**Figure 4(A)** and **Figure 4(B)**) that resulted in more accumulation of H_2O_2 than the red algae. Increase in the activities

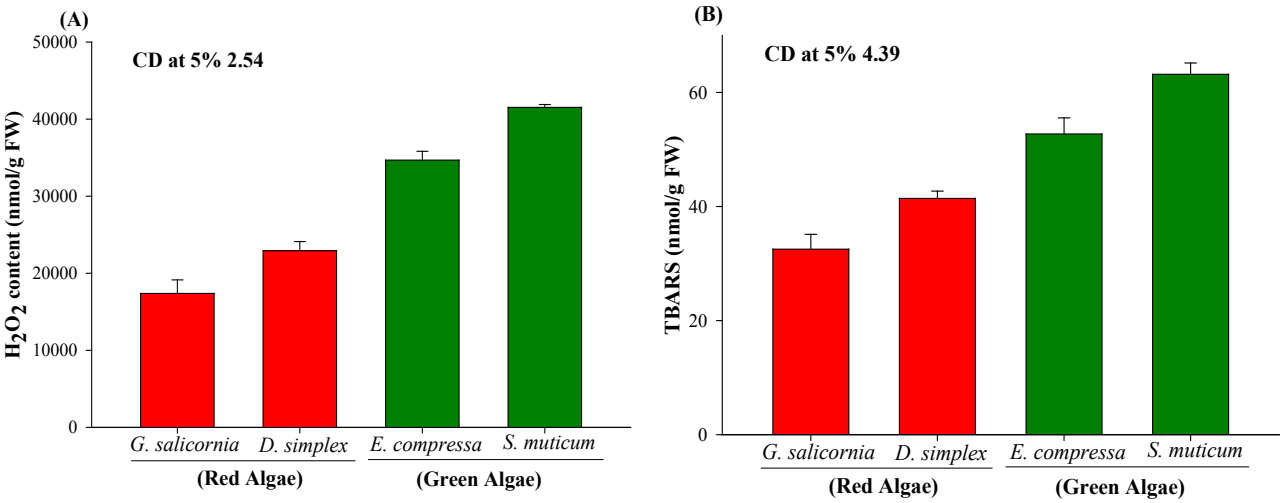

Figure 3. Level of stress markers in red and green algae. (A) H_2O_2 content; (B) Thiobarbituric acid reactive substances. Average of three determinations is presented with CD at 5%, T bars indicating SE.

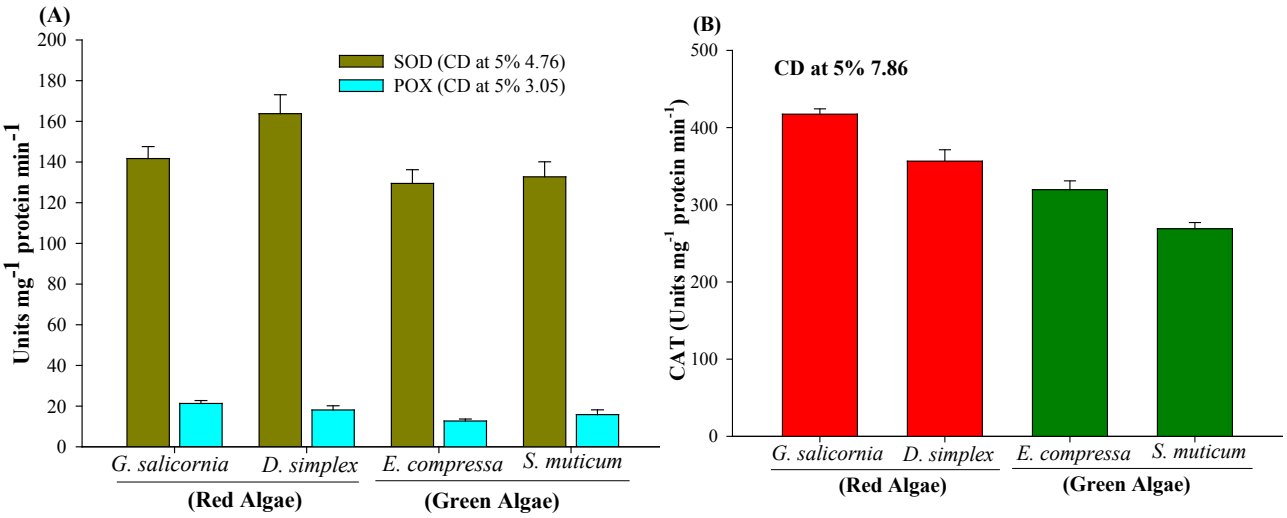

Figure 4. Activity of antioxidant enzymes in red and green algae: (A) activity of superoxide dismutase and peroxidase, (B) activity of catalase. Average of three determinations is presented with CD at 5%, T bars indicating SE.

of antioxidant enzymes in aquatic plants has been reported in response to various environmental stresses (Zou *et al.*, 2014 [43]; Cheng *et al.*, 2016 [38]; Rezayian *et al.*, 2019 [41]). Moreover, the results show that red algae contain more protein that might have contributed to enhanced activities of antioxidant enzymes which ultimately resulted in lower levels of TBARS and H_2O_2 content. It indicates that red algae (*G. salicornia*, *D. simplex*) were more tolerant to various pollutants than the green algae (*E. compressa*, *S. muticum*).

Plants counter osmotic stress by accumulating osmolytes such as Pro and GB. The results of the study show that red algae synthesized more Pro and GB than the green algae and highest level of proline was found in red alga *D. simplex*, whereas lowest value was found in green alga *E. compressa* (**Figure 5(A)** and **Figure 5(B)**). The increased accumulation of sugars, and proline, prevents the damaging effects of stresses by maintaining hydration level, protecting enzyme activity and photosynthesis, and scavenging ROS (Ahanger *et al.*, 2018 [44]; Khan *et al.*, 2017 [45], 2020 [40]). Increased proline accumulation under the stress prevents oxidative stress and protects photosynthesis system, influences the functioning of key enzymes, and improves stress tolerance (Khan *et al.*, 2020 [40]). Therefore, higher accumulation of proline in red algae particularly *D. simplex*, probably caused reduction in H_2O_2 content that resulted in improved photosynthetic pigments and carbohydrate and protein synthesis. It indicates that red algae possess more capacity to counter detrimental effects of excessive concentrations of heavy metals, nutrients, and other environmental stresses than the green algae.

4. Conclusion

The presence of contaminants in water bodies is a menace for the aquatic ecosystem. The present study exhibits that the response of red and green algae to

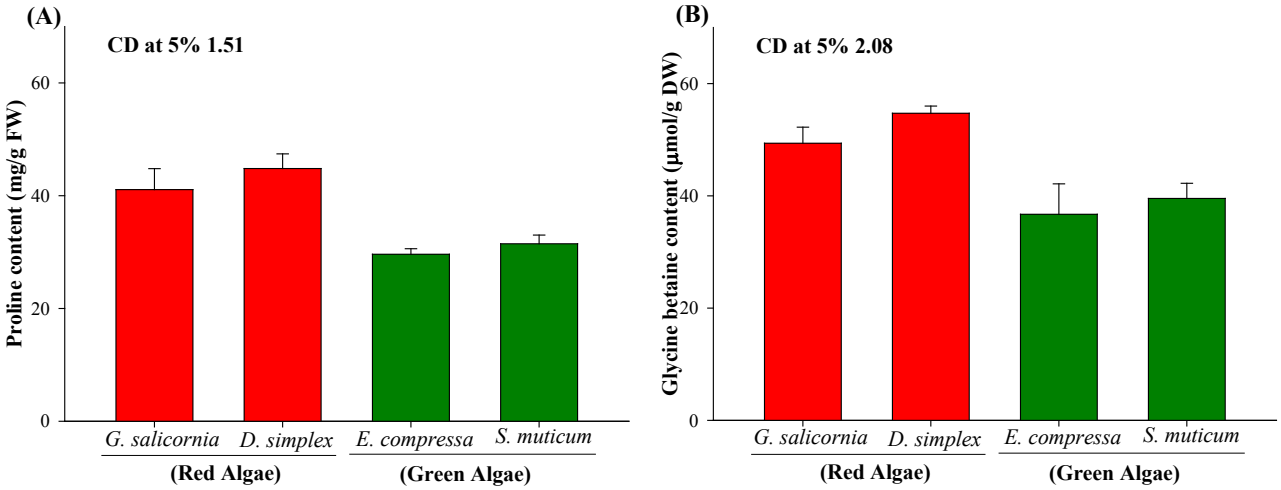

Figure 5. Concentration of osmolytes proline and glycine betaine in red and green algae. Average of three determinations is presented with CD at 5%, T bars indicating SE.

the contaminants present in water of the Red Sea along the Haql coast. The results of the study show 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 algae, whereas, carotenoids, phycocyanin and phycoerythrin were higher in red seaweeds. Higher activities of antioxidant enzymes, and concentration of protein, carbohydrates and protein in red algae show that they possess a strong defense system against the contaminants present in the marine ecosystem. To put all in a nutshell, it can be concluded that red algae were more tolerant of the contaminants than the green algae and the alga *G. salicornia* was found best in having a stronger defense system.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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