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Research Article

Effect of physicochemical parameters of seawater on antioxidant capacity in green, brown, and red macroalgae from the Persian Gulf

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Abstract

The present study aimed to investigate the effect of seasonal variations in physicochemical parameters of seawater including temperature, pH, dissolved oxygen, electrical conductivity, total suspended solids, total nitrogen, and total phosphate, on the antioxidant capacity of the green, brown, and red algae from the northern coasts of the Persian Gulf. The algal collection involved 34 samples (18, 9, and 7 green, brown, and red macroalgae, respectively) from 4 different months of the year 2016 (March, May, October, and December). Antioxidant activity was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. Total phenolic and flavonoid contents were determined using the Folin-Ciocalteu's reagent and the colorimetric method, respectively. Water sampling was performed at two-day intervals on days 6, 4, and 2 before algal sampling. Results revealed that the highest phenolic content and antioxidant capacity were observed in October for the sampled brown algae, whereas for red algae the highest antioxidant capacity was obtained in December. Higher levels of flavonoids were noticed in the green algae in October. In both green and red algae, there was a negative significant correlation between IC_{50} and flavonoids, whereas in the brown algae a negative significant correlation was observed between IC_{50} and phenolic content. In a multivariate regression model, the main and interaction effects of each variable were examined. The antioxidant parameters were influenced by temperature, dissolved oxygen, and total nitrogen. pH at different levels of algal groups showed different effects on IC_{50}, phenolic, and flavonoid contents.

Keywords: Seaweed, DPPH, Phenolic content, Flavonoid, Hydrological parameters

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Introduction

Free radicals like reactive oxygen species (ROS) can cause cell membrane disintegration, damage to membrane proteins, and DNA mutations, which all can lead to the onset or exacerbation of many diseases such as cancer, liver damage, and cardiovascular diseases (Ferdous and Balia Yusof, 2021). An effective way to eliminate ROS is to use antioxidant compounds that act as free radical scavengers. Therefore, special attention has been paid to use antioxidants to protect cells from biological damages (Nishida *et al*., 2020).

Normal growth conditions result in a minimal production of reactive oxygen species; however, environmental stressors raise that production by interfering with cell homeostasis. Stresses such as desiccation, salinity, cold, heat shock, heavy metals, UV, mechanical stress, nutrient deficiency, pathogen attack, intense light, and air pollutants like SO_2 are of these types (Mittler, 2002).

Marine natural products (MNPs) have been one of the most productive chemical sources of inspiration for the development of new drugs such as anti-cancer, antiviral and analgesic ones (Pereira, 2019). Over 3,600 marine compounds from macro and microalgae (or roughly 24% of all newly discovered marine compounds as of 2004) have been identified as antibacterial, antifungal, anti-protozoan, and antioxidant substances (Bhadury and Wright, 2004). The number of these compounds has increased rapidly, such that as of 2016, the marine natural products identified from marine organisms have reached 28,500 (Jimenez, 2018).

Algae are found in numerous types of aquatic environments, ranging from freshwater to brackish lakes, with a high tolerance for pH, temperature, turbidity, oxygen concentration, and carbon dioxide. Seaweeds are frequently subjected to extreme environmental circumstances that can harm their cellular structures, but because a variety of secondary metabolites are produced by the algae to defend it against abiotic and biotic stimuli, the algae are able to tolerate and survive these conditions. The secondary metabolites of seaweed have a wide variety of chemical structures and include compounds such as terpenoids, amino acid derivatives, and polyphenols, some of which act as antimicrobials, UV ray protectants, and repellants against herbivores (Ianora *et al.*, 2006). These various protective compounds have antioxidant effects, the most important of which are phenolic compounds and their subgroups (Shrestha *et al.*, 2021). The levels of secondary metabolites in algae are influenced by many environmental factors such as light intensity, nutrient content, desiccation, salinity, and herbivorous attack, resulting in the induction of the antioxidative defense system within several days to several weeks (Ratkevicius *et al.*, 2003).Numerous studies have focused on the antioxidant activities of seaweeds, and the biologically active compounds of many of these organisms have been identified and isolated (Castejón *et al*., 2021; Silva *et al*., 2021).

Antioxidant capacity is an important tool to study the physiological responses of algae species to environmental factors and also to evaluate the tolerance of species in the face of natural stressors. Several researchers have shown the effect of environmental factors on the antioxidant properties of micro and macro algae in laboratory conditions. For example, Urrea-Victoria *et al.* (2022) have investigated the effect of temperature on the antioxidant capacity of *Pyropia spiralis* and *Sargassum stenophyllum*. Additionally, the response of phenolic compounds to pH in algae shows that they are pH-dependent (Nishida *et al*., 2020). The impact of environmental conditions on antioxidant parameters in the habitat of algae, however, has only been the subject of a few researches. A study on the effects of metal pollution on *Enteromorpha compressa* (*Ulva compressa*) in coastal waters in northern Chile found that copper in the water increased lipoperoxidase levels, indicating damage to cellular structures as a result of oxidative stress in algae (Ratkevicius *et al*., 2003). In other research, Sfriso *et al*. also examined the impact of habitat temperature on the quantity of sulfated polysaccharides. The results showed that green algae produce more sulfated polysaccharides during the hot summer and red algae during the cold season (Sfriso *et al*., 2017).

Due to the variation in the type and amount of protective compounds in different environmental conditions, marine algae have been the target of studies by many scientific groups, whose main focus is on medicinal and biological properties such as antioxidants, anti-inflammatory, anti-fungal, anti-bacterial, as well as the other bioactive compounds of algae (Cotas *et al*., 2020). According to the authors' knowledge, no studies have been done on how seawater's physical and chemical parameters affect algae's active chemicals in their native habitat in Iran. Since the algae from the coastal areas are exposed to very stressful conditions, the present study aimed to investigate the influence of variations in physical and chemical parameters of seawater on secondary metabolites as well as the antioxidant capacity of macroalgae collected from the northern shores of the Persian Gulf. For this reason, species of all three groups of green, brown, and red macroalgae were collected at four different times in the year 2016 and their antioxidant power was evaluated according to the physicochemical parameters of seawater.

Materials and methods

Chemicals

Methanol, gallic acid, ascorbic acid, and Folin-Ciocalteu reagent were obtained from Merck Company (Darmstadt, Germany). DPPH (1,1-diphenyl-2-picrylhydrazyl) and Rutin were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and the other chemicals and reagents were of analytical grade.

Physicochemical parameters of seawater

Sampling was performed at predetermined stations in the year 2016 within 4 different months, including March, May, October, and December.

The geographical coordinates of each sampling site were recorded, and subsequent sampling was performed at the designated location. All algae were collected from the intertidal zone (upper, middle, and lower).

Since it takes a few days for the antioxidant defense system to become active after being subjected to environmental stress, seawater sampling was performed 6, 4, and 2 days prior to the sampling of the algae to demonstrate the conditions to which the algae had been exposed. The temperature and dissolved oxygen (DO) of the seawater were recorded in situ using a portable oxygen meter (DO meter, HQ 40, Hach). Other physical and chemical parameters, including electrical conductivity (EC), acidity (pH), total suspended solids (TSS), total nitrogen (TN), and total phosphorus (TP), which was measured as phosphate (PO_4^{-3}), were determined according to the standard methods for the examination of water (APHA, 1998). Seawater samples were taken from a depth of 50 cm using a Niskin water sampler (Model 1010, 1.2L) in 3 replications. Then, all three samples from one day were mixed and counted as a replicate in subsequent analyses. All containers were tightly closed with parafilm tape and kept in a cool place at 4°C until transferred to the laboratory of the Khuzestan Environment Department.

Electrical conductivity was determined using a conductometer (Metrohm, Swiss) based on millisiemens per centimeter ($mScm^{-1}$). pH was measured using a pH meter (WTW730, Germany). TSS was measured based on the standard operating procedures for total suspended solids. Total nitrogen was measured using the Total Organic Carbon (TOC) Analyzer (Shimadzu, Japan) and was determined based on mg L^{-1} of seawater. Total phosphate was measured as follows: To 100 mL of the sample, 4 mL of nitric acid and 1 mL of sulfuric acid were added. It was then heated in a macro Kjeldahl digestion apparatus (Electrothermal, UK) to different temperatures between 150 and 300°C until NO_2 brown gas was observed. Afterward, it was neutralized using a 4 N NaOH solution to form a purple color. The sample was transferred to a 100 mL flask and the volume was increased to 100 mL with distilled water. Then, 4 mL of ammonium molybdate ($(NH_4)_2MoO_4$) and 0.5 mL of tin chloride ($SnCl_2$) dissolved in glycerin were added, and after 10 minutes, the absorbance was read in a spectrophotometer UV-VIS (Cary100, Varian, Australia) with a wavelength of 690 nm. The amount of total phosphate was determined based on mg L^{-1} of seawater.

Algal sampling

Algal samples were collected manually. Algae collected from the sea were washed with seawater immediately to remove sand and possible epiphytic matter, then washed with distilled water and air-dried in the shade at temperatures below 30 °C. Before beginning the experiments, the samples were kept at -80°C. Voucher algae samples were fixed on the herbarium sheet for further identification and some were kept in 5% formalin solution with seawater for morphological and anatomical examination. The

morphology and anatomy of the cell structures in the samples were investigated using stereomicroscopes and optical microscopes. Algae species were identified using identification keys in the taxonomic publications (Ruangchuay, 2007; Braune and Guiry, 2011; Shams *et al*., 2015). Figure 1 shows the sampling locations along the northern coast of the Persian Gulf.

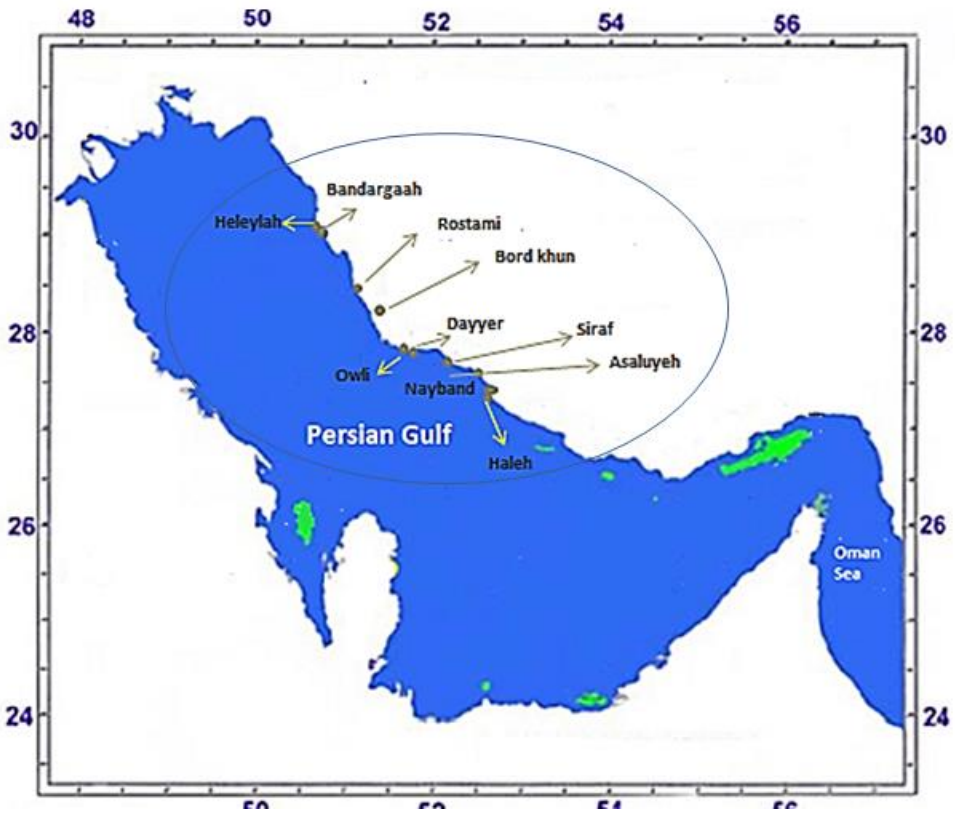

Figure 1: Sampling locations along the northern coast of the Persian Gulf.

Algal Extracting

20 grams of each dried sample powder were extracted with 80% methanol in a 20-minute ultrasonic bath (Bandelin D12207, Sonorex Digitec, Germany). The extracts were then placed in a refrigerated orbital shaker incubator (Axyos, Australia) at 30°C for 20 minutes at 160 rpm. The extracts were centrifuged (Kubota, kN-70, HD8292, Japan) for 15 minutes at 1500 rpm after 48 hours of standing at room temperature, then filtered through a Whatman No. 1 filter paper and dried in an oven at 38°C. Serial dilutions with 80% methanol were used to obtain concentrations ranging from 0.25 to 2 mg mL^{-1}. To prepare the standard curve, ascorbic acid concentrations of 2, 4, 6, 8, and 10 μg mL^{-1} was prepared in 80% methanol.

DPPH radical scavenging assay

DPPH free radical scavenging assay was performed according to the method of Farasat *et al.* (2014). In short, 100 μL of each extract of different concentrations was mixed with 100 μL of 0.16 mM DPPH. The mixture was shaken for 1 min, stood for 30 min in dark, and then, the absorbance was read at 517 nm in an

automated microplate reader (Sunrise-Elisa Reader, Tecan, Swiss). The antioxidant capacity was calculated using the following equation:

$$DPPH\ radical\ inhibition\ (\%) = \left[\frac{\left(A\ control\ -\ (A\ sample\ -\ A\ blank)\right)}{A\ control}\right] \times 100$$

A control is DPPH absorbance without a sample. A sample is the absorbance of a sample-DPPH mixture. A blank is the sample's absorbance without DPPH. IC_{50} (the half-maximal inhibitory concentration) (mg mL^{-1}) was calculated by linear regression analysis and expressed as the mean of three evaluations. IC_{50} of Ascorbic Acid as a positive control was measured as 0.0039 mg mL^{-1}.

Total phenolic and flavonoid contents

Total phenolic content (TPC) was assessed using Folin-Ciocalteu's reagent (Merck, Darmstadt, Germany) based on the method of Antolovich *et al*. (2002). An aliquot of 20 µL of each extract at different concentrations was mixed with 100 µL of 1:10 Folin-Ciocalteu's reagent, then 80 µL of 7.5% sodium carbonate (Na_2CO_3) was added and the mixture was allowed to stand for 2 hours at laboratory temperature. Subsequently, the absorbance was read at 600 nm by a fully automated Elisa reader. Different concentrations of gallic acid were used to generate the standard curve, and the phenolic content of each extract was calculated based on the equivalent of mg of gallic acid per 100 g of dry weight (mg GAE/100g DW).

The total flavonoid content (TFC) of each extract was determined by the colorimetric method proposed by Chang *et al*. (2002). In brief, 20 µL of each concentration of the extracts was mixed with 20 µL of 10% aluminum chloride ($AlCl_3$, $6H_2O$) and 20 µL of 1 M potassium acetate (CH_3COOK) in a 96-cell microplate (SPL,South Korea). After a few minutes, 180 µL of distilled water was added and left for 30 minutes at laboratory temperature. Then, the absorbance at 415 nm was recorded by a fully automated Elisa reader. By using rutin as a reference, the flavonoid content of each extract was determined based on the mg rutin equivalent per 100 g of dry weight (mg RE/100g DW).

Statistical analysis

Data analysis was performed using SPSS 23.0 software. Analysis of variance and post-hoc tests were applied to determine any significant differences between the means. Multivariate analysis of variance was also performed to investigate the single and interaction effects of the variables. Pearson correlation coefficients were calculated for the antioxidant variables. Graphs were drawn using Excel and Past3 software. All experiments were repeated three times for each specimen.

Results

Antioxidant capacity

Over the course of this study, a total of 34 algal samples including 18 green macroalgae (belonged to 5 genera and 10

species), 9 brown macroalgae (belonged to 4 genera and 7 species), and 7 red macroalgae (belonged to 4 genera and 6 species) were collected. The scientific names of algae, habitat (inhabiting zone), sampling time, and location are presented in Table 1.

Table 1: Collected species, habitat, time, and location of sampling

Sample code	Species Name	Habitat	Collection Time	Locality	Latitude (N)	Longitude (E)
Chlorophyta						
GU1	*Ulva clathrata*	UIZ-MIZ	Mar	Rostami	2834676	05140625
GCh1	*Chaetomorpha aerea*	UIZ-MIZ	Mar	Nayband	2723722	05239738
GUc	*Ulva compressa*	MIZ	Mar	Bandargaah	2849347	05054234
GCa	*Chaetomorpha antennina*	UIZ	Mar	Haleh	2724154	05238137
GU2	*Ulva clathrata*	UIZ-MIZ	May	Siraf	2740040	05219711
GCh2	*Chaetomorpha aerea*	UIZ-MIZ	May	Nayband	2723722	05239738
GUi	*Ulva intestinalis*	UIZ	May	Owli	2750316	05153083
GCs	*Caulerpa sertularioides*	UIZ-MIZ	May	Dayyer	2749964	05156178
GCc	*Chaetomorpha crassa*	UIZ	May	Bandargaah	2849347	05054234
GCb	*Chaetomorpha brachygona*	UIZ	May	Heleylah	2850309	05052397
GU3	*Ulva clathrata*	UIZ-MIZ	Oct	Bord khun	2800306	05122437
GCh3	*Chaetomorpha aerea*	UIZ-MIZ	Oct	Dayyer	2749964	05156178
GR1	*Rhizoclonium riparium*	MIZ	Oct	Bord khun	2800306	05122437
GR2	*Rhizoclonium riparium*	MIZ	Oct	Owli	2750316	05153083
GR3	*Rhizoclonium riparium*	MIZ	Oct	Siraf	2740040	05219711
GCm	*Cladophoropsis membranacea*	UIZ	Oct	Asaluyeh	2747617	05259513
GU4	*Ulva clathrata*	UIZ-MIZ	Dec	Dayyer	2749964	05156178
GCh4	*Chaetomorpha aerea*	UIZ-MIZ	Dec	Nayband	2723722	05239738
Phaeophyceae						
BCs	*Colpomenia sinuosa*	LIZ	Mar	Heleylah	2850309	05052397
BSi1	*Sargassum ilicifolium*	MIZ-LIZ	Oct	Rostami	2834676	05140625
BSt1	*Sargassum tenerrimum*	MIZ-LIZ	Oct	Rostami	2834676	05140625
BSc	*Sargassum crassifolium* (now* *Sargassum aquifolium*)	MIZ-LIZ	Oct	Rostami	2834676	05140625
BCm	*Cystoseira myrica* (now *Polycladia myrica*)	MIZ	Oct	Rostami	2834676	05140625
BPa	*Padina australis*	MIZ	Dec	Heleylah	2850309	05052397
BCt	*Cystoseira trinodis* (now *Sirophysalis trinodis*)	LIZ	Dec	Rostami	2834676	05140625
BSi2	*Sargassum ilicifolium*	MIZ-LIZ	Dec	Rostami	2834676	05140625
BSt2	*Sargassum tenerrimum*	MIZ-LIZ	Dec	Rostami	2834676	05140625

Table 2 (continued):

Sample code	Species Name	Habitat	Collection Time	Locality	Latitude (N)	Longitude (E)
Rhodophyta						
RLp1	*Laurencia papilosa*	LIZ	Mar	Heleylah	2850309	05052397
RGc	*Gracilaria corticata*	MIZ-LIZ	May	Rostami	2834676	05140625
RLp2	*Laurencia papilosa*	LIZ	May	Haleh	2724154	05238137
RLs	*Laurencia snyderae*	LIZ	Oct	Owli	2750316	05153083
RLm	*Laurencia majuscula*(now *Laurencia dendroidea*)	LIZ	Oct	Owli	2750316	05153083
RDs	*Digenea simplex*	LIZ	Dec	Bandargaah	2849347	05054234
RAs	*Acanthophora spicifera*	MIZ	Dec	Heleylah	2850309	05052397

UIZ=Upper Intertidal Zone, MIZ=Middle Intertidal Zone, LIZ=Lower Intertidal Zone
*based on https://www.algaebase.org/
The letters G, B, and R at the beginning of the code indicate that they belong to the group of green, brown, and red algae, respectively.

The antioxidant power of algae was evaluated using stable DPPH radicals and reported as IC_{50} of the studied samples. The findings of the current study demonstrated that all studied algae have some level of antioxidant activity; however this capacity varies depending on the influence of environmental factors. In general, compared to the other two groups of red and green macroalgae, brown algae displayed lower values of IC_{50} and had stronger antioxidant activity. In the group of brown macroalgae, *Sargassum ilicifolium* (BSi1), *S. crassifolium* (BSc), and *S. tenerrimum* (BSt1) exhibited the lowest values of IC_{50} (0.105±0.011, 0.113±0.002, and 0.142±0.004 mg mL^{-1}, respectively) all three of which were collected in October. *Colpomenia sinousa* (BCs) showed the highest value of IC_{50} (2.120±0.012 mg mL^{-1}), indicating the lowest antioxidant capacity among the algae in its group. Among the red algae, *Acanthophora spicifera* (RAs) showed the lowest IC_{50} (0.188±0.010 mg mL^{-1}), followed by *Digenea simplex* (RDs) (1.181±0.065 mg mL^{-1}), both of which were collected in December. The highest IC_{50} (2.628±0.034 mg mL^{-1}) was observed for *Gracilaria corticata* (RGc). The red alga *Laurencia papilosa* was found in two different stations in March and May. Generally, red algae showed the lowest IC_{50} in December. However, *L. Papilosa* (RLp1) collected in March showed a lower IC_{50} than the sample collected in May (RLp2). In the group of green algae, *Ulva clathrata* (GU3) showed the lowest IC_{50} (0.734±0.081 mg mL^{-1}) followed by *Cladophoropsis membranacea* (GCm) (0.749±0.005 mg mL^{-1}) and *Rhizoclonium riparium* (GR3) (0.808±0.022 mg.mL^{-1}), all of which were collected in October. The highest IC_{50} (2.643±0.087 mg.mL^{-1}) belonged to *Chaetomorpha brachygona* (GCb). Three samples of *R. riparium* were collected from three stations, including Bord khun (GR1), Owli (GR2), and Siraf (GR3) in October. The sample collected from Siraf (GR3) exhibited a much lower IC_{50}

compared to the GR1 and GR2 samples (1.258±.0.067 and 1.379±.0.131, respectively), and this difference was significant at the level of 5%. Of all the algae studied, only two species of green algae (including *U.clathrata* and *Ch. aerea*) were found in all four sampling months. Additionally, the brown algae *S. ilicifolium* and *S.tenerrimum* were found in two sampling months (October and December). A comparison of the antioxidant capacities of the four mentioned species of green and brown algae suggested that the samples collected in October possessed higher antioxidant capacity with a lower IC_{50} than those of the other sampling months (Fig. 2).

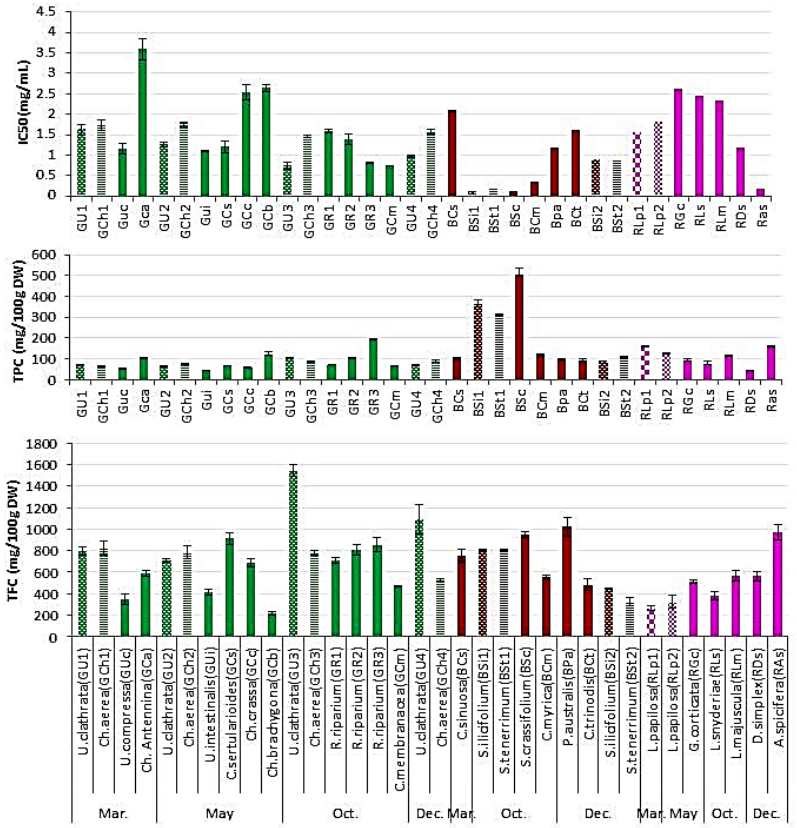

Figure 2: IC_{50}, TPC (Total Phenolic Content), and TFC(Total Flavonoid Content) of the studied Green, Brown , and Red macroalgae. Selected green and brown algae (including *Ulva clathrata, Chaetomorpha aerea, Sargassum ilicifolium,* and *Sargassum tenerrimum*) and also, the red alga *Laurencia papilosa* are shown with different patterns in histogram.

Total phenolic and flavonoid contents (TPC and TFC)

Brown algae showed the highest amount of phenolic compounds (gallic acid equivalent) compared to the other two groups. Among the brown algae, *S.crassifoium* (BSc), *S. ilicifolium* (BSi1), and *S. tenerrimum* (BSt1) showed the highest values of TPC(502.430±30.855, 363.690±18.241 , and 308.810±5.064, respectively). Also, *A. spicifera* and *L. papilosa* (RLp1) in the group of red algae showed the highest values of TPC(158.080±6.455 and 156.920±4.428, respectively). Out of the green algae, *R. riparium* (GR3), *Ch. Brachygona* ,and *U. clathrata* (GU3) exhibited the highest values of total phenolic

contents(190.800±7.958, 120.277±15.619, and 105.087±4.200, respectively). The highest levels of flavonoids were observed in green algae, followed by brown algae, although no significant difference was observed between the two groups (Duncan table is not shown). *U. Clathrata*(GU3, GU4), *Padina australis*, *S. Crassifolium*, and *A. Spicifera* (among green, brown, and red algae, respectively), revealed the highest content of flavonoids. In addition to the antioxidant power, the amounts of phenol and flavonoids of the four species of green and brown groups (GU, GCha, BSi, and BSt) are shown with different patterns in Figure 2. According to the figure, the highest levels of phenolic and flavonoid content in four selected species of green and brown algae were obtained in October. However, in the green alga *Ch. aerea*, the amount of TPC in October and December does not show a significant difference.

The Pearson correlation coefficient was used to examine the correlation between antioxidant variables. A negative significant correlation was seen between IC_{50} and TPC (r= -0.470, p<0.01), IC_{50} and TFC (r= -0.351, p<0.01), and also a positive significant correlation between TPC and TFC (r= 0.207, p<0.05). When the correlation was examined separately for the three groups of algae, the results showed that in the two groups of green and red algae, there was a negative significant correlation between IC_{50} and TFC (r= -0.369, r= -0.576, p<0.01, respectively), whereas in brown algae, such a negative significant correlation was observed between IC_{50} and TPC (r= -0.691, p<0.01). However, brown algae showed a positive significant correlation between TPC and TFC (r=0.531, p<0.01).

A linear regression analysis was carried out between the variables, and the results were drawn in a scattered diagram (Fig. 3). The results of the regression analysis show that, in green algae, there is a stronger relationship between TFC and IC_{50}, whereas in brown algae, this relationship is between TPC and IC_{50}.

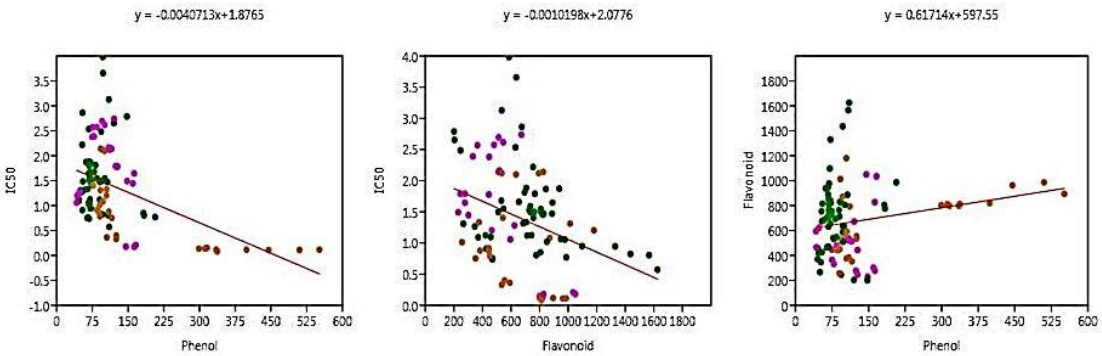

Figure 3: Linear regression between IC_{50}, TPC (Total Phenolic Content), and TFC(Total Flavonoid Content). Each green, brown, and red circular symbol represents a sample of green, brown, and red algae.

Physicochemical parameters of seawater

During the study period, the physical and chemical parameters of seawater were also evaluated. Despite many fluctuations in the seawater status, sampling of water parameters at several-day intervals can

provide a relative estimate of the physicochemical conditions of the seawater. For this reason, seawater parameters were measured 6, 4, and 2 days before algal sampling to determine the hydrological parameters to which the sampled algae were exposed. Table 2 illustrates the physicochemical parameters of seawater for each algal sample collected.

Table 2: Samples and physicochemical parameters of collection area (mean ± SE).

Samples	Temp. (^oC)	pH	DO (mg L^{-1})	EC ($mScm^{-1}$)	TSS (mg L^{-1})	TN (mg L^{-1})	TP (mg L^{-1})	TN:TP
GUc	**20.967**	**7.833**	**9.343**	**55.633**	**1012.270**	**1.973**	**0.507**	**9.953**
	±0.273	±0.067	±0.023	±0.260	±2.186	±0.007	±0.044	±0.343
GU1	**24.400**	**7.900**	**8.340**	**59.067**	**1041.700**	**2.037**	**0.370**	**8.020**
	±0.115	±.057	±0.011	±0.033	±92.294	±0.022	±0.180	±2.585
GCh1	**22.000**	**7.900**	**9.470**	**58.300**	**1049.000**	**2.037**	**0.223**	**9.239**
	±0.057	±0.200	±0.036	±0.252	±21.939	±0.067	±0.017	±0.758
GCc	**30.500**	**8.067**	**9.067**	**60.000**	**1065.300**	**1.997**	**5.097**	**0.490**
	±0.115	±0.033	±0.081	±0.115	±15.387	±0.055	±1.525	±0.176
GCb	**32.500**	**7.800**	**7.003**	**59.200**	**1050.700**	**1.987**	**1.280**	**2.093**
	±0.057	±0.001	±0.003	±0.057	±9.821	±0.064	±0.447	±0.812
GCs	**31.967**	**8.167**	**13.717**	**49.667**	**750.000**	**2.057**	**1.710**	**3.773**
	±1.121	±0.033	±0.132	±0.467	±21.939	±0.128	±1.233	±2.276
GCh2	**26.133**	**7.433**	**1.723**	**62.967**	**1068.700**	**2.107**	**1.137**	**6.553**
	±1.364	±0.033	±0.095	±0.088	±41.155	±0.055	±0.917	±2.974
GUi	**34.833**	**8.133**	**11.477**	**57.067**	**1100.700**	**1.960**	**0.730**	**2.703**
	±0.233	±0.067	±0.618	±0.033	±57.753	±0.011	±0.040	±0.143
GCh2	**36.100**	**8.267**	**8.767**	**48.867**	**876.000**	**2.767**	**0.447**	**8.237**
	±0.493	±0.088	±0.120	±0.841	±67.104	±0.067	±0.179	±2.657
GR1,GU3	**34.133**	**7.967**	**14.450**	**60.933**	**1011.700**	**3.140**	**0.507**	**6.150**
	±0.120	±0.088	±1.299	±0.233	±34.440	±0.025	±0.047	±0.687
RGc	**32.167**	**8.068**	**7.720**	**59.800**	**969.670**	**2.097**	**1.507**	**6.133**
	±0.167	±0.033	±0.042	±0.115	±9.769	±0.075	±1.248	±3.177
GR2,RLs,RLm	**33.400**	**8.333**	**15.347**	**57.700**	**943.000**	**2.910**	**0.940**	**6.763**
	±0.153	±0.067	±1,200	±0.058	±56.027	±0.175	±0.611	±2.902
GR3	**31.133**	**8.067**	**11.737**	**57.100**	**907.330**	**2.953**	**0.886**	**6.269**
	±0.088	±0.033	±0.298	±0.577	±13.544	±0.135	±0.556	±2.316
GU4	**23.433**	**8.067**	**8.287**	**51.270**	**1411.000**	**2.973**	**0.243**	**12.235**
	±0.176	±0.033	±0.077	±0.331	±31.021	±0.018	±0.067	±0.301
GCh4	**18.533**	**7.930**	**8.940**	**52.473**	**1492.700**	**2.967**	**0.320**	**9.657**
	±0.177	±0.115	±0.049	±0.481	±79.780	±0.044	±0.046	±1.369
BCs	**20.467**	**8.000**	**11.120**	**59.400**	**982.670**	**2.097**	**0.193**	**10.851**
	±0.328	±0.115	±0.386	±0.057	±17.892	±0.203	±0.033	±0.205
Bsi1,BSt1,BSc, BCm	**30.534**	**8.467**	**10.527**	**59.332**	**897.000**	**2.650**	**1.887**	**3.510**
	±0.088	±0.067	±0.197	±0.067	±2.346	±0.287	±0.999	±2.356
RDs	**16.200**	**7.767**	**10.973**	**50.270**	**1389.700**	**2.810**	**0.323**	**8.774**
	±0.818	±0.285	±00.812	±0.605	±38.718	±0.021	±0.024	±0.558
BPa,RAs	**13.967**	**7.500**	**8.680**	**51.660**	**1475.000**	**3.087**	**0.217**	**14.442**
	±0.751	±0.115	±0.200	±0.015	±35.303	±0.062	±0.017	±1.218
BCt,BSi2,BSt2	**22.033**	**8.133**	**8.617**	**52.623**	**1432.700**	**2.900**	**0.287**	**10.129**
	±0.410	±0.033	±0.064	±0.024	±52.387	±0.029	±0.009	±0.218
GCa,GU2,GCm, RLp1,RLp2	NA	NA	NA	NA	NA	NA	NA	NA

NA: Not Assessed.

Temp.= Temperature, DO = Dissolved oxygen, EC = Electerical conductivity, TSS = Total suspended solids TN = total nitrogen, TP = Total phosphorus

The surface water temperature ranged from a minimum of 12.7^{o}C in December to a maximum of 36.9^{o}C in October. The lowest pH was recorded in December (7.2) and the highest in October (8.6). Dissolved oxygen fluctuated from a minimum of 1.56 mg L^{-1} in May to a maximum of 16.66 mg L^{-1} in October. The lowest EC was recorded in October (48.700 $mScm^{-1}$), and the highest (63.100 $mScm^{-1}$) in May. The amount of TSS changed from 708 mg L^{-1} in May to 1650

mg L^{-1} in December. TN varied from a minimum of 1.87 mg L^{-1} in May to a maximum of 3.22 mg L^{-1} in October. The lowest TP was observed in March and December (0.19 mg L^{-1}), and the highest amount was measured in May (7.80 mg L^{-1}). Finally, the minimum and maximum values for the ratio of nitrogen to phosphate (TN:TP) were recorded as 0.24 and 16, respectively. According to Table 3, temperature, pH, and DO are higher in October than in other sampling months and show significant differences from the other months of sampling. Also, the amount of TSS in this month is less than in the other sampling months and shows a significant difference with water samples in March, May, and December. In December, temperature and EC were lower and TSS was higher than in other sampling months (Table 3).

Table 3: Physicochemical parameters of seawater in 4 sampling months.

	Temp. (°C)	pH	DO (mg L^{-1})	EC (mScm^{-1})	TSS (mg L^{-1})	TN (mg L^{-1})	TP (mg L^{-1})	TN:TP
Mar.								
Mean	21.958**b**	7.908 **b**	9.568**b**	58.100**a**	1021.500**b**	2.036**b**	0.323**b**	8.016**b**
SE	0.466	0.056	0.312	0.453	22.0525	0.015	0.055	0.964
Minimum	20.000	7.500	8.320	55.200	876.000	1.960	0.190	2.850
Maximum	24.600	8.200	11.840	59.500	1195.000	2.130	0.730	11.210
May								
Mean	31.350**a**	7.944 **b**	8.451**b**	58.117**a**	1000.700**b**	2.034**b**	1.910**a**	3.624**a**
SE	0.692	.0638	0.918	1.010	30.813	0.028	0.501	0.872
Minimum	24.300	7.400	1.560	48.800	708.000	1.870	0.170	0.240
Maximum	35.200	8.200	13.920	63.100	1200.000	2.310	7.800	11.530
Oct.								
Mean	32.530**a**	8.289 **a**	12.504**a**	58.115**a**	929.420 **a**	2.848**a**	1.156**a**	5.558**a**
SE	0.332	0.038	0.470	0.514	12.944	0.059	0.204	0.655
Minimum	30.400	7.800	8.600	48.700	753.000	2.300	0.220	0.860
Maximum	36.900	8.600	16.660	61.300	1060.000	3.220	3.720	12.630
Dec.								
Mean	19.025**c**	7.895 **b**	8.927**b**	51.900 **b**	1442.700 **c**	2.953**a**	0.272**b**	11.242**c**
SE	0.775	0.065	0.188	0.188	16.119	0.022	0.010	0.492
Minimum	12.700	7.200	8.140	49.130	1321.000	2.780	0.190	7.070
Maximum	23.700	8.200	11.940	53.430	1650.000	3.210	0.410	16.000

Means in a column with different letters (a-c) are significantly different at $p \leq 0.05$.
Temp.= Temperature, DO = Dissolved oxygen, EC = Electerical conductivity, TSS = Total suspended solids TN = total nitrogen, TP = Total phosphorus

Multivariate regression analysis

The main and interaction effects of all the variables were investigated in a multivariate regression model. Seawater parameters were used as inputs into the model to predict the effects of the parameters on the dependent variables (IC_{50}, TPC, and TFC). The results of the multivariate analysis are presented in Table 4. According to Table 4, the variables of sampling month, station, algal group, and inhabiting zone (sampling area) all have a significant effect on the IC_{50} value. The sampling month exhibited a significant effect on the antioxidant capacity of algae, as algae collected in March and May showed 1.38 and 0.8 times higher values of IC_{50} than algae collected in December. Although the algae collected in October showed a -0.35 times lower IC_{50} than the samples collected in December, such a difference was not significant (Table 4).

Table 4: Multivariate regression model between IC_{50}, TPC, TFC and sea water parameters.

Variable	Parameter	Level	B±SE	Sig.	95% CI
IC50*	Month	March	1.38±.33	**<0.001**	(.714, 2.051)
		May	.80±.36	**.031**	(.075 , 1.535)
		October	-.35±.35	.333	(-1.066,.368)
	Station	1	-.45±.41	.277	(-1.292 ,.378)
		2	-.09±.38	.807	(-.870 ,.680)
		3	-.46±.42	.281	(-1.307 ,..387)
		4	-1.69±.44	**<0.001**	(-2.596 , -.795)
		5	.01±.43	.979	(-.855 ,.879)
		6	.33±.19	.097	(-.062 ,.722)
		7	-.32 ±.21	.132	(-.751,.100)
	Group	1- Green	-1.45±2.18	.509	(-5.824, 2.921)
		2- Brown	14.92±2.10	**<0.001**	(10.700, 19.139)
	Zone	1	-53.98±9.48	**<0.001**	(-72.980, -34.987)
		2	4.43±1.81	**0.018**	(0.789, 8.072)
		3	-8.11±3.04	**0.010**	(-14.217, -2.011)
		4	-3.28± 2.47	.190	(-8.244, 1.675)
	Temp.	-	.11±.02	**<0.001**	(0.058, 0.161)
	DO	-	-.11 ±.02	**<0.001**	(-0.162, -0.067)
	EC	-	-.06 ±.04	.177	(-0.150, 0.028)
	TN	-	.34 ±.14	**.021**	(0.052, .621)
	Group* pH	Group 1* pH	.11 ±.27	.676	(-0.435, 0.666)
		Group 2* pH	-1.88 ±.27	**<0.001**	(-2.426, -1.341)
	Zone* EC	Zone 1* EC	.90±.16	**<0.001**	(0.575, 1.236)
		Zone 2 * EC	-.08±.03	**.013**	(-0.152, -0.019)
		Zone3 * EC	.13±.05	**.022**	(0.020, 0.246)
		Zone4 * EC	.04±.04	.312	(-0.046, 0.141)
TPC	Station	4	191.28±94.60	**.048**	(1.763, 380.802)
	Group	2- Brown	-995.86±443.40	**.029**	(-1884.123, -107.614)
	Zone	3	1342.87±641.36	**.041**	(58.055, 2627.688)
	Temp.	-	-12.44±5.39	**.025**	(-23.253, -1.631)
	Group* pH	Group2 * pH	125.20±57.01	**.032**	(11.004, 239.412)
	Zone * EC	Zone3* EC	-25.41±11.86	**.037**	(-49.174, -1.649)
TFC	Station	1	-683.71± 244.95	**.007**	(-1174.406, -193.016)
		2	-664.73±227.44	**.005**	(-1120.352, -209.114)
		3	-885.29 ±248.50	**.001**	(-1383.115, -387.472)
		5	-946.78±254.42	**<0.001**	(-1456.447, -437.116)
	Group	1- Green	-3065.01±1282.99	**.020**	(-5635.168 , -494.863)
	Zone	1	-12565.09±5573.54	**.028**	(-23730.237,-1399.949)
		2	-2517.64± 1068.43	**.022**	(-4657.974, -377.320)
		4	-5707.02± 1455.21	**<0.001**	(-8622.172, -2791.873)
	Group* pH	Group 1 * pH	355.40±161.54	**.032**	(31.801, 679.008)
		Group 2 * pH	329.20±159.16	**.043**	(10.357, 648.058)
	Zone * EC	Zone1* EC	220.88±96.89	**.026**	(26.780, 414.993)
		Zone2 * EC	60.08±19.59	**.003**	(20.823, 99.341)
		Zone4* EC	107.13±27.34	**<0.001**	(52.354, 161.924)

Reference: [Month 4=December], [Station 8= Bord khun], [Zone 5=LIZ], [Group 3=Red], [Group =3 * pH] , [Zone5 * EC] **Stations**: [1:Bandargaah, 2: Heleylah, 3: Rostami, 4: Dayyer, 5: Nayband, 6:Owli, 7: Siraf]
Zones: [1:UIZ, 2:UIZ-MIZ, 3: MIZ, 4: MIZ-LIZ]
UIZ=Upper Intertidal Zone, MIZ=Middle Intertidal Zone, LIZ=Lower Intertidal Zone
*Except for the IC_{50}, only variables with significant effect are presented in the table.

Among the seawater parameters, temperature, DO, and TN show a significant effect on the IC_{50} value. The regression model shows that an increase in temperature and TN causes a significant increase in IC_{50}. In contrast, an increase in dissolved oxygen causes a decrease in IC_{50}.

The independent effect of pH on IC_{50} is not significant; however, at different levels of algal groups, pH exhibits an interaction, such that in brown algae, such an effect is significant. The model predicts that as the pH of the seawater increases, a more significant decrease in IC_{50} in this group is expected, compared to the reference group. The parameter of EC at different levels of the sampling zone indicates a significant effect in such a way that, by increasing EC, samples collected from the upper and middle intertidal zones reveal significant differences in their IC_{50} values compared to those of the samples collected from the lower intertidal zone.

Factors that show a significant effect on the amount of phenolic compounds include the month, station, and inhabiting zone, as well as seawater temperature. The interaction of pH with the algal groups, as well as the interaction of EC with the inhabiting zone, have a significant effect on TPC. Factors that show a significant effect on the flavonoid content of algal samples are similar to those affecting phenolic content. The only difference is the temperature of the seawater, which does not exhibit a significant effect on flavonoid content.

The regression analysis model for selected algae (including *U.clathrata*, *Ch. aerea*, *S. ilicifolium*, and *S.tenerrimum*) shows that the increase in three parameters of the seawater, including temperature (B±Se= -0.08±0.02, $p<0.01$), EC (B±Se= -0.172±0.03, $p<0.01$), and TP (B±Se= -0.33±0.14, $p<0.05$) reduces the IC_{50} of the studied species. Also, the rise in the EC of the seawater can cause an elevation in the phenolic content of the selected species (B±Se= 22.34±4.44, $p<0.01$). The regression model also shows that water temperature has a significant effect on the amount of flavonoids among the seawater parameters. As a result, the rising temperature can increase the flavonoid content of the samples (B±Se= 41.87±18.02, $p<0.05$).

Discussion

During the study period, 34 samples of algae were collected from designated stations with the highest number and variety of green algae. In the study of Dadolahi-Sohrab *et al*. (2012), seasonal variations in the composition and biodiversity of algae in the Persian Gulf were examined. Their results showed that biomass, as well as the species composition of seaweeds, largely depends on the season, population structure, and several other ecological factors. The results of the study also suggested that brown seaweed had the highest distribution on the northern coasts of the Persian Gulf. In our study, a diverse number of species of green and red algae were found in the appointed stations in all four sampling months; however, brown algae were found in only three sampling months in the selected stations. The results showed that the mean of all measured seawater parameters was significantly different at disparate times. This difference was also observed between sampling stations. Moreover, a higher antioxidant capacity was observed in October in the two groups of green and brown algae, whereas red algae showed higher antioxidant power in December.

A large number of seaweed species are found in coastal areas and in the intertidal zone. Intertidal macroalgae are periodically exposed to ultraviolet radiation, high temperatures, UV, and desiccation during the low tide period. In this case, antioxidant defense mechanisms will be activated. Accordingly, the species living in the upper tidal zone have adapted to very stressful conditions and are resistant to high stress. These species can lose up to 95% of their water during low tide and reactivate their metabolism after exposure to high tide (Urrea-Victoria *et al*., 2022). In the present work, all the studied algae were collected from the intertidal (littoral) zone, including the upper, middle, and lower littoral zones. Among the studied algae, brown algae indicated more antioxidant power than the other two groups. The brown algae species *S. ilicifolium* (BSi1), *S. crassifolium, S. tenerrimum* (BSt1), and the red algae *A. spicifera* that exhibited the lowest IC_{50}, all were collected from the middle intertidal zone. Furthermore, the three green algae *U. clathrata* (GU3), *C. membranacea*, and *R. riparium* (GR3), which exhibited the lowest IC_{50} among their group, were collected from the middle to upper littoral zones. In a study of 22 macroalgal species, including 5 green algae, 7 red algae, and 10 brown algae from Norway, Aguilera *et al*. (2002) found that green algae had higher antioxidant enzyme activity and ascorbic acid levels than the other two groups. According to their results, species collected from the eulittoral and upper sublittoral zones have revealed higher antioxidant enzyme activities in comparison to species collected from the lower sublittoral zones. Additionally, they showed that the biochemical protection of algae exposed to high-stress conditions, especially ultraviolet radiation, was higher than that of algae that were exposed to lower-stress conditions. In our results, even though brown algae showed more antioxidant power than green algae, mainly, algae collected from upper and middle intertidal areas showed higher antioxidant power than algae collected from deeper areas. This observation is perfectly in line with the findings of Aguilera *et al.* (2002).

In the frame of our experiments, seawater temperature was one of the factors affecting antioxidant variables. When all species of three groups of algae were analyzed in the regression model, temperature with a positive beta slope showed a boosting effect on IC_{50}, indicating that lowering the temperature had an intensifying effect on antioxidant power. When only selected green and brown algae were evaluated in the regression model, the results were slightly different. By increasing the water temperature, lower IC_{50} was observed in these selected algae, such that, the highest antioxidant power was obtained in October and with the highest water temperature. However, according to Tables 1 and 2, three samples of green alga *R. riparium* including GR1, GR2, and GR3 from the three stations of Bordkhun, Owli, and Siraf were harvested in October with mean water temperatures of 34.13, 33.4, and 31.13 ^{o}C, respectively. The *R. riparium* sample from Siraf (GR3) with lower seawater temperature showed

the lowest IC_{50} compared to the GR1 and GR2 samples . Besides, more phenolic and flavonoid contents were observed in the GR3 sample than in the other two samples (Fig. 2). Given that all three samples were collected around the same time, it seems that the reason for the difference in antioxidant capacity was a different factor than what was measured in the present study. Some factors, like the latitude of the place, may have had an effect on the antioxidant parameters of algae due to the change in the intensity of ultraviolet radiation. The GR3 sample was collected from Siraf station, which is closer to the equator than the other two stations based on its latitude. Reports claim that UV radiation is stronger closer to the equator and weaker farther away from the poles (Jablonski and Chaplin, 2010). Additionally, research has demonstrated the protective role of flavonoids in response to UV exposure (Mittler, 2002; Ianora *et al*., 2006). It is clear from the data presented here that the GR3 sample contains more flavonoids than the other two samples. It appears that the latitude of the location, in addition to the seawater temperature, played a role in the difference in IC_{50} of three samples, even if the intensity of ultraviolet light was not evaluated in the current investigation.

In addition to the selected green algae, the selected brown algae also showed the highest antioxidant capacity in October. However, the three species of *Sargassum* (BSi1, BSt1, and BSc), which showed the lowest IC_{50} and the highest phenolic content among all studied algae, were collected from Rostami station with an average temperature of 30.53°C and an alkaline pH of 8.467. Changes in pH were observed at different sampling months and sampling stations. The pH of seawater was above 7 throughout the study period and ranged from slightly alkaline to very alkaline. The lowest pH in all stations was recorded in December and the highest in October. Seasonal changes in temperature affect pH fluctuations by influencing biological production (photosynthesis and respiration). Coastal areas are more prone to runoff and mixing with river water. As a result, pH fluctuations are more pronounced in these areas. The higher pH in October can be attributed to the uptake of carbon dioxide by algae because of their blooming during these periods in the study area. Lower pH in December can also occur as a result of rainfall. According to the regression analysis model, with increasing pH of seawater, brown algae showed a significant decrease in IC_{50} value compared to red algae, which indicates the sensitivity of this group of algae to pH. The results shows that the phenolic content of brown algae has increased in comparison to red algae as a result of the rise in pH. Such an increase in the flavonoid content of both green and brown algae is also observed. Numerous studies have demonstrated that phenolic compounds are pH-dependent, with alkaline circumstances being the most conducive to antioxidant and free radical scavenging activities (De La Coba *et al*., 2008; Nishida *et al*., 2020). High phenolic content, especially in brown algae in October, can be associated with the high pH of seawater.

The electrical conductivity (EC) of seawater was another factor whose impact on antioxidant power was examined. Seawater's electrical conductivity varied considerably between stations and between sample months. The lowest electrical conductivity (mean of all stations) was recorded in December and the highest in May. Electrical conductivity did not significantly affect IC_{50} for all algae in this investigation, but it did significantly affect the selected green and brown algae with a negative beta slope. Decreasing the water temperature reduces the salinity and electrical conductivity, whereas raising the temperature increases the electrical conductivity (Shcherbakov *et al*., 2021) by increasing the surface evaporation of water as well as increasing the salinity. The antioxidant system of the chosen algae from the two groups of green and brown algae appears to have been effectively activated by electrical conductivity, which is itself influenced by seawater salinity. This significant effect of electrical conductivity was also observed in increasing the phenolic content of selected green and brown algae. Previous research has shown the effect of salinity on the activation of the antioxidant defense system and phenolic compounds (Soufiane *et al*., 2022).

The results of the regression model showed that dissolved oxygen had a significant effect on the IC_{50} value, and with increasing DO, a decrease in IC_{50} was observed. Performing chemical and biological processes in water highly depends on the presence of oxygen. Although DO is associated with factors such as temperature and salinity, in estuaries and coastal areas, it mainly depends on the photosynthesis of algae and phytoplankton. The photosynthesis of aquatic plants releases large amounts of dissolved oxygen during the day and consumes oxygen during the night as a result of the respiration process of aquatic growth and microorganisms. During algal blooms, large fluctuations in dissolved oxygen levels are reported (Ssebiyonga *et al*., 2013). According to Rajwa-Kuligiewicz *et al*. (2015), the relationship between water temperature and dissolved oxygen concentration varies on a daily time scale and exhibits a loop-like behavior on the variables plot. Our results showed that with increasing temperatures in October, the amount of dissolved oxygen also increased. Such an increase may be due to the effect of temperature on the growth of algae and phytoplankton, which leads to the release of dissolved oxygen as a result of photosynthesis during the day. It is reported that an increase in dissolved oxygen increases the activity of antioxidant enzymes (Li *et al*., 2020). Also, some correlations between temperature, dissolved oxygen, and antioxidant enzyme activity have been documented in a number of samples of two bivalve species from the Venice lagoon (Irato *et al*., 2007). Our results are consistent with the results of prior research.

Although TSS did not show a significant effect on antioxidant parameters, the amount of TSS varied significantly between sampling months and stations. The highest rate was

observed in December. TSS includes suspended solids and impurities in water. In most cases, these particles are organic and play a role in water pollution. Organic matter in water can be decomposed into ammonia nitrogen or nitrate and nitrite. Such a process of bacterial decomposition reduces the consumption of dissolved oxygen. For this reason, the observed decrease in the dissolved oxygen levels during the month of December can be attributed to the high amount of organic suspended solids and their bacterial decomposition. As the results showed, there was no significant difference in the IC_{50} value of all algae between October and December, even though the water temperature had a decreasing trend (from 32.53°C in October to 19.02°C in December). It should be taken into account that the biochemical composition of algae is not influenced by a single factor. Both high and low seawater temperatures and TSS appear to have affected the biochemical composition of algae. The higher antioxidant power of green and brown algae in October can be due to exposure to higher temperatures and UV rays, which in turn can activate the antioxidant defense system. Red algae live in the lower depths of the sea and are less exposed to direct sunlight and ultraviolet rays. Instead, high concentrations of TSS, which cause seawater turbidity by reducing light penetration, can be considered a stress factor for activating the antioxidant defense system in December. Although TSS did not have a direct and significant effect on antioxidant power, it seems to have an indirect effect on the penetration of sunlight into seawater. It has been reported that the TSS is higher in the rainy season (Cahyono *et al*., 2019). In the Persian Gulf area, changes in weather conditions and, consequently, changes in seawater parameters begin in October. Rainfall typically begins in December. Therefore, observing higher TSS in December is consistent with the results of Cahyono *et al*. (2019).

In the present study, increasing the total nitrogen concentration also had an apparent effect on raising the IC_{50} value. A major part of the nitrogen cycle as a result of human intervention is the use of chemical fertilizers that affect not only the terrestrial ecosystems but also the aquatic ecosystems, especially in the coastal areas. Nitrogen is found in marine environments as nitrate, nitrite, nitric oxide, molecular nitrogen, and ammonium. Nitrogen and phosphorus (as phosphate) play a prominent role in marine ecosystems (Ssebiyonga *et al*., 2013), and both their organic and inorganic forms are used by aquatic organisms. Green algae and cyanobacteria grow in response to nitrogen and phosphorus (Yaakob *et al*., 2021), and one of these two elements is frequently regarded as a factor limiting algae growth (Dong *et al*., 2020). The ratio of total nitrogen to total phosphate is used to detect the limitations of two elements in aquatic environments. Redfield 16N:1P ratio is the initial ratio of resource availability for algae growth in a marine environment without disturbance (Liu *et al*., 2011). A ratio above 16:1 indicates phosphate restriction and below 16:1

indicates nitrogen restriction for production in an aqueous medium. This ratio is typically high in deep-sea areas and is relatively low in coastal areas and estuaries (Downing, 1997). In the current study, this ratio showed a significant difference between the months and sampling stations. The mean of the lowest and highest ratios was recorded in May and December (0.24:1 and 16:1, respectively). In the main measurements, the TN: TP ratio was lower than 16:1, which signifies the nitrogen restriction in the coastal areas of the studied stations.

The allocation of secondary metabolites in macroalgae can be attributed to environmental conditions. There are several examples of algae responding or not responding to environmental changes. For example, the amount of phlorotannins in brown algae is lower when seawater nitrogen is higher, while terpenes are less sensitive to changes in nitrogen concentration (van Alstyne *et al*., 2007). Phlorotannins are polyphenolic compounds and many studies have shown the role of phlorotannins as bioactive compounds with various properties, including antioxidant activity (Shrestha *et al*., 2021; Negara *et al*., 2021).

Coulombier *et al*. (2020) explained that under nitrogen-enriched supply, carotenoid biosynthesis in the green alga *Nephroselmis sp.* increases. They also reported higher antioxidant capacity and more peroxyl radical scavenging activity under nitrogen-enriched conditions than nitrogen deficiency conditions in this green alga. In a different investigation of nitrogen's impact on the production of antioxidant chemicals in two green microalgae (*Chlorella ellipsoidea* and *Dunaliella bardawil*), Kobbia *et al*. (2010) discovered that both algae produced less vitamin E and C when nitrogen levels in the environment were low. However, it has been demonstrated in several research that raising nitrogen up to a certain point boosts the production of antioxidant molecules, whereas any additional increase has the opposite effect. For instance, Ismail (2016) found that 6–18 mM of nitrogen in *Pseudochlorella pringsheimii* algal culture medium increased the production of antioxidants, but excessive nitrogen reduced the production of antioxidant compounds.

According to the TN:TP ratio in our study, all coastal areas were faced with nitrogen restrictions. However, the regression model predicts a positive relationship between total nitrogen and IC_{50}, as the increase in TN decreases the antioxidant power of the studied algae. In the regression analysis model of selected green and brown algae, although there was a positive relationship between TN and IC_{50}, the effect of TN was not significant. Instead, the amount of total seawater phosphate (TP) exhibited a significant and decreasing effect on the IC_{50} value.

In a marine environment, there are different pools of phosphorus, including dissolved inorganic phosphorus, particulate inorganic phosphorus, and dissolved organic phosphorus. ATP is one of these dissolved organic phosphorus compounds whose uptake in aqueous media depends on environmental and

biological factors such as temperature, dissolved inorganic phosphorus, salinity, *Chl*a, and species type (Nausch *et al*., 2018). While nitrogen is a limiting nutrient in marine environments, phosphorus is a key factor whose excessive amount can intensify nutrient diminution (Missimer *et al*., 2021). Numerous studies have shown that high concentrations of phosphate interfere with the uptake of other elements such as iron and zinc by causing toxicity (Xie *et al*., 2019; Rodgher *et al*., 2020). According to Hamouda and Abou-El-Souod (2018), the highest antioxidant power and the highest amounts of phenol and flavonoid of *Scenedesmus obliquus* green microalga occurred at the rate of phosphate 0.007 g L^{-1} (7 mg L^{-1}). Additional phosphate in the culture medium has decreased the antioxidant activity of *Scenedesmus obliquus* green microalga. However, research has shown that deficiencies and limitations of minerals such as phosphorus increase the levels of polyphenols and flavonoids (Munene *et al*., 2017). In the present study, the average amount of phosphate recorded for October and December was 1.156 and 0.272 mg L^{-1}, respectively, and the maximum amount of phosphate (7.80 mg L^{-1}), was recorded in May at the Nayband station. It should be noted that, except for Nayband station, the amount of phosphate was not high in the other stations. However, comparing the two species of *Sargassum* collected from the Rostami station in October and December, October samples (BSi1, BSt1) (with higher levels of seawater phosphate) showed higher levels of TPC as well as higher antioxidant capacity than December samples (BSi2 and BSt2).

Prior research has shown that hydrological parameters of seawater such as salinity, temperature, nitrogen source, nitrogen, phosphorus limitation, DO, pH, heavy metals, UV rays, light quality, and other environmental stressors affect the biochemical composition and antioxidant activity in algae (Wu, 2016). Stressors act as alarms and activate regulatory and adaptive mechanisms by producing various defensive metabolites such as polyphenols (El-fayoumy *et al*., 2021). The results of the present study showed a strong and significant correlation between the antioxidant power and phenolic compounds in brown algae. Our results also showed that there is a strong and negative significant correlation between IC_{50} and flavonoids in the two groups of green and red algae. Marine algae are rich sources of polyphenols like flavonoids with a wide variety of chemicals as they are often exposed to harsh environments such as high salinity or temperature. Many studies have proven the role of phenolic compounds as antioxidant compounds (Sathya *et al*., 2017; Negara *et al*., 2021). The antioxidant activity of the tested brown algae can be attained by the presence of phlorotannins, which have various biological properties, including antioxidant and anti-cancer activities (Vega *et al*., 2020; Negara *et al*., 2021). By isolating antioxidant compounds from the crude extract of brown alga *Cystoseira trinodis* and purifying it using fractionation, Sathya *et al*. (2017) showed that phenolic compounds, particularly

phlorotannins, act as antioxidant compounds in this brown alga. Numerous studies have demonstrated a relationship between phenolic compounds and flavonoids with antioxidant activity (Ismail and Amer, 2020), in spite of reports of fluctuations in the amount of flavonoids under environmental stress circumstances (Ferdous and Balia Yusof, 2021). Despite all of this, some studies indicate that there is no relationship or a poor relationship between flavonoids and antioxidant power (Indradi *et al*., 2017; Muflihah *et al*., 2021).

Antioxidant power can also be attributed to compounds such as sulfated polysaccharides, which are found in the cell walls of marine algae (Cunha and Grenha, 2016). In a study to evaluate the annual variation of sulfated polysaccharides in three red algae (*Gracilariopsis longissima*,*Gracilaria gracilis*,and *G.vermiculophylla*, from Gracilariaceae), and one green alga (*Ulva rigida*), the results showed that the studied red algae produced most of these polysaccharides in the cold season and at temperatures between 13°C and 17°C. The highest amount of ulvan (a sulfated polysaccharide) was produced in green alga in summer and at temperatures above 25°C (Sfriso *et al*., 2017). In our study, the highest antioxidant capacity belonged to the red algae in December with an average temperature of 19°C and to green algae in October with an average temperature of 32.5°C. The results of our study seem to be consistent with the results of Sfriso *et al*. (2017). Also, Urrea-Victoria *et al*. (2022), in a study on two red and brown algae, showed that at low temperature (15°C), the red alga *Pyropia spiralis* has the highest index of total antioxidant capacity as well as high content of mycosporine-like amino acids. At a higher temperature (30°C), the brown alga *Sargassum stenophyllum* displayed a high amount of phlorotannins. Mycosporin-like amino acids (MAAs) are known as strong antioxidants in red algae (Vega *et al.*, 2021), and phlorotannins are also known as polyphenolic compounds with antioxidant properties in brown algae (Negara *et al*., 2021; Shrestha *et al*., 2021). As was already indicated, red algae in the current study demonstrated the highest antioxidant capacity at low temperatures, which may be an indication of the presence of mycosporin-like amino acids in this group of algae. Additionally, at higher temperatures in October, the selected brown algae showed the maximum antioxidant activity and phenolic content, which may be related to phlorotannins, which are the main phenolic compounds in brown algae. These findings concur with those of Urrea-Victoria *et al.* (2022).

Overall, the findings demonstrated that the three groups of algae have distinct antioxidant capacities in response to environmental stresses such high and low temperatures, pH, DO, EC, TSS, TN, and TP. These various reactions might result from the diverse bioactive substances that different species in these groups produce. Depending on the surrounding environment, each species of algae showed a variety of physiological reactions. On the other hand, it appears that variables like temperature, DO, EC, and pH have had the biggest impact on

the antioxidant properties of the examined algae. Additionally, factors like the habitat's latitude might help algae produce protective chemicals. Algal samples obtained in the autumn appear to have stronger antioxidant capacity than others.

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References

Aguilera, J., Dummermuth, A., Karsten, U., Schriek. R. and Wiencke, C., 2002. Enzymatic defences against photooxidative stress induced by ultraviolet radiation in Arctic marine macroalgae. *Polar Biology*, 25(**6**), 432-441. DOI: 10.1007/s00300-002-0362-2

Antolovich, M., Prenzler, P.D., Patsalides. E., Mc Donald, S. and Robards, K., 2002. Methods for testing anti-oxidant activity. *Analyst*, 127:183-198. DOI: 10.1039/B009171P

APHA (American Public Health Association), 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edition, Washington DC: American Public Health Association, USA.

Bhadury, P. and Wright, P.C., 2004. Review-Exploitation of marine algae: biogenic compounds for potential antifouling applications, *Planta*, 219(**4**), 561-578. DOI: 10.1007/s00425-004-1307-5

Braune, W. and Guiry, M.D., 2011. Seaweeds: A Colour Guide to Common Benthic Green, Brown and Red Algae of the World's Oceans: Koeltz Scientific Books, Germany, 601 P.

Cahyono, B.E., Umilailatul, J., Misto Nugroho, A.T. and Subekti, A., 2019. Analysis of Total Suspended Solids (TSS) at Bedadung River, Jember District of Indonesia Using Remote Sensing Sentinel 2A Data, *Singapore Journal of Scientific Research*, 9, 117-123. DOI: 10.3923/sjsres.2019.117.123

Castejón, N., Parailloux, M., Izbebska, A., Lobinski, R. and Fernandes, S.CM., 2021. Valorization of the Red Algae *Gelidium sesquipedale* by Extracting a Broad Spectrum of Minor Compounds Using Green Approaches. *Marine Drugs*, 19, 574- 594. DOI: 10.3390/md19100574.

Chang, C., Yang, M., Wen, H. and Chern, J., 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10, 178-182. DOI: 10.38212/2224-6614.2748

Cotas, J., Leandro, A., Monteiro, P., Pacheco, D., Figueirinha, A., Goncalves, A.M.M., Da Silva, G.A,, and Pereira, L., 2020. Seaweed Phenolics: From Extraction to

Applications. *Marine Drugs*, 18(**8**), 384-431. DOI: 10.3390/md18080384.

Coulombier, N., Nicolau, E., Le Déan, L., Barthelemy, V., Schreiber, N., Brun, P., Lebouvier, N. and Jauffrais, T., 2020. Effects of Nitrogen Availability on the Antioxidant Activity and Carotenoid Content of the Microalgae Nephroselmis sp., *Marine Drugs*, 18(**9**), 453. DOI: 10.3390/md18090453.

Cunha, L. and Grenha, A., 2016. Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications. *Marine Drugs*, 14, 1-42. DOI: 10.3390/md14030042.

Dadolahi-Sohrab, A., Garavand-Karimi, M., Riahi, H. and Pashazanoosi, H., 2012. Seasonal variations in biomass and species composition of seaweeds along the northern coasts of Persian Gulf (Bushehr Province). *Journal of Earth System Science*, 121, 241–250 (2012). DOI: 10.1007/s12040-012-0141-6

De La Coba, F., Aguilera, J., Figueroa, F.L., De Galvez, M.V. and Herrera, E., 2008. Antioxidant activity of mycosporine-like amino acids isolated from three red macroalgae and one marine lichen. *Journal of Applied Phycology*, 21,161-169. DOI: 10.1007/s10811-008-9345-1

Dong, G., Hu, Z., Liu, X., Fu, Y. and Zhang, W., 2020. Spatio-Temporal Variation of Total Nitrogen and Ammonia Nitrogen in the Water Source of the Middle Route of the South-To-North Water Diversion Project. *Water*, 12(9), 2615. DOI: 10.3390/w12092615

Downing, J.A., 1997. Marine nitrogen: phosphorus stoichiometry and the global N: P cycle, *Biogeochemistry*, 37, 237-252. DOI: 10.1023/A:1005712322036

El-fayoumy, E.A., Shanab, S.M., Hassan, O.M.A. and Shakaby, A., 2021. Enhancement of active ingredients and biological activities of Nostoc linckia biomass cultivated under modified BG-110 medium composition. *Biomass Conversion and Biorefinery*, 18,1-18 DOI: 10.1007/s13399-021-01509-7

Farasat. M., Khavari-Nejad, R.A., Nabavi, S.M.B. and Namjooyan, F., 2014. Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from Northern coasts of the Persian Gulf. *Iranian Journal of Pharmaceutical Research*, 13(**1**), 163-170.

Ferdous, U.T. and Balia Yusof, Z.N., 2021. Insight into Potential Anticancer Activity of Algal Flavonoids: Current Status and Challenges. *Molecules*, 26(**22**), 6844. DOI: 10.3390/molecules26226844.

Hamouda, R.A.E. and Abou-El-Souod, G.W., 2018. Influence of various concentrations of phosphorus on the antibacterial, antioxidant and bioactive components of green microalgae Scenedesmus obliquus, *International Journal of Pharmacology*, 14, 99- 107. DOI: 10.3923/ijp.2018.99.107

Ianora, A., Bersma, M., Casotter, R., Fontana, A., Harder, J., Hoffmann, F., Pavia, H., Potin, P., Poulet, S.A.

and Toth, G., 2006. New trends in marine chemical ecology. *Estuaries and Coasts*, 29, 531-555. https://doi.org/10.1007/BF02784281

Indardi, R.B., Fidrianny, I. and Wirasutisna, K.R., 2017. DPPH Scavenging Activities and Phytochemical Content of Four Asteraceae Plants. *International Journal of Pharmacognosy and Phytochemical Research*, 9(**6**), 755-759. DOI: 10.25258/phyto.v9i6.8173

Irato, P., Piccinni, E., Cassini, A. and Santovito, G., 2007. Antioxidant responses to variations in dissolved oxygen of *Scapharca inaequivalvis* and *Tapes philippinarum*, two bivalve species from the lagoon of Venice, *Marine Pollution Bulletin*, 54,1020-1030. DOI: 10.1016/j.marpolbul.2007.01.020

Ismail, M.M.S., 2016. Effect of nitrogen regime on antioxidant parameters of selected prokaryotic and eukaryotic microalgal species, *Acta Physiologiae Plantarum*, 38(**6**), 154-166. https://doi.org/10.1007/s11738-016-2170-2

Ismail, M.M. and Amer, M.S., 2020. Characterization and biological properties of sulfated polysaccharides of *Corallina officinalis* and *Pterocladia capillacea*. *Acta Botanica Brasilica*, 34(**4**), 623-632. https://doi.org/10.1590/0102-33062020abb0121

Jablonski, N.G. and Chaplin, G., 2010. Human skin pigmentation as an adaptation to UV radiation. *Proceedinds of the National Academy of Sciences of the United States of America*, 107, suppl. 2. DOI: 10.1073/pnas.0914628107.

Jimenez, C., 2018. Marine Natural Products in Medicinal Chemistry. *ACS Medicinal Chemistry Letters*, 9(**10**), 959-961. DOI: 10.1021/acsmedchemlett.8b00368

Kobbia, I.A, Khalil, Z., Asker, M.S. and Abd-Elsayed, S.M., 2010. Effect of nitrogen on the biochemical constituents and antioxidant production by two green unicellular algae. *Egyptian Journal of Phycology*, 11, 151-170. DOI: 10.21608/EGYJS.2010.114920

Li, J., Huang, K., Huang, L., Hua, Y., Yu, K. and Liu, T., 2020. Effects of dissolved oxygen on the growth performance, haematological parameters, antioxidant responses and apoptosis of juvenile GIFT (*Oreochromis niloticus*). *Aquaculture Research*, 51, 3079-3090. DOI: 10111/are.14684

Liu, Y., Li, L. and Jia, R., 2011. The Optimum Resource Ratio (N: P) for the Growth of *Microcystis Aeruginosa* with Abundant Nutrients. Procedia Environmental Sciences, 10, 2134-2140. DOI: 10.1016/J.PROENV.2011.09.334

Missimer, T.M., Thomas, S. and Rosen, B.H., 2021. Legacy Phosphorus in Lake Okeechobee (Florida, USA) Sediments: A review and new perspective. *Water*, 13, 39-63. DOI: 10.3390/w13010039

Mittler, R., 2002. Oxidative Stress, Antioxidants and Stress Tolerance. *Trends in Plant Science*, 7, 405-410.

DOI: 10.1016/s1360-1385(02)02312-9.

Muflihah, Y.M., Gollavelli, G. and Ling, Y.C., 2021. Correlation Study of Antioxidant Activity with Phenolic and Flavonoid Compounds in 12 Indonesian Indigenous Herbs. *Antioxidants (Basel, Switzerland),* 10(**10**), 1530-1545. DOI: 10.3390/antiox10101530

Munene, R., Changamu, E., Koriri, N. and Joseph, G.O. 2017. Effects of different nitrogen forms on growth, phenolics, flavonoids and antioxidant activity in amaranth species. *Tropical Plant Research*, 4(**1**), 81-89. DOI: 10.22271/tpr.2017.v4.i1.012

Nausch, M., Achterberg, E.P., Bach, L.T., Brussaard, C.P.D., Crawfurd, K.J., Fabian, J., Riebesell, U., Stuhr, A., Unger, J. and Wannicke, N., 2018. Concentrations and Uptake of Dissolved Organic Phosphorus Compounds in the Baltic Sea, *Frontiers in Marine Science*.5,1-18. https://doi.org/10.3389/fmars.2018.00386

Negara, B.F.S.P., Sohn, J.H., Kim, J-S. and Choi, J-S., 2021. Effects of Phlorotannins on Organisms: Focus on the Safety, Toxicity, and Availability of Phlorotannins. *Foods*, 10(**2**), 452-471. DOI: 10.3390/foods10020452

Nishida, Y., Kumagai, Y., Michiba, S., Yasui, H. and Kishimura, H., 2020. Efficient Extraction and Antioxidant Capacity of Mycosporine-Like Amino Acids from Red Alga Dulse *Palmaria palmata* in Japan. *Marine Drugs*, 18(**10**), 502-520. DOI: 10.3390/md18100502

Pereira, F., 2019. Have marine natural product drug discoveryefforts been productive and how can we improvetheir efficiency? *Expert Opinion on Drug Discovery*. 14(**7**), 717-722. DOI: 10.1080/17460441.2019.1604675.

Rajwa-Kuligiewicz, A., Bialik, R.J. and Rowiński, P.M., 2015. Dissolved oxygen and water temperature dynamics in lowland rivers over various timescales. *Journal of Hydrology and Hydromechanics*. 63, 353–363. DOI: https://doi.org/10.1515/johh-2015-0041

Ratkevicius, N., Correa, J.A. and Moenne, A., 2003. Copper accumulation, synthesis of ascorbate and activation of ascorbate peroxidase in *Enteromorpha compressa* (L.) Grev. (Chlorophyta) from heavy metal-enriched environments in norther Chile. *Plant Cell and Environment*. 26,1599-1608. DOI: 10.1046/J.1365-3040.2003.01073.X

Rodgher, S., Contador, T.M., Rocha, G. and Espindola, E.L.G., 2020. Effect of phosphorus on the toxicity of zinc to the microalga *Raphidocelis subcapitata*, *Anais da Academia Brasileira de Ciencias*, 92(**Suppl. 2**). DOI: 10.1590/0001-3765202020190050

Ruangchuay, R., Lueangthuwapranit, C. and Pianthumdee, N., 2007. Apparent characteristics and taxonomic study of macroalgae in Pattani Bay. *Songklanakarin Journal of Science and Technology*. 29, 893-905.

Sathya, R., Kanaga, N., Sankar, P. and Jeeva, S., 2017. Antioxidant properties of phlorotannins from brown seaweed *Cystoseira trinodis* (Forsskål) C. Agardh. *Arabian Journal of Chemistry*, 10(**Suppl. 2**), 2608-2614. DOI: 10.1016/j.arabjc.2013.09.039

Sfriso, A.A., Gallo, M. and Franco, B., 2017. Seasonal variation and yield of sulfated polysaccharides in seaweeds from the Venice Lagoon. *Botanica Marina*. 6, 339-349. DOI: 10.1515/bot-2016-0063

Shams, M., Afsharzadeh, S. and Balali, Gh., 2015. Taxonomic Study of Six *Sargassum* Species (Sargassaceae, Fucales) with Compressed Primary Branches in the Persian Gulf and Oman Sea Including *S. binderi* Sonder a New Record Species for Algal Flora, Iran. *Journal of Sciences Islamic Republic of Iran*, 26(**1**), 7–16. DOI: 10.22059/JSCIENCES.2015.53212

Shcherbakov, V.V., Artemkina, Y.M., Akimova, I.A. and Artemkina, I.M., 2021. Dielectric Characteristics, Electrical Conductivity and Solvation of Ions in Electrolyte Solutions. *Materials (Basel),* 14(**19**), 5617. DOI: 10.3390/ma14195617.

Shrestha, S., Zhang, W. and Smid, S.D., 2021. Phlorotannins: A review on biosynthesis, chemistry and bioactivity. *Food Bioscience*, 39, 100832. DOI: 10.1016/j.fbio.2020.100832

Silva, A., Rodrigues, C., Garcia-Oliveria, P., Lourenco-Lopes, C., Silva, S.A., Garcia-Perez, P., Carvalho, A.P., Domingue, V.F., Barroso, M.F., Delerue-Matos, C., Simal-Gandara, J. and Prieto, M.A., 2021.Screening of Bioactive Properties in Brown Algae from the Northwest Iberian Peninsula. *Foods*, 10(**8**), 1915. DOI: 10.3390/foods10081915

Soufiane, F., Aasfar, A., Rabie, R., Smouni, A. and El-Arrousi, H., 2022. Salt induced oxidative stress alters physiological, biochemical and metabolomic responses of green microalga *Chlamydomonas reinhardtii*. *Heliyon*, 8, e08811. DOI: 10.1016/j.heliyon.2022.e08811

Ssebiyonga, N., Erga, S.R., Hamre, B., Stamnes, J.J. and Frette, Ø., 2013. Light conditions and photosynthetic efficiency of phytoplankton in Murchison Bay, Lake Victoria, Uganda. *Limnologica*, 43, 185-193. DOI: 10.1016/j.limno.2012.09.005

Urrea-Victoria, V.,Furlan, C.M., Alves Cursino Dos Santos, D.A. and Chow, F., 2022. Antioxidant potential of two Brazilian seaweeds in response to temperature: *Pyropia spiralis* (red alga) and *Sargassum stenophyllum* (brown alga). *Journal* of *Experimental Marine Biology and Ecology*, 549, 151706. DOI: 10.1016/j.jembe.2022.151706

van Alstyne, K.L., Koellermeier, L. and Nelson, T.A., 2007. Spatial variation in dimethylsulfoniopropionate(DMSP)production in *Ulva lactuca* (Chlorophyta) from the Northeast Pacific. *Marine Biology*, 150,1127-1135. DOI: 10.1007/s00227-006-0448-4

Vega, J., Bonomi-Barufi, J., Gómez-

Pinchetti, J.L. and Figueroa, F.L., 2020. Cyanobacteria and Red Macroalgae as Potential Sources of Antioxidants and UV Radiation-Absorbing Compounds for Cosmeceutical Applications. *Marine Drugs*, 18(**12**), 659. DOI: 10.3390/md18120659.

Wu, H., 2016., Effect of Different Light Qualities on Growth, Pigment Content, Chlorophyll Fluorescence, and Antioxidant Enzyme Activity in the Red Alga *Pyropia haitanensis* (Bangiales, Rhodophyta), *BioMed Research International*, 7383918, DOI: 10.1155/2016/7383918.

Xie, X., Hu, W., Fan, X., Chen, H. and Tang, M. 2019. Interactions between phosphorus, zinc, and iron homeostasis in Nonmycorrhizal and Mycorrhizal plants. *Frontiers in Plant Science*, 10, 1172. DOI: 10.3389/fpls.2019.01172

Yaakob, M.A., Mohamed, R.M.S.R., Al-Gheethi, A., Aswathnarayana Gokare, R. and Ambati, R.R., 2021. Influence of Nitrogen and Phosphorus on Microalgal Growth, Biomass, Lipid, and Fatty Acid Production: An Overview. *Cells*, 10(**2**), 393. DOI: 10.3390/cells10020393.