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Antioxidant and photo-physiological acclimatisation in tropical macroalgae at sites with distinct nutrient levels

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Abstract. *Gopeechund A, Bhagooli R, Neergheen VS, Bahorun T. 2023. Antioxidant and photo-physiological acclimatisation in tropical macroalgae at sites with distinct nutrient levels. Indo Pac J Ocean Life 7: 79-90.* The antioxidant efficacy, Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC) were investigated at Gis Gris with Lower Nutrient levels (LN site) and Bain boeuf with Higher Nutrient levels (HN site) around Mauritius Island. A field-based 7-days transplantation manipulation of *Turbinaria ornata*, *Gracilaria salicornia* and *Sargassum obovatum* between HN and LN sites was conducted to test the impact of different nutrient conditions on their physiology. All the species had lower antioxidant efficacies, TPC and TFC at LN compared to HN site. The glutathione peroxidase and catalase were more sensitive at reflecting the lower oxidative stress at LN site and acclimatisation to oxidative stress occurring at the HN site. ETRmax and α were significantly higher in all species at HN site. The quantum yield Fv/Fm increased in *S. obovatum* only, after 7 days of transplantation at HN site, while, *T. ornata* and *G. salicornia* had similar adaptability to photo-physiological stress at both sites. NPQmax decreased in *S. obovatum* but increased in *T. ornata* transplanted to HN site, indicating increased photo-protection at HN site. Lagoons with higher nutrient levels may enhance macroalgal capacity to deal with oxidative stress, thus lowering the need for further photo-protection.

Keywords: Antioxidant efficacy, enzymatic antioxidant, nutrient-dependent variation, phenolic contents, photo-physiology

INTRODUCTION

Most shallow ocean areas receive significant amounts of human-induced chemical inputs and natural environmental conditions (Eklund and Kautsky 2003; Parida and Das 2005; Helmuth et al. 2006; Kannan and Krishnamoorthy 2006). Marine organisms' interaction with pollutants impacts their physiology (Brierley and Kingsford 2009; Dailianis 2010; Morais et al. 2012). Macroalgae may respond to altered environmental conditions in marine areas influenced by human activities by changing their levels of phenolic compounds and antioxidant activities (Orbea et al. 2002; Cunha et al. 2005; Nimptsch et al. 2005; Connan et al. 2006; Scania and Chasani 2021). Collen and Davison (1999) highlighted the exposure of *Fucus* spp. to desiccation or freezing stress increased Reactive Oxygen Species (ROS) production. Increased levels of the enzymatic antioxidant glutathione in the green seaweed *Ulva* sp. correlated with increased levels of dissolved inorganic nitrogen levels (Pereira et al. 2009). Antioxidants have thus been proposed as biomarkers of contaminant-mediated oxidative stress in various marine organisms (Cossu et al. 1997). (Cossu et al. 1997; Gopeechund et al. 2020). However, studies on macroalgae and seagrass antioxidants (Somanah et al. 2012; Ramah et al. 2014; Narrain et al. 2023) and photophysiology (Bhagooli et al. 2021a; Narrain et al. 2023) are limited in the Mauritian waters.

Light plays a key role in triggering different types of stresses in marine organisms. Solar radiation, more specifically short wavelengths (UVB, 280-315 nm) can alter photo-physiological processes in plants, including protective responses (Bischof et al. 2006), DNA damage (Pakker et al. 2000; van de Poll et al. 2001) and growth (Aguilera et al. 2002). Changes in light levels are known to enhance ROS production, for e.g., the production of superoxide increased at photosystem I in the diatom *Thalassia weissflogii* during photosynthesis correlated with changes in the level of light (Milne et al. 2009). There is evidence of phenolic compounds helping in photo-protection. For instance, coumarins in green macroalgae like *Caulerpa* sp. and *Dasycladus* sp. help prevent radiation damage with their high UV absorption properties (Pérez-Rodríguez et al. 2003; Bischof et al. 2006). Intertidal macroalgae are speculated to be physically and physiologically adapted to cope with irradiance fluctuations and maintain optimal conditions for physiological processes such as photosynthesis (Davison and Pearson 1996). Photo-physiological adaptations also occur in response to light induced stress, whereby the photosystem activity is reduced due to strong light (Schagerl and Möstl 2011). Pulse Amplitude Modulation (PAM) fluorometry

uses Rapid Light Curves (RLCs) to provide a measure of chlorophyll a fluorescence (Beer et al. 2006; Campbell et al. 2008; Collier et al. 2009; Bhagooli et al. 2021b). RLC-derived parameters such as maximum quantum yield (Fv/Fm), initial slope (α), ETRmax, and NPQ have been used to indicate stress occurring in marine plants, including algae (Le et al. 2006; Bité et al. 2007; Piniak and Brown 2009; Silverstein et al. 2017). It has also been shown that photophysiological parameters vary during transplantation from one site to another, for example, α and ETRmax decreased in seagrass species post-transplantation to a site with higher freezing stress (Collen and Davison 1999). Marine plants such as seagrasses alter their physiology, morphology and growth after transplantation (Zimmerman et al. 1995; Horn et al. 2009). Marine plants' photosynthetic rate may also be affected by transplantation shock, which may show up in characteristics of chlorophyll *a* fluorescence (Lamote and Dunton 2006; Kahn and Durako 2008; Horn et al. 2009).

Limited studies have experimentally determined the variation of photo-physiological and antioxidant parameters of macroalgae under distinct nutrient levels (Delgado and Lapointe 1994; Teichberg et al. 2013; Moussavou et al. 2014). Given the scarcity of studies describing the nutrient-dependent variation of photo-physiological processes, as well as antioxidant activities and secondary metabolites in macroalgal species, this paper addresses these parameters to assess the status of the macroalgae under prevailing different nutrient conditions. An experimental field trial was conducted to test the changes observed.

MATERIALS AND METHODS

Sample collection

Macroalgal species were collected at two lagoons of Mauritius (Figure 1) in May 2015. Bain Boeuf ("19°59'08"S, 57°36'16"E") was characterized as having High Nutrient level (HN) and Gris Gris ("20°31'28"S, 57°32'02"E") Lower Nutrient level (LN) (Figure 1). The satellite images of these two sites from 2009-2016 was also assessed for environmental changes using Google Earth (Figure 2). In May 2016, the red seaweed *Gracilaria salicornia* and the brown seaweeds *Turbinaria ornata* and *Sargassum obovatum* were collected from the HN and LN sites for transplantation experiment.

Transplantation experiment

Vertical transplantation of *T. ornata*, *G. salicornia* and *S. obovatum* was done between LN and HN sites. Three adult specimens of each species were placed in reticulated, semi-permeable plastic bags and attached using ropes on firm naturally collected blocks. After 5 days of exposure period in shallow areas (1-2m deep), the chlorophyll a stress photo-physiology was measured using the D-PAM fluorometer and the phenolic contents and antioxidant levels were determined.

Photo-physiological parameters

The seaweeds were dark-adapted for about 30 min (Maxwell and Johnson 2000; Schagerl and Möstl 2011; Bhagooli et al. 2021b), after which a saturating pulse was emitted using a Diving-PAM fluorometer to measure the fluorescence. Parameters such as α (Photosynthetic efficiency), ETR_{max}, Fv/Fm and NPQ_{max} were determined. ETR was calculated using the equation ETR= E(Photon irradiance of PAR) × Δ F/F'm (effective quantum yield) (Serôdio et al. 2007) using the light absorption factor of seaweed, that is 4.2. An ETR curve was fitted using an empirical equation of Platt et al. (1980) on SigmaPlot 12.0, to determine ETR_{max}. The initial slope α was determined on the ETR curve. NPQ was calculated using $(F_M - F_M')/F_M'$ (Bilger and Bjorkman 1990), following which the NPQ_{max} was determined. The maximum quantum yield at PSII was determined as Fv/Fm = (FM–F_0)/FM (Genty et al. 1989).

Measurement of physico-chemical parameters

The physico-chemical parameters of seawater at the collection sites were recorded in situ using appropriate apparatus during both periods of collection. Dissolved Oxygen (DO), salinity, pH and temperature were measured using a DO meter (Hanna HI 9142), a refractometer (ERMA), a pH meter (Hanna HI 9024C) and a thermometer (Cormac 314), respectively. The concentrations of nitrate and phosphate in sea water were analyzed in the laboratory using cadmium reduction and ascorbic acid methods, respectively (Greenberg et al. 1992). In the cadmium reduction method, 75 mL of NH_4Cl-EDTA solution was added to 25 mL of water sample and poured in a cadmium column, whereby nitrate present was reduced to nitrite. The collected solution and the end of the column was treated with 2.0 mL of color reagent (85% phosphoric acid and 0.0125 g/mL of sulphonamide dissolved in water) and the color developed was read at 543 nm on a spectrophotometer (Genesys™ 10S, Thermo Scientific, USA) against a distilled water reagent blank. The concentration of phosphate was determined by adding $5cm^3$ of mix reagent (15 mL of 40g/L ammonium heptamolybdate, 15 mL of 3.4g/L potassium antimonyltartrate, 50 mL $5NH_2SO_4$, and 30 mL of 17.6 g/L of ascorbic acid) to $50cm^3$ of water sample. After 15 minutes the absorbance was measured at 880 nm on a spectrophotometer, using distilled deionized water as blank.

Macroalgal extract preparation

Extracts were prepared using protocol of Chakraborty et al. (2013), with modifications in the concentration of methanol used. First, 200g of each seaweed species was collected, freeze-dried and macerated in 70% methanol 1:2 w/v. The filtered extracts were concentrated using a rotary evaporator and then freeze dried into powder. The powdered extracts were then prepared and tested for phytochemicals and investigated for antioxidant efficacy.

Phytochemical analyses

Estimation of total phenolic content

The method of (Singleton et al. 2015) was used for the quantification of total phenolic contents with modifications in volume of reagents used. For the quantification of the total phenol content of the extract using a modified method from the study of Singleton and Rossi (1965) was used with slight modifications. Briefly, 50 µL of extract (0.1-20 mg/mL) was added to 50 µL of distilled water followed by 50 µL of Folin-Ciocalteau reagent. After 3 minutes 200 µL of sodium carbonate was added and the reaction mixture incubated at 40.0°C for 40 minutes. The intensity of the blue coloration was read at 685 nm against a blank (microplate reader: Bio Tek®, USA). A calibration curve of Gallic acid was used to estimate the gallic acid equivalent content of phenolics in milligram per millilitre of extract.

Estimation of total flavonoid content

Quantification of total flavonoids in the extract was done (Lamaison and Carnat 1991). 100 µL of extract (0.1-20 mg/mL), and 100 µL aluminum chloride was incubated at 26°C for 5 minutes. The optical density was read at 440 nm against a blank (microplate reader: Bio Tek® USA). A calibration curve of quercetin was used (20-100 mg/mL) to express results in microgram per gram of the extract. The total flavonoid contents expressed in quercetin equivalent/gFDW.

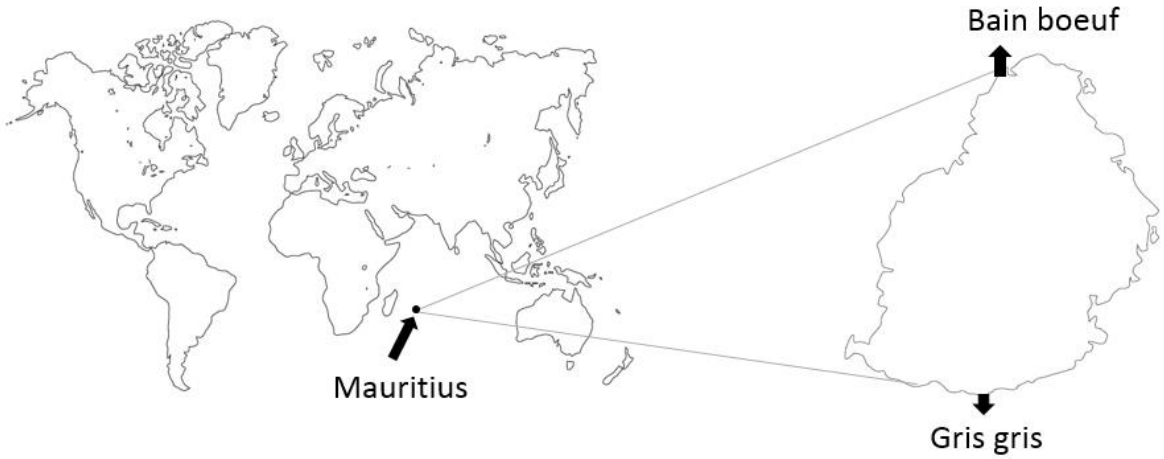

Figure 1. Location of Mauritius in world map and sites of collection in Mauritius: Bain Boeuf and Gris Gris

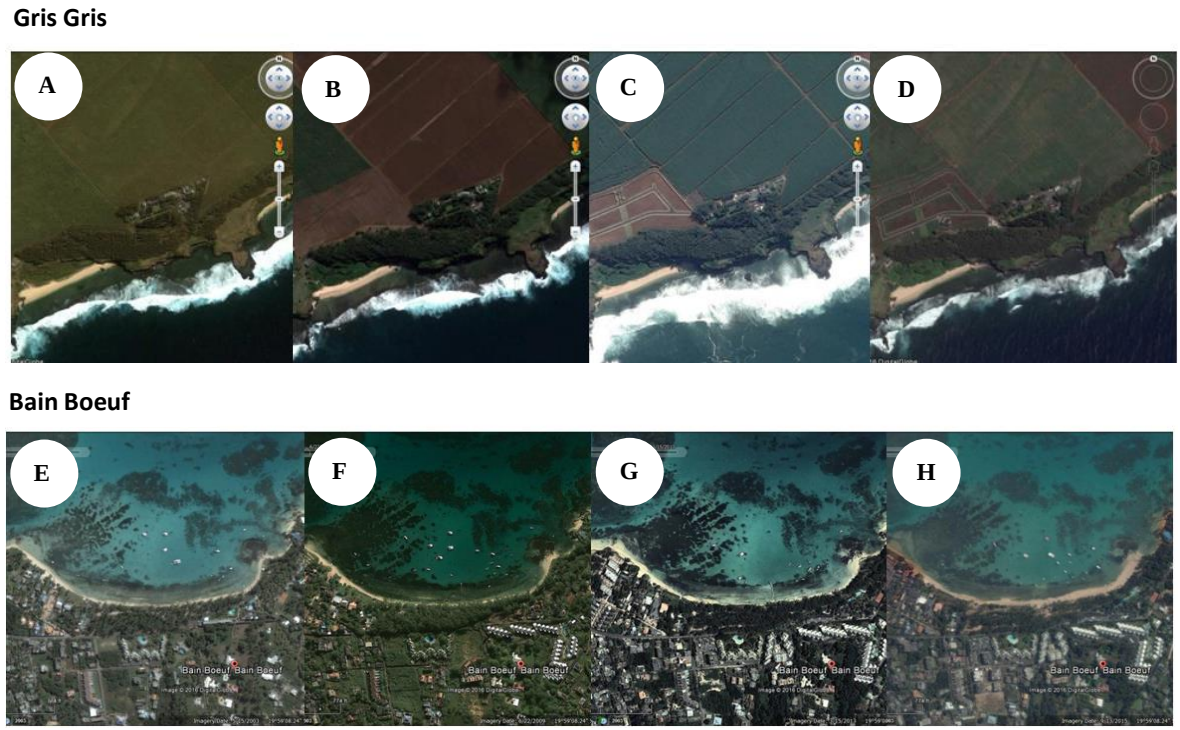

Figure 2. Change in the topography of Gris Gris from 2009-2016 (A-D) and Bain Boeuf from 2003-2015 (E-H). Source: https://www.google.com/earth/

Antioxidant activities

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH radical scavenging assay was used to determine the potential of the extracts to scavenge 2,2-diphenyl-1-picrylhydrazyl radicals (Duan et al. 2007). To 0.1 mL of 2, 2-diphenyl-1-picrylhydrazyl (0.16 mmol/L), 0.1 mL of each extract (0.1-20 mg/mL) was added and the mixture incubated for 30 minutes in darkness at room temperature. The optical density was read at 515 nm (microplate reader: Bio Tek®, USA) and percentage of DPPH radical scavenged was calculated using the formula: DPPH radical Scavenging (%) = $[(A_0 – A_1) / A_0] * 100$. The concentration at which 50 % of the reaction was completed (EC_{50}) was determined. The activities of the species were compared with ascorbic acid, which was used as positive control.

Superoxide scavenging assay

The ability of the extracts to scavenge superoxide anion was determined using the method of Kumar et al. (2011). A total of 1 mL of nitroblue tetrazolium (NBT) solution (156 µM), 1 mL *β*-nicotinamide-adenine dinucleotide, reduced (NADH*;* 200 µM) and 250 µL of each extract (0.1-20 mg/mL) were mixed and the reaction initiated by addition of 100 µL phenazine methosulphate (PMS) solution. The reaction mixture was incubated at 25°C for 30 minutes and the absorbance at 560 nm was measured against a blank (microplate reader: Bio Tek®, USA). The ability of the extracts to scavenge superoxide radicals in the PMS/NADH system was expressed as a percentage using the formula: Scavenging of Superoxide Anion (%) = $[(A_0 – A_1) / A_0] * 100$. Where A_0: absorbance of control (reaction mixture with extract vehicle only) and A_1: absorbance of the sample extract. The concentration at which 50% of the reaction was completed (EC_{50}) was determined. The activities of the species were compared with ascorbic acid, which was used as positive control.

Nitric oxide radical inhibition assay

The nitric oxide radical inhibition assay was used to determine the nitric oxide radical inhibition (Kumar et al. 2008). Greiss Ilosvay reagent was used to quantify nitrite ions produced from the spontaneous oxidation of sodium nitroprusside in aqueous solution. At a final volume of 150 µL, the reaction mixture containing 50 µL sodium nitroprusside (5 mM dissolved in phosphate buffered saline, pH 7) and 50 µL of the extract was incubated at 25°C for 150 minutes. After incubation, 50 µL sulphanilic acid reagent in glacial acetic acid followed by 50 µL N-1-napthylethylenediamine dihydrochloride were mixed and allowed to stand. The absorbance was measured at 546 nm against a blank (microplate reader: Bio Tek®, USA). The scavenging of nitric oxide was calculated using the formula: Scavenging of nitric oxide (%) = [(A0 – A1) / A0] * 100, Where A0: absorbance of control (reaction mixture with extract vehicle only) and A1: absorbance of the sample extract. The concentration at which 50% of the reaction was completed (EC_{50}) was determined. The activities of the species were compared with ascorbic acid, which was used as positive control.

Estimation of Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was used to determine the ability of the extracts to reduce ferric ions (Benzie and Strain, 1996). FRAP reagent was freshly prepared by mixing 2, 4, 6-Tripyridyl Triazine (TPTZ) and ferric chloride in sodium acetate buffer. The FRAP reagent was incubated at 37°C for 30 minutes. The final reaction mixture contained 25 µL of the extract (0.1-20 mg/mL) and 750 µL of FRAP reagent. The intensity of the blue/purple coloration was read at 593 nm (microplate reader: Bio Tek®, USA) against a blank of the buffer. A calibration curve of ferrous sulphate (0.2-2 mmol/mL) was used to express results in mmol Fe^{2+} per mL.

Preparation of extracts for enzymatic antioxidant activities

The enzymes were extracted using a modified protocol of Khan et al. (2015), whereby the 1 g of each macroalgae specimen was crushed in 50 mL of Tris-HCl, EDTA, $MgCl_2$ and PVP buffer and centrifuged at 15000g for 20 minutes at 4°C. Protein content was measured using the protocol of Bicinchoninic Acid (BCA) assay of Smith et al. 1985. To 10 µL of sample/Bovine Serum Albumin (BSA) (different concentration), 200 µL of a mixture of BCA and copper sulphate, followed by incubation at 37°C. The wavelength was read at 562 nm. The BSA curve was constructed, which was used to determine the amount of protein present in the sample.

Enzymatic antioxidant activities

Catalase (CAT) activity assay

The CAT activity was determined using the catalase activity colorimetric assay KIT according to manufacturer's instructions (Biovision, U.S.A). 30 µL samples and 1.5 µL positive control were respectively adjusted to 78 µL with assay buffer. Sample amount of samples and positive are placed in another well, followed by 10 µL stop solution to produce the High Control (HC). An H_2O_2 curve was plotted by measuring the optical density of a range concentration of H_2O_2 (0-10 nmol) at 570 nm (microplate reader: Bio Tek®, USA). Signal change by catalase for the sample i.e $\Delta A = A_{HC}-A_{sample}$. ΔA was applied to the H_2O_2 curve to obtain B, i.e. the nmol of H_2O_2 decomposed by catalase in 30 min reaction. The results were normalized to milligram of proteins used in the assay.

Glutathione Peroxidase (GPx) Activity

The GPx activity was determined using the GPx activity colorimetric assay KIT according to manufacturer's instructions (Biovision, U.S.A). To 30 µL of samples 30 µL of assay buffer was added. To 10 µL of positive control 40 µL of assay buffer was added. To each well the reaction mixture consisting of 33 µL of assay buffer, 40mM NADPH, 3 µL of glutathione reductase and 2 µL of glutathione synthase hydrogenase solutions were added. After fifteen minutes of incubation cumene hydroperoxide was added and was mixed thoroughly. The absorbance A_1 was read at 340nm at T1 and after 5 minutes to obtain A2. The GPx activity was obtained using the following equation: (A1-A2)-(RC_A1-TC_A2) to obtain B. Then, GPx activity = ((B)/(T2-T1)*V)*sample dilution. The

results were normalized to milligram of proteins used in the assay.

Superoxide Dismutase (SOD) assay

The SOD activity was determined using the superoxide dismutase activity colorimetric assay KIT according to manufacturer's instructions (Biovision, U.S.A). To 1 mL of WST solution, 19 mL of assay buffer was added and was mixed well. 15 µL of enzyme solution was then diluted with 2.5 mL of dilution buffer. Twenty (20) µL of sample was placed in well and separately in another well for blank 2, while 20 µL of water was placed in blank 1 and 3. Two hundred (200) µL of WST solution was added to each well. Twenty (20) µL of dilution buffer was added to blank 2 and blank 3. Twenty (20) µL of enzyme working solution was added to all samples and blank 1, and the mixture was incubated at 37°C for 20 minutes, following which the absorbance read at 450 nm (microplate reader: Bio Tek®, USA).

Data analysis

The normality tests were carried out using Shapiro-Wilk statistics. The means among different variables were compared using one-way ANOVA using SPSS 18.0. Tukey LSD as Post Hoc test was used to determine significant differences in mean antioxidant activities and phytochemicals among different species at different sites and during different experimental status.

RESULTS AND DISCUSSION

Variation of physico-chemical parameters and nutrient levels between HN and LN sites

Both at Day 0 and 7 the nutrients, including nitrate and phosphate levels differed among the two study site ($P<0.001$), being lower at the LN site compared to the HN site. Significant differences in photochemical parameters were recorded ($P< 0.001$), whereby pH was higher at HN sites and DO was higher at LN site (Figure 3 and Table 1).

Variation of phenolic contents and antioxidant activities

The non-enzymatic antixodiant activities and the phenolic contents varied significantly within and among the species at the different sites (Two-way ANOVA, $P<0.001$) at day 0 and 7 and before and after transplantation (Three way, $P <0.001$). The enzymatic anoxidant activities differed within and among the species at the two sites at day 0 (Catalase and SOD, Two-way ANOVA $P< 0.001$ and GPx, two-way ANOVA $P<0.05$), day 7 (Two-way ANOVA $P<0.001$) and before and after transplantation (Three-way ANOVA $P<0.01$). The photophysiological parameters differed significantly at the different sites, before and after transplantation (Two-way ANOVA $P<0.001$). No significant interspecific variation in photophysiological parameters was observed during the transplantation experiment (Table 2).

Variation of total phenolic contents and total flavonoid contents before and after transplantation experiment at HN and LN sites

The TPC was lower in all the species at the LN site compared to the HN site. Significant variation ($P < 0.001$) in TPC and TFC was observed among the different macroalgal species before and after transplantation to the LN and HN sites and vice versa. The TPC reduced significantly in all the species transplanted to the LN site and increased in all the species transplanted to the HN site. The TFC was significantly lower in *S. obovatum* at the LN site compared to that in the HN site. The TFC significantly increased in *S. obovatum* transplanted to HN site and decreased in those transplanted to the LN site (Figures 4A and B).

Variation of antioxidant efficacy pre and post-transplantation at sites with different nutrient levels

The DPPH and nitric acid scavenging activity and FRAP was stronger at HN site in all studied species compared to LN site. The DPPH scavenging activity and FRAP significantly increased in macroalgae transplanted to HN site and decreased in those transplanted to LN site.

The nitric oxide scavenging increased in *T. ornata* and *S. obovatum* transplanted to HN site and decreased in specimen transplant to LN site. The nitric oxide scavenging activity of *G. salicornia* increased in *G. salicornia* transplanted to HN site (Figures 5.A-C).

Variation of enzymatic antioxidant activity during transplantation experiment

Enzymatic antioxidant activities varied significantly in the macroalgae after seven days of transplantation (Table 2). The enzymatic antioxidant activities were lower in species at the LN sites compared to the HN site at day 0. At day 7, the GPx and the catalase activities and SOD activities significantly increased in all seaweed species transplanted to the HN site and decreased in all species transplanted to the LN site ($P < 0.01$) (Figures 6 A-C).

Variation of photophysiological parameters during transplantation experiment

Photophysiological parameters including quantum yield (F_v/F_m), ETR_{max}, NPQ_{max} and α (photosynthetic efficiency) and varied before and after transplantation between sites in the species (Figure 7). The quantum yield F_v/F_m increased in *S. obovatum* only after 7 days of transplantation at HN site. ETR_{max} and α were significantly higher in all species at HN site, both prior and after the experiment ($P<0.05$). In *S. obovatum*, NPQ_{max} significantly decreased when transplanted to LN site and increased in the speciemens transplanted to LN site. In *T. ornata*, NPQ_{max} rose after the transplantation in species transplanted to HN site, while in *G. salicornia*, no significant changes in NPQ_{max} were detected.

Correlations among the different parameters

During the transplantation experiment, at day 0, a moderate negative correlation was found between DPPH scavenging activity and pH. The Nitric oxide scavenging activity and total phenolic content positively correlated

with pH. At day 7, only the total phenolic content showed a moderate positive correlation between both nitrate and phosphate. Both at Day 0 and 7, the GPx, catalase and SOD strongly correlated with nitrate, phosphate, pH and DO (r >0.8). The maximum yield, α, ETR and NPQ correlated strongly (r > 0.8) with nitrate, phosphate, pH and DO (r >0.8).

Discussion

Different levels of nutrients, as well as physico-chemical parameters such as pH and DO were found at the two study sites. These may be attributed to dissimilar anthropogenic influences and different hydrodynamic processes at the two study sites. For example, the nitrate level was just 0.8 N mg/mL, the limit of nutrients in a recreational area (MoEHrDE 1999). Most human-induced nutrients enter coastal ecosystems from nearby agricultural sites, rivers and runoffs. Lower nitrate and phosphate levels were recorded at the LN site (Gris Gris) compared to the HN site (Bain Boeuf). The LN lagoon at the LN site is smaller and experiences stronger wave action, water flushing and water current compared to the HN site, which might help maintain lower nutrient levels at the LN site. The effect of some intertidal macroalgae was thus investigated under varied nutrient and physico-chemical conditions.

Table 1. Summary of one-way ANOVA test for variation in physico-chemical parameters and nutrients between HN and LN sites

Variables	Status		SS	DF	MS	F	P Value
Sites	Day 0	Nitrate	1.40	1	1.40	585.4	0.00
		Phosphate	0.01	1	0.01	124.6	0.00
		Salinity	0.10	1	0.10	1.0	0.34
		pH	0.99	1	0.99	738.2	0.00
		Temperature	0.13	1	0.13	2.0	0.18
		DO	1.36	1	1.36	440.5	0.00
	Day 7	Nitrate	1.40	1	1.40	585.4	0.00
		Phosphate	0.01	1	0.01	124.6	0.00
		Salinity	0.10	1	0.10	1.0	0.34
		pH	0.99	1	0.99	738.2	0.00
		Temperature	0.13	1	0.13	2.0	0.18
		DO	1.36	1	1.36	440.5	0.00

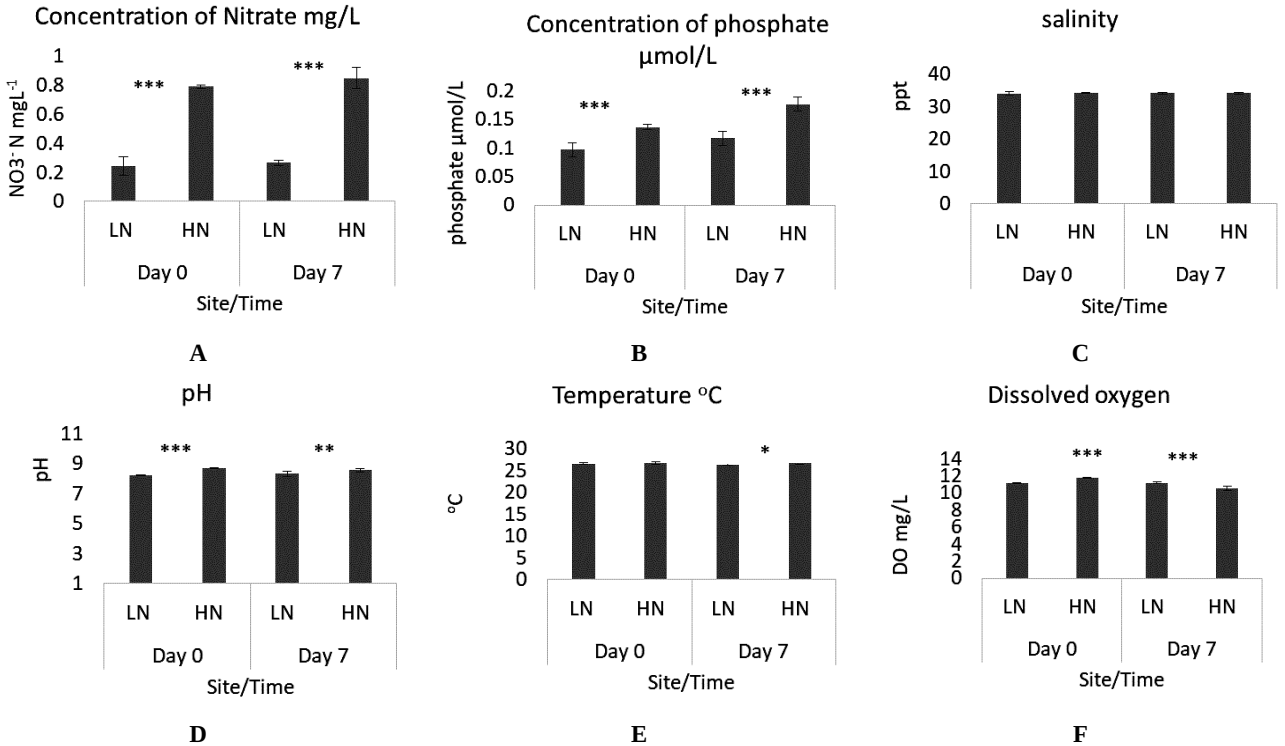

Figure 3. Nutrient levels (A and B) and (C-F) physicochemical parameters (n=3) varying at a different sites. The mean values ± SD are shown in the graphs. *** P < 0.001, **P < 0.01,* P < 0.05. (n=3)

Table 2. Variation of antioxidant activities, phytochemical contents among different species and photo physiological parameters during the transplantation experiment at the different sites. *** P< 0.001,**P < 0.01, *P<0.05 and the double and triple interaction of sites, transfer status and species

ANOVA	Status			SS	DF	MS	F	P value
Species * Site	Day 0	Non-enzymatic antioxidant activities	DPPH	18.8	2	9.4	105.4	***
			NO	338.5	2	169.3	578.5	***
			FRAP	0.1	2	0.1	81.9	***
		Phenolics	TPC	73.7	2	36.8	2443.6	***
			TFC	0.5	2	0.2	58.2	***
		Enzymatic antioxidant activities	GPX	0.4	2	0.2	4.8	*
			Catalase	0.1	2	0.1	46.5	***
			SOD	140.4	2	70.2	95.0	***
		Photophysiological parameters	Yield	0.0	2	0.0	3.8	-
			α	0.0	2	0.0	0.0	-
			ETRmax	43.2	2	21.6	1.6	-
			NPQmax	0.0	2	0.0	0.1	-
	Day 7	Non-enzymatic antioxidant activities	DPPH	15.3	2	7.7	119.6	***
			NO	169.1	2	84.5	2534.9	***
			FRAP	0.1	2	0.0	80.2	***
		Phenolics	TPC	132.0	2	66.0	2797.8	***
			TFC	0.7	2	0.3	1053.3	***
		Enzymatic antioxidant activities	GPX	3.2	2	1.6	17.7	***
			Catalase	0.0	2	0.0	42.9	***
			SOD	167.7	2	83.9	203.9	***
		Photophysiological parameters	Yield	0.0	2	0.0	5.2	*
			α	0.0	2	0.0	12.2	**
			ETRmax	4.4	2	2.2	0.0	-
			NPQmax	0.0	2	0.0	2.1	-
Site Status		Non-enzymatic antioxidant activities	DPPH	28.5	1	28.5	372.3	***
			NO	30.8	1	30.8	177.2	***
			FRAP	0.1	1	0.1	140.6	***
		Phenolics	TPC	60.4	1	60.4	3122.7	***
			TFC	0.0	1	0.0	1.2	-
		Enzymatic antioxidant activities	GPX	459.4	1	459.4	6645.2	***
			Catalase	5.0	1	5.0	5582.7	***
			SOD	591.1	1	591.1	1028.1	***
		Photophysiological parameters	Yield	0.0	1	0.0	0.5	-
			α	1.6	1	1.6	275.4	***
			ETRmax	11346.9	1	11346.9	234.2	***
			NPQmax	4.0	1	4.0	742.2	***
Species * Site * Status		Non-enzymatic antioxidant activities	DPPH	33.4	2	16.7	218.2	***
			NO	483.6	2	241.8	1483.6	***
			FRAP	0.1	2	0.1	103.9	***
		Phenolics	TPC	201.2	2	100.6	5204.5	***
			TFC	0.0	2	0.0	2.6	-
		Enzymatic antioxidant activities	GPX	3.0	2	1.5	21.9	***
			Catalase	0.1	2	0.1	67.3	***
			SOD	10.9	2	5.5	9.5	**
		Photophysiological parameters	Yield	0.0	2	0.0	0.7	-
			α	0.0	2	0.0	2.0	-
			ETRmax	10.8	2	5.4	0.1	-
			NPQmax	0.0	2	0.0	0.1	-

The phenolic contents, antioxidant efficacy, enzymatic antioxidant activities and photophysiological factors varied significantly between Bain Boeuf, characterized by Higher Nutrient level (HN site) and Gris Gris, characterized by Lower Nutrient level (LN site). The phenolic contents and in vitro antioxidant efficacy were higher in most studied macroalgal species at HN site in comparison to LN site. Antioxidant efficacies like ferric reducing antioxidant potential correlated with TPC and TFC. The transplantation of species from HN to LN sites resulted in an increase in non-enzymatic antioxidant activities and phenolic levels, while the reverse trend was observed when the species

were transferred from HN to LN sites. DPPH scavenging activity correlated positively with nitrate, phosphate, pH and DO, indicating antioxidant response of macroalgal species to changing amount of nutrients and physicochemical parameters. The presence of phenolic compounds with antioxidant properties usually increase under stressful condition (Salar et al. 2013). While, increased antioxidant activities and levels of reactive oxygen scavenging enzymes are known to occur as a stress response in macroalgal species (Collen and Davison 1999). Non-enzymatic The conditions at the LN site thus seems to be considerably low, reducing the phenolic level and non-enzymatic antioxidant activities compared to site with higher nutrient occurrence.

Enzymatic antioxidant activities varied significantly during the transplantation experiment. Enzymatic antioxidant enzyme such as Superoxide Dismutase (SOD) are known to act as the first line of defence against oxidative stress by converting oxygen radicals to oxygen and hydrogen peroxide (Alscher et al. 2002), while catalase (CAT) (Bhaduri and Fulekar, 2012) and glutatione peroxidase (Gaetani et al. 1989) catalyse the convertion of hydrogen peroxide into oxygen and water. Levels of intracellular enzymatic antioxidant activities increase under stressful conditions for e.g. CAT and Ascorbate peroxidase (APX) activities increased under nitrogen depleted conditions in the algae *Dunaliella salina* (Yilancioglu et al. 2014). In the current study, the enzymatic activities were initially distinct at the two sites, being higher in HN site and lower in the LN site. Most of the enzymatic antioxidant activities decreased in species transplanted to LN site and increased in species transplanted to HN site. The SOD activity only altered significantly in *S. obovatum* transplanted to HN site, while no significant changes were observed in the SOD activity of *G. salicornia* and *T. ornata*. The GPx and the catalase activities increased in all macroalgal species significantly after transplantation to the HN site and decreased considerably after transplantation to the LN site. Enzymatic antioxidant (GPx and catalase) activities correlated positively with the nitrate, phosphate and dissolved oxygen level, indicating that lower nutrient levels, pH and higher dissolved oxygen level at LN site may have contributed in the lower expression of enzymatic antioxidant activities and phenolic contents in macroalgae. A possible mechanism of increase in antioxidant activities that may occur, as an increase in nutrient levels in marine system is known to entail the limitation of other nutrients, following which levels of antioxidant increase (Turner et al. 2003). At the LN site a balance in all nutrient levels may have thus inhibited a stress state.

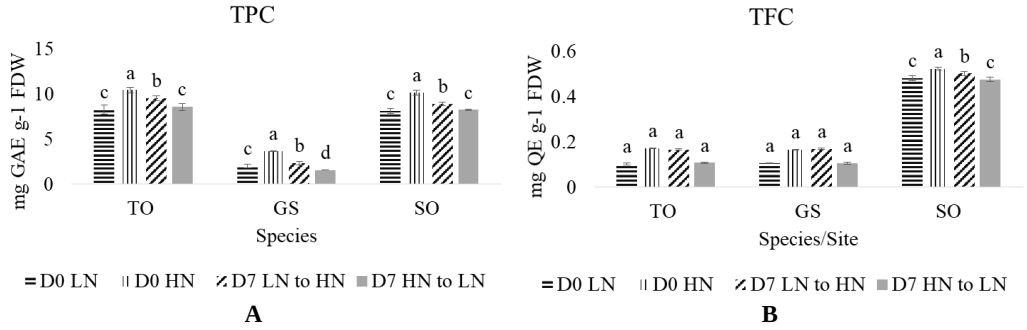

Figure 4. Variation of TPC (A) and TFC (B) in species before transplantation (D0) and seven days after transplantation (D7) to sites with different nutrients level. TO-*T. ornata*, GS-*G. salicorna* and SO-*S. obovatum*. The alphabets indicates differences in a species at a different site and before and after transplantations. (n = 3)

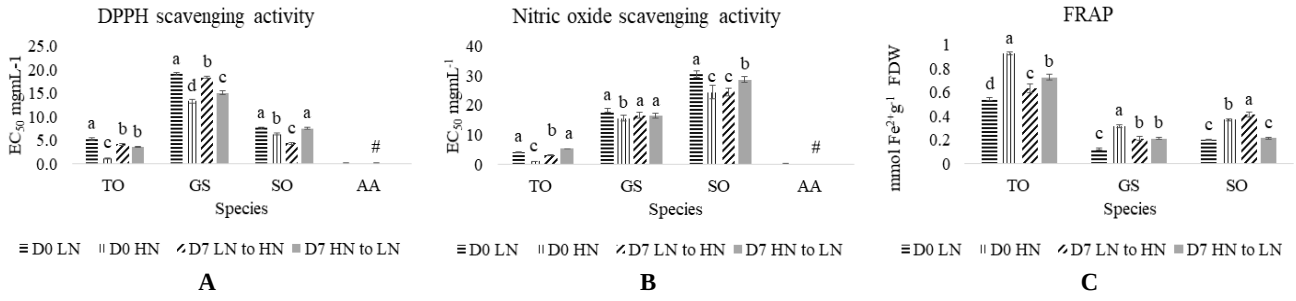

Figure 5. Variation of antioxidant efficacies [DPPH scavenging activity (A), nitric oxide scavenging activity (B) and FRAP (C)]) in species before transplantation (D0) and seven days after transplantation (D7) to sites with different nutrients level. TO-*T. ornata*, GS-*G. salicorna* and SO-*S. obovatum*. LN-Lower Nutrient, HN-Higher nutrient. The alphabets indicates differences in a species at a different site and before and after transplantations. # indicates the difference between the standard and the seaweed species (n = 3)

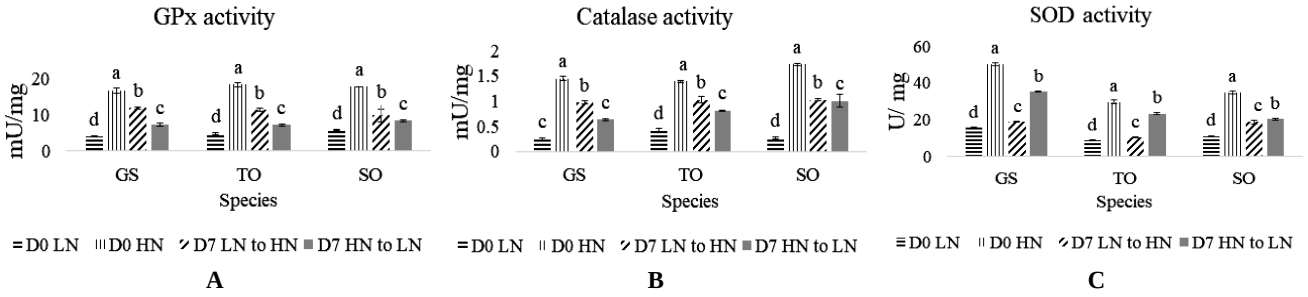

Figure 6. Variation of the enzymatic antioxidant activities, including GPx (A), Catalase (B) and SOD activities (C) in species before transplantation (D0) and seven days after transplantation (D7) to sites with different nutrients level. TO-*T. ornata*, GS-*G. salicorna* and SO-*S. obovatum*. LN-Lower Nutrient, HN-Higher nutrient. The alphabets indicates differences in a species at a different site and before and after transplantations

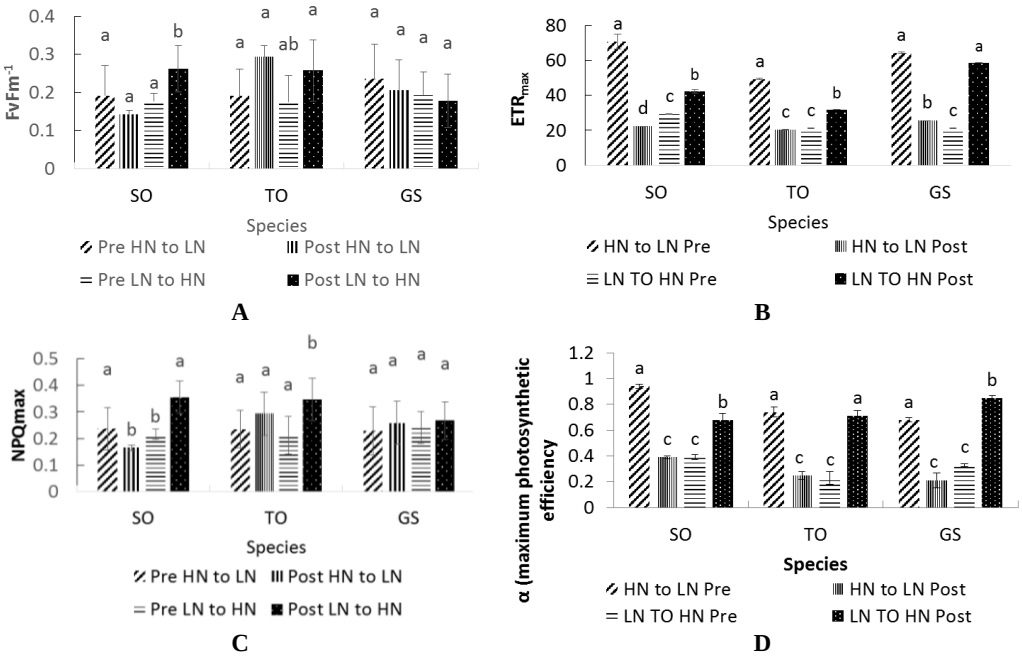

Figure 7. Variation of photophysiological parameters, including Yield (quantum yield) (A) ETR_{max} (B), NPQ_{max} (C) and α (photosynthesic efficiency) (D), in species before transplantation (D0) and seven days after transplantation (D7) to sites with different nutrients level. TO-*T. ornata*, GS-*G. salicorna* and SO-*S. obovatum*. LN-Lower Nutrient, HN-Higher nutrient. The alphabets indicates differences in a species at a different site and before and after transplantations

The highest quantum yield (Fv/Fm) indicates photoinhibition (as the balance of photodamage and repair) and Electron Transport Rate (ETR) photosynthetic capacity (Schreiber et al. 1995). ETRmax and α were significantly higher in all species at HN site, both in resident and transplanted specimens. The quantum yield Fv/Fm increased in *S. obovatum* only after 7 days of transplantation at HN site, while it was similar in the other species before and during other transplantation. This indicates that species like *T. ornata* and *G. salicornia* had better adaptability to photo-physiological stress. The *H. opuntia*'s photosynthetic capacity, efficiency, and photo-protection increased under increased nutrient levels (Teichberg et al. 2013). The maximum yield α, ETR and NPQ correlated strongly and positively with nitrate, phosphate, pH and DO, indicating the possible role of nitrate as well as phosphate, pH and DO in the differences observed. The net photosynthesis rate and Fv/Fm of nitrate-treated Sorghum plants under Cd stress were higher than that of ammonium-treated plants when the pH was unbuffered (Bai et al. 2021). The antioxidant level in watermelon fruits increased three times in plats exposed to 30 mgL^{-1} silver nitrate concentration (Cabrera-De La Fuente et al. 2014). This may suggest the possible investigation of the role of nitrate level in enhancing pH and its effects on photosynthetic efficiency.

The photosynthetic electron transport through PSII under excessive light is limited through non-photochemical quenching (NPQ) (Muller et al. 2001; Schagerl and Möstl 2011). In *S. obovatum*, NPQmax significantly decreased when transplanted to LN site, indicating less photoinhibition, probably due to lesser stress at the site. In

T. ornata, NPQmax rose after the transplantation in species transplanted to HN site, indicating increased photoinhibition due to selective pressure at the site. While in *G. salicornia*, no significant change in NPQmax was detected, possibly indicating its adaptation to growing under varied conditions and suggesting low damage to the photosynthetic reaction center at PSII. ROS production is associated with PAR-driven electron transport (Rijstenbil et al. 2000). ROS production is linked with impairment of the xanthophyll cycle, followed by non-photochemical quenching in samples exposed to the full solar range (Rijstenbil et al. 2000; Krause and Jahns 2004; Asada 2006). In our case, it can be implied that light stress contributes to ROS production in *S. obovatum*. NPQ was not impaired, especially in *G. salicornia* and *T. ornata*, reflecting a successful response to macroalgae stress, possibly by producing antioxidants, preventing damages, e.g., photooxidation of tissues the pigments. These results indicate that macroalgae living under higher nutrient levels have greater adaptation, including that to light stress and are able to dissipate energy produced during light stress. These findings indicate that increased nutrient levels may be linked to increased oxidative stress, followed by enhanced production of photo-protective pigments and secondary metabolites to better cope with oxidative stress. Nevertheless, further studies on oxidative stress are needed, as inferring oxidative stress using antioxidants only can be erroneous (Costantini and Verhulst 2009).

In conclusion, the different sites had distinct environmental conditions that led to their characterization as LN site, with lower nutrients, pH and higher dissolved oxygen levels and HN site, with comparatively higher nutrients, pH and lower dissolved oxygen levels. The phenolic contents and antioxidant activities of macroalgae may be influenced by varied nutrient levels and physico-chemical parameters existing in its habitat. Enzymatic antioxidant activities like glutathione peroxidase and catalase were more sensitive at reflecting the lower oxidative stress and oxidative stress acclimatisation occurring at the HN site, followed by non-enzymatic antioxidant activities and phenolic contents. ETRmax and α were significantly higher in all species at HN site, both in resident and transplanted specimens. The quantum yield Fv/Fm increased in *S. obovatum* only after 7 days of transplantation at HN site, while it was similar in the other species before and during other transplantation. This indicates that species like *T. ornata* and *G. salicornia* had better adaptability to photophysiological stress. In *S. obovatum*, NPQmax significantly decreased when transplanted to LN site, indicating less photoinhibition, probably due to lesser stress at the site. In *T. ornata*, NPQmax rose after the transplantation in species transplanted to HN site, indicating increased photoinhibition due to selective pressure at the site. While, in *G. salicornia*, no significant change in NPQmax was detected, possibly indicating its adaptation to growing under varied conditions and suggesting low damage to the photosynthetic reaction center at PSIILower nutrient levels may thus be linked to reduced oxidative stress, which is followed by the lesser need of photo-protection and secondary metabolites. Macroalgal species may thus play an essential role as a natural indicator of the nutrient levels in of lagoon system.

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