

Seasonal fluctuation of photosynthetic pigments of most common red seaweeds species collected from Abu Qir, Alexandria, Egypt

Fluctuaciones estacionales de los pigmentos fotosintéticos de las algas rojas más comunes de Abu Qir, Alejandría, Egipto

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Resumen. Se investigaron los cambios estacionales en la composición de pigmentos [Clorofila *a* (Cl-*a*), carotenoides totales (Car.), ficobiliproteínas (ficoeritrina (PE), ficocianina (PC), aloficocianina (APC))], en 5 especies de Rhodophyta. Los niveles de clorofila *a*, carotenoides y ficobiliproteínas presentaron altos contenidos durante invierno, y disminuyeron en verano, mostrando decoloración de tejidos de rojo a verde o amarillo. Los resultados muestran que los contenidos de pigmentos variaron estacionalmente con respecto a las especies de algas, y a los cambios de los parámetros físico-químicos, mientras que el pH, oxígeno disuelto, NO₃⁻, NO₂⁻, NH₄⁺, nitrógeno total (TN) y fósforo total (TP) tuvieron un impacto directo sobre el contenido de pigmentos fotosintéticos, y la temperatura tuvo una correlación negativa significativa sobre los pigmentos fotosintéticos.

Palabras clave: Parámetros ambientales, clorofila, ficobiliproteínas, algas rojas, variación estacional

Abstract. Seasonal changes of pigment composition [Chlorophyll *a* (Chl-*a*), total carotenoid (Car.), phycobiliproteins (phycoerythrin (PE), phycocyanin (PC), allophycocyanin (APC))], in the 5 species of Rhodophyta were investigated. Chl-*a*, carotenoids and phycobiliproteins levels showed high contents during winter, whereas decreased in summer showing discoloration tissue from deep red to green or yellow. The results show that the pigment contents were varied seasonally with respect to the algal species and physical and chemical parameters changes, while pH, dissolved oxygen, NO₃⁻, NO₂⁻, NH₄⁺, total nitrogen (TN) and total phosphorus (TP) had direct impact on photosynthetic pigments content, and temperature had significant negative correlation on photosynthetic pigments.

Key words: Environmental parameters, chlorophyll, phycobiliproteins, red seaweeds, seasonal variation

INTRODUCTION

There is a highly diversity in the structure and pigment composition of red algae (Grossman *et al.* 1995, Guan *et al.* 2007). The quantity of macroalgal pigments is mostly used to define algal biomass. There are three different groups of light harvesting and photoprotective pigments in macroalgae, *i.e.*, the chlorophylls, carotenoids and phycobiliproteins (Rowan 1989, Jeffrey *et al.* 2005). Chlorophylls are greenish pigments which contain a porphyrin ring around which electrons are free to migrate because the electrons transfer freely, the porphyrin ring has the potential to gain or lose electrons easily, and thus the potential to provide energized electrons to other molecules. This is the fundamental process by which chlorophyll captures the energy of sun light. On the other hand, carotenoids are usually red, orange, or yellow pigments, don't dissolve in water and must be attached to membranes within the cell. Carotenoids cannot transfer sunlight energy directly to the photosynthetic pathway, but pass their absorbed energy from one chlorophyll molecule to another. For this reason, they are called accessory pigments.

Phycobiliproteins are the major photosynthetic accessory pigments in cyanobacteria (blue-green algae, prokaryotic); Rhodophytes (red algae, eukaryotic); cryptomonads (biflagellate unicellular eukaryotic algae) and cyanelles (endosymbiotic plastid-like organelles). Phycobiliproteins, a group of water-soluble, brightly colored proteins characteristic for red macroalgae (Rowan 1989, Zhao *et al.* 2011), they are structured with an internal core of allophycocyanin (APC), several intermediate packets of phycocyanin (PC), and an external layer of phycoerythrin (PE) (Talarico 1996) that participate in an extremely efficient energy transfer chain in the reaction centers of PSII (Róman *et al.* 2002), and responsible for about 50% of light capturing in cyanobacteria and red algae (Williams *et al.* 1980).

Phycobiliproteins can be divided broadly into 3 classes based on their spectral properties: PE (deep red) (λ_{max} ~565 nm), PC (deep blue) (λ_{max} ~620 nm) and APC (bluish green) (λ_{max} ~650 nm) and a fourth phycobiliprotein known as APC

B ($\lambda_{\text{max}} \sim 670\text{nm}$) (Glazer & Bryant 1975). Light absorbed by PE is efficiently transferred to PC, then to APC and ultimately to chlorophyll (French & Young 1952, Kronick 1986).

Phycocerythrins are the most abundant phycobiliproteins in many red algae and in some unicellular cyanobacteria. Phycocerythrin is associated with chlorophyll in Rhodophyta, and enables them to be photosynthetically efficient in deep water where blue light predominates. The longer wave length red portion of the spectrum that activate green chlorophyll pigments do not penetrate the deeper water of the photic zone, so green algae cannot survive at depth where red algae thrive.

Phycobiliproteins have gained considerable importance in the commercial sector, as they have several applications. The primary potential of these molecules are as natural dyes but a number of investigations have shown their health-promoting properties and broad range of pharmaceutical applications. Phycocerythrin is the most widely used phycobiliproteins in fluorescent probes, pharmaceutical, food industries, immunology, cell biology, flow cytometry, food, and cosmetics colorant (Glazer 1994, Sekar & Chandramohan 2008) and has quantum yields of 82-98% (Oi *et al.* 1982). A number of multinational companies have commercialized antibodies conjugated with PE. C-phycocyanin (C-PC) is used in cosmetics, diagnostics and foods and as nutraceutical or biopharmaceutical (antioxidant, antimutagenic, antiviral, anticancer and immune activation, among others) (Sørensen *et al.* 2013, López-Cristoffanini *et al.* 2015).

Hence, the present study was focused on determination the variation in the pigment contents of 5 species of red seaweeds and their relation to the changes in physical and chemical parameters to determine the most suitable species and season for pigment production.

MATERIALS AND METHODS

COLLECTION OF SEAWEEDS

Five species of marine Rhodophyta were hand-picked seasonally (Viz. February 2014 to January 2015) from Rocky Bay of Abu Qir (longitudes $30^{\circ}03'$ to $30^{\circ}22'$ E and latitudes $31^{\circ}16'$ to $31^{\circ}28'$ N) (Fig. 1). The samples were brought to the laboratory in plastic bags containing seawater to prevent evaporation. Algae were then cleaned from epiphytes and sand particles and given a quick fresh water to remove surface salts. After washing, some of fresh samples were processed as herbarium specimens on the same day of collection; others were preserved in 5% formalin in seawater for taxonomic classification. The collected species were identified as presented in the literature, checked for synonyms and latest accepted names, referred to its systematic groups and described following the methods of Abbott & Hollenberg (1976), Taylor (1960) & Aleem (1993), and confirmed using AlgaeBase website (Guiry & Guiry 2016).

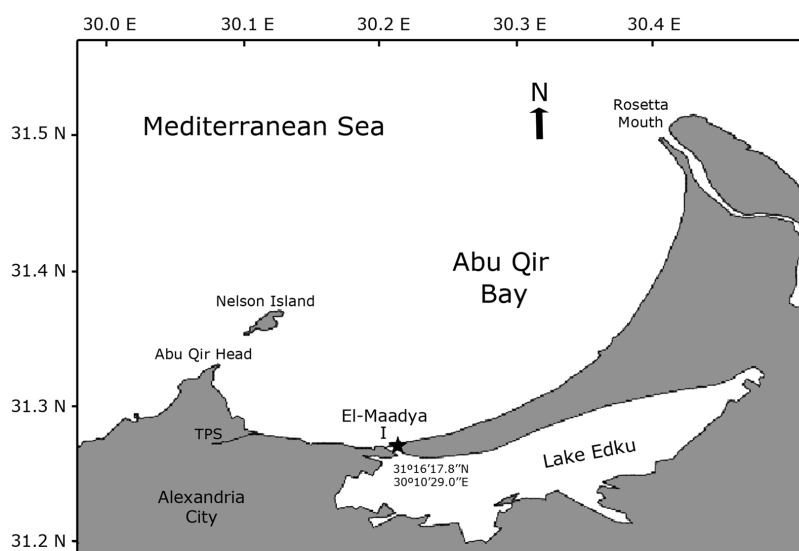


Figure 1. Location of collected samples / Ubicación de las muestras recolectadas

ENVIRONMENTAL PARAMETERS

Water temperature (°C), salinity (PSU), dissolved oxygen (mg L⁻¹) and pH were measured with Hydrolab (Data Sonde 5X). Water samples were collected, filtered (Whatman GF/C), and analyzed for nutrients (NO₂⁻, NO₃⁻, NH₄⁺, TN (total nitrogen) and TP (total phosphorus). The nutrient salts were determined by colorimetry according to the methods of Grasshoff (1976) using a UV-Visible spectrophotometer (Shimadzu double beam Model 150-02).

PIGMENT ANALYSIS

On the day of collection analysis of chlorophyll was extracted in 90% acetone and estimated by spectrophotometry according to the method of Jeffery & Humphery (1975). The level of Chl-*a* and total chlorophyll was calculated using the following formula:

$$\text{Chlorophyll } a = \frac{[12.7(A_{663}) - 2.69(A_{645})] \times \text{vol. of extraction}}{\text{Weight of the sample}} \text{ mg g}^{-1}$$

$$\text{Total chlorophyll} = \frac{[20.2(A_{645}) + 8.02(A_{663})] \times \text{vol. of extraction}}{\text{Weight of the sample}} \text{ mg g}^{-1}$$

where: A₆₆₃ = absorbance at 663 nm, A₆₄₅ = absorbance at 645 nm and Carotenoids were extracted in 90% acetone and estimated by the method of Ridley (1977). The level of carotenoids was estimated using the following formula and it is expressed as mg of carotenoids present in 1 g of fresh tissue.

$$\text{Carotenoids} = \frac{4 \times A_{480} \times \text{vol. of extraction}}{\text{Weight of the sample}} \text{ mg g}^{-1}$$

where: A₄₈₀ = absorbance at 480 nm, and 4 = correction factor

Phycobiliproteins (PC, APC, PE) were extracted with phosphate buffer (0.1 M) and estimated by the method of Padgett & Krogman (1987) and calculated (mg g⁻¹ fresh weight) using the formula:

$$\text{PC} = \frac{(A_{615}) \times (0.475 \times A_{652})}{5.34}$$

$$\text{APC} = \frac{(A_{652}) - (0.208 \times A_{615})}{5.09}$$

$$\text{PE} = \frac{(A_{562}) - (2.41 \times \text{PC}) - (0.849 \times \text{APC})}{9.62}$$

where: A₅₆₂ = absorbance at 562, A₆₁₅ = absorbance at 615 nm and A₆₅₂ = absorbance at 652 nm.

STATISTICAL ANALYSIS

All reported results are the means with standard deviation (±SD) of the 3 replicates. One-way analysis of variance (ANOVA) were carried out using the SPSS for Windows V15 computer statistics program to evaluate significant differences between photosynthetic pigments of some red seaweeds and different seasons.

RESULTS

TAXONOMIC DESCRIPTION OF THE TESTED RED SEAWEEDS

The five species of Rhodophyta collected from Abu Qir Bay were identified as: *Corallina elongata* Ellis et Solander, *Corallina officinalis* Linnaeus, *Jania rubens* (Linnaeus) Lamouroux, *Polysiphonia elongata* (Hudson) Spreng and *Pterocladia capillacea* (S.G. Gmelin) Bornet et Thuret.

TAXONOMIC POSITION

Phylum: Rhodophyta, Class: Florideophyceae,

Order: (1) Corallinales, Family: Corallinaceae,

(1) *Corallina elongata* Ellis et Solander (Fig. 2a)

Synonyms: *Corallina mediterranea* J.E. Areschoug.

Habitat: Epilithic on bed rock in shallow water.

Abundance and season: Dense in all seasons except in summer as compared with other collected species.

Description: Thalli up to 7-10 cm high, colour purple-red-pink, erect, cylindrical, calcified and pinnately dichotomous branch. The stem appears segmented with segments ovoid or triangular.

Remark: Colour of specimen varied from violet grey to white ivory according to season. This species associated with *Jania rubens* and polychaetes.

(2) *Corallina officinalis* Linnaeus (Fig. 2b)

Habitat: Epilithic on bed rock and epiphytic on gastropod shell in shallow water.

Abundance and season: Dense in all seasons.

Description: Thalli up to 10-12 cm high, pink to red, axis cylindrical, erect and dichotomously branched, pinnate, calcareous, lateral branches 2-5 cm and jointed with bone like, branches bear opposite branchlets and the stem appears segmented.

Remark: This species differs from *C. elongata* in the lateral branching not immediately from base. Colour of dried specimen varied from violet grey to white. This species associated with *Ulva fasciata*, *Jania rubens* and polychaetes.

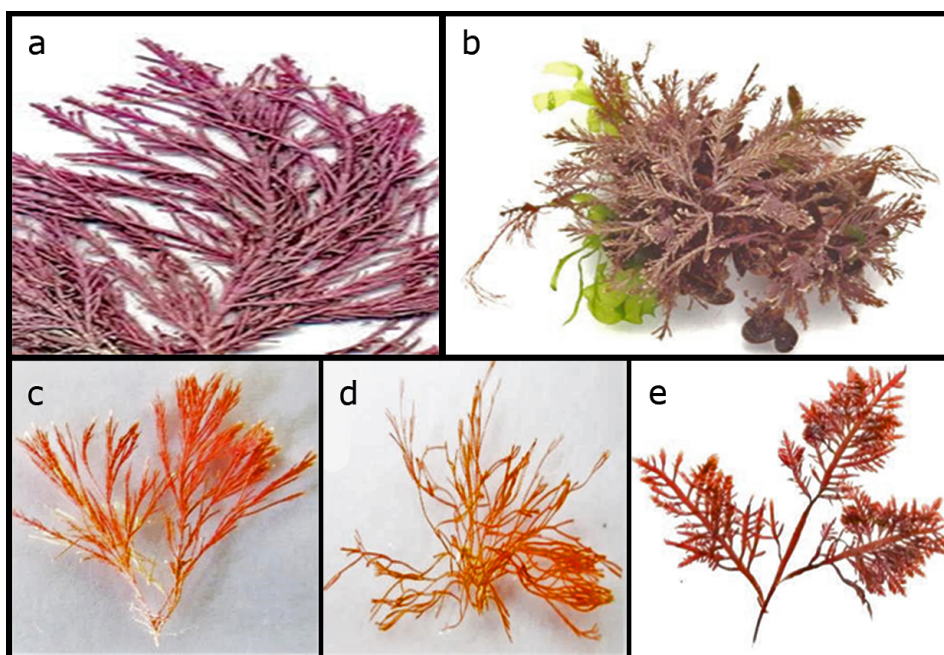


Figure 2. The fronds of five species of Rhodophyta collected from Abu Qir Bay. a) *Corallina elongata*, b) *Corallina officinalis*, c) *Jania rubens*, d) *Polysiphonia elongata*, e) *Pterocladia capillacea* / Frondas de las cinco especies de Rhodophyta colectadas en la bahía de Abu Qir. a) *Corallina elongata*, b) *Corallina officinalis*, c) *Jania rubens*, d) *Polysiphonia elongata*, e) *Pterocladia capillacea*

(3) *Jania rubens* (Linnaeus) Lamouroux (Fig. 2c)

Synonyms: *Corallina rubens* Linnaeus, *Jania corniculata* (Linnaeus) J.V. Lamouroux, *J. spermophorus* J.V. Lamouroux, *C. cristata* Linnaeus, *C. spermophoros* Linnaeus

Habitat: Epilithic on bed rock in shallow water and epiphytic on stipe of *Sargassum* sp.

Abundance and season: Moderate in all seasons.

Description: Thalli 2-4 cm high, pinkish red in colour, calcified clusters, segments and branching repeatedly dichotomously branched and not opposite.

Remark: This species growing attached to *C. officinalis* and *C. elongata*.

Order (2): Ceramiales, Family: Rhodomelaceae,

(4) *Polysiphonia elongata* (Hudson) Spreng (Fig. 2d).

Synonyms: *Conferva elongata* Huds.

Habitat: Epilithic on rock grow near the water level down to a depth of 1 m.

Abundance and season: Moderate in all seasons.

Description: Thalli up to 4 cm, brownish to purplish red in colour, dense bushes on the substratum, cartilaginous, cylindrical, branches alternate to pseudo dichotomous, heterotrichous form, a central axial cell with 4 periaxial.

Order (3): Gelidiales, Family: Gelidiaceae,

(5) *Pterocladia capillacea* (S. G. Gmelin) Bornet et Thuret (Fig. 2e).

Synonyms: *Gelidium capillaceum* (S.G. Gmel.) Kütz., *Pterocladia pinnata* (Hudson.) Papenfuss, *Fucus capillaceus* S.G. Gmel. (Basionym), *Gelidium capillaceum* (S.G. Gmel.) Kütz.

Habitat: Epilithic on bed rock grow near the water level down to a depth of 1 m.

Abundance and season: Moderate in all seasons.

Description: Thalli up to 5-7 cm high, consisting of erect and prostrate dark red axes. Branches alternate on the main axis and the lateral branches reached to 0.5-1.5 cm tall

Remark: It is associated with *Ulva compressa*.

ENVIRONMENTAL PARAMETERS AND PIGMENT CONTENTS

The environmental parameters such as temperature, salinity, pH, dissolved oxygen and nutrient content (*e.g.*, NO₃⁻, NO₂⁻, NH₄⁺, TN and TP) were measured from February 2014 to January 2015 and are presented in Table 1 and 2. All of the measured environmental parameters varied significantly throughout the year. The minimum and maximum temperature values were 18°C during winter and 29°C during summer. Salinity was increased gradually after spring owing to evaporation and atmospheric heating. The minimum value was recorded during winter (35.5 PSU) whereas, the maximum during autumn (37.01 PSU). The

highest values of water dissolved oxygen were recorded during winter (10.58 mg L⁻¹), whereas the lowest during autumn (2.65 mg L⁻¹).

The concentration of nutrients showed significant variation throughout the study. The concentration of NO₃⁻ and NO₂⁻ varied from 11.58 and 1.97 μM in winter to 2.56 and 1.22 μM in summer, respectively. From a seasonal perspective, the NH₄⁺ concentration showed lower values (3.82 μM) in the winter and higher values in spring (15.21 μM). The maximum concentration of TP was recorded in autumn (9.87 μM) and the minimum in spring (22.1 μM).

Table 1. Effect of some physical parameter on red seaweeds algae distribution / Efecto de algunos parámetros físicos sobre la distribución de algas rojas

Season	Scientific name	Abundance	Temperature (°C)	Salinity	pH	DO (mg L ⁻¹)
Winter	<i>Corallina elongata</i>	***	18 ± 0.5	35.52 ± 0.4	8.84 ± 0.2	10.58
	<i>Corallina officinalis</i>	***				
	<i>Jania rubens</i>	**				
	<i>Pterocladia capillacea</i>	**				
	<i>Polysiphonia elongata</i>	**				
Spring	<i>Corallina elongata</i>	***	24 ± 1.0	36.59 ± 0.5	8.57 ± 0.2	10.4
	<i>Corallina officinalis</i>	***				
	<i>Jania rubens</i>	**				
	<i>Pterocladia capillacea</i>	**				
	<i>Polysiphonia elongata</i>	**				
Summer	<i>Corallina elongata</i>	*	29 ± 0.5	36.77 ± 0.3	8.45 ± 0.1	4.74
	<i>Corallina officinalis</i>	***				
	<i>Jania rubens</i>	**				
	<i>Pterocladia capillacea</i>	**				
	<i>Polysiphonia elongata</i>	**				
Autumn	<i>Corallina elongata</i>	***	21.5 ± 1.5	37.01 ± 0.1	8.22 ± 0.1	2.65
	<i>Corallina officinalis</i>	***				
	<i>Jania rubens</i>	**				
	<i>Pterocladia capillacea</i>	**				
	<i>Polysiphonia elongata</i>	**				
	F		68.467**	10.534*	7.992*	196.16**
	P		<0.001	<0.001	<0.001	<0.001

Note: ***=dense, ** =moderate, *=rare. Values represent mean values ± standard deviation (n=3). * Significant $P < 0.05$, **highly significant $P < 0.01$, *** highly significant $P < 0.001$

Table 2. Seasonal average concentrations of NO₃⁻, NO₂⁻, NH₄⁺, total nitrogen (TN) and total phosphate (TP) (μM) in water of Abu Qir Bay / Concentraciones promedio estacionales de NO₃⁻, NO₂⁻, NH₄⁺, nitrógeno total (TN) y fosfato total (TP) (μM) en aguas de la bahía Abu Qir

Season	NO ₃	NO ₂	NH ₄	TN	TP
Winter	11.58 ± 5.62	1.97 ± 0.96	3.82 ± 1.05	302.27 ± 4.12	5.98 ± 0.39
Spring	2.77 ± 1.10	1.22 ± 0.22	15.21 ± 2.51	337.2 ± 3.54	2.51 ± 0.23
Summer	2.56 ± 0.86	1.12 ± 0.23	8.99 ± 2.83	290.17 ± 6.45	2.62 ± 0.74
Autumn	10.96 ± 10.8	1.89 ± 0.98	8.77 ± 2.56	337.72 ± 5.15	9.87 ± 0.54
F	17.836**	1.181*	11.890**	72.685**	139.06**
P	<0.001	<0.001	<0.001	<0.001	<0.001

Values represent mean values ± standard deviation (n= 3)

* Significant $P < 0.05$, ** highly significant $P < 0.01$,*** highly significant $P < 0.001$

Five species of red algae, *Corallina elongata* Ellis et Solander, *C. officinalis* Linnaeus, *Jania rubens* (Linnaeus) Lamouroux, *Polysiphonia elongata* (Hudson) Spreng and *Pterocladia capillacea* (S.G. Gmelin) Bornet et Thuret were analyzed for photosynthetic pigments, Chl-*a*, Car., PE, PC and APC production during different seasons (Figs. 3 and 4).

The chlorophyll *a* concentration in the tested red algae exhibited significant changes in relation to species and environmental factors at different seasons. Chlorophyll *a* content of the tested seaweeds showed maximum and minimum values in the winter and summer, respectively. *Polysiphonia elongata* showed higher percentage of Chl-*a* in all seasons ranged from 2.55 mg g⁻¹ FW in summer to 3.847 mg g⁻¹ FW in winter, while the minimum value was recorded in *C. elongata* (0.49 mg g⁻¹ FW) and *J. rubens* (0.55 mg g⁻¹ FW) in summer.

The pigment content of the tested seaweeds showed maximum and minimum values in the winter and summer, respectively. *Polysiphonia elongata* showed higher percentage of total chlorophyll in all seasons ranged from 3.82 mg g⁻¹ FW in summer to 5.63 mg g⁻¹ FW in winter, while the minimum value was recorded in *J. rubens* (2.73 mg g⁻¹ FW) in winter; *C. elongata* (1.75 & 1.55 mg g⁻¹ FW) in spring and autumn season, respectively and *C. officinalis* in summer (1.69 mg g⁻¹ FW).

Carotenoid also showed a similar pattern as total chlorophyll in the tested seaweeds. *P. elongata* exhibited higher content of 0.609 mg g⁻¹ FW, whereas the lower content (0.28 mg g⁻¹ FW) was recorded in *J. rubens* in winter seasons (Fig. 3).

The variations in the phycobiliproteins content in the tested seaweeds are shown in Fig. 4. During the study period, there were significant differences in PE content in the tested seaweeds

Table 3. The seasonal changes in carotenoid/ Chl *a* ratio in collected seaweeds / Cambios estacionales en la relación carotenoides/ Chl *a* en las macroalgas recolectadas

Species	Car./ Chl- <i>a</i>			
	Winter	Spring	Summer	Autumn
<i>Corallina elongata</i>	0.298	0.455	0.358	0.406
<i>Corallina officinalis</i>	0.117	0.126	0.126	0.223
<i>Jania rubens</i>	0.273	0.102	0.373	0.287
<i>Pterocladia capillacea</i>	0.327	0.370	0.426	0.459
<i>Polysiphonia elongata</i>	0.158	0.166	0.031	0.065
F	6.655*	20.002**	17.929**	73.359***
P	<0.001	<0.001	<0.001	<0.001

Values represent mean values ± standard deviation (n= 3)

* Significant $P < 0.05$, ** highly significant $P < 0.01$,*** highly significant $P < 0.001$

according to the different seasons. *P. elongata* showed highest concentration of PC, APC and PE (2.38, 6.23 and 3.70 mg g⁻¹ FW, respectively), whereas *J. rubens* recorded lowest content of them (0.51, 2.05 and 0.91 mg g⁻¹ FW) in winter season.

The ratios of Car/Chl *a* pigment of different seaweeds are shown in Table 3. Changes in Car/Chl *a* ratio of all tested seaweeds during four seasons showed highly significant different between species and seasons. The highest Car/Chl *a* ratio was noted in *Pt. capillacea* (0.46) in autumn and the lowest ratio was recorded in *P. elongata* (0.03) during summer season.

DISCUSSION

In the present investigation, an attempt has been made to determine the changes in photosynthetic pigments (Chl-*a*, carotenoid and phycobiliproteins (EC, PE and APC)) in most common red seaweeds in Abu Qir Bay, Mediterranean Sea in

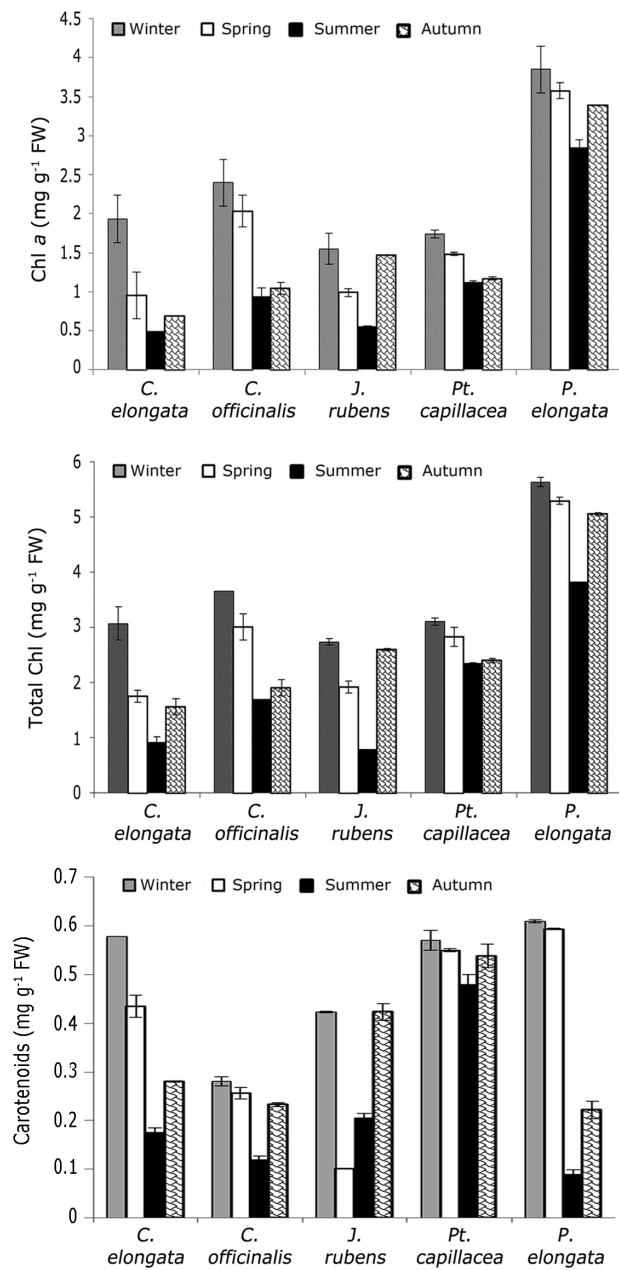


Figure 3. Seasonal variation in chlorophylls and carotenoids content of the common red seaweeds / Variación estacional en el contenido de clorofila y carotenoides de macroalgas rojas comunes

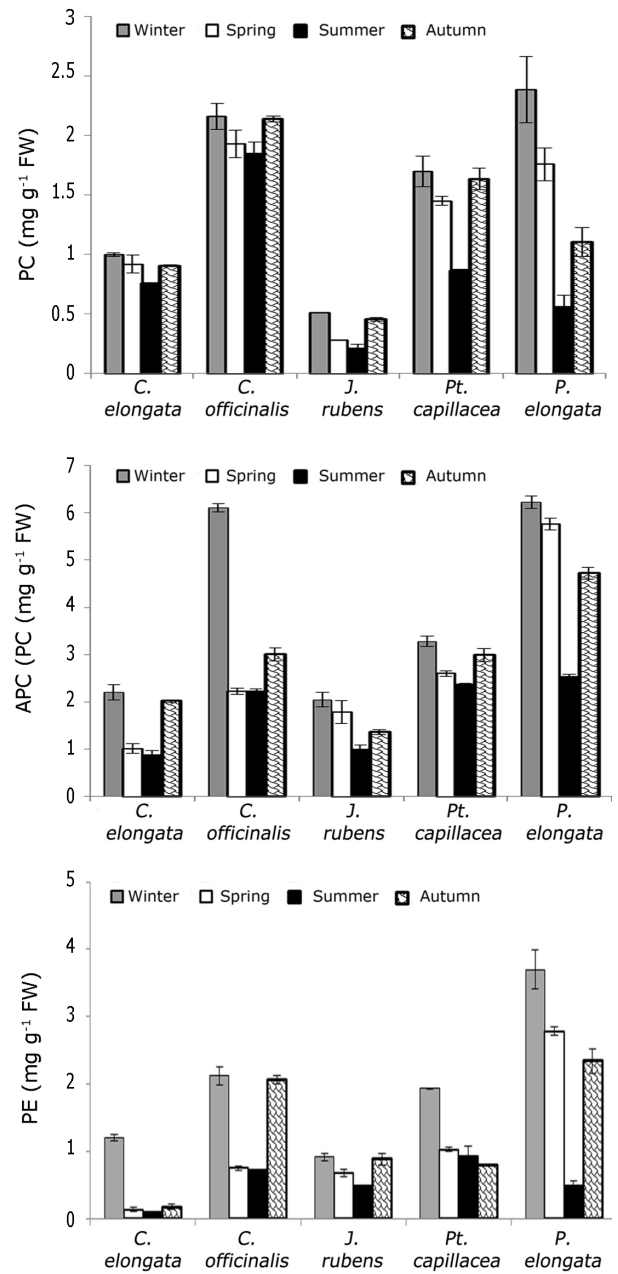


Figure 4. Seasonal variation in phycobiliproteins (PC, APC, PE) content of the common red seaweeds / Variación estacional en el contenido de ficobiliproteínas (PC, APC, PE) de macroalgas rojas comunes

response to species, season and environmental parameters (temperature, pH, salinity, dissolved oxygen, NO_3^- , NO_2^- , NH_4^+ , TN and TP). Our results are similar to those described by Neish *et al.* (1977) who stated that red algae show a characteristic summer discoloration which is regarded as a loss of pigment related to environmental conditions. Schreiber (1979) reported that the phycobilins content vary seasonally in different seaweeds. Paerl (1984) and Rowan (1989) reported that the concentration and distribution of photoprotective plant pigments vary with season in micro- and macroalgae and with tissue type in macroalgal species.

The present data showed that environmental factors especially pH, dissolved oxygen, NO_3^- , NO_2^- , NH_4^+ , TN and TP had positive correlation effect on photosynthetic pigment contents, while seaweeds are efficient in the uptake of nitrate, ammonium, and phosphate from seawater, and the assimilation of these nutrients into nitrogenous compounds (*e.g.* amino acids, proteins, pigments) (Lobban & Harrison 1997). The results showed that all estimated photosynthetic pigments were at the maximum level in winter when the level of the nutrient is at the highest value. On the other hand, the levels of photosynthetic pigments began to decrease in the spring and reached to minimum value in summer then increased again in autumn correlated to the value of these nutrients.

Our results are in line to those described by other authors, who reported an increase in total soluble proteins and phycobiliproteins in winter and a drop in summer (Kosovel & Talarico 1979, Campbell *et al.* 1999, Aguilera *et al.* 2002, Orduña-Rojas *et al.* 2002). The environmental variation in light, temperature, desiccation and nutrients can influence and bring about changes in the concentration of pigments in the thallus (Ramus *et al.* 1976, Contreras-Porcia *et al.* 2011). Mizuta (2002) found that Chl-*a* and PE levels of *Gloiopeltis furcate* and *Porphyra yezoensis* were high during winter, but decreased in late spring or summer. Dere *et al.* (2003) stated that the pigment content may increase in response to the environmental factors such as high nutrient availability. Both PE and PC concentrations showed clear trends of decreasing concentration with increasing temperature (Latham 2008). Pereira *et al.* (2012) stated that the concentration of R-Phycocerythrin (RPE) and Chl-*a* in *Gracilaria bursapastor* is showed maximum values in the winter and minimum values in summer.

The obtained results in this study show that the increase in all photosynthetic pigment may be correlated with nitrogen and phosphorus of seawater due to the vital role of these nutrients in algal metabolic process, while the nutrients nitrate (N) and phosphate (P) are two of the most important elements required for algal growth and metabolism (DeBoer 1981, Lapointe 1987). Nitrogen amount also changes algal pigment composition, particularly the level of PE (Lapointe 1981, Bird

et al. 1982, Lapointe 1985), where phycobiliprotein synthesis depends on the supply of nitrogen in the environment (Liotenberg *et al.* 1996). This observation is similar to that observed by Kolber *et al.* (1988) who stated that nitrogen depletion caused reduction in chlorophyll content, oxygen evolution, carbon dioxide fixation, and tissue production. Lapointe (1981, 1985) reported that a high nitrogen environment results in an increase of PE and Chl-*a* in *G. foliifera*. Amano & Noda (1988) detected that, PE content of *Porphyra yezoensis* was increased five times by nitrogen enrichment. Nitrogen commonly results in an increase in N containing photosynthetic pigments such as chlorophyll and phycobilins (Dawes 1995, Vergara *et al.* 1995). Rico & Fernández (1996) showed that nitrogen and PE in *Gelidium latifolium* were decreased in low ambient nitrate conditions. Nitrogen enrichment increases both Chl-*a* and phycobilin of some Rhodophyta *e.g.*, *Gelidium latifolium* (Rico & Fernández 1996), *Chondrus crispus* (Neish *et al.* 1977) and *Porphyra yezoensis* (Amano & Noda 1987). In another report, Andria *et al.* (1999) demonstrated that PE contents of *Gracilaria* sp. (Rhodophyta) decreased when it was grown under low nitrogen availability, and indicated the role of these compounds as nitrogen storage pools under conditions of nutrient limitation and their allocation for growth. Phycocerythrin content in *Gloiopeltis furcate* (Postels et Ruprecht) J. Agardh, and *Porphyra yezoensis* Ueda correlated significantly with nitrogen content (Mizuta *et al.* 2002). Pereira *et al.* (2012) found that nitrate was assimilated as PC and PE by *Porphyra dioica* Brodie and Irvine and phycocerythrin PE was the main form of nitrogen pool, while, phosphorus participates in the formation of biomolecules such as nucleic acids, proteins and phospholipids. In the same year, Marinho-Soriano (2012) showed the importance of NO_3^- for the biosynthesis of this pigment, demonstrated the positive correlation between them.

The most important role of phosphorus is in energy transfer mediated by ATP and other high energy compounds present in photosynthesis and respiration (Lobban & Harrison 1997). Chlorophyll *a* of *Hypnea cervicornis* (red algae) was stimulated by high phosphate (Bird *et al.* 1982). Lapointe (1986) detected that phosphorus enrichment can stimulate the growth and the photosynthetic rates of some algae, and Martins *et al.* (2011) found that there is a positive correlation between the increase of phosphate in the medium and the content of *Hypnea musciformis* photosynthetic pigments.

Under nitrogen and phosphate limitation, PE concentrations of *G. tikvahiae* showed low values because this pigment was preferentially metabolized to provide nutrients to sustain the algal growth (Bird *et al.* 1982). Our results are in contrast with that of Chopin *et al.* (1995) who found that phosphorus had no significant effect on photosynthesis and photosynthetic pigment contents in *Chondrus crispus*.

Concerning the effect of temperature on photosynthetic pigments of tested seaweeds, the results showed that temperature had significant negative correlation on photosynthetic pigments. It can be concluded that the increase of photosynthetic pigments is correlated with the decrease in temperature as the maximum value of photosynthetic pigments were recorded in winter while the lowest temperature caused increase in the pigment content. Many researchers have reached the same conclusion. For example, Liu & Dong (2001) found that PE and chlorophyll contents of *G. tenuistipitata* decreased if the light intensity was increased due to increase in temperature. Furthermore, Zucchi & Necchi (2001) determined that physical factors, such as light density and quality, photoperiod and temperature can alter pigment contents of red algae. Marinho-Soriano (2012) stated that the RPE content of *G. bursapastoris* (Rhodophyta) was negatively correlated with temperature. Their results were in accordance with that of Vanitha & Chandra (2012) who recorded that temperature and light intensity play a major role in the pigment production of red algae. Ding *et al.* (2013) stated that salinity and temperature have significant or extremely significant effects on photosynthetic pigments (Chl-*a*, Car, PE and PC) in *H. cervicornis*.

In conclusion, the present study demonstrated the seasonal variations in photosynthetic pigments of five red algal species and their relationships to environmental parameters and photosynthetic pigment contents. Our results show that winter season is the most suitable for production of high concentration of photosynthetic pigments of the species collected from the studied location (Abu Qir Bay, Mediterranean Sea, Alexandria).

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