

# Investigation of the Fatty Acid Profile in Some Macroalgae in Relation to the Environmental Conditions for Biodiesel Production

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**Abstract:** In this study, fatty acids profile was determined in four algal species; namely *Ulva fasciata* (Chlorophyta), *Corallina mediterranea*, *Corallina officinalis* and *Pterocladia capillacea* (Rhodophyta) which were collected from two sites (Abu-Qir Bay and Eastern Harbor) located on the Egyptian Mediterranean Sea during the four seasons in the period (2011-2012). In addition, nutrient content in the algal species (total nitrogen and phosphorus) and the environmental conditions of the ambient sea water were measured to find out their effect on the algal fatty acids content. The nitrogen content in algal species was almost equal in both sites. In contrast, the phosphorus content was higher in Abu-Qir (0.33-1.71%) than in Eastern Harbor (0.21-0.74%). The fatty acids profile showed different correlations with water's physico-chemical properties. The fatty acids profile of the four algal species revealed high content of SFAs against MUFA and PUFA in both sites. Consequently, the algae were screened to define the suitable site, season and species for the highest quality and quantity of biodiesel production. Biodiesel physical properties; the Degree of Unsaturation (DU), Long Chain Saturation Factor (LCSF), Cold Filter Plugging Point (CFPP), Iodine Value (IV), Saponification Value (SV), the Cetane Number (CN), Kinematic viscosity ( $\nu$ ), density ( $\rho$ ), the Higher Heating Value (HHV), C18:3% (wt%) and double bond  $\geq 4$ (wt%) were calculated. All the investigated algae showed biodiesel properties that follow the standard values set for optimum biodiesel, with the green alga *U. fasciata* collected from Abu-Qir during winter, as the best choice for producing biodiesel; whereas, the extracted fatty acids from *Corallina officinalis* is more suitable to be used for production of blenders, which can be added to the petroleum diesel in order to increase its quality, oxidative stability and octane number.

**Keywords:** Fatty Acids Profile, Algae, Nutrient Content, Environmental Parameters, Biodiesel

## Introduction

The growing concern on depletion of fossil fuels and their environmental effects of burning, particularly greenhouse gas emissions and consequently climate change, have led to search for viable renewable fuel alternatives, which should be

eco-friendly fuel unlike petrodiesel (Trivedi *et al.*, 2013). Biodiesel is an alternative liquid fuel produced by a chemical reaction between a vegetable oil or animal fat with a short chain alcohol, called transesterification producing fatty acid methyl ester, where it can be used in diesel engines blended with standard gasoil or alone (Marchetti *et al.*, 2007). From

an environmental point of view, biodiesel has several benefits such as the reduction of carbon monoxide (50%) and carbon dioxide emissions (78%). In addition it is a biodegradable, renewable and nontoxic and its use, instead of diesel, reduces fossil fuels consumption (Surendhiran and Vijay, 2014).

Actually, the most widely available forms of biofuel were obtained from corn starch, sugar cane, palm, oilseed rape and soybean. However, these traditional feedstocks for biodiesel are also feed for livestock on farms, as well as a valuable source of food for humans. Because of this, using them in biofuel production increases food crises and increases expenses for both the food and the fuel produced (Abishek *et al.*, 2014). Also, these terrestrial crops may contribute to net greenhouse gas emission and may cause other environmental impacts such as freshwater reducing resources and changing in the water cycle (Demirbas, 2009). Whereas, using algae as a source of biodiesel can alleviate these problems in a number of ways. Firstly, many algae are not used as a primary food source for humans, meaning that it can be used solely for fuel production and there would be little impact in the food industry (Demirbas, 2009). Secondly, many of the waste-product extracts produced during the processing of algae for biofuel can be used as a sufficient animal feed. This is an effective way to minimize waste and a much cheaper alternative to the more traditional corn or grain based feeds (Pienkos and Darzins, 2009). Moreover, they are more productive than terrestrial plants and can succeed in salty or brackish water with only sunlight and available nutrients; they do not need any chemical fertilizers. For all these reasons, recent researches around the world focused on algae for biofuel production; either bioethanol (Goh and Lee, 2010), biogas (Hughes *et al.*, 2012), or biodiesel (Maceiras *et al.*, 2011).

In fact, marine algae are reported to be rich in soluble dietary fibers, carbohydrates, proteins, minerals, vitamins, antioxidants, phytochemicals and polyunsaturated fatty acids with a low caloric value (Polat and Ozgul, 2013). Singh and Gu (2010); John and Anisha (2011) reported that, macroalgal biomass can store large amounts of oil which can be exploited for the production of biodiesel. They are remarkably affected by external factors such as geographical location, environment, season and sampling conditions (Renaud and Luong-Van, 2006).

Fatty acids are one of the most important components in seaweeds. They are classified in three groups; Saturated Fatty Acids (SFA), Monounsaturated Fatty Acids (MUFAs) and Polyunsaturated Fatty Acids (PUFAs) (Polat and Ozgul, 2013). The PUFAs and MUFAs are of particular interest, because they are very important for human health maintenance and they are

synthesized only by plants (Yang *et al.*, 2000; Pulz and Gross, 2004; Le Goff *et al.*, 2013). In contrast, high concentrations of SFA in dietary sources are undesirable, since they have negative effects on human health (Muralidhar *et al.*, 2010), but this group is of special interest in biofuel production (Islam *et al.*, 2013). Many previous researches were concerned with the production of biofuel from a large number of macroalgae such as *Ascophyllum nodosum*, *Codium tomentosum*, *Enteromorpha intestinalis*, *Ulva rigida* and others (Maceiras *et al.*, 2011). In fact, the first step in developing an algal process is to choose the algal species. Pulz and Gross (2004) observed that successful algal biotechnology mainly depends on choosing the right alga with relevant properties for specific culture conditions and products.

However, the properties of biodiesel depend on the type of fatty acids and their saturation levels. Suitability of the biodiesel as a diesel engine fuel is determined by its physical properties to know its feasibility as a diesel engine fuel (Saravanan and Chandrasekar, 2013).

In Egypt, seaweeds grow luxuriantly along the Mediterranean coast and many species are still unexploited, despite their great importance ecologically, economically and industrially (Shams El-Din and El-Sherif, 2012). Most of the literature were interested in seaweeds in medicinal uses (Matloub *et al.*, 2013; El-Kassas and Attia, 2014), antifouling agents (Tadros *et al.*, 2006), bioremediation agents (Abdallah, 2013) and nutritional sources for fish (Wassef *et al.*, 2013) and for human (Shams El-Din and El-Sherif, 2012). However, very scarce studies were carried on seaweeds as a source for biofuel production and the only available literature of Afify *et al.* (2010) has investigated neither the effect of environmental conditions, nor the effect of seasonal variations on biochemical and fatty acids production.

This study aimed to evaluate the potential of different seaweeds for biodiesel production and the impact of the spatial and temporal variations on biodiesel quality. In order to achieve our aim, the study is subdivided into two parts; the first is the determination of the total nutrients contents (nitrogen and phosphorus) in seaweeds in addition to the environmental conditions of the ambient sea water. The second is to determine the quality and quantity of produced biodiesel from different seaweeds at different seasons.

## Materials and Methods

### *The Study Area*

Water and algal samples were collected during 2011-2012 from two sites located on the Mediterranean Sea, Abu-Qir Bay and Eastern Harbor, which were chosen representing different environmental habitats along the Alexandria coast (Fig. 1).

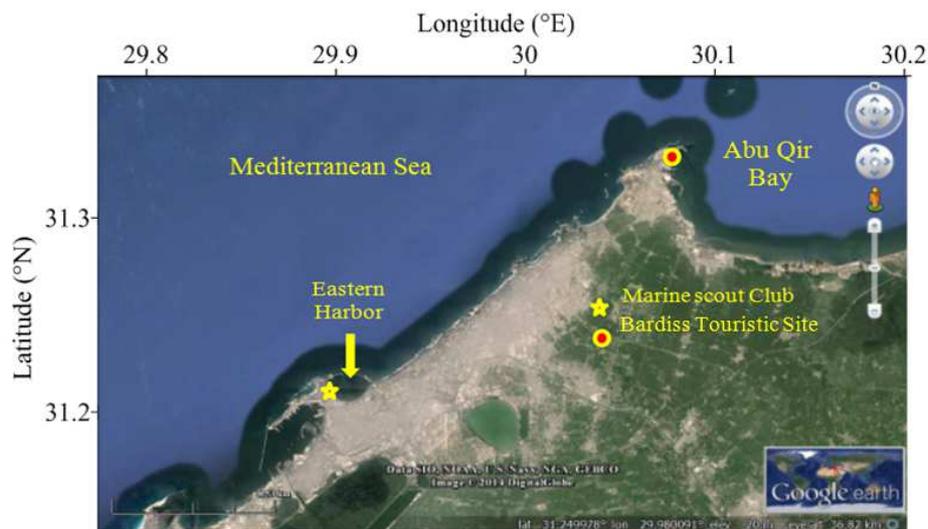


Fig. 1. The study area and sampling station during 2011-2012

The samples were collected from the beach of the touristic site “Bardiss” located at the extremely western head of Abu Qir Bay (A.Q.) at longitudes 30°07' E and latitudes 31°16' N. The bay is a hot spot area since it is subjected properly to discharge of primary treated sewage water, in addition to fishing activities. As well as, a discharge of industrial wastewater through "Tabia Pumping Station" and the agricultural effluents from Lake Edku (Shams El-Din *et al.*, 2014). This area is affected by the eastward wind-induced current (El-Tawil and Khalil, 1983). However, A.Q. site is subjected to wave action and is characterized by exposed rocks extending 100 m from the coast. At the western edge, the substrate consists of chains of natural rocks surrounded by pools (Ismael, 2012). These rocks provide excellent substrata for a rich algal flora (EL-Sarraf, 1995).

The Eastern Harbor of Alexandria (E.H.) is a shallow, semi-enclosed embayment covering an area of about 2.8 km<sup>2</sup>, located along the central part of Alexandria. The southern part of the harbor has been reinforced by concrete blocks and the northern side is protected by an artificial breakwater with two inlets, an eastern and a western inlet. It is bordered to the east by a land projection, El-Silsila and to the northwest by a long causeway (El-Sayed and Khadr, 1999). The bay has always been the recipient of large volumes of domestic waste water from several point sources. In 1996-1997, however, all outfalls except one were closed. The same volume of waste water is now being disposed of through the remaining outfall on the south-west bay margin (Ismael, 2012). In addition, the bay is exposed to pollution due to intensive shipping and fishing activities. The algal

samples were collected from the Scout club located in E.H. at 31°13.3'N latitude and 29°53.10'E longitude. However, the site sheltered four or five common seaweeds, which were recorded previously (Shams El-Din *et al.*, 2007) and were found along the Egyptian Mediterranean coast.

### Sample Collection

#### Water Samples

Water samples were collected seasonally, from the same sites and depths of the algae. The samples were collected directly into 5 liters capacity plastic Jerrycan, which were used for determinations of the studied parameters: Salinity, temperature, nitrite (NO<sub>2</sub>), nitrate (NO<sub>3</sub>), reactive soluble phosphate (PO<sub>4</sub>), silicate (SiO<sub>4</sub>) and oxidizable organic matter (O.M). pH was measured in situ using a Metrohm (827 pH) pH electrode calibrated with TRIS buffer on total scale (pH<sub>T</sub>) following Dickson *et al.* (2007). Measurements of salinity was done using a Bekman Induction Salinometer (Model RS-7C), which is standardized with Standard Seawater Copenhagen, Denmark, of chlorinity 39.377 g l<sup>-1</sup>. The conductivity ratio was measured to the nearest 1×10<sup>-5</sup> and converted to salinity, up to the nearest 0.01 g l<sup>-1</sup> using the tables of salinity/conductivity conversions. The temperature of A.Q. during the four seasons was measured using an ordinary thermometer scale (0-100°C). Whereas, the readings of temperature in E.H. during summer (2011), autumn (2011) and winter (2012) were obtained from the calculations of air temperature. The readings of salinity and temperature in E.H. during spring (2012) were obtained by personal communication (Tarek El Gezery).

Dissolved oxygen was fixed in field and measured according to the modified Winkler's method according to Strickland and Parsons (1972). Nutrient salts (nitrite, nitrate, ammonia, phosphate and silicate) were measured spectrophotometrically in filtered samples, using 6800UV/VIS Spectrophotometer (JENWAY) according to Grasshoff (1976). Oxidizable organic matter was measured according to the method of Carlberg (1972).

### *Collection of Macroalgae*

Algal samples were collected by hand, washed with seawater at the sampling site to remove the adhered sediments and impurities then separated in polyethylene bags. Samples stored under refrigeration 4°C. Quick rinsing of the algae with tap water was carried out in the laboratory on the same day to get rid of the remaining impurities and epiphytes. Herbarium sheets with a preliminary identification of separated species were done and/ or preserved in 4% formalin. Microscopic identification of the investigated algae was carried out according to Aleem (1993).

A total of four algal species were collected from each site at the sub-littoral zone at the depth (0.5-1 m) representing two algal classes: The Chlorophyceae, which was represented by the order Ulvales comprising one family: Ulvaceae, which was represented by one species; *Ulva fasciata* Delile, whereas the second class Rhodophyceae was represented by two orders (Corallinales and Gelidiales) comprising one family each (Corallinaceae and Gelidiaceae), respectively. The family Corallinaceae was represented by two species *Corallina mediterranea* Areschoug and *Corallina officinalis* Linnaeus. On the other hand, the family Gelidiaceae was represented by the species *Pterocladia capillacea* (S.G. Gmelin) Santelices and Hommersand formerly known as *Pterocladia capillacea*. About 250 gm of each represented species at each study area was air dried to constant weight at room temperature (25°C). The total average of three replicas and the standard deviation for nutrient contents of each species were calculated.

### *Extraction and Purification of Total Lipid*

The air dried algal sample was grained manually using mortar and then weighted ( $1g \pm 0.001$ ) into 100 ml screw top bottles. A total of 50 ml solvent was added in a predetermined sequence, with each sample being sonicated for ease of cell wall rupture and oil extraction by a Lab Sonicator Model Cole-Parmer 8852. The process was accomplished by immersing the bottle containing the algal sample with solvent mixture into the Sonicator at 60°C for 15 min.

According to (Folch *et al.*, 1957), lipids were extracted from algae with 30 ml of chloroform/methanol (2/1, v/v) by shaking at 200 rpm and 30°C. This was followed by the addition of a mixture of methanol/water (20 ml, 1:1, v/v) to achieve a final solvent mixture ratio of 2:2:1 for chloroform: Methanol: Water. The bottles were capped and re-shacked for another 30 min. The Supernatants were collected and the residues were re-extracted thrice with 2 ml chloroform/methanol (1/1, v/v) and the combined supernatants were washed with 2 ml of Milli-q water. The lower organic phases were collected after centrifugation; then evaporated till dryness under gentle nitrogen stream.

### *Determination of Fatty Acids*

The extracted total lipid was transferred to 10 ml clean screw top test tubes, with a fresh prepared solution of methanol: Hydrochloric acid: Chloroform (10:1:1 V: V: V ml) for esterification reaction at 90°C for 60 min (Kumari *et al.*, 2011). The obtained fatty acid methyl esters were subjected to gas liquid chromatographic analysis.

A gas chromatograph Model Hewlett Packard (HP) 6890 GC equipped with a FID (Flame Ionization Detector) was used. The separation column was Fused silica capillary column HP-5 (30 m × 0.32 mm ID × 0.25 µm film thickness), packed with (5% diphenyl, 95% dimethyl polysiloxane) at initial temperature 150°C for 2 min. Injector temperature was 220°C, injection volume was 3 µL and detector temperature was 250°C. Nitrogen was used as the carrier gas and the split ratio was 50:1, while the gas flow was 1 ml min<sup>-1</sup>. Individual peaks of FAMES were identified by the comparison of the retention times and equivalent chain length values, using the standard Supelco 37 component FAME Mix, 100mg Neat catalog No. 1819-1AMP with those of authentic standards (Christie, 1988).

### *Measurements of Nutrient Contents (Phosphorus and Nitrogen)*

For phosphorus analysis, algal sub-samples were dried at 60°C to a constant weight, homogenized by crushing each sample in a porcelain pestle and mortar and kept away from metallic materials and dusty conditions to avoid contamination. One gram dry weight of each sample was acid digested in 5 ml concentrated HNO<sub>3</sub> in a Teflonlined vessel in a microwave oven in pressure-controlled conditions. Digested samples were filtered through an acid-washed filter (Whatman GF/C) and diluted to 25 ml with double distilled water (Haritonidis and Malea,

1995; Mohamed and Khaled, 2005). The measurement of phosphorus was based on the reaction of phosphate with molybdate in a strong acidic medium to form a complex (Gamst and Try, 1980). The results of phosphorus were expressed as mg/100 g dry weight.

For measurement of the total organic nitrogen content, the algal samples were dried at 40°C to a constant weight. 1.00 g of the dried sub-sample was completely digested by adding 200 mg of catalyst ( $K_2SO_4$ ,  $CuSO_4$  and  $SeO$  in ratio 100:10:1), respectively and 3 ml of concentrated sulfuric acid then diluted to 75 ml with double distilled water (Grasshoff, 1975). The concentration of total nitrogen content in the digested sample was estimated spectrophotometrically at 240 nm and using ammonium salt as a standard.

### Statistical Analysis

The correlation coefficient between the fatty acids groups with nitrogen and phosphorus content in algal species and with physico-chemical parameters was calculated for each site separately, using the Excel program 13.

### Calculation of Biodiesel Physical Properties

The physical properties of biodiesel products were calculated to investigate the quality of the biodiesel extracted from macroalgal species. The fatty acids methyl ester profiles were used to estimate the Degree of Unsaturation (DU), Long Chain Saturation Factor (LCSF), Cold Filter Plugging Point (CFPP), Iodine Value (IV), Saponification Value (SV), the Cetane Number (CN), kinematic viscosity ( $\nu$ ), density ( $\rho$ ), the Higher Heating Value (HHV), C18:3% (wt%) and weight percent of fatty acids with double bond higher than 4  $Db \geq 4$  (wt%) according to Islam *et al.* (2013) and Saravanan and Chandrasekar (2013).

## Results

### Water Quality

The water quality of sea water samples collected from Abu Qir Bay (A.Q.) and Eastern Harbor (E.H.) during 2011-2012 is shown in Table 1.

Sea water pH lied in the alkaline side during the four seasons in the two sites. pH showed approximately no seasonal variations. The highest pH in A.Q. and E.H. was recorded during both summer and winter seasons (8.45 and 8.48), respectively. Ismael *et al.* (2014) recoded the same results; where the pH values demonstrated negligible variations (8.10-8.30) at both Abu Qir and Eastern Harbor. Water salinity of both sites was lower than that of proper Mediterranean Sea water

and ranged between (37.61 to 38.43‰ in A.Q.) and (38.11 and 38.35‰ in E.H.). The previously recorded salinity by Ismael *et al.* (2014) ranged between 38.10 and 38.90‰ in A.Q. and about 37.4‰ at the Eastern Harbor. The recorded salinity reflects the discharged fresh water finger print in both E.H. and A.Q. Bay. The water temperature was minimum during autumn (15.50 and 18.20°C), while maximum during summer (26.44 and 28.90°C) in A.Q. and E.H., respectively. In general, E.H. showed higher temperature compared to A.Q. in all seasons. The same range of temperature was recorded by Ismael *et al.* (2014) for both sites, since temperature ranged from 18.0 to 29.3°C.

As indicated from the data presented in the Table 1, dissolved oxygen measurements showed that sea water in A.Q. was well ventilated since the dissolved oxygen content showed relatively higher values (4.07-8.97  $mgO_2/l$ ) during all seasons than in E. H., which fluctuated between (3.17-5.23  $mgO_2/l$ ).

The oxidizable organic matter ranged between (0.64  $mgO_2/l$ ) during spring and (1.60  $mgO_2/l$ ) during winter in A.Q., whereas in Eastern Harbor O.M. ranged between (0.80  $mgO_2/l$ ) during winter and (3.90  $mgO_2/l$ ) during summer, with annual average (1.17±0.40  $mgO_2/l$ ) for the former and (2.06±1.46  $mgO_2/l$ ) for the later.

As far as nutrients are concerned, both nitrite and nitrate showed in A.Q. the maximum concentration during spring and winter (1.03 and 1.63  $\mu mol l^{-1}$ ), respectively, whereas, the minimum concentration of the two parameters was recorded during summer (0.40 and 0.85  $\mu mol l^{-1}$ ), respectively. The E. H. showed inverse pattern for nitrite, recording the maximum value during summer (0.95  $\mu mol l^{-1}$ ) and the minimum value during spring (0.40  $\mu mol l^{-1}$ ), whereas the highest concentration of nitrate was recorded during spring (1.68  $\mu mol l^{-1}$ ) and the lowest during winter (0.93  $\mu mol l^{-1}$ ). Inorganic phosphate concentration was relatively low in A.Q. and E.H., except during summer (5.85 and 6.19  $\mu mol l^{-1}$ ), respectively. Regarding the concentration of inorganic silicate, it was high in A.Q., recording a maximum value of (12.30  $\mu mol l^{-1}$ ) during winter and a low value of 3.29  $\mu mole l^{-1}$  during summer. While, the concentration of silicate in E. H. ranged between (3.02  $\mu mol l^{-1}$ ) during winter and (9.81  $\mu mol l^{-1}$ ) during spring.

### The Nutrient Contents (Phosphorus and Nitrogen)

The contents of the two elements; nitrogen and phosphorus displayed wide spatial and temporal variations in the four algal species (Table 2). The corallinate species, *C. officinalis* recorded the maximum value of nitrogen content in A.Q. during autumn

(37.67±5.51 mg g<sup>-1</sup>) and in E.H. during spring (35.00±0.00 mg g<sup>-1</sup>). The lowest value of nitrogen content was recorded in *P. capillacea* in A.Q. (6.67±2.31 mg g<sup>-1</sup>) and in *C. officinalis* in E.H. (7.07±0.60 mg g<sup>-1</sup>) both during winter. The total phosphorus was higher in A.Q. than in Eastern Harbor, with remarkably high

concentration in *U. fasciata* in the former site (1711.00±21.52 mg/100g), which is approximately two folds of that recorded in *U. fasciata* in E.H. during winter. Whereas, the lowest values were recorded in *C. mediterranea* in A.Q. during spring and *C. officinalis* in E.H. during autumn and were approximately equal.

Table 1. The physico-chemical parameters in Abu-Qir Bay and Eastern Harbor during the study period (2011-2012)

Abu-Qir Bay								
Physico-chemical parameters	Summer (2011)	Autumn (2011)	Winter (2012)	Spring (2012)	max	min	average	SD
pH	8.45	8.38	8.45	8.22	8.45	8.22		0.11
Salinity (‰)	-	-	37.61	38.43	38.43	37.61	38.02	0.58
Temperature (°C)	26.44	15.50	16.50	20.50	26.44	15.50	19.74	4.96
D.O. (mgO <sub>2</sub> /l)	4.07	6.26	6.24	8.97	8.97	4.07	6.38	2.01
OM (mgO <sub>2</sub> /l)	1.30	1.12	1.60	0.64	1.60	0.64	1.17	0.40
NO <sub>2</sub> (µmol/l)	0.40	0.75	0.55	1.03	1.03	0.40	0.68	0.27
NO <sub>3</sub> (µmol/l)	0.85	0.98	1.63	1.03	1.63	0.85	1.12	0.35
PO <sub>4</sub> (µmol/l)	5.85	0.17	0.00	0.17	5.85	0.00	1.55	2.87
SiO <sub>4</sub> (µmol/l)	3.29	4.61	12.30	9.01	12.30	3.29	7.30	4.13
Eastern Harbor								
pH	8.47	8.19	8.48	8.12	8.48	8.12		0.19
Salinity (‰)	-	-	38.35	38.11	38.35	38.11	38.23	0.17
Temperature (°C)	28.90	18.20	19.80	22.70	28.90	18.20	22.40	4.72
D.O. (mgO <sub>2</sub> /l)	3.17	4.55	4.41	5.23	5.23	3.17	4.34	0.86
OM (mgO <sub>2</sub> /l)	3.90	2.56	0.80	0.96	3.90	0.80	2.06	1.46
NO <sub>2</sub> (µmol/l)	0.95	0.93	0.45	0.40	0.95	0.40	0.68	0.30
NO <sub>3</sub> (µmol/l)	1.40	1.43	0.93	1.68	1.68	0.93	1.36	0.31
PO <sub>4</sub> (µmol/l)	6.19	0.47	0.26	0.47	6.19	0.26	1.85	2.90
SiO <sub>4</sub> (µmol/l)	5.62	4.77	3.02	9.81	9.81	3.02	5.81	2.88

Note: Dissolved Oxygen = D.O.; Oxidizable organic Matter = OM; Nitrite = NO<sub>2</sub>; Nitrate = NO<sub>3</sub>; Phosphate = PO<sub>4</sub>; Silicate = SiO<sub>4</sub>

Table 2. The nutrient content (nitrogen, mg/g and phosphorus, mg/100 g) and their standard deviation in Abu-Qir Bay and Eastern Harbor during the study period (2011-2012)

Abu-Qir Bay					
Season	Natural content	Summer (2011)	Autumn (2011)	Winter (2012)	Spring (2012)
<i>U. fasciata</i>	Nitrogen	ND	*	14.33±6.51	11.00±1.00
	Phosphorus	ND	*	1711.00±21.52	337.00±13.00
<i>C. mediterranea</i>	Nitrogen	*	*	*	32.97±17.83
	Phosphorus	*	*	*	196.33±4.73
<i>C. officinalis</i>	Nitrogen	*	37.67±5.51	25.00±3.46	ND
	Phosphorus	*	521.00±25.51	351.33±12.34	ND
<i>P. capillacea</i>	Nitrogen	26.33±5.86	ND	6.67±2.31	17.67±4.04
	Phosphorus	328.67±14.22	ND	1363.67±10.26	324.67±6.11
Eastern Harbor					
<i>U. fasciata</i>	Nitrogen	12.00±2.00	8.69±1.05	8.83±1.65	10.33±0.58
	Phosphorus	356.33±23.80	240.33±19.01	738.33±3.06	612.00±21.00
<i>C. mediterranea</i>	Nitrogen	ND	26.93±2.38	9.9±2.09	23.50±1.50
	Phosphorus	ND	360.67±14.01	343.33±5.86	560.0±10.82
<i>C. officinalis</i>	Nitrogen	ND	9.11±1.21	7.07±0.60	35.00±0.00
	Phosphorus	ND	208.00±5.29	262.67±8.62	311.00±13.89
<i>P. capillacea</i>	Nitrogen	8.90±1.39	*	*	*
	phosphorus	499.33±15.18	*	*	*

Note: The \* sign means that the species was not found during this season; ND means that the component was not measured.

Table 3. The concentration ( $\mu\text{g/g}$ ) of fatty acids in *Ulva fasciata* collected from Abu-Qir Bay (A.Q.) and Eastern Harbor (E.H.) during (2011-2012)

Fatty acids	Summer (2011)		Autumn 2011)		Winter (2012)		Spring (2012)	
	A.Q.	E.H.	A.Q.	E.H.	A.Q.	E.H.	A.Q.	E.H.
n-carpoic acid C6:0	15.04	-	*	1.37	27.64	5.97	-	-
Caprillic acid C8:0	1.47	-	*	0.51	8.66	1.71	-	0.09
Capric C10:0	4.01	-	*	0.55	8.38	2.44	-	-
Undecanoic acid C11:0	1.10	-	*	0.35	1.97	2.41	-	-
Lauric acid C12:0	4.81	-	*	0.33	4.70	1.26	-	0.41
Tridecanoic acid C13:0	1.34	-	*	0.28	4.10	1.46	-	0.84
Myristic acid C14:0	26.32	1.62	*	5.36	90.64	20.41	0.77	16.01
Pentadecanoic acid C15:0	16.61	1.15	*	5.86	55.19	16.67	2.93	2.03
Palmitic acid C16:0	1222.31	145.73	*	259.89	4025.55	1331.97	49.41	54.32
Margarinic acid C17:0	13.75	-	*	1.93	15.31	6.58	-	3.81
Stearic acid C18:0	20.72	2.72	*	10.05	74.95	18.45	1.68	2.67
Arachidic acid C20:0	3.53	-	*	0.90	-	-	-	1.94
Heneicosanoic acid C21:0	20.76	-	*	0.92	84.12	15.25	-	2.19
Docosanoic acid C22:0	8.54	-	*	1.43	-	-	-	-
C23:0	6.46	-	*	-	-	-	-	1.53
C24:0	0.49	-	*	1.61	-	-	-	-
$\Sigma$ SFA	1367.26	151.22	*	291.34	4401.22	1424.57	54.79	85.84
Tetradecenoic acid C14:1n-7	1.14	0.25	*	0.54	5.87	1.98	-	0.24
14, Pentadecenoic acid C15:1	2.10	-	*	2.21	15.78	1.32	8.29	35.38
9 Hexadecenoic acid C16:1n-7	9.80	2.29	*	-	-	-	-	-
Heptadecenoic acid C17:1	10.76	-	*	15.32	208.69	90.69	446.72	93.16
Oleic acid C18:1c n-9	64.58	14.22	*	0.71	2.36	1.50	-	1.12
5 Ecosenic acid-11Ecosenic acid C20:1n9	-	-	*	0.40	-	-	-	0.99
13-Docosenoic acid C22:1n-7	4.44	-	*	5.12	67.39	17.90	-	-
$\Sigma$ MUFA	92.82	16.76	*	24.30	300.09	113.39	455.01	130.89
Linoleic acid C18:2c n-6	5.65	2.00	*	-	-	-	-	0.74
$\gamma$ Linolenic acid C18:3n-6	3.05	-	*	1.14	17.63	9.53	-	1.07
Ecosadienoic acid C20:2 n-6	-	-	*	-	-	-	-	-
Ecosatrienoic acid C20:3 n-6	-	-	*	-	-	-	-	0.46
Arachidonic acid C20:4 n-6	-	-	*	-	-	-	-	-
Cis,5,8,11,14,17Ecosapentanoic acid C20:5n-3	66.17	-	*	-	-	-	-	-
Docosadienoic acid C22:2 n-6	-	-	*	-	-	-	-	2.14
Docosahexanoic acid C22:6n-3	-	2.18	*	-	-	-	-	-
$\Sigma$ PUFA	74.87	4.18	*	1.14	17.63	9.53	-	4.41
$\Sigma$ FAs	1534.95	172.16	*	316.78	4718.93	1547.50	509.80	221.14

Note: The \* sign means that the species was not found during this season

### Fatty Acids Composition

The four algal species showed completely different pattern of fatty acids composition spatially and temporally. The fatty acids profile in *Ulva fasciata* revealed higher concentrations in A.Q. bay than that of Eastern Harbor, with a higher value of saturated fatty acid (SFA) than the other two groups during almost all seasons (Table 3). Palmitic acid C16:0 was the dominant fatty acid in the four seasons, recording the highest concentration ( $4025.55 \mu\text{g g}^{-1}$ ) in A.Q. during winter. The second dominant saturated fatty acid was the Myristic acid (C14:0), followed by Stearic acid (C18:0). Considering MUF the Heptadecenoic acid (C17:1) was dominant, with the highest concentration

in A.Q. during spring ( $446.72 \mu\text{g g}^{-1}$ ). The composition of PUFAs group was meager, where many acids were absent. The Cis, 5,8,11,14,17 Ecosapentanoic acid C20:5 was present only in A.Q. during summer ( $66.17 \mu\text{g g}^{-1}$ ), whereas the Docosahexanoic acid C22:6 was present only in E.H. during the same season in very low amount ( $2.18 \mu\text{g g}^{-1}$ ).

The rhodophyte *Corallina mediterranea* was absent in A.Q. during the three seasons; summer, autumn and winter (Table 4). Whereas, comparing the spring season in the two sites revealed much lower concentrations of fatty acids in A.Q. ( $25.08 \mu\text{g g}^{-1}$ ) than E.H. ( $360.54 \mu\text{g g}^{-1}$ ). Regarding the Eastern Harbor, the two seasons; summer and autumn revealed low concentrations of Fatty acids, with SFA higher

than MUFAs and PUFAs. In contrast, the two other seasons; winter and spring revealed higher MUFAs than SFA and PUFAs due to the high concentration of Heptadecenoic acid (C17:1) (801.27 and 281.66  $\mu\text{g g}^{-1}$ ), respectively. Among the SFA group, palmitic acid was dominant, with highest concentration in Eastern Harbor during winter (203.02  $\mu\text{g g}^{-1}$ ). Like in *U. fasciata*, the composition of fatty acids of PUFAs group in *C. mediterranea* was meager, where many acids were absent.

The fatty acids profile in *Corallina officinalis* (Table 5) revealed higher concentrations in A.Q. than that of E.H., except during spring. The Saturated Fatty Acids (SFA) were higher than the other two groups during summer and autumn in both sites. Whereas the opposite pattern was shown during winter and spring in A.Q. and during spring in E.H., where MUFAs were higher than the two other groups; SFA and PUFAs due to the high concentrations of (C17:1)

which represent about 78% of the total fatty acid in A.Q. during winter and 59% in E. H during spring. Among the SFA group, palmitic acid was dominant, with highest concentration in Abu-Qir bay during autumn (235.64  $\mu\text{g g}^{-1}$ ).

The species *Pterocladia capillacea* was absent in Eastern harbor during the three seasons; autumn, winter and spring (Table 6). Whereas, comparing the summer season in the two sites revealed much lower concentrations of total fatty acids in E.H. (390.09  $\mu\text{g g}^{-1}$ ) than in A.Q. (1137.70  $\mu\text{g g}^{-1}$ ). Regarding A.Q., the two seasons; summer and autumn revealed high concentrations of Fatty acids, with SFA higher than MUFAs and PUFAs. In contrast, the two other seasons; winter and spring revealed higher MUFAs than SFA and PUFAs due to the high concentration of Heptadecenoic acid (C17:1) (493.87  $\mu\text{g g}^{-1}$ ) during winter and 9 Hexadecenoic acid C16:1 during spring (458.63  $\mu\text{g g}^{-1}$ ).

Table 4. The concentration ( $\mu\text{g/g}$ ) of fatty acids in *Corallina mediterranea* collected from Abu-Qir Bay (A.Q.) and Eastern Harbor (E.H.) during (2011-2012)

Fatty acids	Summer (2011)		Autumn 2011)		Winter (2012)		Spring (2012)	
	A.Q.	E.H.	A.Q.	E.H.	A.Q.	E.H.	A.Q.	E.H.
n-carpoic acid C6:0	*	-	*	-	*	-	-	4.70
Caprylic acid C8:0	*	-	*	0.46	*	-	-	2.07
Lauric acid C12:0	*	1.83	*	-	*	-	-	-
Tridecanoic acid C13:0	*	-	*	-	*	-	-	-
Myristic acid C14:0	*	43.60	*	2.83	*	11.68	-	-
Pentadecanoic acid C15:0	*	4.10	*	0.75	*	-	0.33	9.32
Palmitic acid C16:0	*	189.63	*	44.71	*	203.02	0.63	4.57
Margarinic acid C17:0	*	2.58	*	-	*	-	-	-
Stearic acid C18:0	*	6.43	*	1.98	*	-	-	-
Arachidic acid C20:0	*	-	*	-	*	-	-	-
Heneicosanoic acid C21:0	*	-	*	-	*	20.18	-	5.09
Docosanoic acid C22:0	*	-	*	-	*	-	-	-
C23:0	*	-	*	-	*	43.81	-	7.41
C24:0	*	-	*	-	*	-	-	-
$\Sigma$ SFA	*	248.17	*	50.73	*	278.69	0.96	33.16
Tetradecenoic acid C14:1n-7	*	10.17	*	0.96	*	30.07	0.42	20.01
14, Pentadecenoic acid C15:1	*	4.74	*	-	*	70.38	1.62	22.59
9 Hexadecenoic acid C16:1n-7	*	-	*	-	*	-	-	-
Heptadecenoic acid C17:1	*	-	*	4.42	*	801.27	21.89	281.66
Oleic acid C18:1c n-9	*	1.03	*	-	*	-	-	-
5 Ecosenic acid-11Ecosenic acid C20:1n9	*	-	*	-	*	-	-	-
13-Docosenoic acid C22:1n-7	*	-	*	-	*	-	-	-
$\Sigma$ MUFA	*	15.95	*	5.38	*	901.72	23.93	324.26
Linoleic acid C18:2c n-6	*	1.39	*	-	*	-	-	-
$\gamma$ Linolenic acid C18:3n-6	*	-	*	-	*	-	-	-
Ecosadienoic acid C20:2 n-6	*	-	*	-	*	-	-	-
Ecosatrienoic acid C20:3 n-6	*	-	*	-	*	5.76	-	-
Arachidonic acid C20:4 n-6	*	-	*	-	*	-	-	-
Cis,5,8,11,14,17Ecosapentanoic acid C20:5n-3	*	-	*	-	*	-	-	-
Docosadienoic acid C22:2 n-6	*	-	*	-	*	29.89	0.19	3.12
Docosahexanoic acid C22:6n-3	*	8.48	*	-	*	-	-	-
$\Sigma$ PUFA	*	9.87	*	-	*	35.65	0.19	3.12
$\Sigma$ SFA	*	273.98	*	56.11	*	1216.06	25.08	360.54

Note: The \* sign means that the species was not found during this season

Table 5. The concentration ( $\mu\text{g/g}$ ) of fatty acids in *Corallina officinalis* collected from Abu-Qir Bay (A.Q.) and Eastern Harbor (E.H.) during (2011-2012)

Fatty acids	Summer (2011)		Autumn (2011)		Winter (2012)		Spring (2012)	
	A.Q.	E.H.	A.Q.	E.H.	A.Q.	E.H.	A.Q.	E.H.
n-carpoic acid C6:0	*	-	2.08	0.72	-	-	-	3.50
Caprylic acid C8:0	*	-	0.79	0.20	-	-	-	1.24
Capric C10:0	*	-	-	0.55	-	-	-	-
Undecanoic acid C11:0	*	-	-	-	-	-	-	-
Lauric acid C12:0	*	-	-	0.76	-	-	-	-
Tridecanoic acid C13:0	*	1.32	-	0.21	-	-	-	-
Myristic acid C14:0	*	25.68	16.84	5.43	1.08	2.16	-	3.17
Pentadecanoic acid C15:0	*	9.13	11.15	2.54	-	2.57	-	1.69
Palmitic acid C16:0	*	82.62	235.64	134.85	38.96	71.27	0.82	88.17
Margarinic acid C17:0	*	-	-	3.56	0.89	-	-	-
Stearic acid C18:0	*	-	21.60	-	1.68	-	-	10.26
Arachidic acid C20:0	*	-	-	-	-	-	-	7.08
Heneicosanoic acid C21:0	*	-	-	-	-	-	-	6.06
Docosanoic acid C22:0	*	-	9.75	15.39	-	15.28	-	-
C23:0	*	-	-	-	6.87	-	0.76	23.98
C24:0	*	-	-	23.40	-	21.78	-	-
$\Sigma\text{SFA}$	*	118.75	297.85	187.61	49.48	113.06	1.58	145.15
Tetradecenoic acid C14:1n-7	*	20.14	9.25	5.56	4.02	3.70	0.50	26.28
14, Pentadecenoic acid C15:1	*	-	-	5.05	-	-	2.17	24.07
9 Hexadecenoic acid C16:1n-7	*	4.80	-	3.84	-	-	-	-
Heptadecenoic acid C17:1	*	-	48.16	5.28	200.33	11.44	21.26	316.60
Oleic acid C18:1c n-9	*	2.27	0.40	-	-	-	0.17	-
5 Ecosenic acid-11Ecosenic acid C20:1n9	*	-	-	-	-	-	-	-
13-Docosenoic acid C22:1n-7	*	-	6.33	-	-	-	-	-
$\Sigma\text{MUFA}$	*	27.21	64.14	19.73	204.35	15.14	24.10	366.95
Linoleic acid C18:2c n-6	*	2.26	-	-	-	-	-	2.15
$\gamma$ Linolenic acid C18:3n-6	*	-	-	-	0.47	-	-	-
Ecosadienoic acid C20:2 n-6	*	-	-	-	-	-	-	5.93
Ecosatrienoic acid C20:3 n-6	*	-	-	-	-	-	-	4.88
Arachidonic acid C20:4 n-6	*	-	-	-	-	-	-	-
Cis,5,8,11,14,17Ecosapentanoic acid C20:5n-3	*	-	-	-	-	-	-	-
Docosadienoic acid C22:2 n-6	*	-	-	-	3.08	-	0.34	12.02
Docosahexanoic acid C22:6n-3	*	18.18	-	-	-	-	-	-
$\Sigma\text{PUFA}$	*	20.44	-	-	3.55	-	-	24.98
$\Sigma\text{FA}$	*	166.40	361.99	207.34	257.38	128.20	26.02	537.08

Note: The \* sign means that the species was not found during this season

Among the SFA group, palmitic acid was dominant, with highest concentration in Abu- Qir bay during autumn ( $1525.72 \mu\text{g g}^{-1}$ ), followed by the Myristic acid (C14:0) and Stearic acid (C18:0). The same pattern of the other algae was followed by *P. capillacea*, where PUFAs group contributed by very low percentage to the total fatty acids.

#### Statistical Analysis

The correlation coefficient between the fatty acids groups in the species (*U. fasciata*, *P. capillacea* and *C. officinalis*) in A.Q. and the species (*U. fasciata*, *C. mediterranea* and *C. officinalis*) in E.H. with

nutrient content in seaweeds and with physico-chemical parameters revealed different effect of these parameters on fatty acids groups (Table 7).

#### Calculation of Biodiesel Physical Properties

The results of the physico-chemical properties of produced biodiesel are shown in Table 8, including the degree of saturation of fatty acids methyl ester (DU), Long Chain Saturation Factor (LCSF), Cold Filter Plugging Point (CFPP), Iodine Value (IV), Saponification Value (SV), the Cetane Number (CN), Kinematic viscosity ( $\nu$ ), density ( $\rho$ ), the Higher Heating Value (HHV), C18:3 (wt%) and double bond  $\text{Db} \geq 4(\text{wt}\%)$ .

Table 6. The concentration ( $\mu\text{g/g}$ ) of fatty acids in *Pterocliadiella capillacea* collected from Abu-Qir Bay (A.Q.) and Eastern Harbor (E.H.) during (2011-2012)

Fatty acids	Summer (2011)		Autumn (2011)		Winter (2012)		Spring (2012)	
	A.Q.	E.H.	A.Q.	E.H.	A.Q.	E.H.	A.Q.	E.H.
n-carpoic acid C6:0	1.39	-	3.95	*	-	*	-	*
Caprylic acid C8:0	0.48	-	1.55	*	-	*	0.61	*
Capric C10:0	0.23	-	-	*	-	*	0.20	*
Undecanoic acid C11:0	-	-	-	*	-	*	1.16	*
Lauric acid C12:0	2.09	1.62	1.86	*	-	*	-	*
Tridecanoic acid C13:0	0.93	1.05	1.33	*	-	*	2.29	*
Myrisitic acid C14:0	97.45	52.21	179.37	*	1.02	*	6.05	*
Pentadecanoic acid C15:0	5.65	6.79	9.14	*	10.78	*	0.12	*
Palmatic acid C16:0	833.39	251.84	1525.72	*	14.78	*	63.29	*
Margarinic acid C17:0	4.90	6.31	16.15	*	-	*	-	*
Stearic acid C18:0	28.27	4.97	46.76	*	1.26	*	3.65	*
Arachidic acid C20:0	1.60	-	-	*	-	*	0.93	*
Heneicosanoic acid C21:0	34.48	-	-	*	1.98	*	-	*
Docosanoic acid C22:0	2.37	-	-	*	-	*	-	*
C23:0	2.20	-	-	*	-	*	5.14	*
C24:0	-	-	-	*	-	*	-	*
$\Sigma\text{SFA}$	1015.43	324.79	1785.83	*	29.82	*	83.44	*
Tetradecenoic acid C14:1n-7	44.50	21.32	38.87	*	13.24	*	18.06	*
14, Pentadecenoic acid C15:1	1.70	2.07	32.56	*	59.48	*	44.74	*
9 Hexadecenoic acid C16:1n-7	8.41	5.89	-	*	-	*	458.63	*
Heptadecenoic acid C17:1	3.52	10.66	252.59	*	493.87	*	-	*
Oleic acid C18:1c n-9	12.70	4.14	-	*	-	*	-	*
5 Ecosenic acid-11Ecosenic acid C20:1n9	1.30	-	-	*	-	*	1.72	*
13-Docosenoic acid C22:1n-7	6.42	-	-	*	-	*	1.24	*
$\Sigma\text{MUFA}$	78.55	44.08	324.02	*	566.59	*	524.39	*
Linoleic acid C18:2c n-6	27.01	10.53	-	*	-	*	-	*
$\gamma$ Linolenic acid C18:3n-6	1.43	1.56	-	*	-	*	-	*
Ecosadienoic acid C20:2 n-6	-	-	-	*	-	*	-	*
Ecosatrienoic acid C20:3 n-6	-	-	-	*	-	*	1.38	*
Arachidonic acid C20:4 n-6	5.16	-	-	*	-	*	-	*
Cis,5,8,11,14,17Ecosapentanoic acid C20:5n-3	4.94	1.51	-	*	-	*	-	*
Docosadienoic acid C22:2 n-6	2.53	-	-	*	0.92	*	3.52	*
Docosahexanoic acid C22:6n-3	2.65	7.62	-	*	-	*	-	*
$\Sigma\text{PUFA}$	43.72	21.22	-	*	0.92	*	4.90	*
$\Sigma\text{FA}$	1137.70	390.09	2109.85	*	597.33	*	612.73	*

Note: The \* sign means that the species was not found during this season

Table 7. Correlation coefficient between the fatty acids groups with nutrient content in algae (nitrogen and phosphorus) and physico-chemical parameters of the surrounding seawater in the two studied sites

Parameters	<i>U. fasciata</i> A.Q.				<i>P. Capillacea</i> A.Q.				<i>C. officinalis</i> A.Q.			
	$\Sigma\text{SFA}$	$\Sigma\text{MUFA}$	$\Sigma\text{PUFA}$	$\Sigma\text{FA}$	$\Sigma\text{SFA}$	$\Sigma\text{MUFA}$	$\Sigma\text{PUFA}$	$\Sigma\text{FA}$	$\Sigma\text{SFA}$	$\Sigma\text{MUFA}$	$\Sigma\text{PUFA}$	$\Sigma\text{FA}$
Nitrogen	0.58	0.86	-0.42	0.64	-0.29	-0.49	0.84	-0.47	0.87	0.57	0.29	<b>1.00</b>
Phosphorus	<b>0.91</b>	0.46	-0.20	<b>0.92</b>	-0.72	0.54	-0.21	-0.68	0.87	0.58	0.29	<b>1.00</b>
NO <sub>2</sub>	-0.56	0.55	-0.81	-0.51	-0.21	0.59	-0.63	-0.08	0.12	-0.19	-0.25	0.00
NO <sub>3</sub>	0.87	0.39	-0.31	0.88	-0.60	0.73	-0.54	-0.50	-0.12	<b>0.97</b>	<b>0.99</b>	0.41
PO <sub>4</sub>	-0.06	-0.39	<b>0.97</b>	-0.08	0.24	-0.89	<b>1.00</b>	0.04	-0.40	-0.56	-0.40	-0.61
SiO <sub>4</sub>	0.65	0.75	-0.48	0.70	-0.83	<b>0.92</b>	-0.62	-0.71	-0.31	0.79	0.86	0.16
OM	0.83	-0.39	0.42	0.78	0.03	-0.12	0.15	0.00	0.04	0.67	0.66	0.38
Chlorophyll	-0.06	-0.38	<b>0.96</b>	-0.08	0.23	-0.88	<b>1.00</b>	0.03	-0.41	-0.56	-0.40	-0.62
pH	0.63	-0.62	0.60	0.57	0.27	-0.43	0.38	0.20	0.11	0.40	0.38	0.30
DO <sub>2</sub>	-0.30	0.74	-0.82	-0.23	-0.47	0.78	-0.70	-0.33	-0.05	0.04	0.03	-0.01
Salinity	0.42	<b>0.94</b>	-0.47	0.49	<b>-0.93</b>	0.89	-0.52	-0.83	-0.50	0.51	0.64	-0.14
Temperature	-0.39	0.47	0.37	-0.33	-0.35	-0.24	0.56	-0.48	-0.74	-0.71	-0.47	<b>-0.97</b>
Parameters	<i>U. fasciata</i> E.H.				<i>C. mediterranea</i> E.H.				<i>C. officinalis</i> E.H.			
	$\Sigma\text{SFA}$	$\Sigma\text{MUFA}$	$\Sigma\text{PUFA}$	$\Sigma\text{FA}$	$\Sigma\text{SFA}$	$\Sigma\text{MUFA}$	$\Sigma\text{PUFA}$	$\Sigma\text{FA}$	$\Sigma\text{SFA}$	$\Sigma\text{MUFA}$	$\Sigma\text{PUFA}$	$\Sigma\text{FA}$
Nitrogen	-0.56	-0.28	-0.13	-0.56	-0.90	-0.14	-0.51	-0.35	0.23	<b>0.96</b>	0.48	<b>0.96</b>
Phosphorus	0.64	0.91	0.89	0.70	-0.67	0.34	-0.12	0.10	0.25	0.54	-0.20	0.53
NO <sub>2</sub>	-0.43	<b>-0.99</b>	-0.66	-0.51	0.04	-0.79	-0.45	-0.65	0.36	-0.61	-0.13	-0.51
NO <sub>3</sub>	<b>-0.95</b>	-0.09	-0.75	<b>-0.92</b>	-0.79	-0.72	<b>-0.92</b>	-0.82	0.49	0.69	0.71	0.78
PO <sub>4</sub>	-0.39	-0.62	-0.15	-0.43	0.46	-0.50	-0.13	-0.30	-0.41	-0.29	0.47	-0.31
SiO <sub>4</sub>	-0.73	0.35	-0.36	-0.67	-0.67	-0.34	-0.63	-0.47	0.17	<b>0.94</b>	0.84	<b>0.95</b>
OM	-0.53	<b>-0.93</b>	-0.56	-0.59	0.17	-0.79	-0.42	-0.62	0.09	-0.47	0.18	-0.41
Chlorophyll	-0.41	-0.61	-0.16	-0.45	0.44	-0.51	-0.15	-0.31	-0.41	-0.26	0.50	-0.28
pH	0.58	-0.22	0.62	0.54	<b>0.99</b>	0.37	0.75	0.58	-0.77	-0.69	-0.23	-0.79
DO <sub>2</sub>	0.04	<b>0.72</b>	-0.01	0.10	-0.70	0.31	-0.15	0.07	0.43	0.67	-0.05	0.69
Salinity	0.58	<b>0.97</b>	0.77	0.64	0.11	0.88	0.60	0.77	-0.45	0.47	0.02	0.36
Temperature	-0.44	-0.30	-0.02	-0.45	0.38	-0.36	-0.12	-0.21	-0.55	0.07	0.75	0.01

Note: The bold values mean significant correlation

Table 8. The physico-chemical properties of biodiesel extracted from collected algae during (2011-2012)

Algae	DU	LCSF	CFPP	IV ( $\text{g}_2100\text{g}^{-1}\text{fat}$ )	SV ( $\text{mgKOHg}^{-1}$ )	CN	TFA wt ( $\mu\text{g/g}$ )	SFAs (%)	MUFA (%)	PUFA (%)	( $\nu$ ) ( $\text{mm}^2\text{s}^{-1}$ )	( $\rho$ ) ( $\text{g m}^{-3}$ )	HHV ( $\text{MJ g}^{-1}$ )	C18:3 (wt%)	Db $\geq$ 4(wt%)	
<i>Biodiesel Standard EN (14214)</i>	-	-	$\leq 5/\leq -20$	$\leq 120$	-	$\geq 51$	-	-	-	-	3.5-5.0	0.86-0.9	NA	$\leq 12$	$\leq 1$	
<i>Biodiesel Standard ASTM D6751-02</i>	-	-	NA	NA	-	$\geq 47$	-	-	-	-	1.9-6.0	NA	NA	-	-	
<i>min/max</i>	max	max	max	max	max	min	min	min	max	max	max	max	min	max	max	
<i>Threshold value</i>	-	-	5.00	120.00	-	47.0	-	-	-	-	-	0.90	-	12.00	1.00	
<i>Ulva fasciata</i>																
Summer	AQ	15.80	9.77	14.21	23.71	206.71	68.96	1534.95	89.08	6.05	4.88	4.27	0.86	39.41	0.20	4.31
	EH	14.59	9.25	12.60	13.31	204.86	72.62	172.16	87.84	9.74	2.43	4.42	0.87	39.99	0.00	0.00
Autumn	AQ															
	EH	8.39	11.77	20.49	7.62	206.65	72.41	316.78	91.97	7.67	0.36	4.38	0.86	39.49	0.36	0.00
Winter	AQ	7.11	9.32	12.82	6.49	207.41	72.36	4718.93	93.27	6.36	0.37	4.35	0.86	39.46	0.37	0.00
	EH	8.56	9.20	12.44	8.01	207.11	72.15	1547.50	92.06	7.33	0.62	4.34	0.86	39.49	0.62	0.00
Spring	AQ	89.25	1.13	-12.91	80.42	199.59	59.17	509.80	10.75	89.25	0.00	4.09	0.86	39.61	0.00	0.00
	EH	63.18	3.94	-4.11	58.49	204.16	62.52	221.14	38.82	59.19	1.99	4.07	0.87	39.75	0.48	0.00
<i>Corallina mediterranea</i>																
Summer	AQ															
	EH	13.03	8.09	8.95	13.73	210.66	71.32	273.98	90.58	5.82	3.60	4.21	0.89	40.35	0.00	0.00
Autumn	AQ															
	EH	9.59	9.73	14.10	8.89	208.69	71.36	56.11	90.41	9.59	0.00	4.24	0.86	39.45	0.00	0.00
Winter	AQ															
	EH	80.01	1.67	-11.23	72.33	198.87	62.44	1216.06	22.92	74.15	2.93	4.34	0.88	40.41	0.00	0.00
Spring	AQ	96.93	0.25	-15.69	86.70	199.23	57.61	25.08	3.83	95.41	0.76	4.02	0.87	39.80	0.00	0.00
	EH	91.67	0.13	-16.08	82.37	199.83	57.31	360.54	9.20	89.94	0.87	3.97	0.87	39.57	0.00	0.00
<i>Corallina officinalis</i>																
Summer	AQ															
	EH	40.92	4.97	-0.88	43.33	209.63	69.70	166.40	71.36	16.35	12.28	4.29	0.95	42.98	0.00	0.00
Autumn	AQ	17.72	13.53	26.04	16.02	206.27	70.77	361.99	82.28	17.72	0.00	4.35	0.86	39.47	0.00	0.00
	EH	9.52	40.21	109.85	9.31	199.51	74.91	207.34	90.48	9.52	0.00	4.92	0.86	39.64	0.00	0.00
Winter	AQ	82.15	1.84	-10.70	73.85	194.42	61.60	257.38	19.22	79.40	1.38	4.27	0.87	40.03	0.18	0.00
	EH	11.81	57.42	163.91	11.07	191.50	77.96	128.20	88.19	11.81	0.00	5.45	0.86	39.91	0.00	0.00
Spring	AQ	92.62	0.32	-15.49	86.28	194.66	58.65	26.02	6.07	92.62	0.00	4.16	0.87	40.01	0.00	0.00
	EH	77.63	3.92	-4.18	70.48	193.33	62.29	537.08	27.03	68.32	4.65	4.33	0.88	40.23	0.00	0.00
<i>Pterocladia capillacea</i>																
Summer	AQ	14.59	9.02	11.86	15.16	206.92	70.84	1137.70	89.25	6.90	3.84	4.30	0.87	39.62	0.13	0.89
	EH	22.18	7.09	5.81	22.70	210.05	69.00	390.09	83.26	11.30	5.44	4.13	0.88	39.99	0.40	0.39
Autumn	AQ	15.36	8.34	9.72	14.25	208.88	70.16	2109.85	84.64	15.36	0.00	4.19	0.86	39.44	0.00	0.00
	EH															
Winter	AQ	95.16	0.35	-15.37	86.85	201.64	57.15	597.33	4.99	94.85	0.15	3.96	0.87	39.57	0.00	0.00
	EH															
Spring	AQ	87.18	1.48	-11.82	82.96	208.24	55.70	612.73	13.62	85.58	0.80	3.68	0.87	39.45	0.00	0.00
	EH															

## Discussion

Fatty acids are influenced by environmental factors such as temperature, light, pH, salinity and nutrients (Aknin *et al.*, 1990; Dembitsky *et al.*, 1991). The temperature is one of the most important environmental factors that influence algal growth rate, cell size, biochemical composition and nutrient requirements (Juneja *et al.*, 2013). Kalacheva *et al.* (2002) stated that, lipid composition is one of the most commonly observed changes with temperature shift. Moreover, Thompson *et al.* (1992) reported that Myristic acids (14:0) increased with higher temperature while PUFA were consistently higher at lower temperature. The reported data for the present study in E.H. followed exactly Thompson *et al.* (1992), where all algae showed the highest PUFA content during winter. In addition, Myristic acid in *C. mediterranea* and *C. officinalis* in E.H. showed the highest concentration during summer except *U.*

*fasciata* which showed inverse pattern. This could be interpreted based on the findings of Converti *et al.* (2009) who found that the effect of temperature on lipid composition varied specifically. Concerning the fatty acid content in algal species in A.Q., it showed different patterns in contrast to Thompson *et al.* (1992). Statistically, fatty acids groups were insignificantly correlated with temperature except the total fatty acids  $\Sigma$ FAs in *C. officinalis* in A.Q. Bay ( $r = -0.97$ ).

Another important factor is pH, since it determines the solubility and availability of  $\text{CO}_2$  and essential nutrients and because it can have a significant impact on algal metabolism (Chen and Durbin, 1994). Alkaline pH increases the flexibility of the cell wall, resulting in an increase in triglyceride accumulation but a decrease in membrane-associated polar lipids (Guckert and Cooksey, 1990). On the other hand, acidic conditions can alter nutrient uptake (Gensemer *et al.*, 1993). Tatsuzawa *et al.* (1996) observed that acidic conditions cause an increase in

saturated fatty acid content, which reduces membrane fluidity and inhibits high proton concentrations. However, in the present study, pH lied in the alkaline side at both sites and it was found that pH was only positively correlated with SFA in *C. mediterranea* in E.H., which antagonize with Tatsuzawa *et al.* (1996).

Salinity is another factor that alters the biochemical composition of algal cells. Exposing algae to lower or higher salinity levels than their natural levels can change growth rate and alter composition such as lipid content. Xu and Beardall (1997) found that a species of *Dunaliella* exhibited an increase in SFA and MUFA fatty acids with an increase in NaCl concentration. In the present study, salinity influenced positively the MUFA in *U. fasciata* in both sites A.Q. and E.H. ( $r = 0.97$  and  $0.94$ ,  $p \leq 0.05$ ), respectively, which agreed with the results of Xu and Beardall (1997), whereas it influenced negatively the SFA in *P. capillacea* ( $r = -0.93$ ,  $p \leq 0.05$ ).

Nitrogen is an important macronutrient for growth and metabolism of algal cells. It is a fundamental element for the formation of proteins and nucleic acids (Harris, 1986) and is available in seawater in the form inorganic nitrogenous compounds (nitrite, nitrate and ammonia). However, Hu (2004) reported that nitrogen accounts for 7-20% of cell dry weight. Inorganic nitrogen taken up by algae is rapidly assimilated into biochemically active compounds and recycled within cells to meet changing physiological needs (Vergara *et al.*, 1995). Major effects of nitrogen deficiency include the enhanced biosynthesis and accumulation of lipids (Demirbas, 2010) and triglycerides (Stephenson *et al.*, 2010) with a concomitant reduction in protein content (Heraud *et al.*, 2005). This, in turn, results in a higher lipid/protein ratio at the expense of growth rate (Converti *et al.*, 2009). In the present study, the nitrogen content in seaweeds was lower in the two sites than the range of Hu (2004), ranging from 0.67 to 3.77% in A.Q. and from 0.71 to 3.50% in E.H. However, nitrogen in tissue was positively correlated with  $\Sigma$ FFA in *C. officinalis* in A.Q. and E.H. and with  $\Sigma$ MUFA in the same species in E.H. Concerning the inorganic nitrogen; nitrate was positively correlated with  $\Sigma$ MUFA and  $\Sigma$ PUFA in *C. officinalis* in A.Q. and negatively correlated with  $\Sigma$ SFA and  $\Sigma$ FFA in *U. fasciata* and with  $\Sigma$ PUFA in *C. mediterranea*, whereas nitrite influenced only negatively  $\Sigma$ MUFA in *U. fasciata* in E.H.

Phosphorus is another important macronutrient, which is available as reactive phosphate in sea water.

It is required for normal growth and development of algal cells (Hu, 2004). Also, it is a key component of phospholipids and an integral part of essential molecules such as ATP and a part of the backbone of DNA and RNA (Harris, 1986). It has been shown that phosphorus, rather than nitrogen, is the primary limiting nutrient for algae in many natural environments (Larned, 1998). Reitan *et al.* (1994) reported that phosphorus limitation leads to accumulation of lipids and reduces the synthesis of n-3 PUFA. According to the Björnsäter and Wheeler's classification (1990) of macroalgal nutrient status based on N:P ratio of tissues, a N:P ratio  $<16$  indicates N-limitation; a N:P ratio 16-24 indicates N and P sufficiency (no limitation) and N:P  $>24$  indicates P-limitation. Applying this classification to our data, we could conclude that they are N-limited in both sites, since in general the ratios in A.Q. did not exceed 8:1, except in *C. mediterranea* during spring (16.79). On the other hand, this ratio in E.H. did not exceed 7.5, except in *C. officinalis* (11.2) during spring. Nascimento *et al.* (2014) recorded a higher range of N:P ratio in tissue from 10:1 to 34:1 with absence of seasonal variations of the two nutrients. The seasonal and spatial variations in tissue nutrients were observed in the present study, with the maximum value of N and P recorded in A.Q. during autumn and winter and in E.H. during winter and spring, respectively. Phosphorus was positively correlated with  $\Sigma$ SFA and  $\Sigma$ FFA of *U. fasciata* and with  $\Sigma$ FFA in *C. officinalis* in A.Q., indicating the role of phosphorus in the synthesis of fatty acids. On the other hand, the inorganic phosphorus was correlated positively with  $\Sigma$ PUFA in *U. fasciata* and *P. capillacea* in A.Q., whereas, inorganic phosphorus had no any influence on the fatty acids groups in the algal species of E.H.

Noticeably, the present study showed great seasonal, specific and spatial variations for lipid profile. Thus, screening for the optimum value for biodiesel properties before its processing is very important to define the suitable site, season and species to obtain the optimum biodiesel.

It was observed that the fuel properties of biodiesel play a significant role in the combustion process and delay period, which in turn affect the engine performance (Sivaramakrishnan and Ravikumar, 2012). The Cetane Number (CN) of the fuel is such an important parameter which is responsible for the delay period. A fuel of higher cetane number gives lower delay period and provides smoother engine operation. Both the British biodiesel standard EN (14214) and

the American biodiesel standard ASTM D6751-02 stated that a cetane number CN should be ( $\geq 51$  and  $\geq 47$ ), respectively. In fact, biodiesel has a higher CN than petrodiesel because of its higher oxygen content (Sivaramakrishnan and Ravikumar, 2012). In the present study, the biodiesel has a CN (based on molecular weight and degree of unsaturation) that exceeds 52 and its value varied within species and seasons. The highest CN was recorded in *C. officinalis* in E.H. during winter (77.96), whereas the lowest CN (55.70) was recorded in *P. capillacea* in A.Q. during spring. Factors that affect the CN in the biodiesel are the number of carbon atoms of the original fatty acids, the number of double bonds, the high amounts of SFA in addition to the type of feed stock (Gopinath *et al.*, 2009; Tesfa *et al.*, 2010).

The degree of saturation plays a significant role in fuel properties. It has been suggested that, the higher the degree of unsaturation of the FAMES of a biodiesel, the higher the tendency of the biodiesel to oxidize (Islam *et al.*, 2013). It also influences largely the kinematic viscosity ( $\nu$ ), Cold Filter Plugging Point (CFPP) and density ( $\rho$ ) (Moser, 2014). The shorter and more unsaturated chains increase the viscosity and flow characteristics at low temperatures, which are undesirable characteristics of fuels. To obtain a biodiesel with appropriate quality characteristics, an appropriate ratio between saturated and unsaturated fatty acids should be maintained (Knothe, 2005). In the present study, the percentage of saturated FAs was almost high, against MUFA% and PUFA%. The maximum percentage was recorded in *U. fasciata* (93.27%) in A.Q. during winter, whereas, the lowest percentage was recorded in *C. mediterranea* (3.83%) in A.Q. during spring.

Kinematic viscosity is a measure of the internal friction or resistance of oil to flow and is the most important physical property of biodiesel since it affects the operation of fuel injection equipment, particularly at low temperatures when the increase in viscosity affects the fluidity of the fuel. High viscosity leads to poorer atomization of the fuel spray and less accurate operation of the fuel injectors (Sivaramakrishnan and Ravikumar, 2012). Kinematic viscosity limits are set by international biodiesel standards to be in the ranges of 1.9-6.0 mm<sup>2</sup> s<sup>-1</sup> and 3.5-5.0 mm<sup>2</sup> s<sup>-1</sup> as per ASTM6751-02 and EN 14214, respectively (Islam *et al.*, 2013). In the present study, the produced biodiesel from all macroalgal species were in the prescribed viscosity range with 3.68-5.54 mm<sup>2</sup> s<sup>-1</sup>, therefore meeting both standards. Sivaramakrishnan and Ravikumar (2012) recorded

viscosity values of biodiesel between 3.6 and 5.7 mm<sup>2</sup> s<sup>-1</sup>, whereas, Islam *et al.* (2013) recorded in some microalgal species viscosity range between 3.44 and 4.20 mm<sup>2</sup> s<sup>-1</sup>.

It is noteworthy to mention that, the fuel injection system supplies fuel by volume not by mass which means denser biodiesel will be injected with greater mass into the combustion chamber, consequently affecting the stoichiometric ratio of air and fuel (Ng *et al.*, 2012). Therefore, density ( $\rho$ ) is another important parameter for biodiesel quality, for which a standard value has been set at 0.86-0.90 g cm<sup>-3</sup> according to EN 14214. Our results are consistent with the standard value, where the density of produced biodiesel in the collected algae ranged between 0.86 and 0.89 g cm<sup>-3</sup> except for *C. officinalis* during summer at A.Q. where the extracted biodiesel recorded a density of 0.95 g cm<sup>-3</sup>.

The Heat of combustion refers to the measure of energy content in the fuel (Sivaramakrishnan and Ravikumar, 2012). Heating value of fuels is an important measure of its releasing energy for producing work. According to Sivaramakrishnan and Ravikumar (2012), the lower heating value of biodiesel is attributed to the decrease in engine power and the energy content of oils which depends on the place where they are grown, the season, the composition and other factors. In fact, the HHV values in the present study (39.41-42.98 MJ kg<sup>-1</sup>) were closed to that of biodiesel blend *Jatropha* (39.17-41.52 MJ kg<sup>-1</sup>) and blend *Karanja* (35.9-41.5 MJ kg<sup>-1</sup>) fuel, where they exceeded the values of oils obtained from some crops (Sivaramakrishnan and Ravikumar, 2012) and the values of some microalgae (36.6-40.4 MJ kg<sup>-1</sup>) (Islam *et al.*, 2013). Ramirez-Verduzco *et al.*, (2012) found that the range of regular biodiesel was between 39.8 and 40.4 MJ/kg, which is normally 10 to 12% less than obtained for petroleum-derived diesel (46 MJ kg<sup>-1</sup>).

The Iodine Index (IV) refers to the tendency of biodiesel to react with oxygen at room temperature. The low iodine index indicates less susceptibility to oxidation by oxygen (Duarte and Maugeri, 2014), which is desirable for biodiesel. According to the biodiesel standard EN (14214), iodine value is  $\leq 120$  gI<sub>2</sub>100g<sup>-1</sup>fat. In the present study, all IV values are within the limits of the standards. It ranged between minimum 6.49 gI<sub>2</sub>100g<sup>-1</sup>fat for *U. fasciata* in A.Q. during autumn to maximum 86.85 gI<sub>2</sub>100g<sup>-1</sup>fat for *P. capillacea* in A.Q. during winter.

Regarding C18:3 wt% and the weight percentage of fatty acid esters containing number of double bonds

higher than 4, they are of the most important property that govern the biodiesel stability. A limit of 12 wt% for this FAME has been set in the European B100 biodiesel standard (EN 14214), which also limits the amount of FAMES with four or more double bonds to 1 wt% (Barabás and Todoruț, 2011). Therefore, polyunsaturated fatty acid content of the biomass, as well as the weighted degree of unsaturation developed by Ramos *et al.* (2009) and the predictive fuel stability calculated from only two FAME contents, linoleate (C18:2) and linolenate (C18:3) can serve as indirect estimates of biodiesel oxidative stability. All algae from this study were much lower than the EN14214 threshold of 12 wt% C18:3. Whereas, the values of wt% of  $\geq$  four double bonds complied with that of biodiesel standard EN (14214) ( $\leq 1$ ), except the values of *U. fasciata* in E.H. during summer was (4.31). The results recorded revealed that the produced biodiesel of all algal species during all seasons at both sites is of highest oxidative stability.

On the other hand, the values of Cold Filter Plugging Point (CFPP) in the present study complied almost with the value set by biodiesel standard EN (14214), ranging from  $\leq 5$ /  $\leq -20$ , with some exceptions, especially the highly exclusive values in *C. officinalis* in the E.H. during autumn (109.85) and winter (163.91). In fact, Islam *et al.* (2013) mentioned that CFPP is of low importance in subtropical and tropical climates. It is noteworthy to mention, that our study region is considered as subtropical, therefore, the weighting of the CFPP parameter was of less importance than other criteria. Based on all these biofuel criteria, the green alga *U. fasciata* collected from A.Q. during winter was the most suitable choice for biodiesel production.

## Conclusion and Recommendations

From the present study it is concluded that, the green alga *Ulva fasciata* collected from Abu Qir Bay during winter can be considered as the most suitable and promising source for biodiesel production. Regarding the biodiesel produced from *Corallina officinalis*, it showed the highest biodiesel quality but its quantity is very low. So it can be used as a blending to the petroleum diesel to increase its quality, oxidative stability and octane number.

Based on all the obtained results we can recommend the following:

- In order to produce a high quality and a large quantity of biodiesel it is recommended to cultivate *Ulva fasciata* during winter

- In order to produce biodiesel of very high quality to be used as an additive to improve petroleum diesel quality, it is better to cultivate *Corallina officinalis* during winter.

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## Author's Contributions

All authors equally contributed in this work.

## Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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