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Existence, seasonal variations and chlorophyll content of some macroalgal flora inhabiting Al Mutrad, Surman, and Sabratha regions on the western Libyan coast.

Aml E. Elnaas[1], Hana A. Abohbell[1], Halemah M. Al-khojah[1], Ola A. Sadek[1], Hoida Ali Badr[2]

1- Botany Department, Faculty of Science, Sabratha University, 250 Sabratha, Libya.
2- Botany & Microbiology Department, Faculty of Science, Sohag University, 82524 Sohag, Egypt.
halimahAL-koja@sabu.edu.ly

Abstract

Studying the algal flora inhabiting the Libyan coast, especially the western Libyan coast, is very neglected by researchers as well as their contents of bioactive compounds. In our study ten algal species belonging to the three main algal divisions (Chlorophyta, Rhodophyta & Phaeophyta) were investigated in three different regions of the western Libyan coast (Al Mutrad, Surman, and Sabratha) seasonally during the period from September 2021 to Jully 2022, the study showed that, during the study period Sabratha region only contained all the ten algal species, while the red alga, J. rubens, was also recorded in Al-Mutrad region, and L. obtusa was also recorded in Surman region. There was no algal flora belonging to chlorophyta were detected in each of Al-Mutrad or Surman regions during the study period. Seasonal environment changes resulted in variation of the algal distribution where some algae were existed only during one season of the year, such as Cladophora, H. filicina, and S. vulgare while J. rubens & P. pavonica were recorded throughout the whole year. Chlorophyll content also was estimated in different algal species and results indicated that brown alga P. pavonica (from Sabratha in summer) had the highest chlorophyll content (20.53 µg/ml) followed by the red alga J. rubens (from Sabratha in summer) (16.53 µg/ml), the high chlorophyll content indicated the economic importance of the investigated marine algal

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species and need further study to can be used in pharmacological or cosmetic fields.
Keywords: Al- Mutrad, chlorophyll, marine algae, Sabratha, Surman, western Libyan coast.

الوجود والتغيرات الموسمية ومحتوى الكلوروفيل في بعض نباتات الطحالب الكبيرة التي تعيش في مناطق المطرد وصرمان وصبراتة على الساحل الغربي الليبي.

أمل الطاهر محمد النعاس[1]، هناء المهدي أبوحبيل[1]، حليمة محمد الخوجة[1]،علا عبدالسلام الصادق[1]، هويدا علي بدر بدر[2]

1- قسم النبات، كلية العلوم، جامعة صبراتة، 250 صبراتة، ليبيا.
2- قسم النبات والأحياء الدقيقة، كلية العلوم، جامعة سوهاج، 82524 سوهاج، مصر

الملخص

إن دراسة الطحالب التي تتواجد في الساحل الليبي، وخاصة الساحل الغربي الليبي، لم تحظى بالاهتمام الكافي من قبل الباحثين وكذلك محتوياتها من المركبات النشطة بيولوجيًا. في دراستنا، تم توثيق عشرة أنواع من الطحالب تنتمي إلى أقسام الطحالب الرئيسية الثلاثة (الطحالب الخضراء و الطحالب البنية والطحالب الحمراء في ثلاث مناطق مختلفة من الساحل الغربي الليبي (المطرد و صرمان و صبراتة) موسمياً خلال الفترة من سبتمبر 2021 إلى جولي 2022، أظهرت الدراسة أنه خلال فترة الدراسة، احتوت منطقة صبراتة على جميع أنواع الطحالب العشرة ، بينما تم تسجيل الطحالب الحمراء، *J. rubens* في منطقة المطرد، كما تم تسجيل*L. Obtusa* في منطقة صرمان و لم يتم اكتشاف أي من الطحالب الخضراء في كل منطقة من مناطق المطرد و صرمان خلال فترة الدراسة. أدت التغيرات البيئية الموسمية إلى تباين توزيع الطحالب حيث كانت بعض الطحالب موجودة فقط خلال موسم واحد من السنة، مثل *Cladophora, H. filicina, and S.vulgare*

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بينما تم تسجيل J. rubens & P. pavonica على مدار العام بأكمله. كما تم تقدير محتوى الكلوروفيل في أنواع مختلفة من الطحالب وأشارت النتائج إلى أن الطحالب البنية *P. pavonica* (من صبراتة في الصيف) كان يحتوي على أعلى محتوى من الكلوروفيل (20.53 آن غرام/مل) تليها الطحالب الحمراء *J. rubens* (من صبراتة في الصيف) (16.53 غ/مل)، يشير المحتوى العالي من الكلوروفيل إلى الأهمية الاقتصادية لأنواع الطحالب البحرية التي تم فحصها ويحتاج إلى مزيد من الدراسة لاستخدامها في المجالات الدوائية أو التجميلية.

الكلمات المفتاحية: الكلوروفيل، الطحالب البحرية، صبراتة، صرمان، المطرد، الساحل الغربي الليبي.

Introduction

Previous studies on the Mediterranean coast: Several lists were drawn up that included marine algae found in the Mediterranean Sea, and among these lists was a list of Fucophyceae found on 16 regions on the Mediterranean Sea coast and included 265 species (Ribera *et al.*, 1992). In addition, a list of green algae that included 214 species (Gallardo *et al*., 1993), and a list of red algae belonging to (Ceramiales, Rhodophyceae) that included 271 species (Gómez *et al*., 2001).

Several reference lists of marine algae were developed in Morocco, including a list of marine algae belonging to Chlorophyceae (Benhissoune *et al*., 2001), the second list includes brown algae in the Mediterranean Sea (Benhissoune *et al*., 2002a), and the third list included algae belonging to red algae, with the exception of algae belonging to the order Ceramiales, (Benhissoune *et al*., 2002b). As for the fourth list, it included red algae belonging to the order Ceramiales (Benhissoune *et al*., 2003).

Another study was conducted in Turkey in the Samsun region, where 20 genera of blue-green algae were recorded, 106 genera of red algae, Gelidium pusillum was recorded as a new species, 27 genera of brown algae, 21 genera of green algae, and two genera of seaweeds (Aysel *et al*., 2008).

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In 2010, the scientist Furnari developed a list of marine algae that existed on the Italian coast from 1950 to 2000. This list included 944 genera, of which 46 genera belong to blue-green algae, 534 genera belong to red algae, 214 genera belong to brown algae, and 150 genera belong to green algae.
In the context of looking for new species in the Mediterranean coast, studies continued, through which some algal species were recorded for the first time on the beaches of some areas bordering the Mediterranean Sea. Among these studies, the first appearance of Culerpascal pelliformis in Lubnan was recorded (Hamel, 1930) and in Palestine (Rayss, 1941), the same alga was also recorded in Syria (Mayhoub, 1976).
During the year 2002, the appearance of Ceramium bisporum of the red algae was recorded for the first time in the Mediterranean Sea (Sartoni & Boddi, 2002), and eight species of the genus Hypnea were recorded in the Mediterranean Sea (Furnari, 1984; Boudouresque and Verlaque, 2002).
Previous studies on the Libyan coast: The first list of Libyan algae was included in an Italian list drawn up by Ardisson in 1893 and a second list was documented that includes Libyan marine algae, where 15 genera and 29 species of green algae, 19 genera and 34 species of brown algae, 76 genera and 112 species of red algae, two genera three species were recorded of blue-green algae. (Nizamuddin *et al*., 1978).
The presence of the genus Dictyota on the Libyan coast was also studied (Nizamuddin, 1981). The distribution of the genus Flabellia, which belongs to Caulerpales (Chlorophyta) on the coasts of the Mediterranean Sea was studied, and this study included some areas of the Libyan coast (Nizamuddin, 1987). The presence of Cladophora psisgerloffii on the Libyan coast was also studied (Nizamuddin, 1988).
In another study that included a survey of the eastern part of the Libyan coast in the area extending from Benghazi to Tobruk, where several samples of Stypopodium tubruqense of the brown algae were collected, and this alga was classified as a new species in the Mediterranean coast (Nizamuddin & Godeh, 1989). After that, some

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lists of Libyan algae were recorded by Nizamuddin & Godeh in the years (1990 a, b, c, 1993), and another list was recorded by Nizamuddin & EL-Menifi in 1993; in this list, a new species of the genus Codium was recorded on the east coast of Libya in Tobruk and this was Codium tobrukense. Also, 168 species of marine algae were collected on the eastern Libyan coast (Godeh *et al*., 1992).
The first appearance of the red alga Nemalion helminthoides was recorded on the Libyan coast in the intertidal zone of the Khoms region in March 2005 (Shtewi & Fetory, 2007).
Chlorophyll content of marine algae: Marine algae are rich sources of various bioactive compounds with many biological activities (Barrow & Shahidi, 2007; Wijesekara & Kim, 2010;). Natural pigments have drawn a lot of interest among the functional components derived from marine algae. The biological benefits of these natural pigments include antioxidant, anticancer, anti-inflammatory, anti-obesity, fascist-angiogenic, and neuroprotective properties. As a result, various natural pigments extracted from marine algae have attracted much attention in the fields of pharmacology, food, and cosmetic (Pangestuti & Kim, 2011). Of marine algal pigments, Chlorophyll or its derivatives were reported to be used as a photodynamic agent in tumor or cancer therapy (Brandis *et al*. 2006).
Our study aimed to investigate the existence and the seasonal variation of some marine algal flora inhabiting three different regions of the western Libyan coast (Al Mutrad, Surman, and Sabratha) and estimation of their chlorophyll content as a very important natural pigment.

Materials & methods

The study was conducted from the September 2021 to July 2022. The coastal study sites in Surman, Sabratha and Al-Mutrad regions were visited several times before starting the work, in order to determine the suitable sites for collecting algal species.

Study areas

The geographical site of the study areas is illustrated in Figure (1), Al-Mutrad lies on the Meditrranean coast about 55 km (34 mi) west

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of Tripoli (the country,s capital), Surman situated about 60 km (37 mi) to the west of Tripoli and Sabratha located about 70 km (43 mi) west of Tripoli.

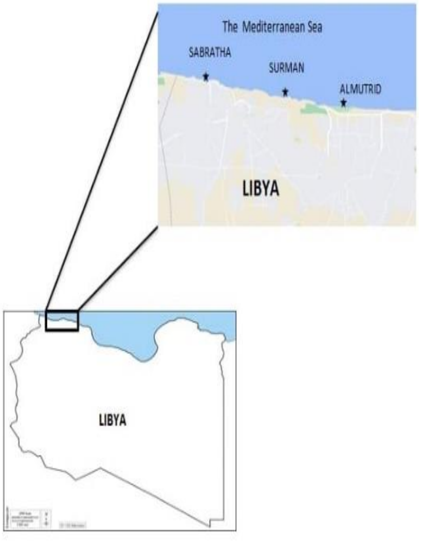

Figure 1. Maps showing the study areas

Equipment and methods used for collection:
Algal samples were collected in the intertidal zone and throughout the year (summer - autumn - winter - spring) from once to twice a month at a distance of half a meter, using polyethylene bags / gloves / jars with a wide mouth and different sizes / knife / Scissors / scraper / boots / note book and Formalin at a concentration of 4-5% in sea water (Nizamuddin & Godeh, 1989).

Identification of algae and preservation of samples
Algae were initially classified in the field of study according to color, after that, algae were identified and classified in the laboratory according to each of the algal divisions that follow them, sections were taken for some types of algae manually and investigated using light microscope, some references related to classification, including (Dawson, 1962; Hiscock, 1984; 1986;

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Taylor, 1972; Verlaque *et al*., 2000; Aleem, 1993) were used to assist in the process of identification of algal species.
Several photos of the collected samples were also taken with a Nikon COOLPIX L19 digital camera& phone camera, see figure (2).

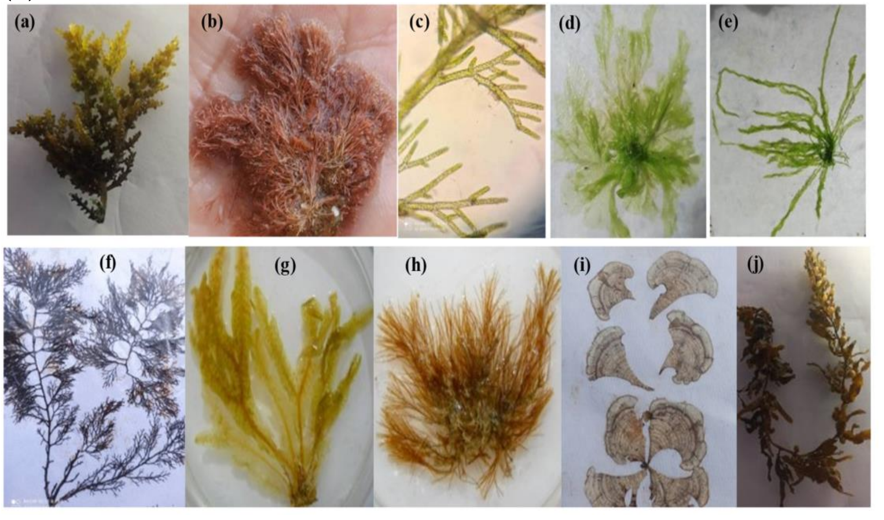

Figure 2. Images of the collected macroalgae (a) Laurencia obtusa, (b) Jania rubens, (c) Cladophora sp., (d) Ulva lactuca, (e) Enteromorpha linza, (f) Cystoseira barbata, (g) Dictyopteris membranacea, (h) Halopteris filicina, (i) Padina pavonica, (j) Sargassum vulgare.

Es Preserving samples
The samples were washed well with tap and distilled waters to remove impurities, epiphytes, sand grains then placed on herbarium sheets in a container of water, and the samples were spread well on the herbarium sheets. In the case of dense algae, scissors were used to reduce the branching, and the water was drained from the sample by placing it diagonally and covering the sample with a piece of cloth so that the sample was between the herbarium sheet and the piece of cloth then the samples were placed in an oven at a temperature of 50 ^{o}C until the samples dried well, after drying well the samples were kept at -20 ^{o}C for future use. Some samples were preserved in a formalin solution at a concentration of 4-5% in sea

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water away from sunlight, which causes the dyes to break down, other samples were kept freshly in refrigerator to be used for taxonomic identification later.**timation of chlorophyll**

Extraction of chlorophyll was done according to (Arnon, 1949), After drying the samples they were grinded to fine powder, one gram of powdered sample was extracted in 20 ml of 80% acetone then centrifuged at 6000 rpm for 15 minutes, then the supernatant was transferred to another tube and the procedure was repeated 4-5 times until the residue becomes colorless, the absorbance of the filtrate was measured at 645 nm and 663 nm and the solvent (acetone) was used as blank.

The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated according to Rajalakshmi and Banu, 2015 using the following equations:

$$\text{Total Chlorophyll: } \frac{20.2(A_{645}) + 8.02(A_{663})}{1000} \times V$$

$$\text{Chlorophyll a: } \frac{12.7(A_{663}) - 2.69(A_{645})}{1000} \times V$$

$$\text{Chlorophyll b: } \frac{22.9(A_{645}) - 4.68(A_{663})}{1000} \times V$$

Where: A645 is Absorbance at wave length =645
A663 is Absorbance at wave length =663
V = Volume of extract (ml)

Results and discussion

Classification& identification of the collected algal samples:

Ten algal species were collected from the three different sites and identified according to (Dawson, 1962; Taylor, 1972; Verlaque *et al*., 2000), and the data shown in table (1) and figure (2). In table (1), we noticed that two species of red algae, three species of green algae, and five genera of brown algae were recorded within the study areas and throughout the year, however, the presence of these

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algae differed from one region to another; where Sabratha region only contained all the ten algal species. The red alga, Jania rubens, was also recorded in the Al-Mutrad region, Laurencia obtusa and Padina pavonica were also recorded in the Surman region, there was no algal flora belonging to chlorophyta were detected in each of Al-Mutrad or Surman regions during the study period.
Although the studied species in the present work were recorded from different regions of the Mediterranean before (Aleem, 1993; Gómez et al., 2001; El-Shoubaky, 2005), they have not been previously recorded in these regions.

Table 1. The recorded algal species in the three regions

Algal species	Al-Mutrad	Surman	Sabratha
1- Red algae			
Jania rubens	+	-	+
Laurencia obtusa	-	+	+
2- Green algae			
Cladophora sp.	-	-	+
Enteromorpha linza	-	-	+
Ulva lactuca	-	-	+
3- Brown algae			
Cystoseira barbata	-	-	+
Dictyopteris membranacea	-	-	+
Halopteris filicina	-	-	+
Padina pavonica	-	+	+
Sargassum vulgare	-	-	+

The nature of algal presence, and the nature of the location in which they are located.
Benthic marine macroalgae require a suitable substratum to attach themselves and survive; these substrata are diverse, such as rock, sand, mud, vascular plants, animals and even other algae (Galicia-García 2017). Large numbers of marine algae can grow epiphytically on other seagrasses or macroalgae (Rindi & Guiry, 2004). In the present study, the red alga *Jania rubens* was recorded as epiphyte and also as epilithic alga, while all of the other species were recorded as epilithic algae.

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In table (2), All the algal species in our study were collected from rocky beaches, with the exception of *Laurencia obtuse* and *Padina pavonica*, which were collected from a rocky beach and some samples from a rocky sand beach, and this was in accordance with what was mentioned in (Shtewi, 2015).

Table 2. The nature of algal presence and the nature of the location in which they are located

Algal species	the nature of algal presence	The nature of the location
1- Red algae		
Jania rubens	Epilithic&Epiphytic on other algae	Rocky
Laurencia obtusa	Epilithic	Rocky& rocky sand
2- Green algae		
Cladophora sp.	Epilithic	Rocky
Enteromorpha linza	Epilithic	Rocky
Ulva lactuca	Epilithic	Rocky
3- Brown algae		
Cystoseira barbata	Epilithic	Rocky
Dictyopteris membranacea	Epilithic	Rocky
Halopteris filicina	Epilithic	Rocky
Padina pavonica	Epilithic	Rocky& rocky sand
Sargassum vulgare	Epilithic	Rocky

Seasonal variations of algal species

From Table (3), it is clear that each of *Jania rubens* alga belonging to the red algae and *Padina pavonica* alga belonging to the brown algae were investigated throughout the whole year, and this was in accordance with (Shtewi, 2015) and this may be due to the

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adaptation of these species to seasonal environment changes. The alga *Cystoseira barbata* and *Laurencia obtusa* were found throughout all seasons except for the summer season. While some algae were existed only during one season of the year, such as *Cladophora sp.* which was recorded only in summer, also *Halopteris filicina* was found in spring only, and *Sargassum vulgare* was investigated in winter only.

Table 3. Detected algal species in the different seasons of the year

Algal species	Summer	Autumn	Winter	Spring
1- Red algae				
Jania rubens	+	+	+	+
Laurencia obtusa	-	+	+	+
2- Green algae				
Cladophora sp.	+	-	-	-
Enteromorpha linza	-	-	+	+
Ulva lactuca	+	-	+	-
3- Brown algae				
Cystoseira barbata	-	+	+	+
Dictyopteris membranacea	-	-	+	+
Halopteris filicina	-	-	-	+
Padina pavonica	+	+	+	+
Sargassum vulgare	-	-	+	-

Estimation of chlorophyll content

Plant chlorophyll concentration is an important parameter that is often studied as a measure of chloroplast content; the production of chlorophyll is largely dependent on sunlight penetration and is the primary source of energy for plants (Srichaikul *et al.*,

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2011).Chlorophyll content was estimated in some collected algae and the data shown in table (4).

Table 4: Chlorophyll content of algal species

Algal species	Place of collection&season of collection	Chl. a (µg/ml)	Chl. b (µg/ml)	Total chlorophyll (µg/ml)
Jania rubens	Al-Mutrad (summer)	2.04	1.19	3.51
Cladophora sp.	Sabratha (summer)	5.96	4.51	11.22
Padina pavonica	Surman (winter)	5.34	3.37	9.89
Dictyopteris membranacea	Sabratha (winter)	7.56	5.01	12.33
Sargassum vulgare	Sabratha (winter)	0.99	0.54	1.05
Laurencia obtusa	Sabratha (winter)	1.32	1.67	2.99
Jania rubens	Sabratha (summer)	5.88	10.64	16.53
Padina pavonica	Sabratha (summer)	7.97	8.73	20.53
Enteromorpha linza	Sabratha (winter)	3.47	0.89	5.00
Halopteris filicina	Sabratha (summer)	9.12	4.09	13.21
Laurencia obtusa	Surman (winter)	1.20	1.61	2.88
Ulva lactuca	Sabratha (summer)	0.69	0.73	1.35
Cystoseira barbata	Sabratha (summer)	6.22	2.11	8.33

The data reviled the difference in the chlorophyll content of the collected algal species according to the collection area. We found that *Jania rubens* alga differed in their content of chlorophyll a and chlorophyll b, and also total chlorophyll according to the collection area and season of collection The sample collected from al-Mutrad region had chlorophylls a and b, and total chlorophyll (2.04-1.19-3.51), respectively, while the sample collected from the Sabratha

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region had chlorophylls a, b, and the total chlorophyll (5.88-10.64-16.53), respectively, as we noticed a high content of chlorophylls in the Sabratha Sea sample, and this may be due to the richness of the Sabratha Sea in organic materials and basic minerals needed to build the body of algae as well as chlorophylls (data not shown), also the difference in the rate of incoming solar radiation according to the difference in the region. This also applies to the alga *Padina pavonica*, where its content of chlorophylls a and b, and the total chlorophyll were (5.34- 3.37- 9.89), respectively for the sample collected from Surman, while for the sample collected from Sabratha they were (7.97-8.73-20.53), respectively. The large difference in the content of chlorophylls between the two samples confirms the previous expectation, similarly the case for the alga Laurencia obtusa, where the content of chlorophylls a, b and the total chlorophyll were (1.20-1.61-2.88) respectively for the Surman sample and (1.32-1.67-2.99) respectively for the Sabratha sample.
In total, we noticed a higher content of chlorophyll a than b for most algal species, and that brown alga *Padina pavonica* had the highest chlorophyll content, followed by the red alga *Jania rubens* then *Halopteris filicina* and *Dictyopteris membranacea* from brown algae, then *Cladophora sp* alga followed by Ulva lactuca from green algae and finally *Sargassum vulgare* had the least chlorophyll content as shown in figure (3).
A study by (Rajalakshmi& Banu, 2015), when estimating both chlorophyll a and b for a total of (9) medicinal plants showed that the concentration of chlorophylls was very different between plant species and genera, and that the highest chlorophyll content was found in mimosa. pudica than other medicinal plants which are used in study.
In a study (Oo *et al*, 2017) to estimate the chlorophyll a and b content of the two microalgae species, Chlorella and Nannochloropsis, it was found that Nannochloropsis alga had more chlorophyll a and b content (9.66 µg/ml, 21.24 µg/ml) respectively, than those of Chlorella which had chlorophyll a & b content (8.45 µg/ml, 4.33 µg/ml) respectively.

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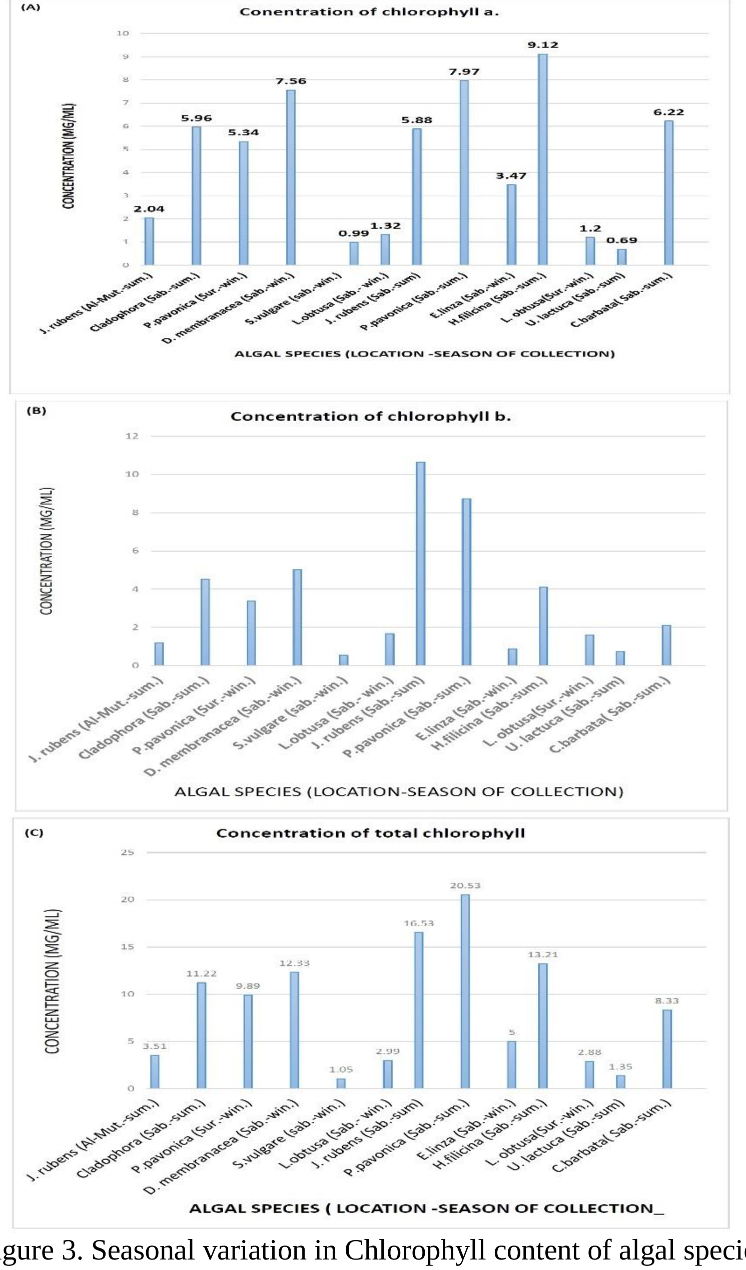

Figure 3. Seasonal variation in Chlorophyll content of algal species collected from different regions: (A): chlorophyll a content; (B): chlorophyll b content; (C): total chlorophyll content.

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In a study to estimate the content of chlorophyll a and b (Arar, 1997) that included 7 unialgal cultures from (Pycnacoccus provasolii, Rhodomonas salina, Selenastrum capricornitum, Amphidinium carterae, Dunaliella tertiolecti, Emiliania huxleyi, Phaeodactylum tricornutum) and the study showed that the highest rate of chlorophyll a was in the alga Amphidinium carterae (7.40 %), and the highest rate of chlorophyll b was in the alga Pycnacoccus provasolii which was (4.45%).

Conclusion

Ten different algal species were recorded in Al-Mutrad, Surman & Sabratha regions on the western Libyan coast in different seasons of the year, most of these species contain high amounts of chlorophyll, the high chlorophyll content indicated the economic importance of the investigated algal species but need further study to can be used in pharmacological or cosmetic fields.

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