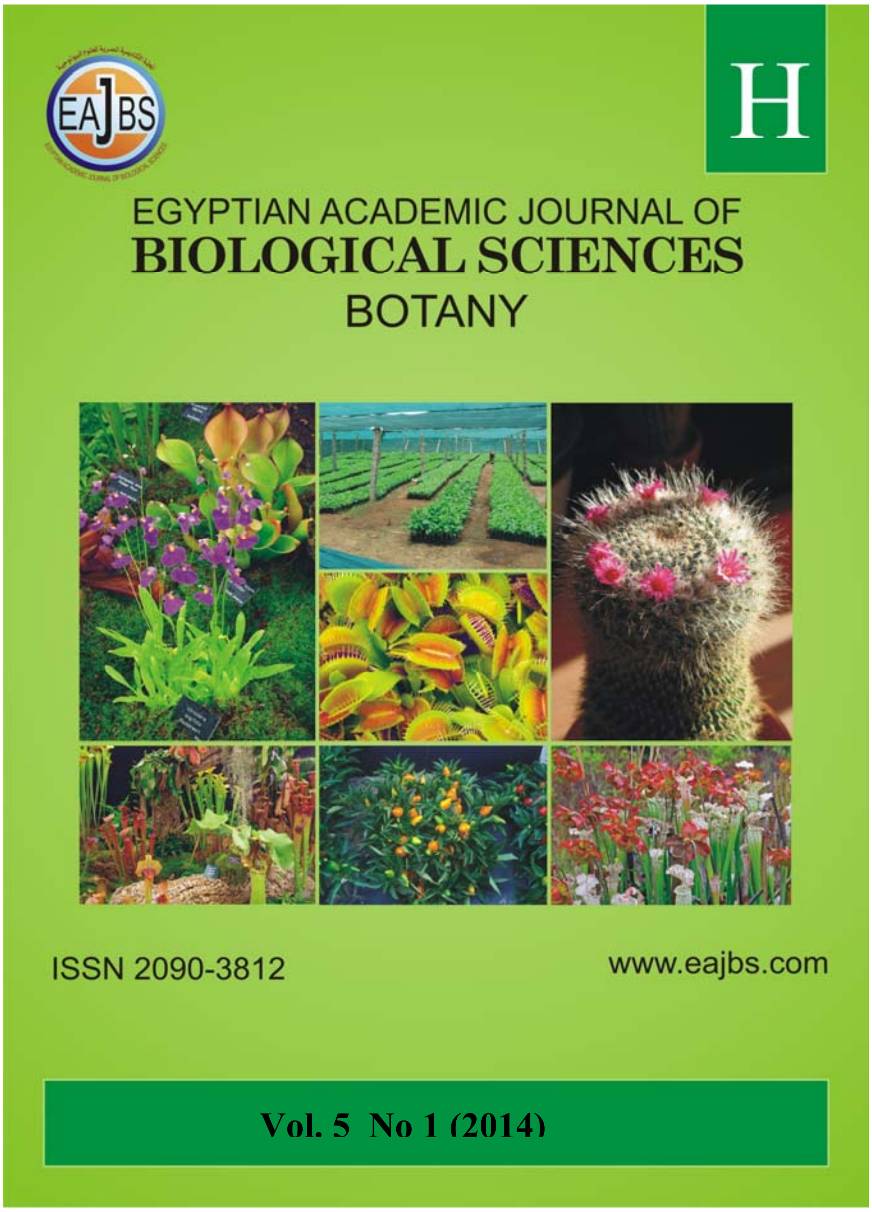

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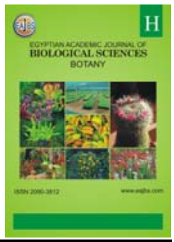

Nutritional evaluation of green macroalgae, *Ulva* sp. and related water nutrients in the Southern Mediterranean Sea coast, Alexandria shore, Egypt

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ABSTRACT

Protein, carbohydrate, lipid, ash, chlorophyll (*a*; *b* , *c* and total chlorophyll), organic carbon and moisture contents of the macroalgae *Ulva* sp. (Chlorophyta) were studied in corresponding to the water nutrient concentrations in the different sites and seasonal variations over one year, 2012. The water and macroalgae samples were collected monthly from rocky and sandy sites, Ras Al-Tin (site A) and El-Muntazah (Site B) along 18.6 Km distance of Alexandria shore, Egypt. The results showed that the studied areas are rich in nutritive elements at high concentrations. As the chemical analysis elucidated that *Ulva* sp. powder was characterized by a high contents of protein (20.7-27.6% DW), lipid (8.7-15.75% DW), carbohydrates (42.31-51.37% DW) and moderate ash content (15.32-20.53% DW), with the peaks of lipid and minerals contents in winter, the peaks of carbohydrates contents in spring, peaks of organic carbon in summer and the peaks of protein and phosphorus contents in autumn. The high nutrients contents of *Ulva* sp. confirm the high nutrient storage capacity of *Ulva* in this area, indicating that, on one hand, it could be used as biofilters in the aquaculture systems and for bioremediation purpose. On the other hand, the proximate composition nominates *Ulva* to be exploited as a good food and energy resource for human; animals and in aquaculture. Also, it could be used in several industries such as food and cosmetics industries, for its high carbohydrate contents. Remarkable seasonal variations were recorded in the contents of the studied parameters in the *Ulva* samples. The N:P and C:P ratios indicated that these algae at the studied areas may experience phosphorus limitation in particular seasons during the annual cycle. Significant positive correlations were noticed between, carbon-nitrogen and carbon-phosphorus as well as between total chlorophyll and both of total phosphorus ($P<0.05$) and total chlorophyll ($P<0.01$).

Key words: green macroalgae, *Ulva* sp., spatial and seasonal influence, biochemical composition, nutritive value of *Ulva* sp.

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INTRODUCTION

Ulva spp. are used as traditional food in many of Asian countries. In Japan, they are included in a variety of dishes such as salads, soups, cookies, meals and condiments as well as a mixed product with other green macroalgaes (Rodríguez *et al*., 2011; Tabarsa *et al*., 2012). The interest in these algae as a novelty food is expanding in the West (Rodríguez *et al*., 2011), and especially in France where they were authorized for human consumption as vegetables (Mabeau and Fleurence 1993; Fleurence 1999). Recently, several studies reported that *Ulva* can be of potential interest for food, development of novel drugs and functional foods, pharmaceutical and agricultural applications (Costa *et al*., 2010; Wijesekara *et al*., 2011).

The nutritional properties of macroalgaes are usually estimated for their chemical composition (Darcy-Vrillon, 1993). Different authors showed that the chemical composition of the macroalgae vary depending on, geographical distribution, habitats, maturity, seasons and the principal environmental conditions, such as water temperature, salinity, light, and nutrients (Kaimoussi *et al*., 2004; Ortiz *et al*., 2006; Messyasz and Rybak 2010). For instance, excessive nitrogen in effluent streams induces changes in the biochemical composition of macroalgae (Faganeli *et al*., 1986; Zhou *et al*., 2006). Moreover, *Ulva* morphology is heavily influenced by age of the thallus, life style, environmental conditions, and nutrient concentrations in their habitats making the recognizing of species by morphological features alone difficult (Messyasz and Rybak 2010; Wolf *et al*., 2012).

The North coast of Egypt, particularly in Alexandria, is rich in macroalgae resources. The green macroalgaes (*Ulva* spp.) are the most abundant in this coastal area yearly (Aleem 1993; Abdallah, 2010). However, the utilization of this macroalgae is very restricted, just as a bait for herbivorous fishes or for the fishes decoration during selling, to communities living in the coastal areas, due to the fact that the knowledge about their nutritional values is absent or at the best still very limited.

For these reasons we proposed to develop this research in order to see the potential for valorization of the *Ulva* sp. algae as human and animal food as well as renewable resource for industrial applications, taking into account this region is characterized by suitable environmental conditions (i.e. sufficient irradiance and temperature) almost year round, free of heavy metals pollutants (Saeed and Moustafa, 2013). Therefore, the present investigation was conducted to evaluate the biochemical composition of *Ulva* sp. macroalgae over one year period, in order to build up extensive information about their nutritional value.

MATERIALS AND METHODS

Area of study

Alexandria city lays on the north coast of Egypt, extending about 32 Km along the coast of the Mediterranean Sea, at latitude 3111.8836 º N and longitude 29,551502ºE. The water and macroalgae samples were collected from two sites namely Ras AL-Tin (A) and AL-Muntazah (B) along 18.6 Km distance, Alexandria coast (Fig. 1).

The site A was selected to represent a polluted region between Eastern Harbor, one of the main Egyptian fishery grounds with an area of 2.53 km^2 and an average depth of 6 m (El-Said and El-Sikaily 2013) and the main Harbor of Alexandria (for export and import goods) as a result of industrial, shipping and/or human activities, while, site B represents unpolluted region (El Muntazah) as described in a previous study (El-Said and El-Sikaily 2013). Site A can also be described as, to some extent, sheltered zone while the site B is fully exposed and affected by the water current from the west to the east.

Fig. 1: Map illustrate the study sites (A and B) (from google earth)

At Ras AL-Tin site, the rocks and cement blocks, which have been settled deliberately around the castle and along the shore for protection against wave's action, contain numerous small and fine curves and caves that afford excellent domains for macroalgae attachment, particularly green macroalgae.

Sampling and preparing:

Water: Water samples were collected monthly from January through December 2012 from the two sites Ras al-Tin (A) and Al-Muntazah (B). At each site, water samples were taken from three points, using PVC tube column sampler at a depth of half meter from the water surface, then mixed in a plastic bucket and a sample of 1 liter was placed in a polyethylene bottle. The samples were directly transferred cold in ice box, using frozen gel cold packs, to the laboratory for analysis.

Macroalgae: Monthly samples of *Ulva* sp. (green macroalgae) were collected by hand from the submerged rocks and substrates at the studied sites and washed with seawater to remove the extraneous matters, sand particles and epiphytes as much as possible. After collection, the samples were immediately transported to the laboratory in an ice box containing frozen gel cold packs. On arrival, the macroalgae samples were gently scrubbed under running tap water to eliminate the other species of macroalgaes as well as adhering epibiota (zooplankton and young bivalves) to the algal surface, sediments and detritus, and briefly rinsed with distilled water. All cleaned algal fronds were individually blotted on towel papers to remove excess water and then dried in the drying oven on 60±2 ºC for 48h, for moisture determination. Then, they were pulverized in a cereal grinder for 5 min and sieved, using a 100 mesh sieve, to obtain a fine and homogeneous powder that was stored in hermetic sealed plastic bags in the refrigerator until chemical analysis.

Laboratory analysis:

a) **Water**: Chemical analysis of dissolved inorganic nitrogen (NO_2-N and NO_3-N mg l^{-1}), total nitrogen (TN), dissolved orthophosphate (PO_4-P) and total phosphorus (TP) were measured according to Grasshoff *et al*. (1983) and the concentrations were expressed as mg/l.

b) **Chemical analysis of macroalgae powder:**

I- Proximate composition: Analyses were performed according to the Association of Official Analytical Chemists AOAC (2000) procedures:

Water content: *Ulva* water content was determined by drying the fresh samples at 60± 2ºC until a constant weight was obtained, after 48h.

Crude protein content: It was determined on the basis of total nitrogen content applying the Kjeldahl method (AOAC, 2000). Nitrogen content is then converted into protein content using the nitrogen conversion factor of 6.25 (AOAC 2000).

Crude fat: Samples lipid content was measured by solvent extraction method in a soxhlet system where petroleum ether was used as solvent.

Total minerals content (**ash**): *Ulva* Minerals content was determined by igniting samples at 550±10 ºC for 3 hours and the residue was determined gravimetrically.

Carbohydrate content was calculated by subtracting the crude protein, crude fat, and minerals (ash) from the whole (100).

II- Total carbon and Total phosphorus: Total carbon was obtained from organic matter which was measured as loss on ignition at 550°C for 3 h, and organic carbon (OC=TC %) was calculated from organic matter (OM) data using the conventional conversion: OC = OM / 1.72 (Bashour and Sayegh, 2007). Total phosphorus (TP) was measured using the dry ash method (Tavares & Boyd, 2003) for digestion then phosphorus was colorimetrically estimated using the vanadomolybdate method (APHA, 1985).

III- Determination of chlorophylls (a, b, c and total): 70-90 mg of fresh *Ulva* sp. samples was ground in a tissue grinder covered with 5-7 ml 90% aqueous acetone solution (90 ml acetone and 10 ml of saturated magnesium carbonate solution), the samples were transferred into centrifuge tubes and completed their volume up to 15 ml using aqueous acetone then incubated overnight in dark at 4°C for complete the extraction process. On the next day the samples were clarified in a centrifuge at speed of 2,000 rpm for 20 min. Chlorophyll concentration in the supernatants were determined spectrophotometrically at wavelengths 750, 664, 647 and 630 nm according to APHA method (1985) and Lichtenthaler (1987).

Statistical analysis

Two-way ANOVA was employed to evaluate the variability of the concentration of each nutrient with respect to different seasons and sites, using the software CoStat ver. 6.311 (CoStat, CoHort software, USA). The analyzed data were expressed as mean ± standard deviation (SD). The relationships between each pair of studied items were performed through Pearson's correlation coefficient matrix. Significant differences are stated at $P<0.05$ and $P<0.01$ (Bailey, 1981).

RESULTS AND DISCUSSION

Chemical characteristics of seawater

There was a remarkable significant seasonal influence over the water quality parameters. Significant differences were noticed in water NO_2, NO_3, total nitrogen (TN), orthophosphate (OP) and total phosphate (TP) among different seasons. The highest ($P<0.05$) values in water nitrite, total nitrogen and orthophosphate were recorded in spring, while the water nitrate and total phosphate values showed the highest ($P<0.05$ and $P<0.01$, respectively) values during autumn. However, there were no significant differences among the studied sites (Table1 & Fig 2).

Nitrite and orthophosphate at both sites had similar seasonal pattern, with maximum concentrations measured during spring and the lowest during autumn. While the total nitrogen total phosphate at both sites as well as nitrate at site (A) had similar seasonal pattern, with the highest concentrations in spring and autumn, respectively, and the lowest concentrations in winter and summer, respectively. At all the measuring times, nitrate showed higher concentrations than nitrite at both sites, due to high oxygenation result from the high currents in this area.

Table 1: Nutrients concentrations (mean ±SD) in water during the four sampling seasons from Ras Al-Tin (A) and Al-Muntazah (B). (n=3)

Season	Nutrients (mg/l)									
	NO_2-N		NO_3-N		TN		OP		TP	
	A	B	A	B	A	B	A	B	A	B
Winter	0.040 ±0.010	0.035 ±0.008	0.332 ±0.06	0.294 ±0.015	3.189 ±0.74	3.234 ±0.172	0.151 ±0.03	0.155 ±0.021	0.254 ±0.06	0.173 ±0.017
	0.038		0.313		3.212		0.153		0.214	
Spring	0.058 ±0.007	0.057 ±0.008	0.527 ±0.07	0.377 ±0.047	4.232 ±0.87	4.777 ±0.901	0.261 ±0.03	0.242 ±0.028	0.366 ±0.03	0.302 ±0.050
	0.058*		0.452		4.505*		0.252*		0.334	
Summer	0.037 ±0.002	0.045 ±0.005	0.221 ±0.016	0.340 ±0.053	2.650 ±0.044	3.054 ±0.661	0.164 ±0.033	0.206 ±0.043	0.208 ±0.031	0.244 ±0.050
	0.041		0.281		2.852		0.185		0.226	
Autumn	0.031 ±0.004	0.030 ±0.002	0.294 ±0.021	0.330 ±0.056	3.012 ±0.391	3.135 ±0.452	0.044 ±0.003	0.041 ±0.008	0.541 ±0.128	0.624 ±0.241
	0.030		0.312		3.074		0.043		0.583**	
O. M."	0.042 ±0.010	0.042 ±0.010	0.344 ±0.081	0.335 ±0.032	3.271 ±0.679	3.551 ±0.753	0.155 ±0.031	0.161 ±0.030	0.342 ±0.048	0.336 ±0.082
	Two-way ANOVA (P-value)									
Season	0.0111*		0.0506		0.0302*		0.0325*		0.0013**	
Site	0.9209		0.1904		0.6619		0.1181		0.4385	

* and ** are significant at (P<0.05) and (P<0.01), respectively. O. M." : overall mean of the study area.

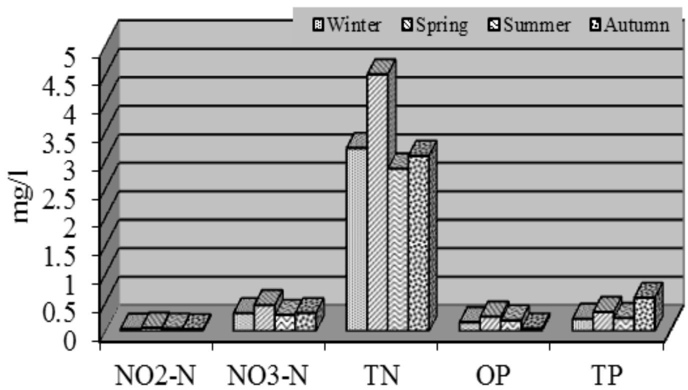

Fig. 2: Seasonal variations of nitrogen and phosphorus in water collected monthly from the north coast of Egypt, Alexandria region.

Based on the nutrient concentrations measured (Table 1) and compared with the previous reported nutrient concentrations in the other eutrophic sites (Table 2), the studied area can be considered a rich area with nutritive elements that would allow proliferating growth in many species of macroalgae.

Table 2: The nutrient concentrations in the present study and in other eutrophicated sites

Studied area	NO2+NO3 mg/L	*DIN mg/L	OP mg/L	Ref
Alexandria shore, Egypt	0.38		0.16	Present study
Northern Adriatic		0.043	0.004	Faganeli *et al*., (1986)
Mombasa city, East of Africa	0.014-0.042		≈0.009	Osore *et al*., 1999
Yangtze River, China	0.52- 0.64	1.1- 1.5	0.04- 0.09	He *et al*., 2008
Dar es Salaam		0.16	0.047	Sjöö and Mörk (2009)
Mombasa	0.014-0.042		0.0093	
Tokyo Bay, Japan	< 1.5	< 3	< 0.3	Yabe *et al*., 2009

*DIN =NO_2 + NO_3 +NH_4.

Chemical characteristics of *Ulva* sp. macroalgae

The biochemical composition (moisture content, protein, lipid, ash, and carbohydrate) of *Ulva* sp. is illustrated in Table (3) and Fig. (3). The moisture content in site A ranged between 80.51±4.54 and 87.03±0.74%, while in site B it ranged from 82.51±3.13 to 87.75±0.9 %. In autumn, the moisture contents were the highly significant (P < 0.01) content among the seasons for *Ulva* in both sites (A and B).

No significant differences were noticed between studied sites. The estimated moisture contents in this study are higher than that (76%) reported in *Ulva rigida* by Satpati and Pal (2011), however, they are within the range that reported for macroalgae (80-90%) by (García-Casal *et al*., 2007).

The protein contents (Table, 3 & Fig. 3) that recorded in the present study ranged from 24.6±4.9 to 27.6±1.3% at site A, and from 20.7±3.1 to 26.3±1.8% at site B. These values are in conformity with those (10-29% DW) reported for *Ulva pertusa*; *Ulva* sp., *Ulva lactuca* and *Ulva rigida* by Fujiwara-Arasaki *et al*., (1984); Fleurence (1999); Ortiz *et al*., (2006) and Valente *et al*., (2006), respectively. However, lower protein contents (7.1 - 8.5% DW) were reported for *Ulva lactuca* in the northeast of Hong Kong by Wong and Cheung (2000) and in Tunisia by Yaich *et al*., (2011). There was a noticeable seasonal pattern, particularly at site A, with the minimum protein content recorded in spring at both sites. On contrary, Khairy and El-Shafay (2013) found that in spring the protein content of *Ulva lacutca*, collected from east Alexandria shore, Egypt, was higher than that in summer and autumn (20.12, 17.9 and 16.8 % DW, respectively). The authors found a significant effect of the seasons on the protein content. According to Fleurence (1999) and Ratana-arporn & Chirapart (2006), the difference among macroalgae species as well as the seasonal period can play an important role in the variation of the algae protein content.

The lipid content of the *Ulva* sp. in the present study showed that, the macroalgae at site B have higher lipid content range (10.8-15.8%) than that of site A (8.7-9.9% DW), but not significantly (Table 3 & Fig, 3). According to the reported values in the literature, most macroalga contained lipid less than 4% DW (McDermid and Stuercke, 2003), however, some macroalga had higher levels of crude lipid, such as *Dictyota acutiloba* (16.1% DW) and *D. sandvicenis* (20.2 % DW) (McDermid and Stuercke, 2003). The measured lipid contents in the present study are in consistency with that reported in *Caulerpa racemosa,* and *Padina gymnospora,* 19.1 %, and 11.4 %, respectively (Rameshkumar *et al*., 2012). However, they are higher than those determined previously for *Ulva lactuca*, (7.87-1.64 % DW) by Yaich *et al*., (2011), and (0.3-1.64% dry weight) by Wong and Cheung (2000) and Ortiz *et al*., (2006). The annual overall mean of the lipid content showed a significant difference ($P<0.05$) between the sites in favor of the site B (12.16±2.2%). A seasonal pattern was observed clearly at site A, however, at site B there was a fluctuation among seasons. The differences in quantity of extracted lipid in this study and those reported in other regions might be due to the methodology of lipid analysis, or geographical and seasonal factors, or climate change as well as the development stage of the macroalgae as stated by Haroon *et al*., (2000) and Ortiz *et al*., (2006).

Table 3: Seasonal mean (±SD) of proximate analysis (on dry weight basis) in *Ulva* sp. during the four sampling seasons from Ras Al-Tin (A) and Al-Muntazah (B).

Season	Item									
	Moisture%		Protein%		lipid%		Ash%		Carbohydrates%	
	A	B	A	B	A	B	A	B	A	B
Winter	86.15	84.16	27.56	22.46	9.93	15.75	16.94	17.72	45.56	44.07
	±2.91	±1.95	±3.89	±4.49	±0.71	±2.08	±3.83	±4.22	±2.16	±2.11
	85.16		25.01		12.84		17.33		44.82	
Spring	86.98	83.67	24.62	20.71	8.69	10.91	15.32	19.17	51.37	49.21
	±0.40	±0.27	±4.88	±3.07	±1.68	±2.34	±1.89	±2.23	±9.40	±3.14
	85.33		22.67		9.80		17.25		50.29	
Summer	80.51	82.51	25.70	26.25	9.92	10.76	15.19	16.79	49.19	46.20
	±4.54	±3.13	±3.70	±1.75	±1.66	±2.17	±0.44	±0.38	±4.58	±5.67
	81.51		25.98		10.34		15.99		47.70	
Autumn	87.03	87.75	27.60	25.96	8.98	11.20	13.91	20.53	49.51	42.31
	±0.74	±0.89	±1.34	±2.67	±0.35	±0.57	±1.39	±0.76	±0.40	±2.86
	87.39**		26.78		10.09		17.22		45.91	
O. M."	85. 2 ±2.61	84.5 ±3.66	26.37 ±3.42	23.85 ±3.64	9.38 ±1.50	12.16* ±2.19	15.34 ±2.91	18.55* ±3.57	48.91 ±2.43	45.45 ±2.97
	Two-way ANOVA (P-value)									
Season	0.0044**		0.2244		0.3081		0.8947		0.2228	
Site	0.5106		0.0903		0.0379*		0.0388*		0.0836	

* and ** are significant at ($P<0.05$) and ($P<0.01$), respectively. O. M.": overall mean of the study area.

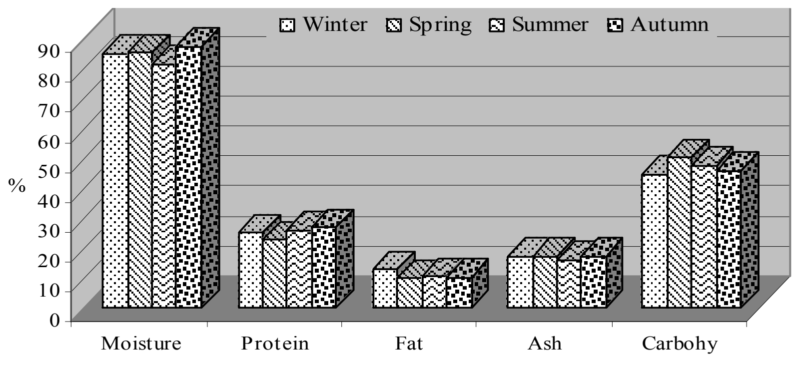

Fig. 3: Seasonal variations of biochemical composition (% DW) of *Ulva* sp. collected monthly from the north coast of Egypt, Alexandria region.

The lipid content of the *Ulva* sp. in the present study showed that, the macroalgae at site B have higher lipid content range (10.8-15.8%) than that of site A (8.7-9.9% DW), but not significantly (Table 3 & Fig, 3). According to the reported values in the literature, most macroalga contained lipid less than 4% DW (McDermid and Stuercke, 2003), however, some macroalga had higher levels of crude lipid, such as *Dictyota acutiloba* (16.1% DW) and *D. sandvicenis* (20.2 % DW) (McDermid and Stuercke, 2003). The measured lipid contents in the present study are in consistency with that reported in *Caulerpa racemosa,* and *Padina gymnospora,* 19.1 %, and 11.4 %, respectively (Rameshkumar *et al*., 2012). However, they are higher than those determined previously for *Ulva lactuca*, (7.87-1.64 % DW) by Yaich *et al*., (2011), and (0.3-1.64% dry weight) by Wong and Cheung (2000) and Ortiz *et al*., (2006). The annual overall mean of the lipid content showed a significant difference ($P<0.05$) between the sites in favor of the site B (12.16±2.2%). A seasonal pattern was observed clearly at site A, however, at site B there was a fluctuation among seasons. The differences in quantity of extracted lipid in this study and those reported in other regions might be due to the methodology of lipid analysis, or geographical and seasonal factors, or climate change as well as the development stage of the macroalgae as stated by Haroon *et al*., (2000) and Ortiz *et al*., (2006).

Different species of macroalgae have been reported to contain ash content within the range between 8 to 40% DW (Mabeau and Fleurence, 1993). In the present study, the ash contents of collected *Ulva* sp. samples were (13.9± 1.39 -16.9± 3.83% DW at site A and 16.79±0.38 -20.5±0.76% DW at site B). The highest ash content was recorded at site A in winter season, while in autumn at site B. The overall mean of the ash content at the site B (18.55± 3.57% DW) was significantly higher ($P<0.05$) than that of site A (15.34± 2.91% DW). Higher values of ash content were reported in the other studies 27.2% DW for *Ulva pertusa* and 27.6% DW for *Ulva intestinalis* (Benjama and Masniyom, 2011), (23.2% DW) for *Enteromorpha flexuosa*, (25.4% DW) for *U. fasciata* (McDermid and Stuercke, 2003), (21.3-24.6 and 24.7% DW) for *Ulva lactuca* and *U. pertusa*, respectively (Behairy & El-Sayed,1983; Wong and Cheung, 2000). However, comparable values were reported in the other studies such 17.58% DW in *Ulva reticulata* (Ratana-arporn and Chirapart, 2006), 19.59±0.51% DW in *Ulva lactuca* in Tunisia by Yaich *et al*., (2011) and 17.6-23.4% DW in *Ulva lactuca* in Egyptian coast by Khairy and El-Shafay (2013). Interestingly, the determined ash contents in the present study are much higher than those of earth plants other than spinach and other vegetables (Ruperez *et al*., 2002).

As shown in Table (3) and Fig (3), the carbohydrate content in *Ulva* sp. ranged between 45.56±2.16 and 51.37± 9.4% at site A and from 42.31±2.86 to 49.21±3.14% at site B. Neither sites nor seasons exert significant influence on the carbohydrate content, in the present study. The carbohydrate contents here are lower than that of previous reported data (61.5-70% DW) for *Ulva lactuca* by Wong and Cheung (2000) and Ortiz *et al*., (2006). However, comparable values of carbohydrate (42.1- 46.5% DW) in *Ulva lactuca* were reported by Khairy and El-Shafay (2013). El-Said and El-Sikaily (2013) studied the carbohydrate in *Ulva lactuca* along the shore of Alexandria, Egypt, and found that it ranged from 10.2 to11.5% dry weight in April 2011. In the present study, there were seasonal changes in the carbohydrate contents with the highest contents recorded in spring at both studied sites. Seasonal changes in carbohydrate contents of *Ulva lactuca* were also observed by Khairy and El-Shafay (2013), but, with the highest value in summer season.

The tissue nutrient concentrations of nitrogen (N), phosphorus (P) and carbon (C) in *Ulva* samples collected from the studied sites are presented in Table (4) and Fig (4). The total tissue nitrogen content in the present study (3.31- 4.42 % DW) are higher than 0.45% DW reported in another study for *Ulva* (Sfriso 1995), although the nitrogen concentration in the ambient water in the present study are lower than that in that study (0.05-1.5 mg N/L). Also, lower nitrogen contents in *Ulva lactuca* were reported by Neori *et al*., (1991), although the ambient water nitrogen concentrations were higher than those found in the present study. This difference may indicate that the *Ulva* sp. macroalgae, in the present study, may have higher nutrient uptake efficiency than those species in those studies.

Table 4: Seasonal mean (±SD) of nutrients (on dry weight basis) and atomic N:P and C:N ratios in *Ulva* sp. during the four sampling seasons from Ras Al-Tin (A) and Al-Muntazah (B).

Season	Item									
	TN%		TP%		N:P		TC%		C:N	
	A	B	A	B	A	B	A	B	A	B
Winter	4.41 ±0.62	3.59 ±0.72	0.24 ±0.03	0.17 ±0.03	40.54 ±7.64	47.80 ±3.79	48.29 ±3.39	47.84 ±4.20	12.87 ±1.11	15.82 ±2.26
	4.00		0.21		44.17		48.07		14.35	
Spring	3.94 ±0.78	3.31 ±0.49	0.24 ±0.02	0.11 ±0.01	37.46 ±9.10	64.28 ±8.03	49.23 ±1.10	46.99 ±1.29	15.05 ±3.58	16.77 ±2.27
	3.63		0.18		50.87		48.11		15.91	
Summer	4.11 ±0.59	4.20 ±0.28	0.25 ±0.02	0.20 ±0.00	37.73 ±8.54	47.85 ±4.32	49.31 ±0.26	48.38 ±0.22	14.21 ±2.27	13.48 ±0.87
	4.16		0.23		42.79		48.85		13.85	
Autumn	4.42 ±0.22	4.15 ±0.43	0.26 ±0.04	0.22 ±0.00	39.06 ±7.86	42.18 ±4.90	50.05 ±0.81	46.21 ±0.44	13.24 ±0.43	13.08 ±1.43
	4.29		0.24**		40.62		48.13		13.16	
O. M."	4.22 ±0.55	3.81 ±0.56	0.25*** ±0.03	0.18 ±0.04	38.70 ±7.47	50.53** ±9.83	49.22* ±1.69	47.36 ±2.07	13.84 ±2.08	14.79 ±2.24
Two-way ANOVA (P-value)										
Season	0.2244		0.0015**				0.8947		0.1500	
Site	0.0903		0.0000***				0.0388*		0.2685	

*, ** and *** are significant at (P<0.05), (P<0.01) and (P<0.001), respectively.
O. M." : overall mean of the study area. N:P and C:N as atomic ratio.

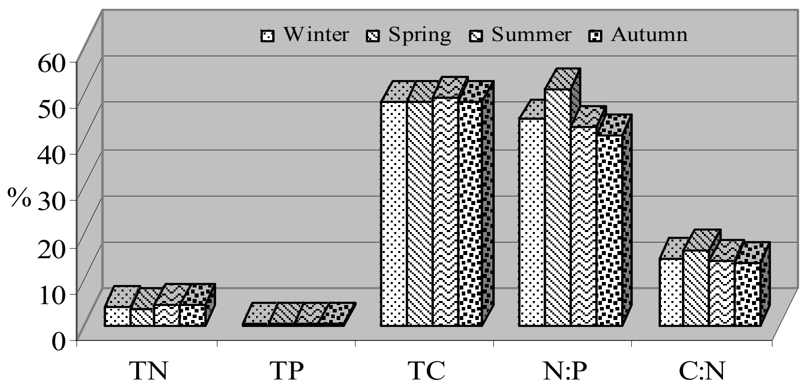

Fig. 4: Seasonal variations of nutrients (nitrogen, phosphorus and carbon) in tissues of *Ulva* sp. collected monthly from the north coast of Egypt, Alexandria region.

As expected the phosphorus contents differed remarkably over time. In autumn, the phosphorus contents were highly significant ($P< 0.01$) than those in the other seasons. The trends of P tissue concentrations were similar at both sites. In general, minimum values (0.24 and 0.11mg/g DW) occurred in spring, then increased in summer and autumn. Seasonal trends were observed for P in *Ulva* and *Eneteromorpha* in north-west of Spain (Villares and Carballeira 2003) and other macroalgae (Lyngby *et al*., 1999). The annual cycle mean contents for each site showed highly significant differences ($P< 0.001$) in favor of site A. In general, the phosphorus contents range in the present study are comparable to those reported for *Ulva* in the other eutrohpicated areas, *Ulva rigida* (0.05-0.35% DW) in Venice Lagoon (Sfriso 1995) and in northern Adriatic (Faganeli *et al*., 1986), and *Ulva* sp. (0.03-0.23% DW) collected from northwest of Spain (Villares and Carballeira (2003). However, the values of phosphorus content at site B, in the present study, are lower, during winter and spring, than those (0.2% DW) proposed as critical concentrations of *Ulva* sp. by (Lyngby *et al*., 1999, Hernandez *et al*., 2005), suggesting P limitation in site B.

N:P ratios in the present study ranged between 37.46 and 40.54 at site A and between 42.18 and 64.28 at site B. Interestingly, at site A the variation in N:P ratio mainly refers to the change in tissue nitrogen content, while at site B the change in tissue phosphorus content exerted the main influence on the N:P ratio. Highly significant differences ($P<0.01$) were noticed between the annual cycle mean of the studied sites, in favor of site A. The lower N:P ratios at site A, compared with those of site B, can be explained by the higher tissue phosphorus content in the plants collected from site A. The phosphorus content in *Ulva* from site A was higher by 47% of that in plants from site B, while the nitrogen content in *Ulva* from site A was higher by only 10% of that in plants from site B.

Sjöö and Mörk (2009) found that N:P ratios in *Ulva* sp. ranged from 9:1 to 40:1. Duarte (1992) calculated the mean N:P ratio as 49:1, for 46 macroalgal species. Other studies (Atkinson and Smith 1983, Villares *et al*., 1999) have also presented ratios that exceed the suggested ratios of 24:1-30:1 (Bjornsater and Wheeler 1990, Larned 1998; Guildford and Hecky, 2000), after which P generally becomes limiting for tropical macroalgae. Several studies have reported P to be the limiting nutrient in tropical areas (e.g. Lapointe 1985, 1987, Littler and Littler, 1990) as well as in the northwestern Spanish coastal waters (Villares *et al*., 1999). Our results in the present study are in agreement with this assumption where the N:P ratios were higher than 30:1.

Concerning the tissue total carbon content, significant differences were noticed between the annual cycle mean at studied sites. The annual mean of TC at site A was significantly ($P<0.05$) higher than that for site B. In general the TC ranged between 46-50% that is higher than the reported values in the other studies. According to Faganeli *et al*., (1986) the C content ranged between 12.2 and 47.6% DW with a mean C content of 26.5% DW, in *Ulva rigida* collected from northern Adriatic. Lower carbon content (20-42% DW) in *Ulva* sp. were reported by Neori *et al*., (1991); Sfriso (1995); Villares and Carballeira (2003) and Nelson *et al*., (2008). In general, no particular seasonal trend was noticed for this element in the present study, as the changes in tissue contents were limited within each site. Similarly, Villares and Carballeira (2003) found no seasonal changes in C tissue content in *Ulva*. The authors attributed the narrow variation in C content to its structural importance.

C:N ratios, in the present study, ranged between 13:1 and 15:1 at site A and from 13:1 to 17:1 at site B, with the highest values recorded in spring season at both sites. There was a remarkable seasonal pattern, where the ratios increased from winter to spring and then started to decrease through summer and autumn at both sites. However no significant differences were noticed among sites or seasons. Comparable C:N ratio (11.1±4.8, on atomic basis) was reported for *Ulva rigida* collected from north Adriatic by Faganeli *et al*., (1986). The present study C:N ratio values (13-17) are also within the range of benthic macroalgae (10-70) reported by Fenchel & Jorgensen (1977). Hernández *et al*., (2002) reported that the C:N ratio of macroalgal tissues might control differences in the nutrient removal efficiency. Therefore, the C:N ratios are a powerful index of the physiological status of macroalgae (Vergara *et al*., 1993) and could be used as an indication of macroalgae healthy status. Generally, C:N ratios show low values when nitrogen is abundant and increase when nitrogen supply is limited (Lahaye *et al*. 1995, Gómez Pinchetti *et al*., 1998). Also, low C:N ratios are characteristic the phases of the rapid biomass increase in the algal growth cycle (Niell, 1976).

The C: P ratios (C:P ratio on atomic basis = (TC/TP) x 2.583), in the present study, (at a range of 431-1107) appear to be quite higher than the C: P ratio range of 200 : 1 (atomic) reported by Fenchel & Jorgensen (1977) as typical for benthic plants. On the other hand, the mean C:P ratios (750 and 523at site A and B, respectively) appear to be similar to the mean C:P ratio (700, atomic) reported by Atkinson & Smith (1983) for 92 benthic plant samples from five phyla collected at nine locations worldwide and comparable to those ratios reported by (Faganeli *et al*., 1986) for *Ulva rigida* from north Adriatic. The average C:P ratios as well as the N:P ratios of *Ulva* sp. collected from Alexandria shore, Egyptian north coastal waters suggest, taking into account Redfield's ratio of phytoplankton (Goldman *et al.,* 1979), that these plants were generally growth-limited by P, which is in consistency with the general notion of the Mediterranean being P limited.

The Chlorophyll "*a*" and "*b*" and total chlorophyll contents (Table, 5 & Fig, 5) showed similar seasonal pattern, where they decreased in spring and increased through summer and autumn. As there were significant differences among seasons in Chlorophyll "*a*" and "*b*", where the significant lowest (P<0.05) chlorophyll contents were noticed in spring. However, the total chlorophyll contents did not show any significant differences neither between sites nor among seasons.

Chlorophyll "*c*" (Table, 5 & Fig, 5) showed an opposite seasonal trend, where it increased in spring and started to decrease through summer and autumn at site A. While at site B it continued increasing until summer season and decreased in autumn. The highest contents were noticed in spring at site A and in summer at site B and the lowest contents in autumn at both sites. But no significant differences were detected.

It was expected that, due to high irradiance intensity during summer season, the chlorophyll content will be at minimum values. However the minimum chlorophyll (*a* and *b*) contents were noticed spring and chlorophyll "*c*" content during autumn. This could be explained by the shading effect of *Ulva* canopy, therefore the effect of high irradiance was exclusive to the upper layer of plants that were bleached and avoided during collection process. Comparable values of chlorophyll "*a*" and "*b*" contents (4.7 and 3 mg /g DW, respectively) and were reported for *Ulva* sp. by Nelson *et al*., (2008). Also, the Chlorophyll "*a*"/ chlorophyll "*b*" ratio in the present study (1.4-1.9 is comparable to that (1.57 mg/g DW) reported by Nelson *et al*., (2008).

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Table 5: Seasonal mean (±SD) of chlorophyll "*a*", "*b*", "*c*" and total chlorophyll (mg/g dry wt.) in *Ulva* sp. during the four sampling seasons from Ras Al-Tin (A) and Al-Muntazah (B).

Season	Item							
	Chl-*a*		Chl-*b*		Chl-*c*		T-Chl	
	A	B	A	B	A	B	A	B
Winter	5.03 ±1.58	3.75 ±0.85	2.74 ±0.923	2.00 ±0.48	0.367 ±0.0.092	0.19 ±0.04	8.14 ±1.58	5.94 ±1.22
	4.39		2.37		0.28		7.04	
Spring	3.00 ±0.14	1.26 ±0.06	1.77 ±0.04	0.89 ±0.07	0.45 ±0.08	0.27 ±0.06	5.22 ±0.36	2.52 ±0.05
	2.13*		1.33*		0.36		3.82	
Summer	3.64 ±0.52	3.04 ±0.71	2.26 ±0.39	1.98 ±0.45	0.30 ±0.07	0.31 ±0.06	6.20 ±1.30	5.34 ±1.08
	3.34		2.12		0.31		5.77	
Autumn	3.94 ±0.83	4.45 ±0.82	2.59 ±0.54	2.87 ±0.47	0.25 ±0.05	0.18 ±0.04	6.78 ±1.90	7.50 ±1.33
	4.20		2.73		0.22		7.14	
O. M."	3.90 ±0.85	3.13 ±1.37	2.34 ±0.43	1.93 ±0.81	0.34* ±0.87	0.24 ±0.06	6.59 ±1.21	5.30 ±2.13
	Two-way ANOVA (P-value)							
Season	0.0225*		0.0313*		0.1593		0.0532	
Site	0.1404		0.2034		0.0232*		0.1577	

* are significant at (P<0.05). O. M.": overall mean of the study area.

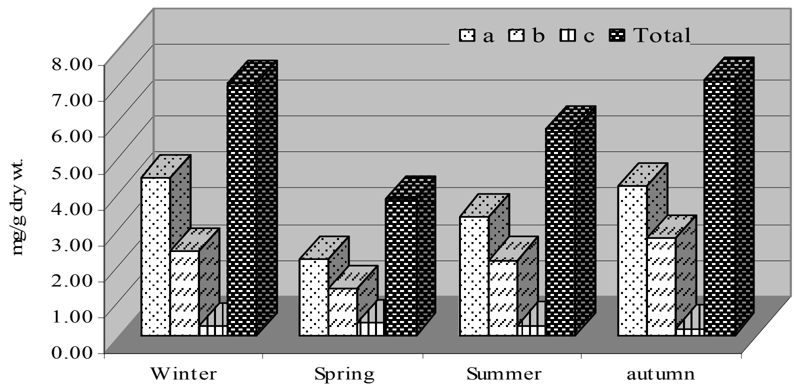

Fig. 5: Seasonal variations of chlorophyll (*a*, *b*, *c* and total mg/g dry wt.) in *Ulva* sp. collected monthly from the north coast of Egypt, Alexandria region.

The data of correlation coefficient between each two pair of nutrients in *Ulva* sp. were investigated and presented in Table (6). There were significant positive correlations between, C-N and C-P as well as between P and total chlorophyll (P<0.05), and between N and total chlorophyll (P<0.01). Villares and Carballeira (2003) found significant (P<0.001) correlation between C-N in *Ulva* and *Enteromorpha* and between N-P in *Enteromorpha* (P<0.01). The relationship between the metabolism of N and C in algae was explained by McGlathery (1992), N-limitation may affect C fixation through synthesis of necessary proteins for the CO_2 carboxylation. Also, it had been showed that N-depletion may limit inorganic phosphorus assimilation by means of low protein content and enzyme activity (Pedersen and Borum, 1996). On the side, nitrogen metabolism in *Gracilaria tikvahiae* may be influenced by P limitation through limited energy molecules synthesis (i.e. ADP and ATP) (Lapointe, 1987).

Table 6: Correlation coefficient matrix (r) between concentrations of paired items in green macroalgae (*Ulva* sp.)

Item	Moisture %	Fat %	Ash %	Carboh.%	TC %	TN %	TP %	N:P	C:N	T. chl
Moisture %	1.0									
Fat%	0.0792	1.0								
Ash%	-0.0129	-0.1335	1.0							
Carboh. %	-0.1437	-0.0251	0.2243	1.0						
TC%	0.0129	-0.1335	-1.0	-0.2243	1.0					
TN%	0.0105	-0.01871	-0.4621*	-0.2700	0.4621*	1.0				
TP%	-0.2031	-0.3354	-0.4050*	-0.3528	0.4050*	0.4641	1.0			
N:P	0.2599	0.1842	0.1726	0.2470	-0.1726	-0.0145	-0.8993***	1.0		
C:N	0.0296	0.1214	0.2133	0.2700	-0.2133	-0.9548***	-0.3978	-0.0302	1.0	
T. chl	0.2267	-0.0768	-0.2944	-0.1527	0.2037	0.6057**	0.4622*	-0.2588	-0.3815**	1.0

*, **and *** are significant correlation at (P<0.05), (P<0.01) and (P<0.001), respectively.

As there were significant negative correlations between, ash content and both of total nitrogen and total phosphorus (P<0.05), total nitrogen and C:N ratio and between total phosphorus and N:P ratio (P<0.001). Also, there were negative highly significant correlation between total chlorophyll and C:N ratio (P<0.01).

Interestingly, the correlation between total nitrogen and N:P ratio and between total carbon and C:N ratio, are negative, on contrary with what was expected. However, this could be explained by that the N:P and C:N ratios, in the present study, depend mainly on the second element, which exerted the main influence. Similarly, Durate (1992) and Villares and Carballeria (2003) found large variation in the levels of P followed by N, whereas those of C were more stable. These data may suggest that the storage capacity of carbon is higher than nitrogen storage capacity which in its turn is higher than that of phosphorus, so the changes in the tissue total phosphorus content are more rapid than the changes in the tissue nitrogen content, and that the later are more rapid than the changes in total carbon content.

CONCLUSION

The present study demonstrated that, the studied sites are rich in nutritive elements that proliferates many species of macroalgaes. It also revealed that *Ulva* sp. are characterized with high contents of protein, lipid, carbohydrates and minerals. That confirms the high nutrient storage capacity of *Ulva*, indicating that, on one hand, it could be used as biofilters in the aquaculture systems as well as for bioremediation of eutrophicated shores. On the other hand, the proximate composition nominates this macroalgae to be exploited as a good food and energy resource for human, animals and in aquaculture. Also, it could be used in cosmetics industries, for its high carbohydrate contents. It is also evident that there are pronounced seasonal variations in the biochemical composition of *Ulva* sp. Also, the results showed that the *Ulva* macroalgae may suffer from phosphorus limitation in particular seasons, e.g. winter, spring and summer in site (B).

RECOMMENDATIONS

Ulva sp. in the studied area is a good candidate for bioremediation of polluted sites and bio-filtration of aquaculture effluent. It also could be considered as a good food and energy source for human, animals and in aquaculture, as it may be seen as promising raw material for several industries, such as cosmetic industries. However, further studies are needed to investigate the digestibility and quality of proximate composition of *Ulv*a, such as amino acids, fatty acids and polysaccharides content.

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ARABIC SUMMARY

التقييم الغذائي للعشب الأخضر الأولفا بالساحل الجنوبى للبحر الأبيض المتوسط ـ شاطئ الإسكندرية- مصر

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لقد تم دراسة المحتوى من البروتين، الكربوهيدرات، الدهون، الرماد، الكلوروفيل(الكلوروفيل الكلى وأنواعه)، الكربون العضوى و الرطوبة فى العشب البحرى الأولفا وعلاقاتها بتركيز العناصر فى المياه من منطقتين مختلفتين و التغيرات الموسمية خلال عام 2012. حيث تم جمع عينات المياه و طحالب الأولفا شهريا من المناطق الخاضعة للدراسة – منطقة رأس التين (موقع أ) و المنتزة (موقع ب) و يفصل بينهما مسافة 18.6 كم على طول شاطئ الأسكندرية. أوضحت النتائج أن المناطق محل الدراسة غنية بالعناصر الغذائية بمستويات أعلى من تلك المذكورة للمناطق الاخرى الملوثة. كما أوضح التحليل الكيميائي أن مسحوق طحالب الأولفا يتميز بإرتفاع محتواه من البروتين (20.7-27.6% من الوزن الجاف) الدهون (8.7-15.75% من الوزن الجاف الرماد (15.32-20.53% من الوزن الجاف) و الكربو هيدرات (42.31-51.37 من الوزن الجاف) ، مع أعلى محتوى للدهون و المعادن فى فصل الشتاء، أعلى محتوى للكربوهيدرات فى فصل الربيع، أعلى محتوى للكربون العضوى فى فصل الصيف وأعلى محتوى للبروتين و الفوسفور فى فصل الخريف. كذلك فإن المحتوى العالى من العناصرفى طحلب الأولفا يؤكد السعة التخزينية العالية لطحلب الأولفا فى هذة المنطقة من العناصرو هو ما يشير الى إمكانية إستخدامه كمرشح فى أنظمة الإنتاج السمكى وفى معالجة المناطق الملوثة- و من ناحية اخرى فإن التركيب الكيميائى للطحلب يشير الى إمكانية إستغلال طحلب الأولفا كمصدر غذائى جيد و مصدر للدهون للإنسان و الحيوانات و فى إستزراع الأسماك – كما يمكن إستخدام هذا الطحلب فى صناعات مساحيق التجميل نظرا للمحتوى العالى من الكربوهيدرات. ولقد كانت هناك إختلافات موسمية واضحة فى مستوى العناصر المقدرة فى أنسجة الطحالب. و لقد أشارت نسبة النيتروجين الى الفوسفور و كذلك نسبة الكربون الى الفوسفور الى أن هذة الطحالب فى تلك المناطق ربما تعانى من محدودية الفوسفور فى مواسم معينة من السنة. كما لوحظت علاقات إرتباط إيجابية معنوية بين محتوى العشب من الكربون الكلى و كل من محتوى النيتروجين و الفوسفور و كذلك بين الكلوروفيل الكلى و كل من الفوسفورو النيتروجين.