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Seasonal variation of biochemical content and nutritional composition of the newly recorded alga *Grateloupia gibbesii*, Alexandria, Egypt

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ABSTRACT

In the present study, the newly recorded red alga *Grateloupia gibbesii* was collected from the Eastern Harbor, Alexandria, Egypt in order to determine the biochemical content, total nitrogen and phosphorus content during winter and spring 2012 in addition to summer 2016. The study moisture, ash content, amino-acids profile, macro-elements (calcium, sodium, potassium, magnesium), and trace elements (copper, zinc, iron, cobalt, manganese, selenium, cadmium, lead, nickel and mercury) were addressed during spring 2015 & summer 2016. The concentration of the total carbohydrates, protein, nitrogen, and phosphorus content in the alga was relatively high during summer 2016 (394.30, 268.20, 39.10 mg/g and1680 mg/100 g, respectively). However, the lipid content of *Grateloupia gibbesii* experienced low values during the study period. The fatty acids profile showed the highest concentration during winter 2012 (922.09 µg/g**)**. The moisture and ash content of *Grateloupia gibbesii* recorded the highest percentages (24.01 and 27.72%) during spring 2015, respectively. The total amino acids (EAA) attained a maximum of 732.20 mg/g during spring 2015. The macro-elements and trace elements mostly showed higher content during spring 2015 compared to summer 2016. The results of ANOVA test revealed that the seasonal variation in all the biochemical contents recorded a highly significant difference ($P< 0.0001$). The moisture and ash content showed a significant difference ($P< 0.0001$) during spring 2015 & summer 2016. For the macro-elements, the values of potassium and magnesium were significantly different during spring 2015 & summer 2016, while sodium and calcium were not significantly different during the two seasons. All the trace metals were not affected by seasonal variation, except for nickel, iron, selenium and mercury ($P< 0.0001$).

INTRODUCTION

Macroalgae are one of the most important groups in marine environment. They play a key role in ecosystems, as they provide nutrition, and shelter for diverse communities. In addition, they provide the other organisms with the oxygen necessary for their activities and their life maintenance **(Chopin & Sawhney, 2009)**. Thus, macroalgae are significant biotic components maintaining the stability of ecosystem **(Dere *et al.*, 2003)**. On the other hand, seaweeds have been used as food for a long period in the Far East, especially China, Japan and Korea **(Dere *et al.*, 2003)**. Remarkably, the consumption of seaweed products has surged in the western

countries. In Europe, about 15-20 species are now being marketed for food consumption due to their richness in carbohydrates, proteins, polyunsaturated fatty acids, minerals and vitamins; they contain a wide variety of essential amino acids **(Anis *et al.*, 2017)**. Physiologically, these bioactive substances are classified into non-absorbed high-molecular materials like dietary fibers and low-molecular materials, which are absorbed and directly affect the maintenance of human homeostasis **(Turan *et al.*, 2015)**. Therefore, seaweeds are considered as functional ingredients in many industrial applications such as functional foods that provide specific health advantages beyond basic nutrition, pharmaceuticals and cosmetics **(Wijesinghe & Jeon, 2012)**. It is worthy to mention that, seaweeds are used in medicine and pharmacology for their antimicrobial, antiviral, antitumor and anticoagulant activity **(Anis *et al.*, 2017)**. However, many studies were conducted on the biochemical and nutritional composition of various seaweeds to investigate their nutritional value **(Polat & Ozogul, 2013; Turan *et al.*, 2015; Aroyehun *et al.*, 2019)**.

In Egypt, seaweeds luxuriantly grow along the Mediterranean coast, among which many species were investigated for different purposes such as medicinal uses **(El-Kassas & Attia, 2014)**, antifouling agents **(Ibrahim *et al.*, 2019)**. Moreover, they were addressed as bioremediation agents **(Abdallah, 2013)**, nutritional sources for fish **(Wassef *et al.*, 2013)** and bioindicators of pollution **(Shams El-Din *et al.*, 2014)**. Recently, many studies focused on the nutritional value of seaweeds for the sake of human beings **(Shams El-Din & El-Sherif, 2012; El-Shafay, 2014; Shams El-Din & Shaltout, 2015; Alwaleed, 2019; Fouda *et al.*, 2019)**, especially that the Egyptian population has been witnessing a tremendous increase aligned with the increasing demand for qualitative and quantitative food supply. Notably, the red alga *Grateloupia gibbesii* Harvey (Family Halymeniaceae, order Haymeniales) was newly introduced in the Egyptian Mediterranean Sea **(Rodríguez-Prieto *et al.*, 2021)**.

Thus, the aim of this study was to investigate the seasonal variation of the biochemical content and nutritional composition of the newly recorded alga *Grateloupia gibbesii.*

MATERIALS AND METHODS

2.1. The study area:

Samples of *Grateloupia gibbesii* were collected from the Eastern Harbor, Alexandria, Egypt during winter 2012, spring 2012, spring 2015 & summer 2016 (Fig. 1).

The Harbor is a shallow, semi-enclosed embayment covering an area of about 2.8 km^2, located along the central part of Alexandria, the Mediterranean Sea. Large volumes of domestic waste water have always been dumped in the bay **(Ismael, 2012)**. Furthermore, the bay is polluted as a result of heavy shipping and fishing activities. The algal samples were collected from the Scout club located in the Eastern Harbor (E.H.) at 31°13.3`N latitude and 29°53.10`E longitude (Fig. 1).

Figure 1. The study area and the sampling station (Marine Scout Club) during 2016

2.2. Collection of the alga

The samples of the alga were handpicked whole from their bases, scraping the substrata on which they were attached and washed with seawater at the sampling site to remove sediments and impurities and were then put in polyethylene bags. The samples were stored under refrigeration (4°C). At the laboratory, algal samples were immediately rinsed with tap water to get rid of the remaining impurities and epiphytes. The newly introduced alga is identified in the study of **Rodríguez-Prieto *et al.* (2021)** as *Grateloupia gibbesii* Harvey (Fig.2). The species is currently regarded as the synonym of *Phyllemenia gibbesii* **(Rodríguez-Prieto *et al*,. 2022)**.

During every season, about 250g of the species was air dried to constant weight at room temperature (25°C). Excluding trace metals (2 replicas), the total average of three replicas and the standard deviation for each constituent of the alga, fatty acids and amino acids (one replica) were calculated.

2.3. Measurements of moisture and ash content of the alga

The moisture content of the algae was determined by drying 2g of samples in a thermo-regulated incubator (Lab Companion IB- G Series air, CHINA) at 105°C until reaching a constant weight **(AOAC, 1997)**. Moisture content (%) was determined by subtracting the wet weight of the alga from oven-dried weight.

Ash content was determined by incineration of one gram of the samples in a muffle furnace (Muffle furnace, 1200, CHINA) at 500°C overnight **(Munier *et al*., 2013)**. The ash content is expressed as a percentage of dry weight.

Fig. 2. Fresh material of *Grateloupia gibbesii* collected from the Eastern Harbor, Alexandria, Egypt in 2016

2.4. Measurements of biochemical contents

2.4.1. Total carbohydrates and total protein

The air- dried algal sub-samples were ground to fine powder using a mortar. Total carbohydrates (TCH) content was estimated according to the method of **Dubois *et al*. (1959)**. Measurements of total protein (TPr) content was performed spectrophotometrically at 650nm according to the method of **Lowry *et al*. (1951)**, using a salt-free bovine serum albumin as a standard. The results of the two components were expressed as mg/g dry weight.

2.4.2. Extraction and purification of total lipid

The air-dried algal sample was manually grained in a mortar before being weighed (1.00 g) into 100 ml screw-top vials. A total of 50 mL solvent was added in a specified order, and each sample was sonicated with a Lab Sonicator Model Cole-Parmer 8852 to facilitate cell wall rupture and oil extraction. The procedure was accomplished by immersing the bottle containing the algal sample with solvent mixture in the Sonicator for 15 minutes at 60°C **(Shaltout & Shams El-Din, 2015)**. According to **Folch *et al*. (1957)**, lipids were extracted from algae with 30ml 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 with a ratio of 2:2:1 for chloroform: methanol: water. The bottles were caped and re-shacked for another 30 minutes. 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.

2.4. 3.Determination of calorific content

For the evaluation of the nutritive value of *Grateloupia gibbesii* as a source of food for human being, the calorific content of the species was calculated, using the following known conversion values to convert the organic content into calorific values: fats (9.45), carbohydrates (4.10) and protein (5.65 K cal/g) **(Brody, 1945)**.

2.4.4. Determination of fatty acids

The extracted total lipid was transferred to 10ml clean screw top test tubes, with a freshly prepared solution of methanol: hydrochloric acid: chloroform (10:1:1 v :v:v ml) for esterification reaction at 90°C for 60 minutes **(Kumari *et al.*, 2011)**. FAMEs were then extracted by using hexane/ chloroform (4:1, v/v), where the hexane layer with extracted FAMEs was transferred to 10 ml screw top glass tube and evaporated till dryness. Afterwards, FAMEs were re-dissolved in 1.0ml of hexane at the time of measurement and were analyzed and characterized via gas liquid chromatography.

A gas chromatograph Model HP (Hewlett Packard) 6890 GC, equipped with 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 of 150oC for 2min. Injector temperature was 220oC, injection volume was 3µl, and detector temperature was 250oC. Nitrogen was used as the carrier gas and the split ratio was 50:1, while the gas flow was 1.0 ml.min-1. Individual peaks of FAMEs were identified by the comparison of the retention times and the 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)**.

2.4.5. Estimation of amino acids

The determination of amino acids was accomplished according to the method adopted in the study of **Sánchez-Machado *et al.* (2003)**. First, the amino acids were hydrolyzed by hydrochloric acid (HCl), derivatized and analyzed on the high-pressure liquid (HPLC).

2.4.5.1. Extraction of total amino acids (AAs)

A weight of 100 mg of dry algal biomass is hydrolyzed with 10 ml of HCl (6M) containing 1% phenol was then added in a small vial. Then, the vial was sealed and placed in a conventional oven at 110°C for 24h, cooled at room temperature, and their contents were vacuum-filtered through Whatman no. 41 paper. The filtrate was diluted to 25 ml with de-ionized water in a volumetric flask and 1.0 ml of the resulting liquid was membrane-filtered (Millipore 0.45 µm) to obtain the hydrolysates **(Sánchez-Machado *et al.*, 2003)**.

2.4.5.2. Derivatization with PITC:

The derivatization procedure was a modification of the method of González-**Castro *et al.* (1997)**. Amino acid standard solution or sample hydrolysate (30 µL) was placed in a tube and dried in an oven for 20 min at 42^{o}C. Methanol–water–TEA (2:2:1, v/v; 50 µL) was then added to the residue and the resulting solution was vacuum-dried for 20 min at room temperature (25 ^{o}C). Methanol–water–TEA–PITC (triethylamine (TEA) and phenylisothiocyanate (PITC)(7:1:1:1, v/v; 50 µL) was then added, and the tubes were vortex for 15s, and then left for 20 min at room temperature.The resulting solution was dried for 100 min at room temperature (25^{o}C).

After derivatization, Na_2HPO_4 (5 mM containing 5% acetonitrile; 100 µL) was added as diluent, with vortex mixing for 15 s.

The mobile phase was prepared from two solutions, A and B. Solution A was 0.14 M ammonium acetate buffer containing 0.05% (v/v) TEA (pH adjusted to 6.4 with glacial acetic acid). Solution B was acetonitrile- water 60:40 (v/v). The relative abundance of each peak and the comparison to amino acid standards allows for the quantification of each amino acid in the algal sample.

2.4.6. Measurements of macro-elements (phosphorus, nitrogen, calcium, sodium, potassium and magnesium):

For measurement of the total organic nitrogen content, the algal samples were dried at 40^{o}C to a constant weight. A weight of 1.0 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 75ml with double distilled water **(Grasshof, 1975)**. The concentration of total nitrogen content in the digested sample was estimated spectrophotometrically at 240 nm and using ammonium salt as a standard.

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. A weight of 1.00 g dry weight of each sample was acid digested in 5 ml concentrated HNO_3 in a Teflon lined 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 *et al*., 1983; 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/100g dry weight.

The macro-elements sodium, potassium, magnesium, and calcium were estimated as described by **(EPA, 2007)**, where 1.0 g of sample was digested for 75 min in concentrated 6 ml of HNO_3 (65%) at 95 °C. After which, dissolution of organic matter was achieved with the addition of 6 ml of hydrogen peroxide (30%). Samples were then digested for 1.0 h at 95 °C in concentrated (10 ml HCl), and then made up to volume with 10 ml of deionized water. The stock solutions were prepared in deionized water at the initial concentrations of 100.0 mg/l and 500.0 mg/l for each element. The concentrations of minerals in the samples and the standard solutions for each element were determined by Stat-Lab spectrometer (SS-147, CHINA) by using element-specific wavelengths 589.0, 766.5, 285.2, 422.7nm, for sodium, potassium, magnesium, and calcium, respectively. The results were within the concentration range of the series solution of standard for each element. The concentration of each element was expressed as mg/100g dry weight.

2.4.7. Estimation of trace metals:

For measurement of heavy metals in algae, the samples were oven-dried at 37 °C for 2 days to a constant weight and homogenized manually into powder. From each sample, two replica subsamples were prepared and digested in Teflon vessels with 4 ml HNO_3 and 2 ml $HClO_4$ for 12 h at room temperature and heated subsequently at 100 °C for 2 h. After cooling, solutions were then made up to 25 ml with double-distilled water **(Schuhmacher and Domingo, 1996)**. The analyses of metals in all samples were performed by PerkinElmer (AAnalyst 100) atomic absorption spectrophotometer. Hg concentration was measured by cold vapor technique using Mercury Hydride System MHS-10, while all the other metals were determined by flame technique. The detection limits of the studied metals were the

following: Cu (1.5 ppb), Zn (0.85 ppb), Cd (0.18 ppb), Pb (9.7 ppb), Ni (2.06 ppb), Co (8.8 ppb), Fe (4.6 ppb), Mn (0.84 ppb), and Hg (0.26 ppb). Accuracy and precision were verified by using reference materials for biota (MA-A-2/TM) provided by the International Atomic Agency (IAEA). Analytical results of the quality control samples indicated a satisfactory performance of heavy metal determination within the range of certified values with 95–111% (for biota) recovery for the metals studied.

Selenium concentration was determined by Stat-Lab spectrometer (SS-147, CHINA) at optical density of 685 nm. A weight of 100 mg dried algal sample was digested with concentrated nitric acid HNO_3 and H_2O_2 (3:1 v/v) at 180ºC for 3 h. The samples were allowed to cool, dissolved in 4 ml of 0.6% HNO_3 and filtered through Whatman filter paper **(EPA, 2007)**. The volume of each sample was maintained up to 10 ml with 0.6% HNO_3 and then the total Se content was analyzed and expressed as µg/g. The stock solution was prepared in deionized water at the initial concentrations of 100.0 mg/l and 500.0 mg/l for the metal ion.

3. Statistical analysis:

Analysis of variance (ANOVA-one way) was performed using **XLStat program (version 2020.3)** at confidence limit of 95% to find out the effect of seasonal variation on the biochemical contents, major elements and trace metals.

RESULTS

The total carbohydrates, total protein, total lipids, calorific content, total nitrogen, phosphorus content and fatty acids content of *Grateloupia gibbesii* during (winter 2012, spring 2012 and summer 2016), moisture, and ash content, macro-elements, trace elements, and amino acids contents of the alga during (spring 2015 and summer 2016) are shown in Tables (1-4) in addition to their comparison with that of other *Grateloupia* spp. from different localities (Table 5).

3.1. Biochemical contents:

3.1.1. Total carbohydrates, total protein and total lipids:

The total carbohydrates content in the alga showed differences during the three seasons (winter 2012, spring 2012, and summer 2016), where the concentration was exclusively high during summer 2016 (394.83±1.30mg/g), while the contents during winter 2012 and spring 2012 were comparable (60.67±17.50 and 69.67±2.52 mg/g), respectively. The total protein content showed great variations during the three seasons, with the maximum content (268.20±2.70 mg/g) during summer 2016, while the lowest content (40.00±4.58 mg/g) was recorded during spring 2012 (Table 1). However, the lipid content of *Grateloupia gibbesii* experienced low values during the three seasons; winter 2012, spring 2012 and summer 2016, ranging from (7.97- 12.04 mg/g) (Table 1).

Table (1): The chemical composition of *Grateloupia gibbesii* during (2012 &2016) and the P value of the ANNOVA-test.

Chemical composition	Winter (2012)	(%)	Spring (2012)	(%)	Summer (2016)	(%)	*P* value
Total carbohydrates (TCH) mg/g	60.67±17.50	6.07	69.67±2.52	6.97	349.83±1.3	34.93	**< 0.0001***
Total protein (TPr) mg/g	113.33±20.84	11.3	40.00±4.58	4.00	268.20±2.7	26.82	**< 0.006***
Total lipids (TL) mg/g	7.97±0.06	0.80	12.03±0.31	1.20	12.04±0.08	1.20	**< 0.0001***
Caloric value (Kcal/g)	0.97	-	0.63	-	3.25	-	-
Total nitrogen (TN) mg/gm	14.68±1.01	1.47	8.24±1.23	0.82	39.10±2.03	3.91	**< 0.0001***
Total phosphorus (TP) mg/100gm	258.33±26.35	0.26	1110.33±15.0	1.11	1680±122.9	1.68	**< 0.0001***

Note: The asterisk means significant

3.1.2. Fatty acids:

Like the total lipids, the total concentration of the fatty acids was low during the study period (winter 2012& spring 2012 and summer 2016), with low number (5-14). The fatty acids profile revealed higher concentrations during winter 2012(922.09 µg/ g**)** than that of spring 2012 (504.32 µg/g), whereas the concentration of fatty acids was meager during summer 2016 (21.58 µg/g). The peak of the fatty acids during winter 2012 and spring 2012 was due to the excessively high value of the monosaturated fatty acid (MUFA) heptadecenoic acid (C17:1) (757.05 and 469.50 µg/g), respectively. This fatty acid recorded a negligible concentration during summer 2016, causing the drop in the total concentration. Considering the saturated fatty acids (SFA), palmitic acid (C16:0) was the dominant one in the three seasons, recording the highest concentration (82.16 µg/g) during winter 2012. The composition of PUFAs group was meager, where many acids were absent. The docosadienoic acid (C22:2 n-6) was present during winter 2012 and summer 2016, with low concentration (1.24 and 1.88 µg/g), respectively. On the other hand, arachidonic acid (C20:4 n-6) and ecosapentanoic acid (EPA)(C20:5n-3)were only present during summer 2016, recording low concentrations of 2.35 and 1.46 µg/g, respectively (Table 2).

Table (2): The concentration (µg/g alga) and percentage (%) of fatty acids in *Grateloupia gibbesii*collected from Eastern Harbor during (2012 & 2016).

	Winter (2012)		Spring (2012)		Summer (2016)	
	Concentrat -ion	**% of total FA.**	**Concentrat -ion**	**% of total FA.**	**Concentrat -ion**	**% of total FA.**
n-carpoic acid C6:0	-	-	-	-	-	-
Caprilic acid C8:0	0.26	0.03	-	-	-	-
Capric acid C10:0	1.19	0.13	-	-	0.62	2.87
Undecanoic acid C11:0	2.14	0.23	-	-	-	-
Lauric acid C12:0	-	-	-	-	0.21	0.97
Myrisitic acid C14:0	0.30	0.03	-	-	0.41	1.90
Pentadecanoic acid	0.55	0. 06	7.37	1.46	-	
Palmitic acid C16:0	82.16	8.91	8.17	1.62	1.95	9.04
Margarinic acid C17:0	-	-	-	-	-	
Stearic acid C18:0	3.14	0.34	-	-	0.97	4.49
Heneicosanoic acid	0.84	0.09	-	-	-	
ΣSFA	**90.56**	**9.82**	**15.55**	**3.08**	**4.16**	**19.27**
Tetradecenoic acid	65.20	7.07	12.90	2.56	-	-
14, Pentadecenoic acid	5.64	0.61	6.38	1.27	-	-
9 Hexadecenoic acid	-	-	-	-	2.03	9.41
Cis-10 Heptadecenoic acid C17:1n-7	757.05	82.10	469.50	93.09	1.44	6.67
Oleic acid C18:1c n-9	1.49	0.16	-	-	2.88	13.35
13-Docosenoic acid	1.52	0.16	-	-	5.38	24.93
ΣMUFA	**830.90**	**90.11**	**488.78**	**96.92**	**11.73**	**54.36**
Arachidonic acid C20:4	-	-	-	-	2.35	10.89
Cis,5,8,11,14,17Ecosapentanoic acid C20:5n-3	-	-	-	-	1.46	6.77
Docosadienoic acid	1.24	0.13	-	-	1.88	8.72
ΣPUFA	**1.24**	**0.13**	**-**	**-**	**5.69**	**26.37**
ΣFA	**922.09**		**504.32**		**21.58**	

3.1.3. Calorific content:

The calorific content of *Grateloupia gibbesii* showed great seasonal variation. It was very low (0.97 & 0.63 Kcal/g) during winter 2012 and spring 2012, while it attained a maximum of 3.25 Kcal/g during summer 2016.

3.1.4. Amino acid composition:

The amino acid contents of the *Grateloupia gibbesii* are illustrated in Table 3. The total amino acids (EAA) were more rich (732.2 mg/g) during spring 2015 than that during summer 2016 (546.4 mg/g). However, the cystine was not detected during spring 2015, while tryptophane was not detected during both seasons (spring 2012 and summer 2016). The level of essential amino acids ranged from 7.80 to 97.40 mg/g during spring 2015 and from 4.1 to 57.7 mg/g during summer 2016. The alga was rich in arginine (97.40 mg/g) followed by lysine (56.70 mg/g) during spring 2015 and rich in arginine (41.6 mg/g) and leucine (57.7 mg/g) during summer 2016. The non-EAA ranged from 26.70 to 86.40 mg/ g during spring 2015 and from 19.5 to 68.5 mg/ mg during summer 2016. The alanine was the dominant one (86.40 mg/g) followed by glycine (75.50 mg/g) during spring 2015, while glutamic acid was dominant during summer 2016 (68.5 mg/g) followed by aspartic acid (68.3 mg/g).

Table (3): The concentration of amino acids (mg/g of dry weight) of *Grateloupia gibbesii* during spring (2015) and summer (2016).

Amino acid	Spring (2015)	Summer (2016)	FAO/WHO (1991) requirement pattern		
			Child (2-5 years)	Child (10-12 years)	Adult
Alanine[b]	86.40	39.4			
Arginine[a]	97.40	41.6			
Aspartic acid[b]	73.90	68.3			
Cystine[a]	ND	4.1			
Glutamic acid [b]	59.40	68.5			
Glycine [b]	75.50	38.5			
Histidine [a]	7.8	4.5	19.00	19.00	16.00
Isoleucine[a]	32.80	33.6	28	28	13
Leucine[a]	49.30	57.7	66	44	19
Lysine[a]	56.70	28.3	58	44	16
Methionine[a]	12.50	9.6	Methionine + cystine (25)	22	17
Phenylalanine[a]	32.60	29.4	Phenylalanine + tyrosine (63)	22	19
Proline [b]	28.70	31.7			
Serine [b]	26.70	19.5			
Threonine [a]	36.90	29.4	34	28	9
Tryptophane [a]	ND	ND	11	9	5
Tyrosine [a]	17.30	13.8			
Valine [a]	38.30	28.5	35	25	13
Total AA	**732.2**	**546.4**	339	241	127
EAA	**381.6**	**280.5**			
Non-EAA	**350.6**	**265.9**			
EAA/Non-EAA	**1.09**	**1.05**			
EAA/ Total AA	**0.52**	**0.51**			

[a] EAA, Essential amino acid. [b] Non-EAA, Non essential amino acid.
ND not detected

3.2. Moisture and ash content:

The moisture content of *Grateloupia gibbesii* was 24.01 and 12.76%, while the ash content was 27.72 and 9.69% during spring 2015 and summer 2016, respectively (Table 4).

3.3.1. Macro-elements:

However, the total nitrogen in the alga showed great variations during the three seasons, with the highest concentration (39.10 ±2.03 mg/g) during summer 2016, while the lowest concentration was recorded during spring 2012 (8.24±1.23 mg/g). Concerning the total phosphorus, it showed the maximum concentration during

summer 2016 (1680±122.88 mg/100 g) against the lowest one (258.33±26.35 mg/100 g) during winter 2012 (Table 1).

The calcium content was relatively low, with comparable values (130.00±17.32 and 126.67±15.28 mg/100g) during spring 2015 and summer 2016, respectively. The sodium and potassium content displayed relatively high concentrations during spring 2015 and summer 2016, with low N/K ratio of 0.86 and 0.60, respectively. There was a difference in magnesium content, with Ca/Mg ratio of 0.56 and 0.37 during both seasons, respectively (Table 4).

3.3.2. Trace metals:

The trace metals contents in *Grateloupia gibbesii* (copper, zinc, cadmium, lead, and iron) experienced relatively higher concentrations during summer 2016 (9.91±0.26, 184.50±57.42, 1.00±0.27, 4.50±0.32, and 126.30±0.14 µg/g), respectively compared with that of spring 2015 (9.61±0.44, 78.86±7.78, 0.73±0.06, 4.29±0.00, and 80.37±0.16µg/g) respectively, while nickel, manganese, selenium, and mercury showed inverse pattern. On the other hand, the cobalt content was comparable during both seasons (Table 4).

Table (4): Moisture, ash content and the concentration of some elements in dry *Grateloupia gibbesii*during spring (2015) and summer (2016), and the P value of the ANNOVA-test.

Mineral composition	Spring (2015)	Summer (2016)	*P* value
Percentage (%)			
Moisture content	24.01±0.00	12.76±0.10	**< 0.0001***
Ash content	27.72±0.01	9.69±0.13	**< 0.0001***
Macro-elements (mg/100g)			
Calcium	130.00±17.32	126.67±15.28	0.815
Sodium	1170±45.83	993.33±155.0	0.131
Potassium	1360±62.45	1660.00±65.5	**0.005***
N/K ratio	0.86	0.60	-
Magnesium	233±40.41	343.33±35.12	**0.024***
Ca/Mg ratio	0.56	0.37	
Micro-elements (µg.g^{-1})			
Copper	9.61±0.44	9.91±0.26	0.495
Zinc	78.86±7.78	184.50±57.42	0.123
Cadmium (Cd)	0.73±0.06	1.00±0.27	0.300
Lead (Pb)	4.29±0.00	4.50±0.32	0.442
Nickel (Ni)	4.24±0.05	3.94±0.07	**0.041***
Cobalt	2.84±0.05	2.86±0.01	0.730
Iron	80.37±0.16	126.30±0.14	**< 0.0001***
Manganese	6.41±2.18	4.80±0.02	0.436
Selenium	0.77±0.06	0.53±0.06	**0.008***
Mercury	1.81±0.16	0.87±0.02	**< 0.0001***

Note: The asterisk means significant

Table (5): comparative analysis of the biochemical and minerals contents of *Grateloupia gibbesii* with that of *Grateloupia* spp. in the previous studies.

Algal content	*G. gibbesii* (present study)	*G. doryphora* (Perfeto 1998)	*G. doryphora* Caliceti et al. (2002)	*G. doryphora* Lourenço et al.(2006)	*G. Lithophila* (Sivakumar and Arunkumar, 2009)	*G. trutru* (Denis et al. 2010)	*G. Lithophila* (Manju et al. 2012	*G. trutru* (Munier et al., 2013)	*G. trutru* (Cecile et al. 2016)	*G. gibbesii* (Shabaka andMoawad, 2021)
Ash content (percentage)										
Ash content	9.69-27.72	6.98-11.85				18		14.44-15.59	30.96	6.73
Biochemical contents (mg/g)										
Total carbohydrates	60.67- 349.83	418.20-547.2	-	-	-		55.20	16.05-41.57	-	63.70
Total protein (TPr)	40.00-268.20	229-290	-	-	-	230	305.00	161.58-217.75	-	47.90
Total lipids (TL)	7.97-12.04	8.10-13.0	-	-	-	26.00	18.00	28.07-54.35	32.50	12.40
Total nitrogen (TN)	8.24-39.10	-	-	52.30-93.50	-				36.20	-
Macro-elements (mg/100g)										
Calcium	126.67-130.00	-	-	-	-	-	-	-	-	651
Sodium	993.33-1170	-	-	-	6558	-	-	-	-	1438
Potassium	1360-1660	160-1270	-	-	3818	-	-	-	-	502
Magnesium	233-343.33	-	-	-	-	-	-	-	-	515
Phosphorus	258.33-1680	75-200	-	260-740	-	-	-	-	-	115
Na/K ratio	0.60-0.86									4.85

Micro-elements (µg/g)										
Copper	9.61-9.91	-	6.00-14.00	-	-	-	-	-	-	15.00
Zinc	78.86-184.50	-	163-300	-	-	-	-	-	-	350.00
Cadmium	0.73-1.00	-	<0.1-0.3	-	-	-	-	-	-	9.00
Lead	4.29-4.50	-	0.60-5.60	-	-	-	-	-	-	2.50
Nickel	3.94-4.24		0.60-4.30			-	-	-	-	14.75
Cobalt	2.84-2.86	-	-	-	-	-	-	-	-	-
Iron	80.37-126.30	-	190-448	-	-	-	-	-	-	873
Manganese	4.80-6.41	-	-	-	-	-	-	-	-	16.00
Selenium	0.53-0.77	-	-	-	-	-	-	-	-	nd
Mercury	0.87-1.81	-	-	-	-	-	-	-	-	-

4. Statistical analysis:

The results of ANOVA test revealed that the seasonal variation in all the biochemical contents was highly significantly different (P value at confidence limit 95% < 0.0001-0.006) (Table1). The moisture and ash content showed significant difference (P< 0.0001) during (spring 2015 & summer 2016, Table 4). For the major elements, the values of potassium and magnesium were significantly different (P= 0.005 & 0.024, respectively), while that of sodium and calcium were not significantly different during (spring 2015 &summer 2016) (Table 4). All the trace metals were not affected by seasonal variation, except for nickel (P value = 0.041), iron (P value = < 0.0001), selenium (P = 0.008), and mercury (P< 0.0001) (Table 4).

DISCUSSION

The red alga *Grateloupia gibbesii* is an endemic species of the West Atlantic. It was recorded in Florida, USA **(Leichter *et al.*, 2008)** and in Albama at Gulf of Mexico **(Priest and Lopez-Bautista, 2012).** The species is recently reported from the Eastern Harbor, Egyptian Mediterranean Sea (**Rodríguez-Prieto *et al.*, 2021).** To the best of our knowledge, there is no available literature on the biochemical and nutritional composition of the native strains of this alga.

The protein content of *Grateloupia gibbesii* varied from 4.00 to 26.82% during the three seasons (winter 2012, spring 2012 and summer 2016). **Fleurence (1999)** reported that the protein content was within the range of 10-47% for green and red seaweeds. In the present study, the range of the protein content was lower than that of *Grateloupia doryphora* **(Perfetto, 1998)**, *Grateloupia truturu* **(Denis *et al.*, 2010; Munier *et al.*, 2013)** and *Grateloupia lithophila* **(Manju *et al.*, 2012**) which was due to the remarkably low value during spring 2012 in the current study. In contrast, the total carbohydrates content in *Grateloupia gibbesii* was excessively higher (60.67-349.83 mg/g) than that of *G. lithophila* (55.20 mg/g) **(Manju *et al.*, 2012)** and that of *G. turutru* (16.05-41.57 mg/g) **(Munier et al. 2013)**. While, **Perfetto (1998)** recorded higher carbohydrates content (418.20-547.20 mg/g) in *G. doryphora* than that of the current study, which in consequence showed decreased values of protein/carbohydrates ratio. Our results agreed with **Perfetto (1998)**, where the ratio oscillated between 0.57-1.87, indicating that the alga is nitrogen limited **(Turpin, 1991)**.

Generally, seaweeds have little total lipids content, ranging from 1-5% of dry weight (El Shoubaky *et al.*, 2008), which is in agreement with our results (0.80-1.20%). These values were comparable with that of *G. doryphora* **(Perfetto, 1998)**, but lower than that of previous studies on *G. truturu* **(Denis *et al.*, 2010; Munier *et al.*, 2013; Cecile *et al.*, 2016)**, *G. lithophila* **(Manju *et al.*, 2012)**, and *G. gibbesii* **(Shabaka and Moawad, 2021)**. However, the biochemical composition of *Grateloupia gibbesii* of the current study during spring coincided with that of *Grateloupia gibbesii* collected from the same site during this season **(Shabaka and Moawad, 2021).** The variation in the level of biochemical contents as well as

minerals contents is attributed to the seasonality **(Shiener *et al.*, 2015)** which was confirmed by the statistical analysis in the current study, differences in algal species, locality and environmental conditions such as temperature, salinity, and nutrient concentration **(Marinho-Soriano *et al.*, 2006)**. For gross energy, *Grateloupia gibbesii* displayed particularly low values during winter 2012 and spring 2012, recording a maximum of 3.25 Kcal/g during summer 2016, which was comparable to some edible seaweeds such as Nori, Dulse, Kombu, Wakame and Hijiki (2.32-3.49%) **(Philpott and Bradford, 2006)**. In fact, **Rupérez and Saura-Calixto (2001)** attributed the low energy content in seaweeds to low crude lipid content, high protein content, as well as non-digestible polysaccharides, which designate them as a potential source of healthy food.

Despite the low percentage of lipids, most of marine algae are rich in polyunsaturated fatty acids (PUFAs) **(Kayama *et al*,. 1989)** and include essential fatty acids, important in the nutrition of humans and animals **(Van Ginneken *et al.*, 2011)**. Saturated fatty acids, according to **Ulbricht and Southgate (1991)**, raise relative risks of diseases such coronary heart disease, atherosclerosis, fatty liver disease, inflammatory diseases, and Alzheimer's disease. Unsaturated fatty acids, on the other hand, reduced the risk of many disorders **(Simopoulos, 1991)**. In fact, fatty acid chain length is also used for the diagnosis of many diseases **(Poulos *et al.*, 1985)**, where the propagation of very long chain fatty acids (>22 carbon length chain) is indicative of these disorders **(Izai et al., 1992)**.

In the present study, the SFAs contributed by low percentages (3.08-19.27%), with the dominance of palmitic acid (1.62-9.04%). The PUFAs was represented only by docosadienoic acid (C22:2 n-6), arachidonic acid C20:4 n-6 and ecosapentanoic acid C20:5n-3 (EPA) in very low amounts. In fact, the fatty acids profile of *Grateloupia gibbesii* was very rich in monosaturated fatty acid (MUFAs), which is due to the presence of *cis*-10-heptadecenoic acid (C17:1) in considerable amounts (757.05 and 469.50 µg/ g) during winter and spring 2012, respectively. In contrast, the concentration of this fatty acid was negligible during summer 2016, which may be attributed to seasonal variability associated with temperature shift **(Shaltout and Shams El-Din, 2015)**. Actually, *cis*-10-heptadecenoic acid (C17:1) is considered as a member of omega-7 fatty acids, which are rare in the plant kingdom and even scarcer in the animal world. A small daily dose of omega-7 functions as a lipokine; a signaling molecule that reduces inflammation and promotes lipid profile stabilization, resulting in a net reduction in cardiovascular and diabetes risk **(Foryst-Ludwig*et al.*, 2015)**. Omega-7 may minimize the risk of early death or disability related to overweight, obesity, or disturbed lipid profiles **(Farias de Queiroz *et al.*, 2009)**. In addition, *Cis*-10-heptadecenoic acid (C17:1) was tested for anticancer efficacy and found to inhibit HL-60 cell growth with an IC50 of 302 µM and to prevent LPS-induced tumor necrosis factor generation in mice macrophages **(Fukuzawa *et al.*, 2008)**.

Nitrogen plays a significant role in growth and metabolism of algal cells. It is an essential element required for the production of proteins and nucleic acids (Angell et al., 2014). In the current study, the percentage of nitrogen content in *Grateloupia*

gibbesii was (0.82-3.91%), which was much lower than that of *Grateloupia doryphora* (5.23-9.35%)(Lourenço et al., 2006). According to Hu (2004), nitrogen makes up 7% to 20% of the dry weight of algal cells.

The essential amino acids found in *Grateloupia gibbesii* accounted for 51-52% of the total amino acids, which was higher than that of **Wong and Cheung (2000)**(42.1-48.4%). In the present study, the level of the essential amino acids fulfilled to those of the **FAO/WHO (1991)** requirements, particularly that of children (10-12 years) and adults. Accordingly, *Grateloupia gibbesii* can be able to contribute adequate levels of total EAA for human being. In most analyses of amino acid composition in marine algae, glutamic acid and aspartic acid have the greatest quantities of amino acids **(Siddique *et al.*, 2013)**. These amino acids occur as protein constituents and as free amino acids or their salts and are responsible for the special flavor and taste **(Mouritsen, 2013)**, in addition to alanine and glycine **(Holdt and Kraan, 2011)**. In the current study, *Grateloupia gibbesii* contained large amount of aspartic acid and glutamic acid during summer 2016. Similar results have been obtained in previous studies **(Gressler *et al.*, 2010; Benjama and Masniyom, 2012)**. Considering the essential amino acids, arginine was present in considerable amount, particularly during spring 2015. It is involved in cell division, wound healing, ammonia removal from the body, immunological function, and hormone release **(Witte and Barbul, 2003)**. It is a precursor for the synthesis of nitric oxide (NO), hence it's crucial for blood pressure control **(Dong *et al.*, 2011)**. In the present study, the concentration of leucine (4.93- 5.77 %) during the two seasons far exceeded many food sources such as soybeans (2.87%), beef (1.67%), fish and salmon (1.62%), whereas it was lower than concentrate powder of whey protein (10.00-12.00%), soy protein (7.5-8.5%), and pea protein (6.60%)**(NNDSR, 2015)**. However, lysine concentration attained a value of (5.60%) during spring 2012, corresponding with food containing significant proportions of lysine (5.73-9.19%) such as beef, chicken, soybean, and legumes. Actually, a food is considered to have sufficient lysine if it has at least 5.10% **(Institute of Medicine, 2005)**.

The moisture content during spring (2015) was double than that of summer 2016 (24.01 and 12.76%, respectively). This may be attributed to the higher temperature during summer 2016 (29.0^{o}C) than that of spring 2015 (21.0^{o}C), respectively **(Abd El-Ghany, 2016)**. Furthermore, the species grows on the rocky shore, which is exposed to high rate of evaporation and light intensity, particularly during summer season, resulting consequently to higher desiccation of the alga and lower moisture content. This significant difference in the two values of moisture was confirmed statistically ($P< 0.0001$). However, there is no available literature on the moisture content on *Grateloupia* spp. The ash content of *Grateloupia gibbesii* was (9.69-27.72%) which was higher than that of *Grateloupia gibbesii* during spring (6.73%) **(Shabaka and Moawad, 2021)**, *Grateloupia doryphora* (6.98-11.85%) **(Perfetto, 1998)**, *Grateloupia trutru* (14.44-15.59%) **(Munier *et al.*, 2013)** and (18.00%) **(Denis *et al.*, 2010)**, but was lower than that of *Grateloupia trutru* (30.96%)**(Cecile *et al.*, 2016)**. **Siddique *et al.*, (2013)** found that high level of ash was

coupled with the amount of mineral elements. However, ash content of seaweed accounts for 8 and 40% DW **(Rupérez, 2002)**. The variation in ash content depends on seaweed species, geographical origins, and their method of mineralization **(Sanchez-Machado *et al*., 2004).** In this trend, **Pavia *et al*., (2018) and Ansari and Ghanem (2019)** attributed the variations in the ash content to changes in environmental conditions and seasonality. Our results agreed with these findings, where seasonal variation affected significantly the ash content ($P< 0.0001$). On the other hand, the ash content in the current study, particularly during spring was higher than the common edible vegetables (5-10%), such as potatoes (10.4%), carrots (7.1%) and tomatoes (7.1 %) **(Rupérez, 2002)**, but comparable to the spinach (20.4%) **(USDA, 2001)**.

The valuable mineral contents of seaweeds grant them great potential for human nutrition and for application in the food industry as new ingredients for the development of numerous functional food products **(Circuncisão *et al*., 2018)**. For instance, seaweeds contain considerable amounts of calcium, which can fulfill the requirements of human being, particularly for expectant mothers, adolescents and elderly **(Burtin, 2003)**. They also contain high levels of potassium (K), sodium (Na), phosphorus (P) and magnesium **(Bocanegra *et al.*, 2009)**. In the present study, magnesium content was 233.00-343.33 mg/100g, while calcium content was 126.67-130.00 mg/100g. Comparing the content of these two elements with that of **Shabaka and Moawad (2021)**, our results were much lower. The Ca/Mg ratio was 0.37-0.56, which showed that *Grateloupia gibbesii* is calcium limited. An appropriate Mg intake may decrease blood pressure, since it acts as a calcium antagonist on smooth muscle tone, thus causing vasorelaxation **(Bo and Pisu, 2008)**. It's worth noting that the Ca/Mg ratio is a predictor of calcium absorption, since a lack of magnesium can lead to an excessive accumulation of calcium in soft tissues, resulting in kidney stones formation and arthritis **(Blaine *et al*., 2015)**. Considering the calcium dietary recommended intake (DRI) for male and female (800 mg/100g per day)**(Ratana-arporn and Chirapart, 2006)**, its content in the present study was lower than these values.

Sodium and potassium content in *Grateloupia gibbesii* was excessively lower than that of *Grateloupia lithophila* **(Sivakumar and Arunkumar, 2009)**, whereas the second element was higher than that of *Grateloupia doryphora* **(Perfetto, 1998)**. In the present study the Na/K ratio was 0.86 and 0.60 during spring 2012 and summer 2016, respectively, which was much lower that of **Shabaka and Moawad (2021)**(4.8). Furthermore, these results were lower than that reported by **Abdallah (2008)** and **Shams El-Din and El-Sherif (2012)** in some Egyptian seaweeds. This is due to the seasonal variations of these elements in the algae **(Ansari and Ghanem, 2019)**. Notably, the Na/K ratio recommended by the World Health Organization (WHO) is close to one, so consumption of food products with this proportion or below should be considered for healthy cardiovascular purposes **(Blaustein *et al*., 2012)**.

Previous studies of **Perfetto (1998) and Lourenço *et al.* (2006)** on *Grateloupia doryphora* revealed relatively low range of phosphorus content (75-200

mg/100g) and (260-740 mg/g), respectively compared with that of *Grateloupia gibbesii* (258.33-1680 mg/100g). Furthermore, our results were much higher than that of **(Shabaka and Moawad, 2021)**(115 mg/100g), which can be attributed to the difference in time of harvesting of the alga and the effect of different environmental conditions surrounding the alga.

Essential trace elements are namely; copper, zinc, iron, manganese, cobalt, molybdenum, selenium, and iodine **(Circuncisão et al., 2018)**. They play a significant role for health maintenance. Fe is included in oxygen transport, electron transfer, and oxidase activities, while Mn is bounded with many metalloenzymes and is associated to amino acid, lipid, and carbohydrate metabolism **(Mišurcová *et al.*, 2011)**. Likewise, Cu and Zn are involved in neurotransmitter synthesis and energy metabolism **(Osredkar and Sustar, 2011)**. Furthermore, Co is essential for vitamin B12 synthesis **(Bocanegra *et al.*, 2009)**. Essential trace element imbalances, whether minor or severe, can be considered risk factors for a variety of disorders of public health concern **(Nugta, 2010)**. Large concentrations of selenium salts, for example, are poisonous, although traces are required for cellular activity in many organisms. Selenium is a constituent of the antioxidant enzymes glutathione peroxides and thioredoxin reductase. Recent research has revealed that selenium-containing deiodinase enzymes are responsible for the conversion of thyroxine to 3,5,3'-triiodothyronine (the metabolic active form)**(Mehri and Marjan, 2013)**, which has many beneficial effects on human health **(Gelfand *et al*,. 1987)**.

In the current study, most of the trace elements revealed lower concentrations than that of the study on *G. gibbesii* by **(Shabaka and Moawad, 2021)**, which may be attributed to temporal variations and the fluctuations of the environmental conditions. Furthermore, our results were lower than that of *G. doryphora* **(Caliceti *et al.*, 2002)**. Considering *Grateloupia gibbesii* as a potential food for human being, the concentrations of essential trace elements were adequate for fulfilling human dietary intake. For instance, the selenium content was low (0.55-0.73 µg/g) in respect to the dietary intake, where **Rayman (2012)** recommended for selenium intake an average of 60 µg per day for men and 53 µg per day for women. In addition, the concentration of copper and zinc was relatively low, ranging from (9.61-9.91µg/g) and (78.86-184.50µg/g), respectively. According to the Institute of Medicine, the daily intake of copper and zinc for an adult should not exceed 2 and 10 mg, respectively **(Trumbo et al., 2001)**. However, seaweeds have an advantage over terrestrial plants in that they contain lower levels of phytic acid **(Oliveira *et al.*, 2009)**, which is especially significant when it comes to zinc consumption because the human body does not store this mineral, necessitating daily consumption **(Rink and Gabriel, 2000)**.With zinc concentrations greater in the current study than that of many staple **foods (kenneth *et al.*, 2003)**, *Grateloupia gibbesii* may be beneficial in reducing zinc insufficiency **(Charney, 2012)**.

There is a seasonal variation in iron content, where the summer 2016 showed higher content than that of spring 2015. This was confirmed by the statistical analysis (P value = < 0.0001). In general, the iron content in *Grateloupia gibbesii* surpassed

that of the terrestrial crops (20 - 40 μg.g^{-1}) **(Coultate, 2009)**. The estimated daily intakes of iron was 18 mg/day **(FNB /IOM, 2001)** in women (19–50) years, assuming consumption of 8 g dried seaweed for an average adult (70 kg) in Asian cuisines (cited in Aroyehun et al. 2019). In the current study, *Grateloupia gibbesii* contributed by (642.96-1010.40 μg.g^{-1}) of the recommended daily average allowance.

There is no available literature on the defined limits of cobalt in the genus *Grateloupia* to be used for human consumption. The content of cobalt in *Grateloupia gibbesii* was high during (2.84-2.86 μg/g), which was relatively close to the edible *Gracilaria* spp. (1.5 μg/g)(Neto et al. 2018), lower than that of the edible *Phymatolithon calcareum* (7.00 μg/g)**(Desideri *et al.*, 2016)** and much higher than that of *Chondrus crispus* (0.1 μg/g)**(Ruperez, 2002)**. On the other hand, the content of manganese in *Grateloupia gibbesii* (4.80-6.41 μg/g) was lower than that of these three edible algae and lower than that of *Grateloupia gibbesii* (16 μg/g) **(Shabaka and Moawad, 2021)**.

High level of mercury, lead and other hazardous metals limits safe consumption of macroalgae as food source for human being **(Balina *et al.*,2016; Desideri *et al.*, 2016)**. Considering Cd, Pb, and Ni content in *Grateloupia gibbesii*, they far exceeded the values of that of previous studies (0.062-0.54, 1.00, 2.60 μg/g), **(Squadrone *et al.*, 2018; Real Decreto, 1978; Nilka de Oliveira *et al.*, 2009)**, respectively. Mercury content was particularly high than that of many European edible seaweeds (0.006 - <1 μg/g)**(Circuncisão *et al.*, 2018)**.

CONCLUSION

The newly recorded red alga *Grateloupia gibbesii* is a good source for carbohydrates, proteins, amino acids and fatty acids. The alga is enriched also with macro-elements and essential micronutrients, which showed great seasonal variations. The spring (the growing season) showed the highest concentrations, except for biochemical contents. On the other hand, the high concentration of toxic metals limits safe consumption of the alga as food source for human being. However, bioremediation can be a viable option in this case. On the other hand, it is highly recommended to set legislations in Egypt to define the limits of toxic elements in seaweeds, which are used as fertilizers for terrestrial plants, additive foods for fish, and livestock in our country. It is noteworthy to mention that Egypt should adopt seaweeds farming as a new trend, which will guarantee safe seaweeds consumption.

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