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INFLUENCE OF DRIFTING ON AMINO ACID PROFILES, MINERALS, PHENOLIC CONTENTS AND ANTIOXIDANT ACTIVITY OF THREE MARINE BROWN ALGAE

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Abstract

Effect of drifting on amino acids profile and mineral content in attached and drift marine algae: *Dictyota dichotoma*, *Sargassum vulgare* and *Cystoseira spinosa* (phaeophyta) was investigated as well as, their antioxidant activity in relation to their total phenolic content. All algae tested displayed similar amino acid patterns, with predominance of aspartic and glutamic acids in their tissues. Total phenolic contents varied from a minimum of 0.22 mg g^{-1}dw in drift *Cystoseira* samples, to a maximum of 0.93 mg g^{-1}dw in attached *Dictyota* samples. The results revealed that drifting negatively affect the phenol content of studied species. The extract of attached and drift *D. dichotoma* found to have the most potent antioxidant activity (45.38 and 33.61%), respectively. Cu and Na were presented in low values, especially in *Dictyota dichotoma*, while Fe and K were the most abundant elements in all algae analyzed. All micro and macro elements values were negatively affected by drifting, except those values of K, Ca and Mg in *Cystoseira* samples.

Keywords: Drifting**,** algae, antioxidant activity, phenolic content, minerals.

Introduction

Seaweeds have been used as food, fertilizers and for medicinal purposes for a long time. Over the last several decades, seaweeds or their extracts have been studied as novel sources which have been shown to produce a variety of inorganic and organic substances, which probably have been reported to possess biological activity of potential medicinal value (Hou & Yan, 1998; Jiménez-Escrig & Goni, 1999; Fallarero *et al.,* 2006; Satoru *et al.,* 2003). Free radicals are responsible for aging and causing various human diseases. Several studies showed that antioxidant substances which scavenge free radicals have been obtained from marine seaweeds (Burtin, 2003; Lim *et al.,* 2002; Zhao *et al.,* 2004). The antioxidants mainly identified as phenolic compounds (Kuda *et al.,* 2005a; Lim *et al.,* 2002). The high phenolic, amino acid and mineral contents in seaweeds, especially brown algae, make them nutritionally and pharmaceutically valuable

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(Jiménez-Escrig and Sanchez-Muniz, 2000; Portugal et al., 1983; Zahra *et al.*, 2007).

Drying may affect the types and proportions of the chemical constituents of algae. As seaweeds can deteriorate rapidly, it is important to transport them to laboratory soon after harvest; so they can be stored for a long time without significant loss of their chemical constituents (Naylor, 1976; Wong & Cheung 2001a). In general, slow drying under high temperature and exposure circumstances (drifting) may cause chemical and physical degradation in seaweed tissues (Fellows, 1988; Zubia *et al.*, 2003). Drifting could be an important factor affecting the nutritional value of seaweeds either through chemical modifications or direct losses of the nutrients. However, information concerning the effect of drifting on the chemical composition of different seaweeds is limited (Wong & Cheung 2001a; Zubia *et al.*, 2003).

Among marine algae spread along seashore of Alexandria, brown algae constitute one of the most abundant groups of economic interest. The present work was undertaken to investigate the effect of drifting on some chemical nutritional constituents of three common brown seaweeds: *Dictyota dichotoma*, *Sargassum vulgare* and *Cystoseira spinosa*, in relation to their antioxidant activity.

Materials and Methods

Study site:

Abu-Qir Bay is a semi-enclosed basin located about 36 km east of Alexandria. It lies between 30°05'- 30°22' E and 31°16'- 31°21' N. This western inshore area of the bay is a shallow region (with average depth of 3.8 m), interrupted with several rocky islets.

Sampling:

Attached samples of three brown marine algae *Dictyota dichotoma*, *Sargassum vulgare*, *Cystoseira spinosa* were randomly collected and then were brought to the laboratory in dark plastic bags just after harvest. In addition, several samples of drift algal species were cautiously collected and transported to laboratory in dark plastic bags. Then all collected samples were identified, washed with distilled water to remove epiphytes and impurities and then weighed. The algal samples were dried at 60 ^{0}C till constant weight. The dried samples were weighted and ground into a fine powder. All chemical analyses were conducted in triplicate on dried ground material. All values were reported relative to the dry weight of the seaweed. Mean values and standard error were calculated.

Determination of total amino acids:

Seven ml of HCl with mercapto-ethanol (5µ L / 10 ml acid) were added to 0.04 mg sample in test tube. The test tubes containing acidified samples were suspended in a vacuum assembly with purified nitrogen purge and evacuated and purged with N three times, then sealed off. The samples then hydrolyzed at 110°C for 22 hrs. The test tubes were cooled to room temperature and scibed with a tube cutter, filtered and transferred to 10 ml volumetric flask using deionized water. One ml of filtrate was dried in a vacuum desiccators in the presence of NaOH. Then the dried sample residues were dissolved in 2 ml diluting sodium citrate buffer (pH 2.2) and filtered through filter paper according to the method described by Moore and Stein (1958).

Determination of total phenolic compounds:

Total phenolic compounds in the fresh and drift brown algae were analyzed in triplicates according to the standard Folin-Ciocalteu method (Velioglu *et al.,* 1998). One ml aliquot of each algal sample placed in a test tube contained 1.5 ml de-ionized water as well as 0.5 ml of 0.1 M Folin-Ciocalteu reagent was added. After 1 min, 1.0 ml 20% Na_2Co_3 was added to the mixture followed by vortex-mixing. The reaction mixture was allowed to stand for 60 min in darkness. The control contained all the reaction reagents except the algal sample. The total phenolic compounds were determined colorimetrically at 750 nm using a spectrophotometer, and compared to a gallic acid calibration curve.

Antioxidant activity for DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity:

Scavenging activity of algal samples towards DPPH free radical was performed according the method of Yen and Chen (1995). Two ml aliquot of tested algal samples (in methanol: chloroform, 2:1) were added to 2.0 ml of 0.16 mM DPPH methanolic solution. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in the dark, then the absorbance was read at 517 nm. The capability to scavenging the DPPH radical was calculated using the following equation according to (Zhang *et al*., 2006):

Scavenging effect (%) = {1- ($A_{sample} - A_{sample\ blank}$) / $A_{control}$} X 100

Where $A_{control}$ is the absorbance of control (DPPH without algal samples), A_{sample} is the absorbance of algal sample with DPPH, and $A_{sample\ blank}$ is only the absorbance of algal sample (without DPPH).

Determination of reducing power:

The reducing power of algal samples was determined as described by Dorman *et al.,* (2003). One ml of each sample was mixed with 1.0 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% aqueous potassium hexacyanoferrate solution. The reaction mixture was left for 30 min at 50˚C, then 1.5 ml of 10%

trichloroacetic acid were added. The mixture was then centrifuged at 2500 rpm for 10 min. Finally distilled water and 0.5 ml of 0.1% aqueous $FeCl_3$ were added, and the absorbance was measured at 700 nm. The data were presented as ascorbic acid equivalent (AscAE) in milligrams of ascorbic acid per gram of extract.

Determination of elements:

Part of each algal samples were oven dried at 65˚C till constant weight obtained. Three grams of each dry sample were dried in muffle at 550˚C for an hour, digested using concentrated HCl and HNO_3, then completed to a definite volume and analyzed for their metal content according to the method of Martin (1979) using Atomic Absorption or emission spectrophotometer.

Results

The effect of drifting on amino acid profiles are presented in Table 1. The amounts of amino acids of all drift samples were significantly ($p<0.05$) lower than those of the attached algal samples. Moreover, due to drifting, the damage of the amino acids seemed to be non-specific, since the differences on the level of individual amino acids between the attached and the drift algal samples showed no particular trend (Table 1). The tested algae displayed similar amino acid patterns, with a predominance of aspartic and glutamic acids in their tissues.

Total phenolic contents of the three tested algae were presented in Figure 1A. It varied from a minimum of 0.22 mg g^{-1}dw in drift *Cystoseira* samples, to a maximum of 0.93 mg g^{-1}dw in attached *Dictyota* samples. Attached and drift samples of *D. dichotoma* exhibited the maximum phenolic content among the other algal samples analyzed. From Figure 1A, it was obvious that drifting negatively affect the phenol content of studied algal species. *Dictyota dichotoma* was the least species affected by drifting, where it lost only 16.13 % of its phenolic content, while *Sargassum* and *Cystoseira* lost about 23.89 and 51.11 %, respectively of their phenolic content.

All algal samples possessed the antioxidant ability to various degrees (Figure 1B). Of the tested algae, the extract of *Dictyota* (attached and drift samples) found to have the most potent antioxidant ability (45.38 and 33.61%). Extract of *Sargassum* and *Cystoseira* showed nearly equal scavenging values towards DPPH for attached (17.5 & 17.0 %) and drift samples (10.97 & 10.03 %), respectively (Figure 1B). In general, the higher total phenolic content of algal samples resulted in higher antioxidant capacity. Reducing power of the tested algae was represented in Figure 1C. On the basis of ascorbic acid equivalent (AscAE) values, extract of attached *Dictyota* samples showed the maximum (2.64 mg g^{-1}) reducing power rather than other studied algal extract. From Figure 1C, it

Table 1. Influence of drifting on amino acid profiles (mg g^{-1}dw) of the brown algae *Dictyota dichotoma, Sargassum vulgare* and *Cystoseira spinosa* from Alexandria.

Amino Acids (mg g-1 dw)	*Dictyota dichotoma*		*Sargassum vulgare*		*Cystoseira spinosa*	
	Attached	**Drifted**	**Attached**	**Drifted**	**Attached**	**Drifted**
Aspartic acid	7.70	5.70	6.88	4.90	5.33	3.98
Therionine	**1.37**	**1.30**	3.11	2.70	2.00	1.72
Serine	**1.20**	**1.21**	1.79	1.60	1.55	1.44
Glutamic acid	8.00	5.00	8.03	7.07	6.31	6.01
Proline	1.27	0.98	**2.72**	**2.70**	**1.92**	**2.00**
Glycine	3.02	2.64	**4.10**	**3.91**	2.74	2.56
Alanine	3.91	2.70	4.01	3.93	2.00	2.01
Valine	2.78	2.10	**3.00**	**2.95**	**2.00**	**1.82**
Methionine	0.62	0.43	0.71	0.56	0.73	0.81
Isoleucine	2.07	2.14	2.76	2.51	1.54	1.86
Leucine	3.89	3.51	4.21	4.00	3.02	2.64
Tyrosine	0.76	0.53	**0.70**	**0.51**	0.87	0.77
Phenylalanine	1.93	1.60	2.00	1.61	1.84	2.11
Histidine	2.31	2.20	2.42	1.90	**1.90**	**1.90**
Lysine	0.61	0.55	0.69	0.57	0.76	0.53
Arginine	2.86	2.24	3.00	2.53	**1.75**	**1.60**

Bold numbers means that the difference between attached and drifted amino acid values are not significant ($p < 0.05$). Tryptophan and cystine are not detected.

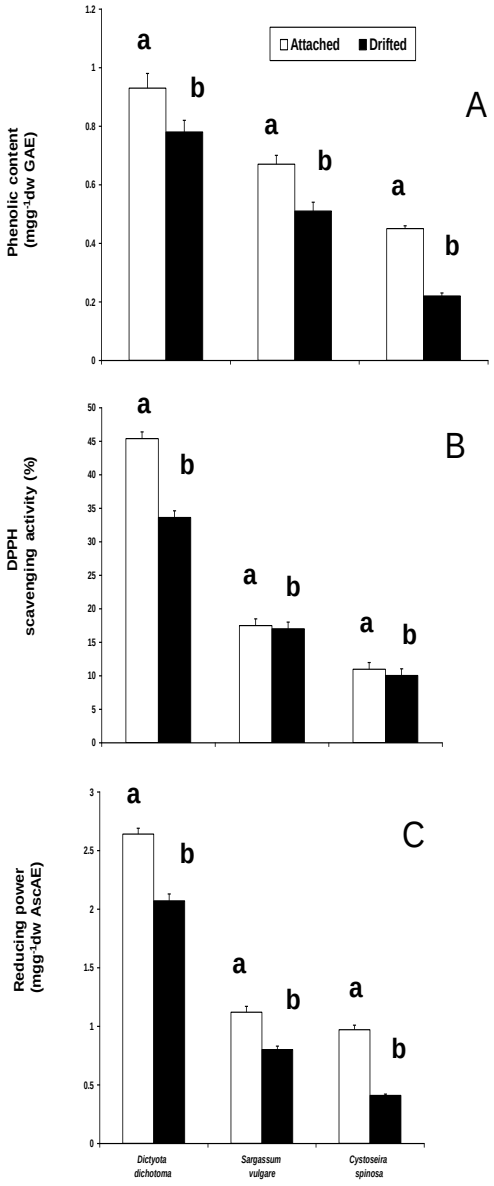

Figure 1. Effect of drifting on total phenolic content (mg g^{-1} dw) (A), DPPH Scavenging activity (%) (B), and reducing power (%) (C) of three brown algae. Different letters on Columns for each species means significant at $p < 0.05$.

appeared clearly that, drifting significantly decrease the reducing power ability of all analyzed algal samples.

Figure 2 & 3 represent the macro and micro elements concentrations measured in attached and drift algal samples. Cu and Na were present in low concentrations in all species tested, especially in *Dictyota dichotoma*. Fe and K were the most abundant elements, where the maximum Fe concentration (1801 µg g^{-1}dw) was measured in drift sample of *Sargassum*, while the maximum K value (10.2 % dw) was recorded for drift *Cystoseira* samples. High concentrations of macro elements were observed in attached and drift tissues of *Cystoseira spinosa*, while high values of micro elements were measured in attached and drift samples of *Sargassum vulgare*. Mineral contents of tested algae varied in all attached and drift algal samples analyzed, without particular trend. In general, all micro and macro elements values were negatively affected by drifting, except those values of K, Ca and Mg in *Cystoseira* samples.

Discussion

The non-specific degradation of amino acids by drifting on the studied brown algae agrees with the findings of Chan *et al*. (1997). In the present work, the analyzed species contained all the essential amino acids (except tryptophan) in different proportions. Similar results were obtained from other brown algae by other workers (Qasim, 1991; Behairy & El-Sayed, 1983). The dominance of aspartic and glutamic acids in all tested algae were in agreement with the findings reported by several researchers (Fleurence, 1999; Wong & Cheung 2001a). In our study, the concentrations of Methionine, Tyrosine and Lysine were low, which is agreement with the work of Portugal *et al.,* (1983) on several brown seaweeds. The brown algae in general appear to be deficient in sulphur-containing amino acids (Fleurence, 1999; Zubia *et al.,* 2003). Furthermore, the individual amino acid values recorded for the three tested species were comparable to the values recorded for other brown algae by several workers (Zubia *et al.,* 2003; Wong & Cheung, 2001a).

Phenolic compounds have been highly regarded for their important dietary roles as antioxidative and chemopreventive agents (Bravo, 1998; Ismail & Hong, 2002; Kuda *et al.,* 2005a). The results obtained indicated that the three analyzed algal species possess considerable phenolic content in their tissues. These findings appear useful in leading to the development of therapeutic products from Egyptian marine algae. Different methods for determination of antioxidant activity have been developed and used to screen various plant samples (Zhang *et al.,* 2006). In our study, the DPPH radical scavenging assay method was successfully used to assess the total antioxidant capacity of the marine algal extracts, being simple, fast, and reliable. The extract of *Dictyota* showed the

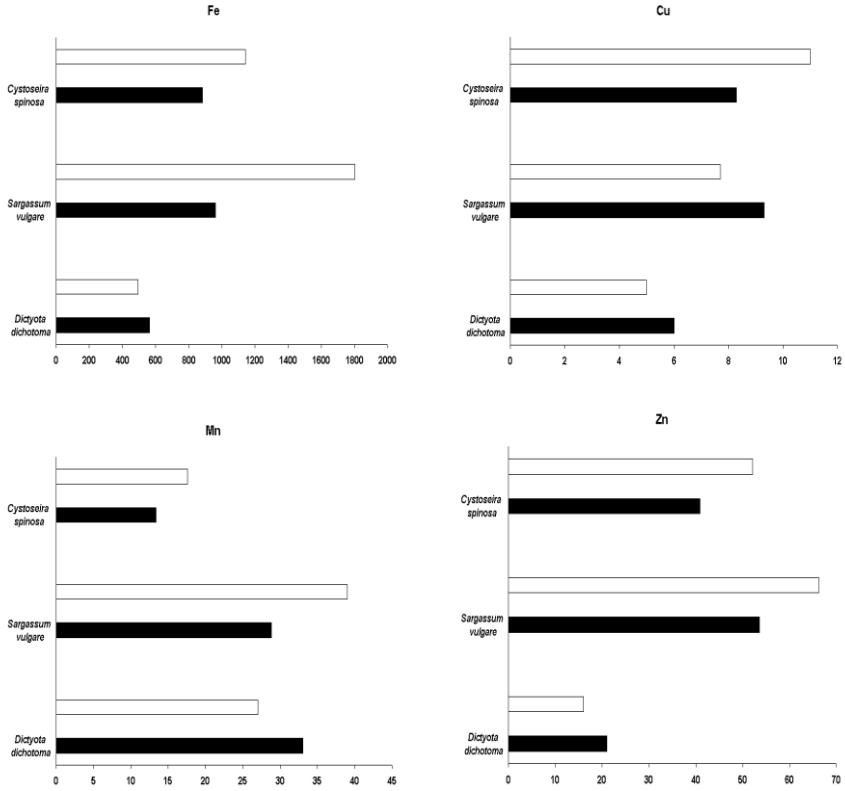

Figure 2. Microelement concentrations in attached (white columns) and drift (black columns) brown algae.

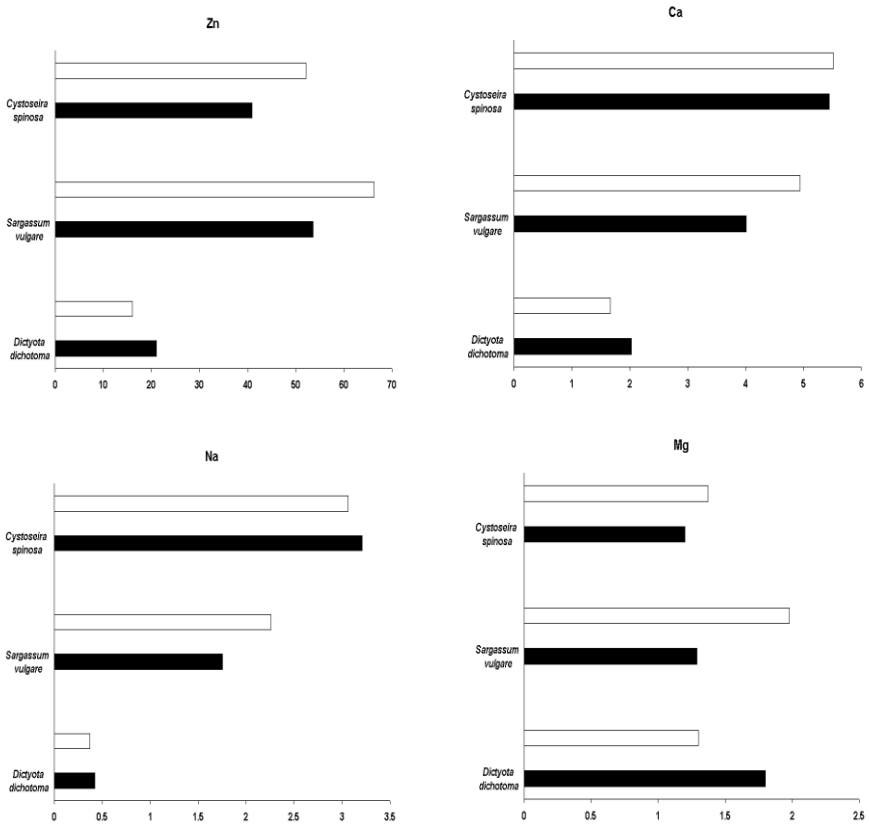

Figure 3. Macroelement Concentrations (% dw) in attached (white columns) and drift (black columns) brown algae.

highest antioxidant activity, as well as, the highest content of phenolic components. However, the previous results were not confirmed statistically, where there was not significant association between the total phenolic content and DPPH radical scavenging activity. Previous reports mentioned that there was a direct relationship between the antioxidant activity and the total phenolic content in plants (Velioglu *et al.,* 1998). However, there were also some reports, which indicated no such positive relationship (Kähkönen *et al.,* 1999). A possible explanation for these disparate different results is that, the total phenolic content did not include all the antioxidants. However, it could be suggested that some other antioxidants, which are structurally different from phenolics are present in the tested materials (Sun and Ho, 2005; Zhang *et al.,* 2006). In the present study the statistical analysis proved that drifting negatively affect the total phenolic compounds in all tested algae, which argue with the findings reported by Chan *et al.,* (1997) working on other algae, and was in agreements with the findings of Jiménez-Escrig *et al.,* (2001), who suggested that drifting and exposure circumstances could decrease the antioxidant activity. Furthermore, the phenolic content, antioxidant activity and reducing power of the three tested algae were comparable to values reported for other seaweeds species (Zhang *et al.,* 2006; Shanab, 2007; Ismail & Hong, 2002; Zahra *et al.,* 2007).

Concentrations of Fe, Mn, Zn, K and Ca were high in all analyzed algal samples, which is consistent with the results obtained by other reporters (Chan *et al.,* 1997; Hou & Yan, 1998; McDermid & Stuercke, 2003). Such observations could suggest the use of the algae under investigation as natural agricultural fertilizers due to their richness in mineral content. Drifting significantly causes most of the investigated minerals to be accumulated in the tissue of analyzed algal species. This may suggest using attached, as well as, drift marine seaweeds as natural mineral source in agricultural fertilizers, also may be cautiously used in pharmaceutical industries due to their high mineral content.

Conclusion

1. The three analyzed brown algae contain considerable amounts of amino acids in their attached as well as in drifted samples, which may favored their using in some nutritional and pharmaceutical natural products.

2. Due to their richness in elements, they may used as fertilizers, but caution must be taken when using them as food supplements, especially for hypertensive persons.

3. All analyzed algae possessed considerable antioxidant activity, which favors using them in pharmaceutical industry as natural anti-aging.

4. In general, a complete screening is needed to screen algal flora along northern (Mediterranean Sea) and eastern (Red Sea) coasts of Egypt, which aimed at detailed investigation and characterization of their phenolic content in relation to their antioxidant activity.

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تأثير الانجراف على نمط الأحماض الأمينية و العناصر المعدنية و المحتوى الفينولي و النشاط المضاد للتأكسد لثلاثة طحالب بنية بحرية

علاء فتحي

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تمت دراسة أثر الانجراف على نمط الأحماض الأمينيّة و محتوًى المعادن في الطحالب البحريّة المتصلة و المنجرفة: ديكتيوتا دايكوتما، سارجاسم فولجير و سيستوسييرا سبينوسا (طحالب بنية)، هذا بالإضافة إلى دراسة العلاقة بين نشاطهم المضادّ للتّأكسد بمحتوًاهم الفينولي الكلّيّ. عندما اختبرت أنماط الأحماض الأمينيّة اظهرت كلّ الطّحالب تشابه في تركيب أحماضهم الأمينيّة، وخاصة الحمض الأمينيّ أسبارتيك و الحمض الأمينيّ جلوتاميك في أنسجتهم. وقد تباينت الكميات الفينولية الكلّيّة من الحدّ الأدنى 0.22 ملجم لكل جم وزن جاف في عيّنات سيستوسييرا المنجرفة، إلى الحدّ الأقصى 0.93 ملجم لكل جم وزن جاف في عيّنات ديكتيوتا المتصلة . كما دلت النّتائج أن الانجراف له أثّر سلبيً على المحتوًى الفينولي للأنواع محل الدراسة . وقد أظهر مستخلص ديكتيوتا دايكوتوما المتصلة والمنجرفة نشاطا مضادا للتّأكسد الأكثر فاعليّة (45.38 و 33.61 %)، على التّوالي . وقد كانت تركيزات عنصري النحاس و الصوديوم منخفضة للغاية، خاصة في طحلب ديكتيوتا دايكوتوما، في حين كانت تركيزات عنصري الحديد و البوتاسيوم هما الأعلى في أنسجة جميع الطحالب محل الدراسة. كما أظهرت الدراسة أن جميع العناصر التي تم تقديرها قد تأثرت سلبيا بعامل الانجراف فيما عدا عنصري البوتاسيوم و الكالسيوم في عينات طحلب سيستوسيرا.