Temporal variation in chemical composition of *Dictyota dichotoma* (Hudson) J.V. Lamouroux (Dictyotales, Phaeophyceae) from Red Sea Coast, Egypt

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**Objective:** To relate the chemical composition of *Dictyota dichotoma* (Hudson) J.V. Lamouroux (*D. dichotoma*) to variation of water characteristics at Hurghada, Egypt.

**Methods:** Sea water and *D. dichotoma* fronds were collected from Hurghada shores, during autumn of 2014. Water samples were analyzed and fronds of *D. dichotoma* were biochemically quantified.

**Results:** Sea water was characterized with high levels of salinity, P and heavy metals but with low content of nitrogen. The confined nature and the high evaporation of the Red sea contribute to the high salinity while the mining and transportation of phosphatic ore in the nearby region contribute to the high load of P and heavy metals. With the progress of season from September to November, water temperature was markedly reduced whereas pH and ionic content of water was reduced to a lesser extent, with marked alteration in ionic composition. With the progress of season towards winter, there was marked reduction in mineral composition of the fronds, shift in frond composition in favor of carbohydrates at the expense of proteins, lipids and alginate; and shift in the pigment composition in favor of chlorophylls at the expense of carotenoids and fucoxanthin.

**Conclusions:** These facts point to active growth and photosynthetic activity during the cold period; that is, *D. dichotoma* is a cold water alga. *D. dichotoma* had moderate nutritive value, with moderate contents of carbohydrates, proteins, lipids and minerals. The mineral accumulation capacity of the fronds was more evident for N, P and K than for Ca, Mg and heavy metals.

**Keywords:** Alginates, Minerals, Pigments, Red sea

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1. Introduction

Seaweeds are divergent group of algae that inhabit both fresh and marine waters. They usually attain huge size which approached up to 60 m in length as a result of the very rapid growth. On the basis of pigmentation, seaweeds are classified into three major groups: brown algae (Phaeophyceae), green algae (Chlorophyceae) and red algae (Rhodophyceae)[1]. Seaweeds constitute the base of the food chain in the marine environment and provide nourishment as well as shelter and habitat for many coastal animals[2]. Seaweeds are valuable sources of proteins, fibers, vitamins, polyunsaturated fatty acids and trace elements in addition to other important bioactive compounds[3]. Substantial amounts of macronutrients such as potassium, calcium, phosphorus and magnesium in addition to appreciable amounts of microelements such as iron, zinc, manganese, boron, copper, iodine and chlorine occur in seaweeds[4]. Therefore, these algae represent a valuable and economic option for the nutrition of humans and animals. Due to their high nutritive value, fresh and dry seaweeds are extensively consumed by people living in the coastal areas, particularly the south east of Asia[5].

Nevertheless, the nutrient composition of seaweeds differs depending on the species, habitat, developmental stage, geographical location and environmental conditions[1]. In addition, seaweeds can produce valuable medicinal components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and antitumors[6].
Seaweeds are exclusively utilized for the production of commercially and industrially important phycocolloids such as alginic acids which are used in food and pharmaceutical industries as thickening and gelling agents[1].

In order to meet the increasing demand upon macroalgal products, the temporal variation in their chemical composition needs thorough investigation. The present work aimed to monitor the changes in chemical composition of the brown alga *Dictyota dichotoma* (Hudson) J.V. Lamouroux (*D. dichotoma*) during the months of autumn at Hurghada, Red sea coast of Egypt. *D. dichotoma* is one of the most promising seaweeds, with appreciable nutritive and therapeutic value. This species is of widespread occurrence and has been defined as the only cosmopolitan species of the genus *Dictyota*[7]. The alga grows along the Red sea coast of Egypt throughout the year, but flourishes enormously in autumn.

2. Materials and methods

2.1. Study area and algal sampling

Fronds of the brown alga *D. dichotoma*, a member of class Phaeophyceae and family Dictyotaceae, were collected by hand-picking from shores of Hurghada, Red sea coast of Egypt during autumn (September, October and November) 2014. The study site which is situated between 27°13’ N and 33°45’ E presents one of the most favorable environments for algal growth along the Egyptian coasts (Figure 1).

2.2. Estimation of physicochemical characteristics of sea water

Temperature, EC and pH of sea water were measured *in situ* by using a glass thermometer, Jenway conductivity meter model 470 and Horizon Co 5995 pH meter, respectively. Alkalinity, dissolved oxygen (DO), biochemical oxygen demand (BOD), CO₂, CO₃²⁻ and HCO₃⁻, hardness, and the contents of K, Ca, Na, P and N in water were measured according to the method adopted by the American Public Health Association[8]. Mg, Fe, Mn, Zn, Cu, Ni, Co and Cr were estimated using a Perkin-Elmer 2380 atomic absorption spectrophotometer.

2.3. Preparation and analysis of *D. dichotoma* fronds

After collection, the fronds were directly washed with seawater to remove the extraneous foreign particles and epiphytes, followed by successive washings with tap water and distilled water to remove salts from the surface. An aliquot of the frond (0.5 g) was kept in 95% ethanol and stored frozen for estimation of pigments. Fronds were spread on blotting paper to remove excess water, shade-dried and then oven-dried at 60 °C for 4 h. The dried algal material was ground to pass 2 mm mesh prior to analysis.

2.3.1. Estimation of total carbohydrates

Total carbohydrates content of the fronds was determined spectrophotometrically using anthrone as described by Hedge and Hofreiter[9]. A known weight (about 100 mg) of the algal powder was incubated with 5 mL of 2.5 mol/L HCl in a boiling water bath for 3 h. After cooling, the extract was neutralized with solid Na₂CO₃ and the volume was made up to 100 mL with distilled water. An aliquot (0.1 mL) of the supernatant was taken and made up to 1 mL with distilled water, to which 4 mL of the anthrone reagent was added and the mixture was heated for 8 min in a boiling water bath. After cooling, the optical density was measured at 630 nm against water blank. Total carbohydrates content was determined from a standard curve using glucose in the range of 0–100 μg/mL.

2.3.2. Estimation of proteins

Protein content of the fronds was estimated according to the method of Bradford[10]. Proteins were extracted by incubating a known weight (about 100 mg) of the powdered algal material with 4 mL of 1 mol/L NaOH for 24 h at 4 °C. The residue was removed by centrifugation and to 0.1 mL of the supernatant 5 mL of the Coomassie brilliant blue G-250 reagent was added and the mixture was heated for 8 min in a boiling water bath. After cooling, the optical density was measured at 560 nm against water blank. Protein concentration was determined from a standard curve using bovine serum albumin in the range of 0–100 μg/mL.

2.3.3. Estimation of lipids

Lipid content of the alga was estimated gravimetrically according to Egan *et al.[11]*. To 10 g of the algal powder, 16 mL water were added, followed by 40 mL methanol and 20 mL chloroform; the mixture was macerated for 2 min. Further 20 mL chloroform was added and maceration continued for 30 s, followed by addition of 20 mL water and maceration was repeated for another 30 s. The mixture
was centrifuged at 5000 r/min for 10 min and the lower chloroform layer was drawn off and filtered through filter paper. Chloroform was evaporated to dryness and weight of lipids was recorded.

2.3.4. Estimation of alginites

Alginites were extracted according to the procedure adopted by Fenorodosoa et al.[12]. A known weight (25 g) of the algal powder was soaked in 800 mL of 2% formaldehyde for 24 h at room temperature, washed with water and then added to 800 mL of 0.2 mol/L HCl. After 24 h in the acid, the sample was washed again with distilled water and the alginites were extracted with 2% Na₂CO₃ at 100 °C for 3 h. The soluble fraction was collected by filtration and alginites were precipitated using three volumes of 95% ethanol. The precipitated alginites were washed twice by 100 mL of acetone and dried at 65 °C.

2.3.5. Estimation of pigments

The pigment content of the fronds was estimated following the method of Ritchie[13]. About 0.5 g of the fresh algal material was homogenized in 10 mL of 95% ethanol and the homogenate was centrifuged at 9 500 r/min for 15 min. Absorbance of the supernatant was measured at 470, 581, 630, 647, 664 and 691 nm. The concentrations of pigments (μg/mL) were estimated according to the equations of Ritchie[13].

The results were expressed as μmol pigment per gram of frond fresh weight.

2.3.6. Estimation of minerals

Algal material was digested according to the method of Sudharsan et al.[14]. About 1 g of the algal powder was incubated overnight at room temperature with 20 mL concentrated HNO₃. Then 10 mL of a mixture of concentrated nitric and perchloric acids (4:1) was added and the mixture was heated at 120 °C until the volume was reduced to about 5 mL. The residue was then made up to 20 mL with 20% nitric acid. After filtering through Whatman No. 1 filter paper, the contents of Na, Ca and K in the digest were determined using a Jenway PFP 7 flame photometer. The concentrations of Mg, Fe, Mn, Zn, Cu, Ni, Co, and Cr in the digest were estimated using a Perkin-Elmer 2380 atomic absorption spectrophotometer. Phosphorus and nitrogen were estimated according to APHA[8].

2.4. Statistical analysis

Data were analyzed using SPSS version 22 and ANOVA was done to verify the effect of sampling time on chemical composition of both the sea water and the alga. Mean separation was performed using the Duncan’s multiple range test at P ≤ 0.05.

3. Results

The sea water at the study site (Hurghada, Red sea coast of Egypt) during autumn was characterized with moderate alkaline reaction (pH of 7.8), relatively high levels of salinity (EC of 57.1 dS/m, 522 mmol/L Na) and macro-elements (P in particular), substantially high content of microelements but low content of nitrogen (Table 1). With the progress of season from September to November there was progressive decline in water temperature from 28 °C to 23 °C; and this was associated with marked alteration in some of the physicochemical characteristics of water while some others were subjected to marginal alterations. Water salinity in terms of EC as well as the levels of pH, biological oxygen demand (BOD), free CO₂, HCO₃⁻, CO₃²⁻, total hardness, Ca hardness, Mg hardness, K, Ca, N, Na, Mn, Zn, Cu, Ni, Co and Cr in sea water exhibited significant and progressive temporal reductions. By contrast, the temporal reductions in total alkalinity, dissolved oxygen (DO) and the levels of P, Mg and Fe were non-significant. Among the significantly reduced characteristics, the magnitude of reduction was relatively great (an average of 15%) in water temperature, alkalinity, DO, BOD and the levels of HCO₃⁻, CO₃²⁻, Ca hardness, K, Zn, Cu, Co and Cr but relatively low (about 5%) for water pH, EC, free CO₂, total hardness, Mg hardness and the levels of Ca, P, Mg, N, Na, Fe, Mn and Ni. Among the macro-elements, K experienced the greatest temporal reduction (17%) compared with only 5% reduction in Na, Ca, N, P and Mg. Among the heavy metals, the temporal reductions in Zn, Cu, Co and Cr (an average of 15%) were greater than those in Fe, Mn and Ni which averaged around 5%.

**Table 1**

| Characteristic | September | October | November |
|---------------|-----------|---------|----------|
| Temperature (°C) | 28 | 26 | 23 |
| pH | 8.0 ± 0.06<sup>a</sup> | 7.9 ± 0.10<sup>b</sup> | 7.6 ± 0.06<sup>c</sup> |
| EC (dS/m) | 58.5 ± 0.10<sup>a</sup> | 57.2 ± 0.10<sup>b</sup> | 55.7 ± 0.06<sup>c</sup> |
| Alkalinity (meq/L) | 2.8 ± 0.10<sup>a</sup> | 2.7 ± 0.10<sup>b</sup> | 2.4 ± 0.12<sup>c</sup> |
| DO (mg/L) | 12.5 ± 0.10<sup>a</sup> | 12.3 ± 0.06<sup>a</sup> | 12.2 ± 0.10<sup>b</sup> |
| BOD (mg/L) | 1.74 ± 0.01<sup>a</sup> | 1.71 ± 0.01<sup>a</sup> | 1.55 ± 0.01<sup>a</sup> |
| Free CO₂ (mg/L) | 19.6 ± 0.10<sup>a</sup> | 19.1 ± 0.10<sup>a</sup> | 18.7 ± 0.15<sup>a</sup> |
| HCO₃⁻ (mg/L) | 0.76 ± 0.02<sup>a</sup> | 0.69 ± 0.01<sup>a</sup> | 0.63 ± 0.01<sup>a</sup> |
| CO₃²⁻ (mg/L) | 2.12 ± 0.08<sup>a</sup> | 1.97 ± 0.03<sup>a</sup> | 1.82 ± 0.01<sup>a</sup> |
| Tot. Hardness (g/L) | 4.66 ± 0.02<sup>a</sup> | 4.50 ± 0.01<sup>a</sup> | 4.20 ± 0.01<sup>a</sup> |
| Ca Hardness (g/L) | 1.50 ± 0.03<sup>a</sup> | 1.30 ± 0.03<sup>a</sup> | 1.20 ± 0.02<sup>a</sup> |
| Mg Hardness (g/L) | 3.16 ± 0.02<sup>a</sup> | 3.20 ± 0.02<sup>a</sup> | 3.00 ± 0.03<sup>a</sup> |
| Total K (mg/L) | 470 ± 10.0<sup>a</sup> | 420 ± 15.3<sup>a</sup> | 390 ± 11.55<sup>a</sup> |
| Total Ca (mg/L) | 409 ± 7.2<sup>a</sup> | 390 ± 2.89<sup>a</sup> | 378 ± 2.00<sup>a</sup> |
| Total Na (mg/L) | 12.27 ± 0.02<sup>a</sup> | 12.10 ± 0.08<sup>a</sup> | 11.80 ± 0.04<sup>a</sup> |
| P (mg/L) | 2.81 ± 0.033<sup>a</sup> | 2.74 ± 0.040<sup>a</sup> | 2.73 ± 0.047<sup>a</sup> |
| N (mg/L) | 1.932 ± 0.023<sup>a</sup> | 1.909 ± 0.020<sup>a</sup> | 1.813 ± 0.023<sup>a</sup> |
| Mg (mg/L) | 2.69 ± 0.111<sup>a</sup> | 2.64 ± 0.040<sup>a</sup> | 2.57 ± 0.049<sup>a</sup> |
| Fe (mg/L) | 1.74 ± 0.042<sup>a</sup> | 1.67 ± 0.011<sup>a</sup> | 1.64 ± 0.012<sup>ab</sup> |
| Mn (mg/L) | 1.93 ± 0.039<sup>a</sup> | 1.89 ± 0.014<sup>a</sup> | 1.82 ± 0.006<sup>a</sup> |
| Zn (mg/L) | 0.56 ± 0.010<sup>a</sup> | 0.51 ± 0.004<sup>a</sup> | 0.47 ± 0.004<sup>a</sup> |
| Cu (mg/L) | 1.68 ± 0.015<sup>a</sup> | 1.57 ± 0.015<sup>a</sup> | 1.45 ± 0.015<sup>a</sup> |
| Ni (mg/L) | 1.78 ± 0.015<sup>a</sup> | 1.74 ± 0.015<sup>a</sup> | 1.65 ± 0.016<sup>a</sup> |
| Co (mg/L) | 0.62 ± 0.005<sup>a</sup> | 0.58 ± 0.004<sup>a</sup> | 0.55 ± 0.008<sup>a</sup> |
| Cr (mg/L) | 0.77 ± 0.011<sup>a</sup> | 0.73 ± 0.004<sup>a</sup> | 0.69 ± 0.013<sup>a</sup> |

Each value is the mean of three replicates ± SE. Means with common letters are not significantly different at P ≤ 0.05.
Most of the *D. dichotoma* biomass was contributed by alginates which accounted for 57.7% of the frond dry weight (as an average of the three sampling dates), followed by carbohydrates (30%) and proteins (11.1%); with only 1.3% lipid content. The progress of season from September to November was associated with a progressive increase in carbohydrate content of the fronds, concomitant with progressive decrease in the contents of proteins, lipids and alginates (Table 2). In general, most of the pigment content of *D. dichotoma* frond was contributed by Chl a (55% of the total pigment content), followed by Chl c (34%) with minor proportions of carotenoids (8.5%) and fucoxanthin (only 2.5%). The progress of season from September to November was associated with progressive decline in the contents of carotenoids and fucoxanthin, an increase in Chl c but non-significant decrease in Chl a content of the frond (Table 3). These changes led to temporal variation in pigment composition of the frond; which was manifested as increases in the proportions of Chl c from 0.318 to 0.347 at the expense of reductions in the proportions of Chl a from 0.562 to 0.549, of carotenoids from 0.091 to 0.080 and of fucoxanthin from 0.109 to 0.100.

**Table 2**

| Constituent          | September | October | November |
|----------------------|-----------|---------|----------|
| Total carbohydrates  | 107.6 ± 1.31 | 110.2 ± 0.95 | 126.3 ± 0.55 |
| Proteins             | 45.6 ± 0.30 | 43.2 ± 0.12 | 38.9 ± 0.12 |
| Lipids               | 5.23 ± 0.15 | 4.70 ± 0.10 | 4.64 ± 0.06 |
| Alginates            | 229.2 ± 1.68 | 223.7 ± 2.13 | 209.9 ± 1.15 |

Each value is the mean of three replicates ± SE. Means with common letters are not significantly different at $P ≤ 0.05$

**Table 3**

| Constituent          | September | October | November |
|----------------------|-----------|---------|----------|
| Chlorophyll *a*      | 2.19 ± 0.011 | 2.17 ± 0.017 | 2.17 ± 0.011 |
| Chlorophyll *c*      | 1.24 ± 0.016 | 1.34 ± 0.033 | 1.37 ± 0.019 |
| Carotenoids          | 0.354 ± 0.002 | 0.325 ± 0.004 | 0.318 ± 0.002 |
| Fucoxanthin          | 0.109 ± 0.001 | 0.100 ± 0.003 | 0.094 ± 0.001 |

Each value is the mean of three replicates ± SE. Means with common letters are not significantly different at $P ≤ 0.05$

Generally, most of the mineral composition of the frond was contributed by K and Na, followed by Ca and P and the least contributing macroelement was Mg. The progress of season from September to November was associated with progressive decrease in the mineral content of *D. dichotoma* fronds. The temporal reductions were most pronounced in P (27%); followed by K and Na (21% each) and Ca, N and Mg (with an average reduction of 14%). These changes resulted in temporal reductions of 16% in the P/N ratio, 5% in the Ca/Mg ratio, with almost no change in the K/Na ratio (Table 4). The relationship between N and protein contents of *D. dichotoma* fronds is a saturable one (Figure 2), with a linear increase in protein content over the low range of N resources and a tendency towards saturation as N content exceeded a certain limit (about 2.2 mmol/g DW). The different mineral elements exhibited different accumulation ratios. The accumulation ratio was exceptionally high (350–400) for N, high (35–50) for P, moderate (4–6.5) for K and Ca, mild (around

**Figure 2.** Relationship between the contents of protein and nitrogen in the fronds of *D. dichotoma* collected from Hurghada, Red sea coast of Egypt during September, October and November 2014.

Most of the microelements content of the frond (45.4%) was contributed by Mn, with lesser contributions of Fe, Cu, Ni and Cr (12% each) and minor fractions of Zn and Co (3% each). Similar to macro-elements the content of micro-elements in the frond decreased with the advance of season from September to November (Table 4). The temporal reductions were greatest (34.5%) in Fe, moderate (26.7%) in Cu, mild (17.5%) in Zn and Co and least (9.4%) in Mn, Ni and Cr. These changes led to temporal alteration in the microelement composition of the frond; where the proportions of Mn and Ni in the total microelement content increased by 12% at the expense of reductions in the remaining microelements which varied from 23.3% in Fe, 13% in both Cu and Cr and 4% in both Zn and Co.

**Table 4**

| Mineral          | September | October | November |
|------------------|-----------|---------|----------|
| K                | 1.087 ± 0.008 | 0.951 ± 0.004 | 0.854 ± 0.005 |
| Ca               | 0.671 ± 0.003 | 0.643 ± 0.001 | 0.561 ± 0.005 |
| Na               | 1.026 ± 0.007 | 0.883 ± 0.007 | 0.813 ± 0.005 |
| P                | 0.063 ± 0.0005 | 0.055 ± 0.0003 | 0.046 ± 0.0007 |
| N                | 0.803 ± 0.007 | 0.706 ± 0.013 | 0.700 ± 0.019 |
| Mg               | 0.0024 ± 0.00006 | 0.0023 ± 0.00004 | 0.0021 ± 0.00004 |
| K/Na ratio       | 1.059 ± 0.014 | 1.078 ± 0.0103 | 1.050 ± 0.0119 |

| Mineral          | September | October | November |
|------------------|-----------|---------|----------|
| Fe               | 0.521 ± 0.018 | 0.392 ± 0.019 | 0.341 ± 0.019 |
| Mn               | 0.572 ± 0.007 | 0.554 ± 0.0058 | 0.512 ± 0.019 |
| Zn               | 0.092 ± 0.015 | 0.091 ± 0.009 | 0.077 ± 0.008 |
| Cu               | 0.408 ± 0.016 | 0.329 ± 0.011 | 0.299 ± 0.018 |
| Ni               | 0.385 ± 0.008 | 0.360 ± 0.0058 | 0.357 ± 0.020 |
| Co               | 0.138 ± 0.003 | 0.122 ± 0.001 | 0.112 ± 0.002 |
| Cr               | 0.097 ± 0.002 | 0.093 ± 0.002 | 0.087 ± 0.002 |

Each value is the mean of three replicates ± SE. Means with common letters are not significantly different at $P ≤ 0.05$.
1.5) for Mg and extremely low (around 0.1) for Na. The K/Na, Ca/ Mg and P/N ratios of the frond were about 50 times, 3 times and 0.1 times respectively that of sea water. The accumulation ratios of all the microelements except Cr (those of Fe, Mn, Zn, Cu, Ni and Co) averaged around unity; only that of Cr was low (about 0.5). With the progress of season from September to November there was a general progressive significant reduction in the accumulation ratio of the different elements, except with K, Mn, Zn, Cu, Ni, Co and Cr where the temporal reductions were non-significant (Table 5).

**Table 5**

Accumulation ratio (internal concentration/external concentration) of the macro- and micro-elements in the frond of *D. dichotoma* collected from Hurghada, Red sea coast of Egypt during September, October and November 2014.

| Mineral     | September | October | November |
|-------------|-----------|---------|----------|
| K           | 6.20 ± 0.13<sup>a</sup> | 6.08 ± 0.201<sup>b</sup> | 5.87 ± 0.150<sup>c</sup> |
| Ca          | 4.51 ± 0.096<sup>a</sup> | 4.53 ± 0.039<sup>b</sup> | 4.07 ± 0.058<sup>c</sup> |
| Na          | 0.132 ± 0.001<sup>a</sup> | 0.115 ± 0.001<sup>b</sup> | 0.109 ± 0.001<sup>c</sup> |
| P           | 47.87 ± 0.759<sup>a</sup> | 42.81 ± 0.680<sup>b</sup> | 35.86 ± 0.666<sup>c</sup> |
| N           | 398.86 ± 6.99<sup>a</sup> | 354.88 ± 10.17<sup>b</sup> | 354.22 ± 3.57<sup>c</sup> |
| Mg          | 1.48 ± 0.082<sup>a</sup> | 1.42 ± 0.023<sup>b</sup> | 1.36 ± 0.046<sup>c</sup> |
| K/Na ratio  | 47.08 ± 1.212<sup>a</sup> | 52.94 ± 1.695<sup>b</sup> | 54.12 ± 0.891<sup>c</sup> |
| Micro-elements |
| Fe          | 1.147 ± 0.068<sup>a</sup> | 0.898 ± 0.048<sup>b</sup> | 0.798 ± 0.050<sup>c</sup> |
| Mn          | 1.115 ± 0.031<sup>a</sup> | 1.102 ± 0.017<sup>b</sup> | 1.058 ± 0.040<sup>c</sup> |
| Zn          | 0.741 ± 0.131<sup>a</sup> | 0.799 ± 0.085<sup>b</sup> | 0.735 ± 0.081<sup>c</sup> |
| Cu          | 1.059 ± 0.038<sup>a</sup> | 0.912 ± 0.038<sup>b</sup> | 0.895 ± 0.055<sup>c</sup> |
| Ni          | 0.872 ± 0.015<sup>a</sup> | 0.829 ± 0.010<sup>b</sup> | 0.870 ± 0.057<sup>c</sup> |
| Co          | 0.898 ± 0.025<sup>a</sup> | 0.849 ± 0.008<sup>b</sup> | 0.819 ± 0.017<sup>c</sup> |
| Cr          | 0.450 ± 0.005<sup>a</sup> | 0.455 ± 0.006<sup>b</sup> | 0.452 ± 0.014<sup>c</sup> |

Each value is the mean of three replicates ± SE. Means with common letters are not significantly different at *P* ≤ 0.05.

### 4. Discussion

Sea water at the study site (Hurghada, Red sea coast of Egypt) during autumn was of unique composition; with relatively high levels of salinity, P and heavy metals but with lower content of nitrogen. This unique composition of the Red sea water at the study site can be related to several factors. The high salinity, for example, can be accounted for on the basis of the fact that the Red sea is a relatively small, almost confined water body with limited supply of fresh water and high rate of evaporation as a result of the prevailing hot dry weather. Likewise, the high levels of P and heavy metals can be attributed to the enhanced mining activities for phosphatic ore in the nearby regions; which might contribute to the high input of phosphatic dust along with its load of heavy metals to sea water. The contribution of phosphatic fertilizers in contamination input of phosphatic dust along with its load of heavy metals to sea water. The contribution of phosphatic fertilizers in contamination input of phosphatic dust along with its load of heavy metals to sea water. The contribution of phosphatic fertilizers in contamination input of phosphatic dust along with its load of heavy metals to sea water. The contribution of phosphatic fertilizers in contamination input of phosphatic dust along with its load of heavy metals to sea water. The contribution of phosphatic fertilizers in contamination input of phosphatic dust along with its load of heavy metals to sea water.

Consequently, the micro- and macro-elements in the frond and the increase in chlorophyll content at the cold period might point to active photosynthetic activity at this time of the year. This enhanced photosynthetic activity might partly explain the lowered inorganic carbon content (CO<sub>2</sub>, CO<sub>3</sub><sup>−</sup> and HCO<sub>3</sub>) of sea water during the cold period as a result of consumption in photosynthesis.

The above mentioned findings mean that *D. dichotoma* is a...
cold water alga, that is the alga experiences vigorous growth and metabolic activity during the cold season and undergoes a stage of lowering activity during the unfavorable summer period. It is well-established that Phaeophyceae is cold water algae; that they flourish in the intertidal belt and the upper littoral region and dominate these regions in colder waters, particularly in the Northern Hemisphere[23]. The relatively high lipid content of D. dichotoma fronds during the warm period which coincides with the slow growth stage of the alga supports the conclusion that D. dichotoma is a cold water alga. In this context, Juneja et al.[22] reported that the decrease in lipid content of the green alga Botryococcus braunii was associated with accumulation of polysaccharides; and Converti et al.[23] found that increasing the growth temperature from 20 to 25 °C doubled the lipid content of Nannochloropsis oculata.

Macronutrients play a prominent role in cycling and storing of nutrients in coastal waters, where their nutrient content presents a good indicator of ambient nutrient conditions within the water column[24]. The nutrient demand and hence uptake of minerals by seaweeds are expected to be related to environmental conditions such as light, temperature, salinity and pH of water, as well as age or growing stage of the fronds, which in turn are dependent on the environmental conditions[25]. The progressive decline in mineral content of D. dichotoma fronds as the season advances towards winter can, thus, be viewed as a dilution effect arising from the vigorous growth of the alga during the favorable cold period.

Algae are often termed as super food because of their high content of valuable nutrients such as protein, minerals and unsaturated lipids. The protein content of D. dichotoma fronds of the present study (4.3%), although being moderate, is comparable to that reported by Tabarsa et al.[26] for D. dichotoma collected from the Persian Gulf and the carbohydrate content was more than twice that reported by Ozgun and Turan[27] for the alga collected from the Northeastern Mediterranean coast of Turkey. Thus, the protein and carbohydrate contents of an alga may vary not only between habitats but also according to stage of maturity and time of the year.

The saturable relationship between N and protein contents of D. dichotoma fronds means that D. dichotoma utilizes the limited internal N resources during the cold period in the manufacture of proteins; but a greater proportion of the surplus N resources during the warm period was invested as non-protein N; probably free amino acids, amides and secondary metabolites. The shift in biomass composition of D. dichotoma in favor of carbohydrate at the expense of protein during the cold favorite period of growth can point to a transient state of N deficiency during winter; or, more precisely, faster growth rate than the rate of N uptake. In support to this conclusion, Msanne et al.[28] reported an increase in total carbohydrate content, concomitant with a progressive decrease in protein content of several algae under shortage of nitrogen.

Macronutrients have the ability to accumulate essential mineral elements as well as heavy metals[29]. Seaweeds can, therefore, be used to assess the levels of heavy metals in the marine environment. The rank of accumulation ratios of the different metals in the fronds of D. dichotoma, which followed the order N > P > K > Ca > Mg > heavy metals > Na, might suggest that nitrogen is the most limiting nutrient for algal growth followed by P while the levels of the other macronutrients and micronutrients are in the adequate range. Only Na and Cr might approach such high levels in the sea water that the alga has to restrict their entry into the cell either by blocking of their respective channels or through their extrusion outside the cell. The operation of Na exclusion mechanism along with active accumulation of K has been suggested to aid in thriving of brown algae in sea water[30]. Osmoregulation in marine algae are mainly maintained by Na/K+ pump operating between seawater and cell sap and the turgor pressure changes are caused by variable ionic composition of vacuoles[31]. The high K/Na ratio of D. dichotoma fronds (an average of unity) relative to that of the sea water (0.02) points to the high K/Na selectivity of the alga (about 50). Potassium is an essential macronutrient required for the growth and metabolic activities of plants in general and seaweeds in particular. Most of the brown algae along the coast of Mannar Gulf, India accumulate more K+ than Na[32]; however, the K content of D. dichotoma fronds at Hurghada, Red sea (about 1 mmol/g DW = 39 mg/g DW) was higher than that reported for the algae of Mannar Gulf[32] which amounted to 29.9 mg/g DW.

D. dichotoma, a brown alga, is a valuable seaweed because of its promising nutritive and industrial potentialities. The alga grows along the Egyptian shore of the Red sea and flourishes during autumn, approaching climax during the cold period. The temporal variation in water temperature was linked to marked alteration in composition of water and the alga, with maximum content of carbohydrate but minimum contents of protein, lipids and alginate during the cold season.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Balboa EM, Conde E, Moure A, Falqué E, Dominguez H. In vitro antioxidant properties of crude extracts and compounds from brown algae. Food Chem 2013; 138: 1764-85.
[2] Carneiro JG, Rodrigues JAG, Teles FB, Cavalcante ABD, Benevides NMB. Analysis of some chemical nutrients in four Brazilian tropical seaweeds. Acta Sci Biol Sci 2014; 36: 137-45.
[3] Hamid N, Ma Q, Boulos S, Liu T, Zheng Z, Balbas J, et al. Seaweed

minor constituents. In: Tiwari BK, Troy DJ, editors. Seaweed sustainability food and non-food applications. Amsterdam: Elsevier-Academic Press; 2015.

[4] Nazini P, Renuga. Minerals composition of the selected brown seaweed from Mandapam, Gulf of Mannar region, Tamilnadu. Int J Res Mar Sci 2015; 4(1): 1-5.

[5] Nedumaran T, Arulbalachandran D. Seaweeds: a promising source for sustainable development. In: Thangavel P, Sridevi G. editors. Environmental sustainability role of green technologies. India: Springer; 2015, p. 65-88.

[6] Herman TS, Aravindan N. Anti-pancreatic cancer deliverables from seaweed. J Environ Sci 2012; 70: 1-9.

[7] Rabanal M, Poncea NMA, Navarro DA, Gómez RM, Stortza CA. The system of fucoidans from the brown seaweed Dictyota dichotoma: chemical analysis and antiviral activity. Carbohydrate Polymers 2014; 101: 804-11.

[8] American Public Health Association (APHA). Standard methods for the examination of water and wastewater. Washington DC: American Public Health Association; 1992, p. 18.

[9] Hedge JE, Hofreiter BT. Carbohydrate chemistry. New York: Academic Press; 1962, p. 163-201.

[10] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 27: 131-7.

[11] Ritchie RJ. Universal chlorophyll equations for estimating chlorophylls a, b, c, and d and total chlorophylls in natural assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents. Photosynthetica 2008; 46(1): 115-26.

[12] Sudharsan S, Seedevi P, Ramasamy P, Subhapradha N, Vairamani S, Shanmugam A. Heavy metal accumulation in seaweeds and sea grasses along southeast coast of India. J Chem Pharm Res 2012; 4(9): 4240-4.

[13] Mamdouf AF, Laila MA, Ahmed MA, Mohamed A, Aly-Eledeen, Ehssan M, et al. Evaluation of the quality for the Egyptian red sea coastal waters during 2011-2013. J Environ Prot 2016; 7: 1810-34.

[14] El-Taher A, Althoyba. Natural radioactivity levels and heavy metals in chemical and organic fertilizers used in Kingdom of Saudi Arabia. Appl Radiat Isot 2012; 70: 290-5.

[15] Aoun M, El Samrai AG, Martiges BS, Kazpard V, Saad Z. Releases of phosphate fertilizer industry in the surrounding environment: investigation on heavy metals and polonium-210 in soil. J Environ Sci 2010; 22(9): 1387-97.

[16] Attia OEA, Ghrefat H. Assessing heavy metal pollution in the recent bottom sediments of Masabias Bay, North Hurghada, Red Sea, Egypt. Environ Monit Assess 2013; 185(12): 9925-34.

[17] Mahmoud AD, Fatma AF, Afif ME, Hussain MN. The effects of land-based activities on the near-shore environment of the Red Sea, Egypt. Environ Earth Sci 2016; 75: 188.

[18] Juneja A, Ceballos RM, Murthy GS. Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: a review. Energies 2013; (6): 4607-38.

[19] Converti A, Casazza AA, Ortiz EY, Perego P, del Borghi M. Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production. Chem Eng Prog 2009; 48: 1146-51.

[20] Tang Q, Zhang J, Fang J. Shellfish and seaweed mariculture increase atmospheric CO2 absorption by coastal ecosystems. Mar Ecol Prog Ser 2011; 424: 97-104.

[21] Tipnie S, Ramaraj R, Unpaprom Y. Nutritional evaluation of edible freshwater green macroalgae Spirogyra varians. Emerg Life Sci Res 2015; 1(2): 1-7.

[22] Tabarsa M, Rezai M, Ramezanpour Z, Waaland JR, Rabiei R. Fatty acids, amino acids, mineral contents, and proximate composition of some brown seaweeds. J Phycol 2012; 48: 285-92.

[23] Ozgun S, Turan F. Biochemical composition of some brown algae from Iskenderun Bay, the northeastern Mediterranean coast of Turkey. J Black Sea Mediterranean Environ 2015; 21(2): 125-34.

[24] Msanne J, Xu D, Konda AR, Casas-Mollano JA, Awada T, Cahoon EB, et al. Metabolic and gene expression changes triggered by nitrogen deprivation in the photoautotrophically grown microalgae Chlamydomonas reinhardtii and Coccomyxa sp. C-169. Phytochemistry 2012; 75: 50-9.

[25] Mouradi A, Benossier L, Gloaguen V, Mouradi A, Zidane H, Giveraud T. Accumulation of heavy metals by macroalgae along the Atlantic coast of Morocco between El Jadida and Essaouira. World J Biol Res 2014; 6(1): 1-9.

[26] Yancey PH. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. J Exp Biol 2005; 208(15): 2819-30.

[27] Eisler R. Accumulation of zinc by marine biota. In: Nriagu JO, editor. Zinc in environment Part II: health effects. New York: John Willey & Sons; 1980, p. 259-351.

[28] Sivakumar SR, Arunkumar K. Sodium, potassium and sulphate composition in some seaweeds occurring along the coast of Gulf of Mannar, India. Asian J Plant Sci 2009; 8: 500-4.