Introduction

Assortments of human influences have improved meaningfully in coastal ecosystems all over the world through the past centuries (Murphy et al., 2019). The coastal area gets nutrients from many sources either natural marine or atmospheric sources were reported by Jickells (1998) and Baker (2003). Ben Chekroun et al. (2014) provide an overview of the biomonitoring of organic contaminants used by microalgae in aquatic water bodies and their environmental impact. In addition, nutrients from anthropogenic sources such as finfish farming, aquaculture, and urban use are added to the marine environment.

Marinho et al., (2015) have a hopeful attitude to reduce eutrophic effluents through the cultivation of macroalgae, which lead to eliminating inorganic nutrients through its
growth. Macroalgae can accumulate nutrients over seawater levels by a factor of up to 105 and maintain specific mechanisms to store enormous quantities of nitrogen and phosphorus in their tissues (Lobban & Harrison, 1997). Furthermore, by improving internal stores, macroalgae can react to increase nutrient availability (Corey et al., 2013). Dissolved inorganic phosphorus (DIP) and Inorganic Dissolved Nitrogen (IDN) are vital macronutrients to preserve the metabolism and development of marine algae. Nitrogen is considered the major limiting nutrient in marine environments for algal growth. The success of algal farming, therefore, requires knowledge of the algae’s nitrogen supplies (Corey et al., 2013). The supreme important sources of nitrogen for macroalgae are ammonium, nitrate, and to some extent, nitrite (Lobban & Harrison, 1997). Theoretically, ammonium has to be countless because it can be directly assimilated into amino acids, while nitrate must be reduced before amino acid production (Barsanti & Gualtieri, 2006). The formation of biomolecules, such as phospholipids, proteins, and nuclear acids, is facilitated by phosphorus. Phosphorous plays a significant role in photosynthesis and respiration because of its conversion to high-energy compounds (Lobban & Harrison, 1997). The provision of phosphorus can stimulate algal growth and photosynthetic rates (Martins et al., 2011). The survival of Fucus vesiculosus L. could be limited to 50% by phosphorus (Bergstrom et al., 2003). Macroalgae can be easily harvested, have the efficiency to accumulate nutrients and pollutants. Another advantage is that the biomass of seaweed can be also used to extract bio-based chemicals (Neveux et al., 2015).

De Clerck et al. (2006) claimed that Dictyota Lamouroux is a tropical and subtropical marine species. While, D. dichotoma (Hudson) Lamouroux is one of the most dominant species of the Red Sea coast of Egypt and grows all over the year. The flourishing season of this species was recorded during winter (El-Manawy, 2008; Farghaly & El-Shoubaky, 2015; Deyab et al., 2017; El-Manawy et al., 2019). Furthermore, Bakar et al. (2019) found that sterol compounds isolated from D. dichotoma exhibit antibacterial and antifouling activities, and therefore, it was selected for this study.

This research aimed to assess the capacity of D. dichotoma to convert these nutrients into soluble protein, soluble carbohydrates, and lipids under varying concentrations of ammonium and phosphate to absorb and assimilate them, as well as, to maximize their efficacy in their nutrient removal.

Materials and Methods

Site description

Location of the selected site situated in a coastal lagoon of the National Institute of Oceanography and Fisheries (NIOF). This located at about 5km of NIOF between latitude 27° 17’ 13’’ N and Longitude 33° 46’ 21’’ E (Fig. 1). This site is characterized by the widely distributed reef flats that extended for about 5km seaward with depth variation between 1.5m and 6m. The inshore zone of this site suffers from underground wastewater seepage.

Collection and identification of Dictyota dichotoma

Dictyota dichotoma has been stored in marine bags to prevent evaporation after collected directly and transported to the laboratory inside the icebox (Fig. 2). A portion of this sample was fixed in 4% formaldehyde, and the other part was distributed for taxonomic identification on herbarium sheets. Alga was identified by morphological characters like the pigment, structure of the vegetative thallus with reproductive organs, and other characters using taxonomic references (Aleem, 1978; Coppejans & Beeckman, 1990; Sahoo,
2001). The name of this species was identified according to Guiry & Guiry (2019).

To remove small epiphytes, sand, surface debris, and invertebrates, healthy fronds of collected fresh seaweeds were washed in seawater. It is necessary to properly clean the plants for consistent results (Buapet et al., 2008). Wet weight of 20± 2g D. dichotoma fronds were grown in 20L sterile seawater aerated glass jars. The nutrient media levels were 0.4μmol/L for phosphate, 0.6μmol/L for ammonia, 0.135μmol/L for nitrate, and 0.011μmol/L for nitrite in the incubation water.

**Experimental design**

Experiments were performed with a ratio of 2g of algal biomass per 1L culture medium in Erlenmeyer flasks (1L). Treatments consisted of sterilized seawater supplemented by modified medium (Von Stosch media, this medium with iron salts, Manganese, EDTA, and three vitamins, not including phosphate and nitrate), according to Ribeiro et al. (2017). Ammonium (NH₄Cl) and phosphate (Na₂HPO₄·12H₂O) were added to the Von Stosch modified medium in order to obtain a phosphorus/ nitrogen (P/N) ratio of 1:10. The control of these experiments lacks ammonium or phosphate but contains vitamins and trace elements. Ammonium concentrations in various treatments were 0, 8, 20, 40, 60 and 80μmol/L. On the other hand, the phosphorus concentrations were 0, 0.8, 2, 4, 6, and 8μmol/L. Control and different treatment either by nitrogen or phosphorus, were in three replicates. The light intensity was 20 μmol photons m⁻² sec⁻¹ with 16: 8hrs light: dark period for 12 days. The flasks were aerated by air pump. The cultures were refreshed every 4 days. Temperature, pH, Salinity and dissolved oxygen were measured daily by Multi-parameter Instrument (YSI ProODO).

**Measurement of nutrient uptake**

The sampling periods from the flasks under experiments were 0, 1, 4, 8 and 24hrs. Dissolved ammonium (NH₄⁺) and dissolved inorganic phosphate (PO₄³⁻) were determined spectrophotometrically according to the methods described by APHA (2005). For each nutrient, the Nutrient Uptake Efficiency (NUE) was determined using Kang et al. (2011) formula as follows:

\[
\text{NUE} = \left( \frac{c_i - c_f}{c_i} \right) \times 100
\]

where, NUE is Nutrient Uptake Efficiency in percentage. \(c_i\) and \(c_f\) are the initial and the final concentrations (μmol/L) of each nutrient.

Macrophytes nutrient uptake was measured on the basis of reduced concentration in the culture medium of a given nutrient (Tremblay-Gratton et al., 2018).

**Growth rate of Dictyota dichotoma**

The fresh weight of D. dichotomias were determined at the start of the experiment and every 2 days until the end after the drying using tissue paper and the specific growth rate (SGR, % d⁻¹) was calculated as follows:

\[
\text{SGR} = \frac{(\ln W_i - \ln W_{i-1}) \times 100}{t}
\]

where: \(W_i\) represents the fresh weight collected at time \(i\), \(W_{i-1}\) represents the fresh weight collected at time \(i-1\), \(t\) represents days (d) between \(i\) and \(i-1\).

**Determination of nutritional composition of Dictyota dichotoma**

The total soluble carbohydrates (TSC) were estimated according to Hedge & Hofreiter (1962). On the other hand, the total soluble proteins (TSP) were measured according to AOAC (2000).
Statistical analysis
One-way ANOVA and Fisher’s 95% significance level grouping test were used to check the influence of enrichment of different nutrient concentrations on the growth rate, the absorption of nutrients, and the nutritional content of *D. dichotoma* (Cambell, 1989). All statistical analyses were carried out using Minitab ® software (Version 16).

Results

**Taxonomic description of Dictyota dichotoma**

Algal sample was morphologically identified as presented in the literature. The latest accepted name was *D. dichotoma* (Hudson) J.V. Lamouroux.

**Physical and chemical conditions of the culture**

Culture temperature, pH, salinity, and dissolved oxygen were measured during the 11 days of experiment and the results were illustrated in Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (˚C)</td>
<td>25.6±0.6</td>
</tr>
<tr>
<td>pH</td>
<td>8.4±0.5</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>41.2±0.7</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>4.4±0.6</td>
</tr>
</tbody>
</table>

Each value represents the average ± SD

**Bioremediation of ammonium and phosphate by Dictyota dichotoma**

The different concentrations of ammonium (8, 20, 40, and 60μmol/L) and phosphate (0.8, 2, 4, and 6μmol/L) were decreased with time until reaching their minimum values of 0.001μmol/L at 24hrs. The highest concentration of NH₄⁺ (80μmol/L) and PO₄³⁻ (8μmol/L) was reached at 24hrs to 21.23± 0.32 and 0.75± 0.07, respectively (Fig. 3). The uptake of different concentrations of NH₄⁺ and PO₄³⁻ were significantly different at P< 0.05. The highest uptake efficiency of NH₄⁺ at 24hrs was 99.98± 0.01% occurred for the conc. of 8μmol/L, while the lowest uptake efficiency of NH₄⁺ was 69.18 ± 3.2% occurred for the conc. of 80μmol/L (Fig. 4).

Phosphate concentration levels were close to fluctuations in concentrations of ammonium. The highest uptake efficiency of PO₄³⁻ at 24hrs was 100 ± 0.01% occurred for the conc. of 0.8μmol/L, while the lowest uptake efficiency of PO₄³⁻ was 90.7 ± 3.2% occurred for the conc. of 8μmol/L (Fig. 4).

**Effect of phosphate and ammonium (P/N) concentrations on the growth of Dictyota dichotoma**

The obtained results displayed that dissimilarities in ammonium and phosphate concentrations have an effect on the growth of *D. dichotoma*. The biomass of *D. dichotoma* increased as PO₄³⁻/NH₄⁺ concentration increased from 0μmol/L to 6/60μmol/L until the end of the experiment (12 days of incubation), while at 8/80 μmol/L the biomass was decreased since the second day of treatment. The highest SGR was 9.3± 0.2% day⁻¹ at 6/60μmol/L while the lowest was 5.11 ± 0.3% day⁻¹ at 0 μmol/L (Fig. 5). Furthermore, the results indicated that significant differences (P< 0.001, F= 11.3) were observed in SGR values of untreated *D. dichotoma* (without nutrient addition) and different concentrations of nutrients.

**Effect of Phosphate and Ammonium (P/N) Concentrations on the nutritional content of Dictyota dichotoma**

TSC of the *D. dichotoma* was increased with the addition of ammonium plus phosphate after ten days of the experiment compared with its initial content. The highest average of TSC (9.79± 0.45% dry wt.) was observed at the concentration of 6/60 μmol/L, while the lowest average (5.65± 0.42% dry wt.) was observed at 0/0μmol/L (Fig. 6).

TABLE 1. Physical and chemical conditions of the culture during the experiment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (˚C)</td>
<td>25.6±0.6</td>
</tr>
<tr>
<td>pH</td>
<td>8.4±0.5</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>41.2±0.7</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>4.4±0.6</td>
</tr>
</tbody>
</table>

Each value represents the average ± SD

**Bioremediation of ammonium and phosphate by Dictyota dichotoma**

The different concentrations of ammonium (8, 20, 40, and 60μmol/L) and phosphate (0.8, 2, 4 and 6μmol/L) were decreased with time until reaching their minimum values of 0.001μmol/L at 24hrs. The highest concentration of NH₄⁺ (80μmol/L) and PO₄³⁻ (8μmol/L) was reached at 24hrs to 21.23± 0.32 and 0.75± 0.07, respectively (Fig. 3). The uptake of different concentrations of NH₄⁺ and PO₄³⁻ were significantly different at P< 0.05. The highest uptake efficiency of NH₄⁺ at 24hrs was 99.98± 0.01% occurred for the conc. of 8μmol/L, while the lowest uptake efficiency of NH₄⁺ was 69.18 ± 3.2% occurred for the conc. of 80μmol/L (Fig. 4).

Phosphate concentration levels were close to fluctuations in concentrations of ammonium. The highest uptake efficiency of PO₄³⁻ at 24hrs was 100 ± 0.01% occurred for the conc. of 0.8μmol/L, while the lowest uptake efficiency of PO₄³⁻ was 90.7 ± 3.2% occurred for the conc. of 8μmol/L (Fig. 4).

**Effect of phosphate and ammonium (P/N) concentrations on the growth of Dictyota dichotoma**

The obtained results displayed that dissimilarities in ammonium and phosphate concentrations have an effect on the growth of *D. dichotoma*. The biomass of *D. dichotoma* increased as PO₄³⁻/NH₄⁺ concentration increased from 0μmol/L to 6/60μmol/L until the end of the experiment (12 days of incubation), while at 8/80 μmol/L the biomass was decreased since the second day of treatment. The highest SGR was 9.3± 0.2% day⁻¹ at 6/60μmol/L while the lowest was 5.11 ± 0.3% day⁻¹ at 0 μmol/L (Fig. 5). Furthermore, the results indicated that significant differences (P< 0.001, F= 11.3) were observed in SGR values of untreated *D. dichotoma* (without nutrient addition) and different concentrations of nutrients.

**Effect of Phosphate and Ammonium (P/N) Concentrations on the nutritional content of Dictyota dichotoma**

TSC of the *D. dichotoma* was increased with the addition of ammonium plus phosphate after ten days of the experiment compared with its initial content. The highest average of TSC (9.79± 0.45% dry wt.) was observed at the concentration of 6/60 μmol/L, while the lowest average (5.65± 0.42% dry wt.) was observed at 0/0μmol/L (Fig. 6).

The findings of the ANOVA showed a significant difference (P< 0.05) between the different concentrations and the initial content.

The tendency TSP of the *D. dichotoma* in this study showed an increase in culture media up to 6/60μmol/L with increases in phosphate and ammonium. The highest average of TSP (16.3± 0.32% dry wt.) was observed at the concentration of 6/60 μmol/L, while the lowest average (8.5± 0.52% dry wt.) was observed at 0/0μmol/L (Fig. 6). The differences between TSP content in different concentrations of PO₄³⁻/NH₄⁺ were statistically significant (P< 0.05). Concerning *D. dichotoma* lipid content, similar PO₄³⁻/NH₄⁺ implementation increased its total lipid compared to control group.
Fig. 3. Nutrients concentration (μmol/L) during 24hrs of treatment; (A) Ammonium and (B) phosphate.

Fig. 4. Uptake efficiency (%) for nutrient; (A) Ammonium and (B) phosphate. The letters indicate significant differences (p < 0.05) among different experimental treatments.

Fig. 5. Specific growth rate (SGR) of *D. dichotoma* cultured for 10 days under supply of different PO4³⁻/NH4⁺ concentrations (μmol/L). Different letters indicate significant differences (P< 0.05) among different experimental treatments.
Fig. 6. Nutritional content (%) of *D. dichotoma* cultured for 10 days under supply of different PO$_4^{3-}$/NH$_4^+$ concentrations (μmol/L) [Different letters indicate significant differences (P< 0.05) among different experimental treatments].

The total lipid content of *D. dichotoma* treated with P/N 2/20, 4/40 and 6/60 μmol/L concentrations were 7± 0.24, 6± 0.16, and 8± 0.30% dry wt., which are 34.6%, 15.38%, and 53.9% higher than their relevant initial lipid content.

In addition, the results indicated that a significant difference in the total lipid content of untreated *D. dichotoma* was observed (P< 0.05) if compared to all phosphate and ammonium concentrations (Fig. 6).

**Discussion**

**Bioremediation of ammonium and phosphate by Dictyota dichotoma**

It is well established that, seaweeds have significant attention for their high bioremediation capabilities (Ben Chekroun et al., 2014). This study revealed that two nutrients, (NH$_4^+$ and PO$_4^{3-}$), have been significantly removed at different levels by seaweed *D. Dichotoma* with increased efficiency in biofiltration. Over a 24hrs period, 69.2–99.9% of the available ammonium and 90.7–100% of the available phosphate were taken up by Dictyota dichotoma. It means that this species has the highest capacity to accumulate ammonium and phosphate in its vacuoles. Previous studies using other macroalgae such as *Ulva lactuca*, it showed ammonia-N and phosphate removal rate of 84.74% and 41.1%, respectively (Al-Hafedh et al., 2012).

Carneiro et al. (2011) found that Gracilaria cervicornis was able to reduce NH$_4^+$ concentrations by 74% (2hrs) and PO$_4^{3-}$ by 95.1% (10hrs) in wastewater treatment using seaweed. Hernández et al. (2006) reported mean filtration efficiencies of 93.2% for NH$_4^+$ and 62.2% for PO$_4^{3-}$ after 7hrs of incubation in an experiment using Gracilariopsis longissima. On the other hand, the lowest uptake efficiency was obtained at high concentration of ammonium and phosphate of 80 and 8 μmol/L, respectively after 24 h of incubation (69.18± 3.2% for NH$_4^+$ and 90.7±3.2% for PO$_4^{3-}$) and this may be related to nutrient storage in the algal tissues as reported by Yang et al. (2006).

**Biomass and growth of Dictyota dichotoma**

Dissolved Inorganic Phosphorus (DIP) and Dissolved Inorganic Nitrogen (DIN) were the essential macronutrients for maintaining macroalgae metabolism and development. The main constituents of phospholipids, nucleic acids, adenosine triphosphate (ATP) are phosphorus (P), and nitronine (N) and they also participate in enzyme reaction adaptation and metabolic pathway regulation (Lubsch & Timmermans, 2019). In this research, growth rate analysis found a significant effect on a specific growth rate (SGR) of *D. dichotoma* on the treatment of PO$_4^{3-}$/NH$_4^+$ at different concentrations. The results
of this study showed that D. dichotoma biomass increased from 0μmol/L to 6/60μmol/L until the end of the experiment (10 days of the incubation), as PO$_4^{3-}$/NH$_4^+$ concentration rose. The maximum SGR at 6/60μmol/L was 9.3± 0.2% day$^{-1}$ while the lowest at 0μmol/L was 5.11± 0.3% day$^{-1}$. The results reported were consistent with Joniyas et al. (2016) findings.

In the same context, the SGR of Hypnea cervicornis J. Agardh increased linearly with the addition of ammonium (Ribeiro et al., 2013). Sometimes, some reports related to nutrients and growth of macroalgae claimed that the requirement of nutrients depends on the algal species and its physiology. The biomass was decreased on the second day of treatment at 8/80μmol/L, which maybe because of the high concentrations of ammonium. Ribeiro et al. (2017) stated that high concentrations of ammonium (50-80μmol/L) could be toxic for some seaweed.

**Effect of phosphate and ammonium (P/N) concentrations on the nutritional content of Dictyota dichotoma**

Seaweed has a physiological mechanism to obtain, utilize, and accumulate various forms of nitrogen from the media. This study revealed that TSC, TSP, and total lipids of the tested D. dichotoma were significantly increased with the increase of PO$_4^{3-}$/NH$_4^+$ concentration. The highest averages of TSC (9.79± 0.45% dry wt.), TSP (16.3± 0.32% dry wt.) and total lipid (8± 0.30 % dry wt.) were observed at the concentration of 6μmol/L for PO$_4^{3-}$ and 60μmol/L for NH$_4^+$ after 12 days of incubation and greater than their respective initial content. Zhang et al. (2010) found that soluble protein content of Potamogeton crispus increases when it is fertilized with NH$_4^+$-N compared with untreated seaweeds. Ismail & El-Sheek (2017) discussed the nitrogen content in culture media stimulate the biosynthesis of carbohydrates, protein, and pigment of the seaweed. Furthermore, the increased PO$_4^{3-}$/NH$_4^+$ concentration will led to an increase in total soluble proteins (Joniyas et al., 2016). On the other hand, the data obtained by Suthar et al. (2019) contradict our results. They found that, addition of different concentrations of P/N didn’t significantly affect lipid synthesis in Ulva fasciata. Their results about biochemical analysis of biomass were 9.30± 0.32% for proteins, 20.2± 2.51% for carbohydrates and 6.28± 0.84% for dry weight.

**Conclusion**

The macro-algae are of considerable nutrition value and play an important role in the marine food chain for marine organisms. D. Dichotoma considered being a good candidate for bioremediation of contaminated sites and biofiltration of aquaculture effluent, canning effectively, and efficiently depleting ammonium and phosphate in the aquatic environment. Our consequences demonstrated that D. dichotoma was largely assimilated ammonium and phosphorus with a percentage of 69.2–99.9% of the available ammonium and 90.7–100% of the available phosphate. Nutrient concentration in the growth media of D. dichotoma affects on the growth rate, where nutritional composition (lipids-carbohydrate and protein) increases with the increase of ammonium and phosphates. On the other hand, at 8/80 μmol/L PO$_4^{3-}$/NH$_4^+$ concentration, the biomass was decreased. High concentrations of the nutrient are toxic and slow down the growth and may lead to macroalgae death.

**Conflict of interests:** The authors declare no conflict of interest.

**Authors contribution:** All are equally.

**Ethical approval:** Not applicable.

**References**


Egypt J. Bot. 60, No.3 (2020)
المعالجة الحيوية للأمونيوم والفوسفات وتأثيرها على الطحالب الكبيرة من نوع الدكتيوتا دايكوتومس

(1) أمانى مدكور، (2) سارة رشدي، (3) حسين طليبه

(1) معمل التلوث البحري - قسم البيئة البحرية - المعهد القومي لعلوم المحيطات والمصايد - محافظة البحر الأحمر، مصر
(2) معمل علم الأحياء المنوي - قسم البيئة البحرية - المعهد القومي لعلوم البحر والصيد، فرع الجيزة - جمهورية مصر العربية
(3) معالم علم الأحياء المائية - قسم البيئة البحرية - المعهد القومي لعلوم البحار والمصايد، فرع الجيزة - جمهورية مصر العربية

والآداب، جامعة عين شمس. القاهرة. مصر.

تم إجراء تجربة معملية استغرقت أحد عشر يوما على تأثير الأمونيوم والفوسفات الذائبين على امتصاص ونمو طحلب الدكتيوتا دايكوتومس وذلك لتحديد طبيعتها كمرشح حيوي مغذي. تم إضافة 80/8 و60/6، 40/4، 20/2، 8/0 (8) مكرومول/لتر من الأمونيوم والفوسفات على النحو التالي، خلال فترة 1:10 ميكرومول/لتر (إلى مياه البحر المعقمة. كانت نسبة الفوسفات بالنسبة للنيتروجين المولي 100% من الفوسفات المتاح بواسطة 100% من الأمونيوم المتاح و 99.9% من الفوسفات المتاح بواسطة طحلب الدكتيوتا دايكوتومس. تم تسجيل أعلى معدل نمو الطحلب هو 9.3 ± 0.2% ليوم وذلك عند تركيز 60/6 مكرومول/لتر. بينما كان أقل معدل نمو هو 5.11 ± 0.3% ليوم عند تركيز قدره صفر مكرومول/لتر. على الجانب الآخر عند تركيز قدره في 80 ميكرومول/لتر فصل النباتات الحيوية مشتملة في المواد الكربوهيدراتية الكلية ذاتية وكذلك البروتينات ذاتية وأيضا الدهون الكلية لطحلب الدكتيوتا دايكوتومس وذلك بزيادة نوعية من تركيز الفوسفات والأمونيوم مقارنة بمحتواها عند بدء التجربة. تم تسجيل أعلى نسبة للفوسفات المذابة للفوسفات و 60 ميكرومول/لتر للفوسفات و 60 ميكرومول/لتر للأمونيوم. بناء على هذه النتائج فإن طحلب الدكتيوتا دايكوتومس يتمتع بقدرة عالية على امتصاص الأمونيوم والفوسفات وتم استخدامه كعلاج حيوي مقصور في البيئات ذات التركيزات العالية من هذه العناصر.