



Bioremediation of Ammonium and Phosphates and their Effect on the Macroalgae *Dictyota dichotoma* (Hudson) Lamouroux Native to the Red Sea Coast, Egypt

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IN LABORATORY experiments for eleven days, the effect of dissolved ammonium and phosphate on the absorption, growth, and nutritional content of *Dictyota dichotoma* was examined to determine their potential application as a nutrient biofilter. Five combinations of phosphates and ammonium concentration (0.8/8, 2/20, 4/40, 6/60, and 8/80 μmol/L) were added to Sterile seawater. Molar phosphorus/ nitrogen ratio was 1:10. Over a 24hrs period, 69.2–99.9% of the available ammonium and 90.7–100% of the available phosphate were taken up by *D. dichotoma*. The highest specific growth rate was $9.3 \pm 0.2\%$ day⁻¹ at 6/60 μmol/L while the lowest was $5.11 \pm 0.3\%$ day⁻¹ at 0 μmol/L. On the other hand at 8/80 μmol/L the biomass was decreased in the second day of treatment. Total soluble carbohydrate, total soluble protein, and total lipids of the tested *D. dichotoma* were significantly increased with the increase of PO₄⁻³/NH₄⁺ concentration and greater than their respective initial content. The highest averages of total soluble carbohydrate (TSC) ($9.79 \pm 0.45\%$ dry wt.), total soluble protein (TSP) ($16.3 \pm 0.32\%$ dry wt.) and total lipid ($8 \pm 0.30\%$ dry wt.) were observed at the concentration of 6 μmol/L for PO₄⁻³ and 60 μmol/L for NH₄⁺. Based on these results, *D. dichotoma* has a strong absorption capacity for ammonium & phosphates, and as bioremediator in eutrophic environments can be a promising applicant.

Keywords: Ammonium and phosphate nutrients, Biomass, Bioremediator, *Dictyota dichotoma*, Nutrient uptake, Red Sea, Specific growth rate, Total soluble carbohydrate (TSC), Total soluble protein (TSP).

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

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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. Phosphorus 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 $27^{\circ} 17' 13''$ N and Longitude $33^{\circ} 46' 21''$ 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.



Fig. 1. Site of collection.

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).



Fig. 2. *Dictyota dichotoma*.

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 ± 2 g *D. dichotoma* fronds were grown in 20L sterile seawater aerated glass jars. The nutrient media levels were $0.4 \mu\text{mol/L}$ for phosphate, $0.6 \mu\text{mol/L}$ for ammonia, $0.135 \mu\text{mol/L}$ for nitrate, and $0.011 \mu\text{mol/L}$ 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 (NH_4Cl) and phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) were added to the Von Stosch modified medium in order to obtain a phosphorous/ 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 $80 \mu\text{mol/L}$. On the other hand, the phosphorus

concentrations were 0, 0.8, 2, 4, 6, and $8 \mu\text{mol/L}$. Control and different treatment either by nitrogen or phosphorus, were in three replicates. The light intensity was $20 \mu\text{mol photons m}^{-2} \text{sec}^{-1}$ 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 (NH_4^+) and dissolved inorganic phosphate (PO_4^{3-}) 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 :

$$\text{NUE} = [(c_i - c_f) / c_i] \times 100$$

where, NUE is Nutrient Uptake Efficiency in percentage. c_i and c_f are the initial and the final concentrations ($\mu\text{mol/L}$) of each nutrient.

Macroalgae 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. dichotomas* 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 1. Physical and chemical conditions of the culture during the experiment.

Parameters	Measurements
Temperature (°C)	25.6±0.6
pH	8.4±0.5
Salinity (ppt)	41.2±0.7
DO (mg/L)	4.4±0.6

Each value represents the average ± SD

Bioremediation of ammonium and phosphate by *Dictyota dichotoma*

The different concentrations of ammonium (8, 20, 40, and 60 µmol/L) and phosphate (0.8, 2, 4 and 6 µmol/L) were decreased with time until reaching their minimum values of 0.001 µmol/L at 24hrs. The highest concentration of NH₄⁺ (80 µmol/L) and PO₄³⁻ (8 µmol/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 8 µmol/L, while the lowest uptake efficiency of NH₄⁺ was 69.18 ± 3.2% occurred for the conc. of 80 µmol/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 0.8 µmol/L, while the lowest uptake efficiency of PO₄³⁻ was 90.7± 3.2% occurred for the conc. of 8 µmol/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 0 µmol/L to 6/60 µmol/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 6/60 µmol/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 0/0 µmol/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 6/60 µmol/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 0/0 µmol/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.

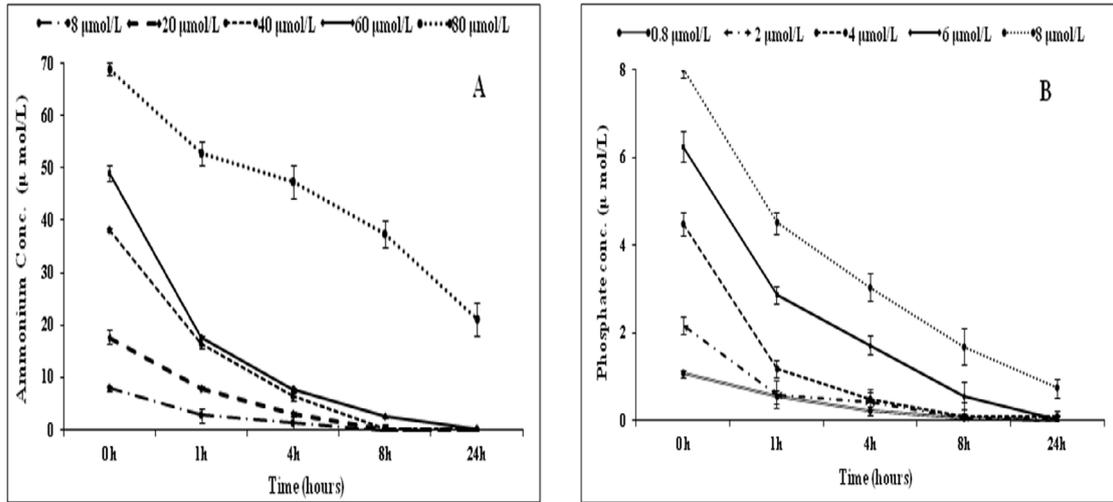


Fig. 3. Nutrients concentration (μmol/L) during 24hrs of treatment; (A) Ammonium and (B) phosphate.

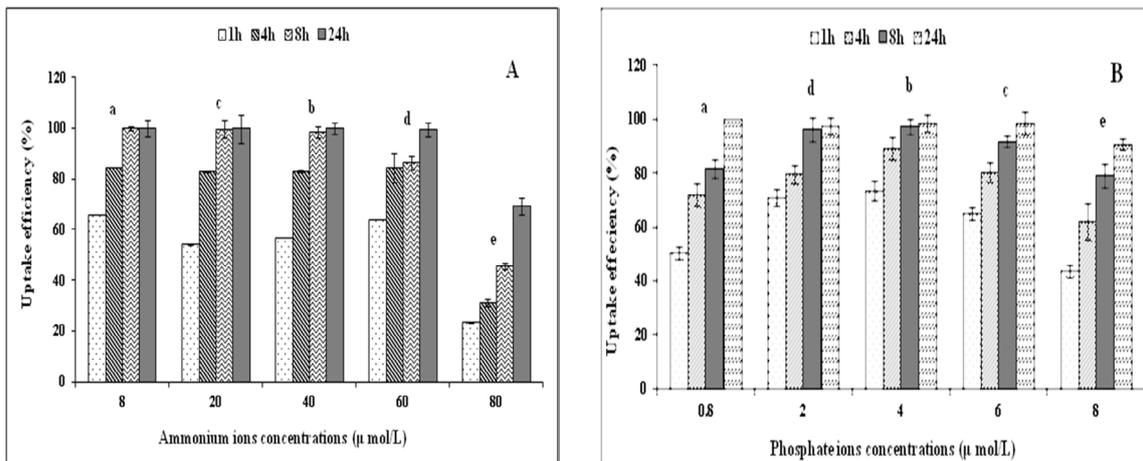


Fig. 4. uptake efficiency (%) for nutrient; (A) Ammonium and (B) phosphate [The letters indicate significant differences (P < 0.05) among different experimental treatments].

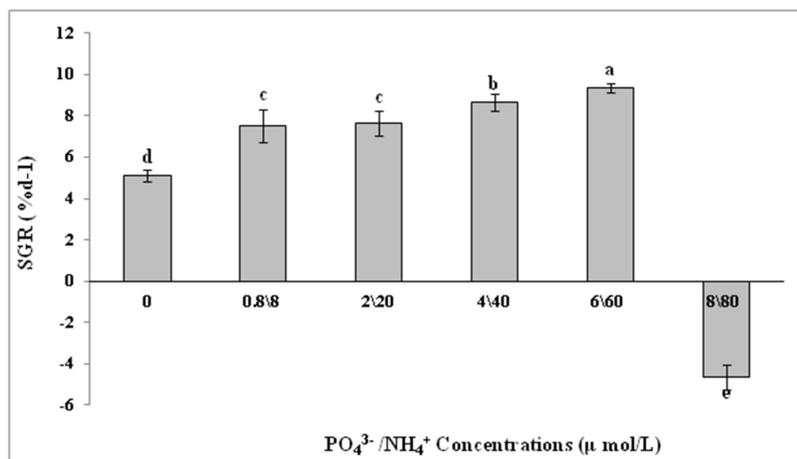


Fig. 5. Specific growth rate (SGR) of *D. dichotoma* cultured for 10 days under supply of different PO₄³⁻/NH₄⁺ concentrations (μmol/L) [Different letters indicate significant differences (P < 0.05) among different experimental treatments].

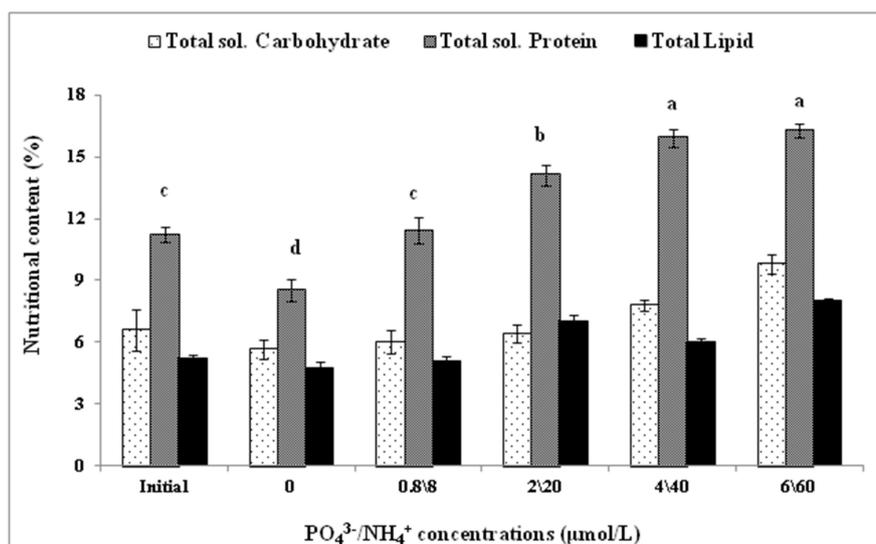


Fig. 6. Nutritional content (%) of *D. dichotoma* cultured for 10 days under supply of different PO₄³⁻/NH₄⁺ concentrations (µmol/L) [Different letters indicate significant differences ($P < 0.05$) among different experimental treatments].

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 $8 \pm 0.30\%$ 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, (NH₄⁺ and PO₄³⁻), 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 NH₄⁺ concentrations by 74% (2hrs) and PO₄³⁻ by 95.1% (10hrs) in wastewater treatment using seaweed. Hernández et al. (2006) reported mean filtration efficiencies of 93.2% for NH₄⁺ and 62.2% for PO₄³⁻ 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 ($69.18 \pm 3.2\%$ for NH₄⁺ and $90.7 \pm 3.2\%$ for PO₄³⁻) 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 nitrogen (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 PO₄³⁻/NH₄⁺ at different concentrations. The results

of this study showed that *D. dichotoma* biomass increased from 0 $\mu\text{mol/L}$ to 6/60 $\mu\text{mol/L}$ until the end of the experiment (10 days of the incubation), as $\text{PO}_4^{3-}/\text{NH}_4^+$ concentration rose. The maximum SGR at 6/60 $\mu\text{mol/L}$ was $9.3 \pm 0.2\% \text{ day}^{-1}$ while the lowest at 0 $\mu\text{mol/L}$ was $5.11 \pm 0.3\% \text{ day}^{-1}$. 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 8/80 $\mu\text{mol/L}$, which maybe because of the high concentrations of ammonium. Ribeiro et al. (2017) stated that high concentrations of ammonium (50-80 $\mu\text{mol/L}$) 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 $\text{PO}_4^{3-}/\text{NH}_4^+$ concentration. The highest averages of TSC ($9.79 \pm 0.45\% \text{ dry wt.}$), TSP ($16.3 \pm 0.32\% \text{ dry wt.}$) and total lipid ($8 \pm 0.30\% \text{ dry wt.}$) were observed at the concentration of 6 $\mu\text{mol/L}$ for PO_4^{3-} and 60 $\mu\text{mol/L}$ 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 $\text{NH}_4^+\text{-N}$ 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 $\text{PO}_4^{3-}/\text{NH}_4^+$ 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 $9.30 \pm 0.32\%$ for proteins, $20.2 \pm 2.51\%$ for carbohydrates and $6.28 \pm 0.84\%$ 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 $\mu\text{mol/L}$ $\text{PO}_4^{3-}/\text{NH}_4^+$ 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.

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المعالجة الحيوية للأمونيوم والفوسفات وتأثيرها على الطحالب الكبيرة من نوع الدكتيوتا دايكوتوموس (*Dictyota dichotoma* (Hudson) Lamouroux)، المتوطن في ساحل البحر الأحمر – مصر

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تم إجراء تجربة معملية استغرقت أحد عشر يوماً على تأثير الأمونيوم والفوسفات الذائبين على امتصاص ونمو وكذلك الحالة الغذائية لطحلب الدكتيوتا دايكوتوموس وذلك لتحديد تطبيقها كمرشح حيوي مغذي. تمت إضافة خمس تركيزات مشتركة من اللامونيوم والفوسفات على النحو التالي (8,0/8، 20/2، 40/4، 60/6 و 80/8 ميكرومول/لتر) إلى مياه البحر المعقمة. كانت نسبة الفوسفات بالنسبة للنيتروجين المولي 1:10. خلال فترة 24 ساعة، تم استهلاك 69.2 - 99.9% من الأمونيوم المتاح و 90.7 - 100% من الفوسفات المتاح بواسطة طحلب الدكتيوتا دايكوتوموس. تم تسجيل أعلى معدل لنمو الطحلب هو $0.2 \pm 9.3\%$ لليوم وذلك عند تركيز 6/60 مول/لتر، بينما كان أقل معدل نمو هو $0.3 \pm 5.11\%$ لليوم عند تركيز قدره صفر ميكرومول/لتر. على الجانب الآخر عند تركيز قدره في 8/80 ميكرومول/لتر تم نقص الكتلة الحيوية مئتمله في المواد الكربوهيدراتية الكلية الذائبة وكذلك البروتين الذائب وأيضا الدهون الكلية لطحلب الدكتيوتا دايكوتوموس وذلك بزيادة نوعيه في تركيز الفوسفات/الامونيوم مقارنة بمحتواها عند بدء التجربة. تم تسجيل أكبر نسبة للمواد الكربوهيدراتية القابلة للذوبان ($0.45\% \pm 9.79$ وزن جاف)، البروتين الكلي القابل للذوبان (0.32 ± 16.3 وزن جاف) وكذلك الدهون الكلية (0.30 ± 8 وزن جاف) وذلك عند تركيز قدره 6 ميكرومول/لتر للفوسفات و 60 ميكرومول/لتر للأمونيوم. بناءً على هذه النتائج فإن طحلب الدكتيوتا دايكوتوموس يتمتع بقدرة عالية على امتصاص الامونيوم والفوسفات ويتم استخدامه كمعاج حيوي خاصه في البيئات ذات التركيزات العالية من هذه العناصر.