



Identification of culturable microalgae diversity in the River Nile in Egypt using enrichment media

Amal A. Badr¹ and Walid M. Fouad^{1,2*}

¹Biotechnology Program, School of Sciences and Engineering, The American University in Cairo, New Cairo 11835, Egypt.
E-mail: Amal.badr@aucegypt.edu

²Department of Biology, School of Sciences and Engineering, The American University in Cairo, New Cairo 11835, Egypt.
E-mail: wfouad@aucegypt.edu

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Abstract

This study aimed to morphologically identify the highest possible microalgae biodiversity in a sample collected from the River Nile using the culture-based method. Water samples were cultured on the two commonly used media BG-11 and BBM media to obtain a broad spectrum of microalgae biodiversity. Likewise, the effects of nutrients concentration and vitamin supplements to BG-11 and BBM for supporting the maximum diversity of culturable microalgae were tested. Cell counts and morphology-based identification were conducted to evaluate the population diversity. A total of 35 species have been identified using the two media combined. The standard BG-11 and BBM media enriched 25 and 27 species, respectively, while the reduced nutrient concentration of BG-11 and BBM had a species richness of 19 and 24, respectively. The vitamin-enriched media each sustained BG-11^{+vit} (23) and BBM^{+vit} (20). We found that some algae species were uniquely identified on the reduced nutrient concentration or vitamin-enriched media. The results of this study report the current algae diversity in the Nile demonstrating that the usage of single-culture media for algal enrichment would result in underestimating the species richness. The diversity identified can be used as a reference for continuous monitoring of the River Nile microalgae diversity in Cairo.

Keywords: River Nile, Freshwater, Biodiversity, Morphological identification, BG-11 medium, BBM medium

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1. Introduction

Microalgae are rich with value-added by-products that are useful for many industrial applications, scientists so far have only been able to identify and examine about 30,000 species (Frac *et al.*, 2010). Moreover, the significance and potential of microalgae remain mostly untapped as a result of the theoretical and methodological restrains in the study of microalgal biodiversity (Sharma and Rai, 2011). According to May (2002), about 30% of taxonomical work on plant biodiversity and less than 2% of the workforce are engaged in microalgae diversity research (May, 2002). This leads to us having insufficient records of the biodiversity of the planet's microalgae and the loss of their potential usages (Magurran and Dornelas, 2010).

* Corresponding author: Walid M. Fouad, Department of Biology, School of Sciences and Engineering, The American University in Cairo, New Cairo 11835, Egypt. E-mail: wfouad@aucegypt.edu

The River Nile in Egypt, the longest river in the world, yet, the available reports on microalgae populations had a low emphasis on diversity. Limited reports, however, indirectly addressed microalgae biodiversity while screening for high-lipid producing microalgae from the River Nile (Abd El-Karim *et al.*, 2016; and Abdo *et al.*, 2013). Others confronted the microalgae biodiversity in polluted locations with the aim of identifying microalgae that have properties of biosorption of heavy metals (Shanab *et al.*, 2012). In the previous study mentioned, the water samples from heavy metal polluted locations were cultured on BG-11 and BBM to obtain unialgal species; three species were then isolated and tested on different heavy metal-containing media. Therefore, only algal species capable of growing on the standard BG-11 or BBM were identified and, therefore, further tested. The Rosetta branch and Damietta region are relatively the most studied regions of the River Nile in Egypt for microalgae related research. Some reports studied the biodiversity (Abd El-Karim *et al.*, 2016; and Gharib, 2006) by analyzing the fatty acid composition of the algal cells and concluded that microalgae are the chief contributors of carbon cycling and organic matter in surface sediments in the Damietta and Rosetta Branch of the River Nile. However, no microalgae identification was performed prior to fatty acid composition analysis. To our knowledge, microalgae biodiversity in the River Nile has not been investigated in greater Cairo. In terms of the usage of culture media to enrich microalgae diversity from environmental samples several studies have been previously reported in the literature with the aim of isolation, identification (either microscopically or molecular level identification) and maintenance of microalgae species (Abou-Shanab *et al.*, 2011; Cho *et al.*, 2013; Edwin *et al.*, 2019; Ilavarasi *et al.*, 2011; and Lee *et al.*, 2014). Both Blue-Green Medium (BG-11) and Bold's Basal Medium (BBM) are two media commonly used for identification and maintaining population diversity due to its ability to sustain a wide range of species.

Therefore, in this study, our aim was to investigate the microalgal biodiversity present in the River Nile in greater Cairo governorate through the enrichment of the collected sample using BG-11 and BBM as an easy and inexpensive approach for microalgae biodiversity analysis. Furthermore, our aim was to examine the effect of nutrients concentrations alongside media enrichment with vitamins on algae population diversity. We found that the usage of multiple algal growth media approach for algae enriching from environmental samples revealed a more comprehensive range of diversity compared to the usage of the single-media approach.

2. Material and methods

2.1. Sampling site and collection

The environmental sample collected for this investigation was from the River Nile in the Cairo governorate, Egypt (30°00'55.4"N; 31°13'41.8"E). Two liters of surface water were collected from the location; one liter of the sample has been utilized for water physicochemical analysis. The temperature of the samples was measured at the sampling site using a thermometer, and the pH of the water samples was measured in the lab using a pH meter. The samples were kept in the refrigerator (4°C) until further analysis. The experiments were repeated twice using samples from the same location.

2.2. Physical and chemical analysis

The physicochemical analysis was performed to assess the composition of the natural habitat of the microalgae identified in the water samples from each location. Salinity, pH and electrical conductivity (EC) were measured using a multimeter-probe (330i, WTW, Germany), and the presence of cation and anions such as sodium, chloride, sulfate, potassium, and calcium were measured according to standard methods (ASTM D 4327, 2011). Total ammonia, nitrate, and phosphorus were measured based on standard methods (Rice and Franson, 2005).

2.3. Experimental design and culturing conditions

All the media components have been purchased from PhytoTechnology Laboratories® (Shawnee Mission, Kansas, USA). The media was prepared according to the manufacturer recommendations unless stated otherwise, and the pH of each media was adjusted before autoclaving as following: BG-11 (7.15) and BBM (6.6). Finally, the media was autoclaved at 121 °C for 20 min. For algae growth conditions, transparent 600 ml plastic bottles were used to support a culturing volume of 400 ml. Each Nile water sample was separated into three testing conditions, further details will be mentioned below, and each condition was performed with three replicates. Hence, each replica was inoculated with 40 ml of the River Nile water sample. All cultures were maintained for three weeks in a JSR-Growth Chamber 3-Side Illumination (model JSPC-960C2) at 22 ± 1 °C. The light/dark regime was adjusted to 12:12 h at a light intensity of 28 μmol photons m⁻²s⁻¹.

We tested the impact of three different factors on supporting algal population growth and diversity from the Nile water sample. The chemical composition of the growing media was examined, and we have chosen the two commonly used algae growth media BG-11 (Andersen *et al.*, 2005) and BBM (Nichols and Bold, 1965). Detailed chemical composition of both BG-11 and BBM are listed in Table 1. To reduce the dominance of the competitive species and provide an intermediate ground for the less competitive species to coexist in the population, we have reduced the nutrient concentration to 0.1× of the original media concentration as the second tested factor (Graham and Duda, 2011). Therefore, treatments denoted as BG-11^{0.1×} and BBM^{0.1×} represent 0.1× concentrations of BG-11 and BBM media, respectively.

Table 1: Chemical composition of the BG-11 and BBM media used for microalgae population growth. The concentration of salts in mg L⁻¹

Components	BG-11	BBM
Boric Acid	2.86	11.42
Calcium Chloride, Anhydrous	27.18	18.87
Citric Acid, Anhydrous	6	-
Cobalt Nitrate • 6H ₂ O	0.049	0.49
Cupric Sulfate • 5H ₂ O	0.079	1.57
EDTA, Disodium Salt	1	63.61
Ferric Ammonium Citrate	6	-
Ferrous Sulfate • 7H ₂ O	-	4.98
Magnesium Sulfate • 7H ₂ O	75	-
Magnesium Sulfate, Anhydrous	-	36.63
Manganese Chloride • 4H ₂ O	1.81	1.44
Potassium Hydroxide	-	31
Potassium Phosphate, Dibasic	40	75
Potassium Phosphate, Monobasic	-	175
Sodium Carbonate, Anhydrous	20	-
Sodium Chloride	-	25
Sodium Molybdate • 2H ₂ O	0.39	1.19
Sodium Nitrate	1500	250
Zinc Sulfate • 7H ₂ O	0.222	8.82

Algae are assumed to depend on the bacterial synthesis for vitamins such as vitamin B₁ (thiamine), vitamin B₇ (biotin) and vitamin B₁₂ (cobalamin) (Barra *et al.*, 2014; Croft *et al.*, 2006; Hiibel *et al.*, 2015; and Krohn-Molt *et al.*, 2013, 2017). Hence, vitamins-enrichment of the normal nutrient salt concentration of BG-11 and BBM media was also tested and designated as BG-11^{+vit} and BBM^{+vit}, respectively. The vitamin mix was added to reach the following final concentrations: D-biotin 0.05 mg L⁻¹, folic Acid 0.5 mg L⁻¹, glycine (Free Base) 2 mg L⁻¹, myo-inositol 100 mg L⁻¹, nicotinic acid (free acid) 5 mg L⁻¹, pyridoxine•HCl 0.5 mg L⁻¹ and thiamine • HCl 0.5 mg L⁻¹. Media of normal concentration, containing no vitamin, was run in parallel for comparison.

2.4. Growth analysis

Microalgae population growth was monitored by performing a cell count every four days using a Neubauer Improved Hemocytometer counting chamber. Cell count was performed under Zeiss Axiostar plus light microscope at 400× magnification based on (Edler and Elbrächter, 2010; and Reza Moheimani *et al.*, 2013). Briefly, each bottle was well mixed by shaking, and 500 μL was transferred from each bottle into a 1.5 ml Eppendorf, and 10 μL was loaded onto the hemocytometer. Each replica was sampled twice for counting using the center region of the hemocytometer, and an average was used to calculate the cell density of each replica.

2.5. Identification and classification of microalgal

The identification of microalgae was based on the morphological characteristics observed under a bright-field microscope (Bellinger and Sigee, 2010; and Bock, 1971). The strains were examined under a light microscope (Leica) using the software LAS EZ (Leica DM500). Briefly, each bottle was mixed by shaking, and a volume of 500 μL was transferred from each bottle into a 1.5 ml Eppendorf, a few drops were loaded onto a slide and was observed under 400× magnification. Identification of the microalgae species was performed per microscopic fields in the multiple of 10 when no new species have been identified after the 10 fields; the diversity analysis is completed.

2.6. Alpha diversity indices

The relative abundance of each genus was used in calculating the diversity indices. The total number of microalgae was summed up and represented as Species richness (S), diversity index was calculated using the Shannon-Wiener index (H') (Equation 1) (Shannon, 1948), and dominance concentration were evaluated by the Simpson's diversity index (D) (Equation 2) (Hillebrand and Sommer, 2000; Simpson, 1949). In the equations below, (i) represents the species proportion relative to the total species number (p_i). Species Evenness (E) was calculated using (Equation 3) (Davari *et al.*, 2011).

Equation 1. Shannon's Diversity Index (H')

$$H' = - \sum_{s=1}^s (p_i * \ln p_i)$$

Equation 2. Simpson's Diversity Index (D)

$$D = \frac{1}{\sum_{i=1}^s p_i^2}$$

Equation 3. Species Evenness (E)

$$E = \frac{H'}{\ln(S)}$$

2.7. Statistical analysis

All cell cultures were performed in triplicate. Growth curves and statistically significant differences between treatments were computed by t -test using GraphPad Prism version 7.00 (La Jolla California, USA).

3. Results

3.1. Physical and chemical characteristics of the environmental water sample

The composition of the environmental water sample was assessed to evaluate the natural conditions of the water sampled from the studied site of the River Nile in the Cairo governate (Table 2). The pH for the sample obtained was about 7.50. The temperature measured in-situ was 21°C. The electrical conductivity measured was 450 $\mu\text{S cm}^{-1}$. Sodium and chloride were the highest minerals in the water with a concentration of 28.5 and 78.1 mg L^{-1} , respectively, on the other hand, potassium was the lowest mineral present in the water sample (5.1 mg L^{-1}). Nitrate and ammonia levels were 4.97 and 2.03 mg L^{-1} , respectively, while sulfate was 49.95 mg L^{-1} .

Table 2: Physicochemical analysis of the water samples collected from Cairo governate, Egypt	
Physicochemical Parameters	
pH	7.50
Electrical Conductivity ($\mu S/cm$)	450
Total Dissolved Solids (ppm)	287.0
Chemical Parameters	mg L⁻¹
HCO ₃ ⁻	92.1
Cl ⁻	78.1
SO ₄ ⁻²	49.95
Ca ²⁺	22.6
Mg ²⁺	27.0
Na ⁺	28.5
K ⁺	5.1
NO ₃ ⁻	4.97
NH ₄ ⁺	2.03
Iron	0.066
Phosphorus	<1.5 [†]
Manganese	<0.5 [†]
Zinc	<0.2 [†]
Copper	<0.2 [†]
Boron	<0.3 [†]
Note: [†] Unit ($\mu g L^{-1}$)	

3.2. Effect of manipulating media on microalgae growth and population diversity

3.2.1. Effect of the standard media composition

The standard concentration of the commonly used media in microalgae culturing and diversity analysis in research (BG-11 and BBM) were tested to evaluate the capability of each media in sustaining the maximum algal diversity from a freshwater environmental sample. Identification was performed based on morphological features by microscopic visualization of the microalgae; representative images of some microalgae are presented in Figure 1. Both BG-11 and BBM supported similar population cell densities and were not significantly different ($p > 0.05$) (Figure 2A). However, BBM maintained the growth of a slightly diverse population of 27 species compared to 25 species in BG-11, (Table 3, Figure 3A). Apart from the 21 species that were commonly identified in both BG11 and BBM media, each media supported the growth of unique species. BG11 exclusively supported the growth of *Botryococcus sudetica*, *Golenkinia sp.*, *Melosira sp.* and *Pseudanabaena limnetica*. On the other hand, *Mirocystis sp.*, *Actinastrum hantzschii*, *Chlamydomonas sp.*, *Coelastrum microporum*, *Cylindrospermum sp.* *Lagerheimia ciliate* and *Mirocystis sp.* were uniquely identified on BBM media (Table 3) In total 31 species were identified using the two media combined (Figure 3A).

The Shannon-Wiener's index (H'), although similar between BG-11 and BBM, 1.92 and 1.73, respectively. BG-11 had a higher H' index and evenness (E) 0.60 (Table 4). Moreover, the Simpson's diversity index (D), also



Figure 1: Micrographs of some of the identified microalgae species in the River Nile under 400×. (a) *Actinastrum hantzschii*; (b) *Monoraphidium litorale*; (c) *Aphanothece smithii*; (d) *Chlorella vulgaris*; (e) *Coelastrum microporum*; (f) *Coelosphaerium kuetzingianum*; (g) *Crucigenia tetrapedia*; (h) *Botrydiopsis*; (i) *Dictyosphaerium ehrenbergianum*; (j) *Euglena wangi*; (k) *Lagerheimia ciliate*; (l) *Leptolyngbya fragilis*; (m) *Merismopedia tenuissima*; (n) *Micractinium pussillum*; (o) *Mougeotia rotundagulata*; (p) *Nacivula sp.*; (q) *Oocystis marssonii*; (r) *Oscillatoria sp.*; (s) *Pediatrum angulosum*; (t) *Scenedesmus dimorphus*; (u) *Scenedesmus quadricauda*; (v) *Selenastrum capricornutum*; (w) *Staurastrum gracile*; (x) *Tetradron tumidulum*. Scale bar: 10µm.

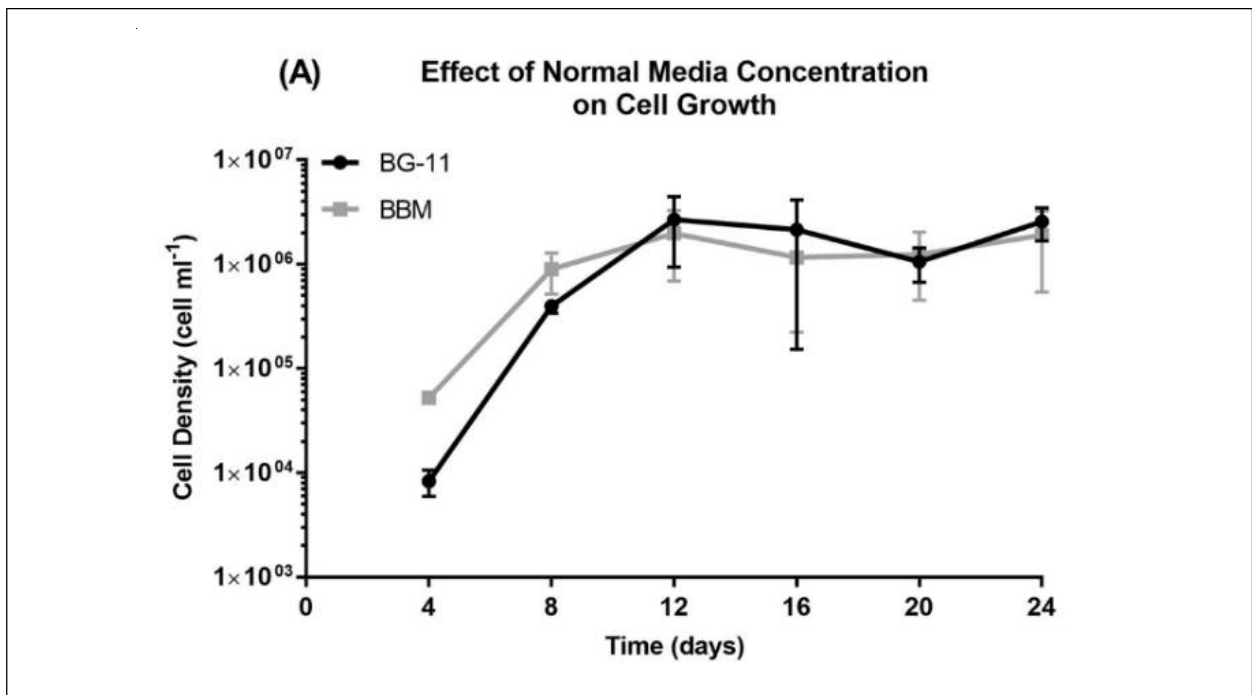


Figure 2: Growth curves studying the effect of media on microalgae growth. Monitoring of microalgae growth in culture. (A) Growth curve for the 1x of the tested media: BG-11 (black) and BBM (grey). (B) Growth curve of reduced media BG-11^{0.1x} and BBM^{0.1x}. (C) Growth curve of the media enriched with vitamins. Data represent means ± SD of three replicas per medium. Samples were cultured in an environmentally controlled growth chamber under a light intensity of 28 µmol m⁻²s⁻¹ with 12:12 h, light:dark cycle at 22 ± 1 °C.

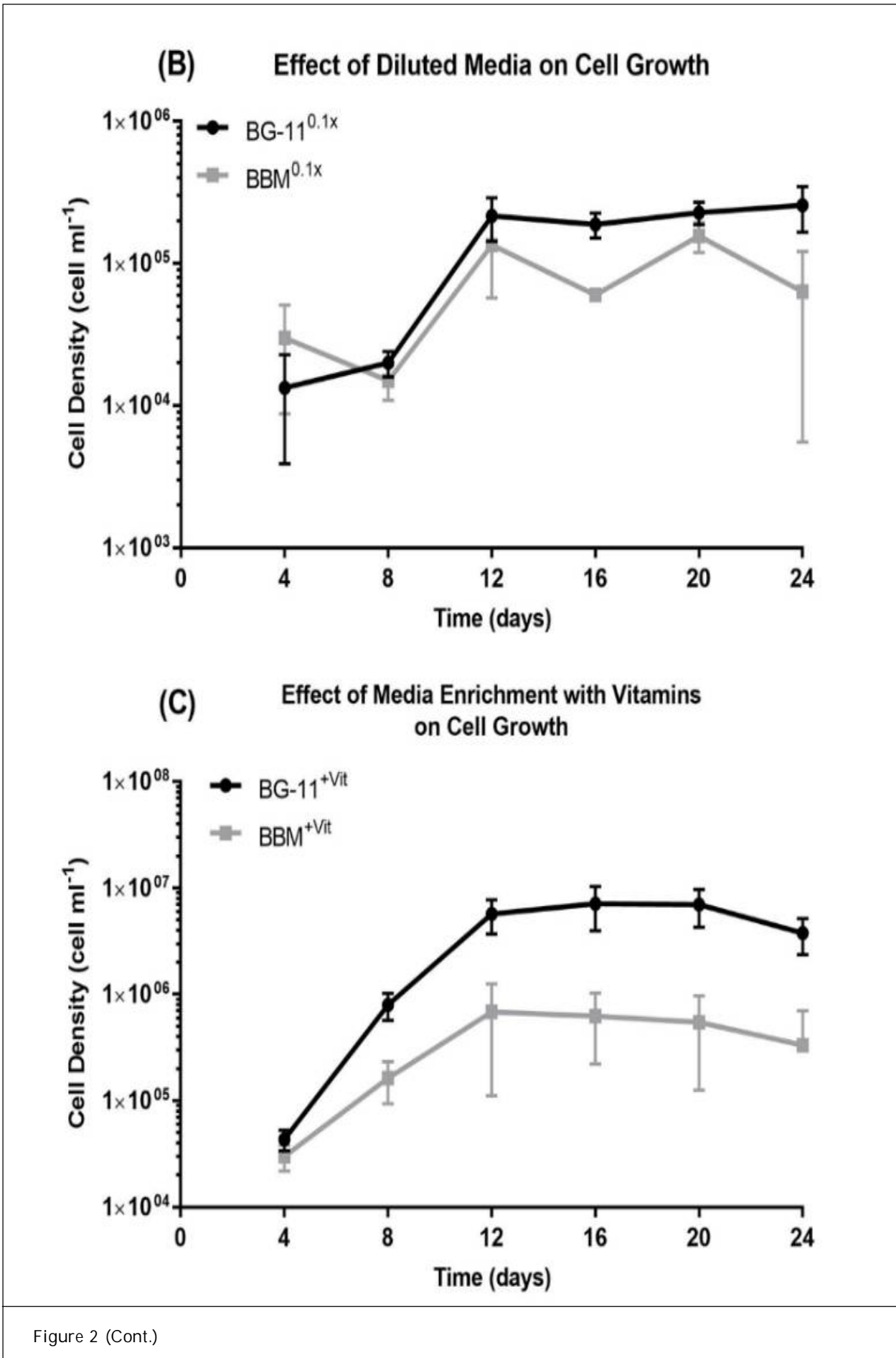


Figure 2 (Cont.)

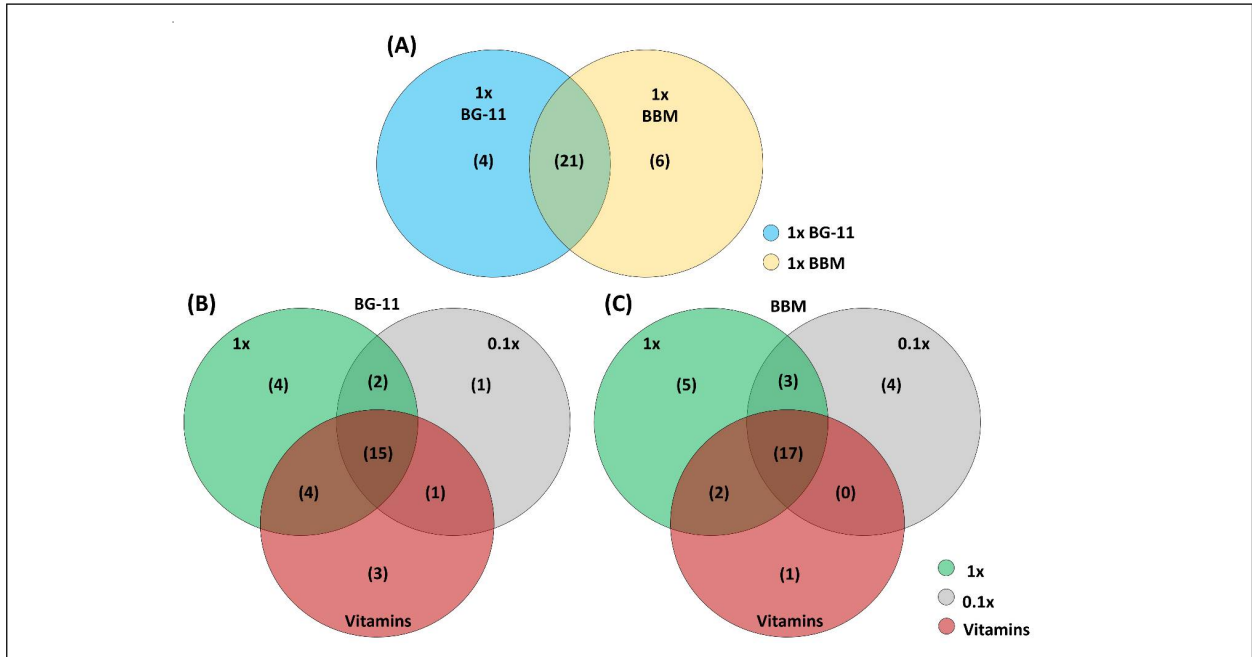


Figure 3. Venn diagram illustrating the shared/unique species between the tested conditions: normal media concentration (1 × media), reduced nutrient media (0.1 × media), and media enriched with vitamins (Vitamins). (A) Venn diagram summarizing the number of species identified using the standard BG-11 and BBM media only, (B) Venn diagram for the shared and unique species identified using BG-11 media and the three tested conditions, (C) Venn diagram for the shared and unique species identified in all tested BBM conditions.

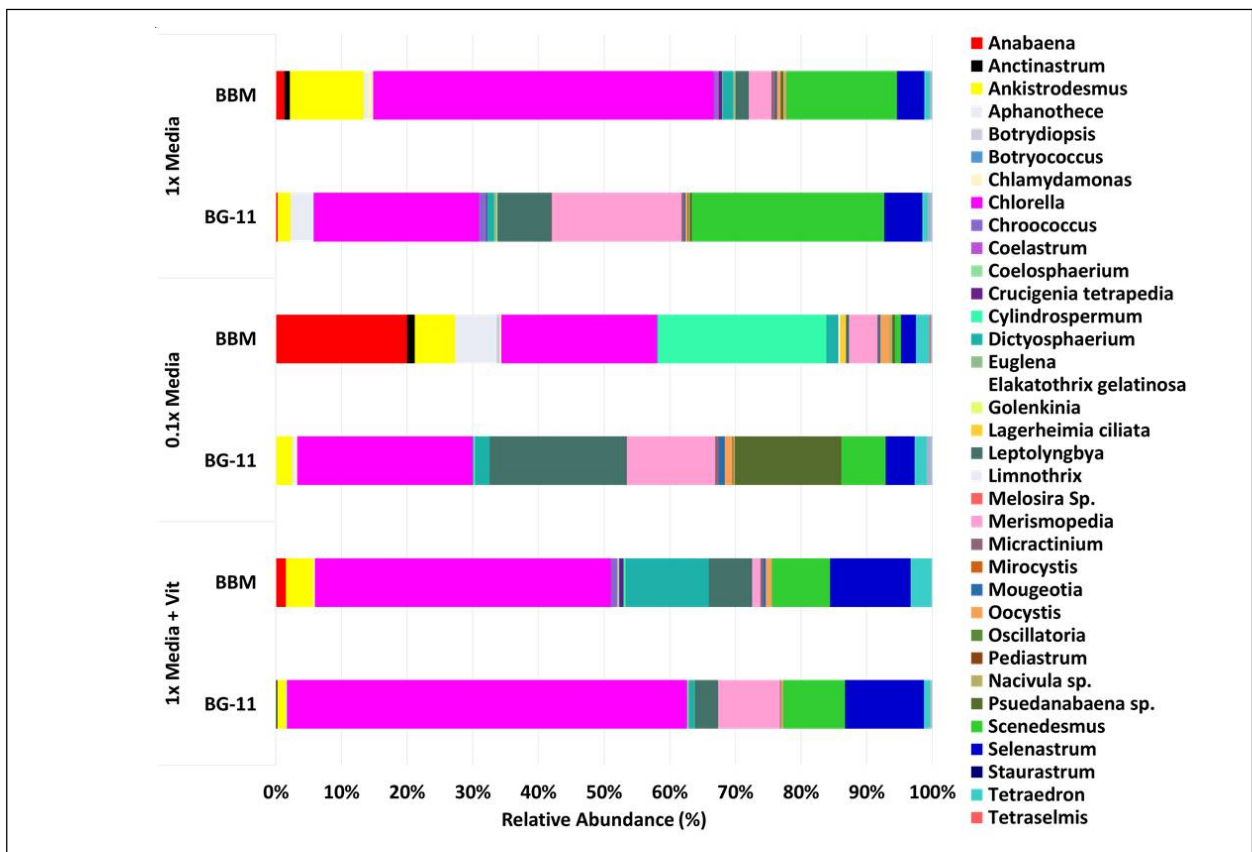


Figure 4. Species relative abundance graph comparing between 1 × Media, 0.1 × Media and vitamin-enriched media of BBM and BG-11

Table 3: List of the morphologically identified algal species across the six deferent media

Sp.	Species	1 × Media		0.1 × Media		Vitamin Enriched Media	
		BG-11	BBM	BG-11	BBM	BG-11	BBM
1	<i>Anabaena sp.</i>	+	+	-	+	+	+
2	<i>Actinastrum hantzschii</i>	-	+	-	+	+	-
3	<i>Monoraphidium litorale</i>	+	+	+	+	+	+
4	<i>Aphanothece smithii</i>	+	+	+	+	+	-
5	<i>Botrydiopsis arhiza</i>	-	-	-	+	-	-
6	<i>Botryococcus sudetica</i>	+	-	-	-	-	-
7	<i>Chlamydomonas sp.</i>	-	+	+	+	-	+
8	<i>Chlorella vulgaris</i>	+	+	+	+	+	+
9	<i>Chroococcus sp.</i>	+	+	+	+	-	+
10	<i>Coelastrum microporum</i>	-	+	-	-	+	+
11	<i>Coelosphaerium kuetzingianum</i>	-	-	-	-	+	+
12	<i>Crucigenia tetrapedia</i>	+	+	-	-	+	+
13	<i>Cylindrospermum sp.</i>	-	+	+	+	+	+
14	<i>Dictyosphaerium ehrenbergianum</i>	+	+	+	+	+	+
15	<i>Elakatothrix sp.</i>	-	-	-	+	-	-
16	<i>Euglena wangi</i>	+	+	-	-	+	-
17	<i>Golenkinia sp.</i>	+	-	-	-	+	-
18	<i>Lagerheimia ciliata</i>	-	+	-	+	-	-
19	<i>Leptolyngbya fragilis</i>	+	+	+	+	+	+
20	<i>Melosira sp.</i>	+	-	-	-	-	-
21	<i>Merismopedia sp.</i>	+	+	+	+	+	+
22	<i>Micractinium pussillum</i>	+	+	+	+	+	+
23	<i>Mirocystis sp.</i>	-	+	-	-	-	-
24	<i>Mougeotia rotundagulata</i>	+	+	+	+	+	+
25	<i>Oocystis marssonii</i>	+	+	+	+	+	+
26	<i>Oscillatoria sp.</i>	+	+	-	-	-	-
27	<i>Pediastrum angulosum</i>	+	+	+	-	-	-
28	<i>Nacivula sp.</i>	+	+	+	+	+	+
29	<i>Pseudanabaena limnetica</i>	+	-	+	+	+	-
30	<i>Scenedesmus sp.</i>	+	+	+	+	+	+
31	<i>Selenastrum capricornutum</i>	+	+	+	+	+	+
32	<i>Staurastrum gracile</i>	+	+	-	-	-	-
33	<i>Tetraedron tumidulum</i>	+	+	+	+	+	+
34	<i>Tetraselmis sp.</i>	-	-	-	+	-	-
35	<i>Tetraspora sp.</i>	+	+	+	+	+	+
Total number of species identified		25	27	19	24	23	20

Alpha Diversity Index	1 × Media		0.1 × Media		Vitamin-Enriched Media	
	BG-11	BBM	BG-11	BBM	BG-11	BBM
<i>S</i>	25	27	19	24	23	20
<i>H'</i>	1.92	1.73	2.07	2.12	1.38	1.87
<i>E</i>	0.60	0.53	0.70	0.67	0.44	0.62
<i>D</i>	0.20	0.31	0.17	0.17	0.40	0.25

Note: (*S*), Species richness index; (*H'*), Shannon-Wiener diversity index; (*E*), Species evenness index; and (*D*) Simpson's diversity index.

commonly known as a dominance index, is lower in BG-11 (0.20) than in BBM (0.31), reflecting the abundance and the dominance of a species in BBM. Figure 4 illustrates the relative abundance of microalgae genera in 1× BG-11 and BBM, with the genus *Chlorella* being the most dominant species in BBM, followed by *Scenedesmus* sp. BG-11 as well had few dominant species, such as *Chlorella vulgaris*, *Merismopedia* sp., and *Scenedesmus* sp.

3.2.2. Influence of reduced nutrient salts

The aim of testing a low nutrient concentration of the media was to provide an intermediate nutrient disturbance and provide enough enrichment through the addition of low concentration of the tested culture media BG-11 (BG-11^{0.1×}) and BBM (BBM^{0.1×}) to support the growth of less competitive species. BG-11^{0.1×} enriched 19 species, while BBM^{0.1×} supported the growth of 24 species (Table 3). The cell density of both the tested media had no statistical differences observed ($p > 0.05$). The aim of using the reduced nutrient media was not to obtain high biomass. However, the population growth was evaluated with the sole purpose of making sure that the cell growth was not hindered by the alteration of nutrient concentration (Figure 2B). Eighteen microalgae species were commonly identified on BG-11^{0.1×} and BBM^{0.1×} media (Table 3). Six species were only identified on BBM^{0.1×}, and one species (*Pediastrum*) was found only on BG-11^{0.1×} and not BBM^{0.1×} (Table 3). When compared to the standard BG-11 and BBM media, two species, *Chlamydomonas* sp. and *Cylindrospermum* sp., appeared on BG-11^{0.1×} and were missing on BG11 media (Figure 3B). Similarly, four species, *Botrydiopsis arhiza*, *Elakatothrix* sp., *Pseudanabaena limnetica*, and *Tetraselmis* sp., appeared on BBM^{0.1×} and were not present in the standard BMM media concentration (Figure 3C). Out of the two reduced media tested, BBM^{0.1×} had a higher Shannon index (*H'*), (2.12), over BG-11^{0.1×} (2.07). Will the evenness (*E*) was higher in BG-11^{0.1×} (0.70) than BBM^{0.1×} (0.67) (Table 4). When comparing the evenness of the two reduced media and the standard media used, the higher evenness observed in the reduced media can be attributed to the more evenly distributed of relative abundance of species in the reduced media over the standard media (Figure 4). Moreover, the reduced media had the highest calculated *H'* and *E* values, and the lowest *D* index, when compared between the standard and vitamin-enriched media.

3.2.3. Effect of vitamin enrichment

The effect of vitamin enrichment of the original concentration of BG-11 and BBM on the cell density and environmental sample diversity identification was studied. The algae population growth observed on the BG-11^{+vit} was higher than that of the BBM^{+vit} (Figure 2C). The diversity indices of the BG-11^{+vit} and BBM^{+vit} indicated that BG-11^{+vit} supported a higher genera richness (23), while, BBM^{+vit} enriched 20 genera. Comparing BG-11^{+vit} with BBM^{+vit}, 18 genera were commonly identified on both media. While five genera were exclusively identified on BG-11^{+vit}, only two genera were uniquely identified on BBM^{+vit} (Table 3). A total of 18 microalgae species were commonly identified on both the standard 1× BG-11 and BG-11^{+vit}. While BBM media lacked eight species that were previously identified on the standard BBM, the *Coelosphaerium kuetzingianum* was only identified on the +vit media. BG-11^{+vit} solely helped identify four microalgae species over the standard BG-11 media, out of which *Cylindrospermum* sp. was commonly identified in the BG-11^{0.1×} media (Figure 3B). On the other hand, six genera were missing from the BG-11^{+vit} and were identified on 1× BG-11 (Figure 3B, Table 3). Even though BG-11^{+vit} had a higher genera richness over the BBM^{+vit} media, the values of Shannon-Wiener (*H'*) and Species evenness (*E*) index were relatively low in the BG-11^{+vit}, indicating the absences of heterogeneity between the number of individual species. This is also supported by the high Simpson's index (*D*) of 0.40

(Table 4) which reflects the presence of a dominant species in the BG-11^{+Vit} as observed in (Figure 4), with *Chlorella* being the dominant genus.

4. Discussion

We have selected the two commonly used algae growth media BG-11 (Andersen *et al.*, 2005) and BBM (Nichols and Bold, 1965) to assess their strength and limitations in supporting the growth of freshwater microalgae from an environmental sample. The water analysis of the water sample indicated that the River Nile water quality and nutrients content are suitable to encourage the life of a great diversity of aquatic organisms. However, the nutrients' content is not high to cause algal bloom or the loss of species diversity due to any toxicity in the water (Behar, 1996; and UNESCO/WHO/UNEP, 1996).

A combined total of 35 culturable microalgal species were microscopically identified from the River Nile location in Cairo using the six different media conditions. Thirteen species were commonly present in all media conditions, while 22 other species displayed differential growth based on the media composition. Using either of the standard BG-11 or BBM media alone would have resulted in missing 10 and eight microalgae species, respectively. However, a total of 31 species would have been identified if both BG-11 and BBM media were used together. An approach that is not usually practiced, since most reported studies utilized either one of these to media to isolate microalgae from an environmental sample as an initial step followed by culturing the isolates colonies on different media to obtain axenic cultures (Bajwa *et al.*, 2017; Rizza *et al.*, 2017; and Yee *et al.*, 2019). Another approach utilizes the screening of microalgae using solidified media (Lee *et al.*, 2014; and Liu *et al.*, 2018), which can be complicated because using a solidified media can hinder growth due to influence by neighboring colonies, as well as the limitation caused by the available surface area of the culturing plate (Yee *et al.*, 2019).

Taking a closer look at the media composition of the two-standard media used, BG-11 and BBM, BG-11 contains a higher nitrogen content, while BBM is richer in phosphorus and potassium content. Additionally, BBM media contain about 10 times more cobalt than BG-11, as well as two times more cupric sulfate. In contrast, BG-11 contains citric acid and citrate as a carbon source for microalgae, and BBM does not contain a carbon source. Research conducted on media impact usually investigates the effect of macro- and micronutrients of BG-11 and BBM on biomass and lipid production and not on diversity enrichment (Gour *et al.*, 2018; and Zhang *et al.*, 2019). Furthermore, several reports promote BG-11 due to its high nitrogen concentration media over BBM (Bajwa *et al.*, 2017; and Sharma *et al.*, 2016) since BG11 contains about six times more nitrate. The high nitrogen concentration in BG11 leads to the increase of biomass, as observed in Figure 2. However, this increase in biomass does not necessarily mean an increase in the enrichment of biodiversity. Previous reports indicate that nitrogen uptake by microalgae is negatively affected as a result of phosphorus limitation (Xin *et al.*, 2010). Therefore, the high nitrogen content in BG-11 may not be efficiently up-taken by the microalgae due to the low P concentration in the media. While BBM media has a relatively lower N content, the P content is higher than BG-11, which could lead to the effective uptake of N in microalgae and hence the support of growing various species. This was observed in the diversity analysis, where the total number of species identified on BBM media is higher compared to the BG-11 at the standard and the reduced nutrients. Micronutrients such as cobalt and copper in BBM are 10 and 20 times higher, respectively and could be another reason for the increase in higher S (species richness) in BBM over BG-11, as cobalt and copper are also considered limiting nutrients (Zhang *et al.*, 2019).

The calculated Shannon index (H'), although similar between a 1× BG-11 and 1× BBM, 1.92 and 1.73, respectively indicate the presence of rich diversity and a better distribution of individuals of each taxon. When compared to a previous study on the River Nile in Cairo district in 1976 and 1982, the H' index reported was between 0.18-1.06 throughout the study period (Shehata and Bader, 1985). However, Shehata and Bader (1985) performed diversity analysis directly from the water sample using a microscope and Sedgwick-Rafter cell without algal population enrichment. The reported H' index in the current study range from of 1.38-2.12 and higher across all tested media compared to the previously reported H' index between 1976 and 1982 which might be due to an increase in eutrophication of the River Nile from farmlands due to agriculture practices, hence increasing the nutrient content in the River Nile (Kim and Chae, 2016; and Mitchell and Buzzell, 1971). The physicochemical analysis of the water samples used in the current study confirmed at least 10 times nitrate and ammonia concentration compared to that reported between the years 1978 and 1979 (Shehata and Bader, 1985).

For both BG-11 and BBM, *Chlorella* and *Scenedesmus* were the abundance species; however, these species were distributed more evenly in BG-11; hence the evenness index calculated for BG-11 was higher than for

BBM media. *Scenedesmus* and *Chlorella* have been previously reported to be used in a *Chlorella/Scenedesmus* microalgae consortia to treat wastewater due to their similar growth rate pattern of under various nutrient concentrations (Koreivienė *et al.*, 2014; and Wan *et al.*, 2016). However, changing the culturing condition of both BG11 and BBM significantly influenced *Scenedesmus* and *Chlorella* dominance, discussed below:

Furthermore, the culturing of the environmental samples on a reduced nutrient media and vitamin-enriched media lead to the identification of three species exclusively in BBM^{0.1×} media, and one species was present only in response to vitamins addition to either of BG11 or BBM medium. These four species, *Botrydiopsis arhiza*, *Coelosphaerium kuetzingianum* *Elakatothrix sp.*, and *Tetraselmis sp.*, would have been completely overlooked if only the standard BG11 and BBM were used. To provide support to the less competitive species through an intermediate nutrient disturbance, yet, provide enough nutrients, the addition of low concentration of the BG-11 (BG-11^{0.1×}), and BBM (BBM^{0.1×}) was studied. Species such as *Psuedanabaena limnetica*, consistently appeared in the BG-11 media of all tested conditions; however, *Psuedanabaena limnetica* and *Tetraselmis sp.* have been previously reported to grow and withstand low nutrient conditions, and hence they were able to grow under the decreased dominance of the Chlorophyta in BG-11^{0.1×} and BBM^{0.1×} (Fabregas *et al.*, 1984; and Gao *et al.*, 2018). The appearance of certain species in the reduced nutrient media could be due to the presence of an even distribution of species, and this was supported by the diversity indices. The calculated Shannon-Weiner Index (*H'*) and Evenness (*E*) were higher in the 0.1× media than in the 1× media tested. The even distribution of genera in the 0.1× media can also be clearly observed in Figure 4. The decrease of the dominance of specific genera, such as, *Chlorella vulgaris*, *Merismopedia sp.* and *Scenedesmus sp.* that were observed in the 1× concentration of BG-11 and BBM, contributed to the decrease of the dominance index (*D*), in BG-11^{0.1×} (0.17) and BBM^{0.1×} (0.17), when in comparison to the dominance index of the standard BG-11 (0.20) and BBM (0.31). The reduced media may have enabled other less competitive species to coexist and grow as the competition with dominant genera is reduced due to less preferred conditions (Dickinson and Murphy, 1998; Fraser *et al.*, 2014; and Graham and Duda, 2011). The species identified in the reduced nutrient concentration indicates that they adapted to the current experimental nutrient profile. Therefore, they can be used as potential strains that can be utilized in limited nutrients applications and would reduce the need for adding nutrients, reducing the cost of culturing microalgae for applications (Hiibel *et al.*, 2015).

On the other hand, The enhancement of BG-11 and BBM media with vitamins to provide the microalgae with the necessary micronutrients that are usually produced by their bacterial partner (Ji *et al.*, 2018) did not drastically improve the culture-based method in diversity identification. Even though all species identified using the vitamins-enriched media were previously identified either on the standard or reduced media, both BG-11^{+vit} and BBM^{+vit} exclusively identified *Coelosphaerium kuetzingianum*.

5. Conclusion

In conclusion, the results demonstrated that the culture-based method for the identification of microalgae has great potential, and the utilization of several media compositions can help reveal a greater diversity that is already present in the environmental samples studied. Moreover, our work also reveals that there is still room for further media recipe modification to aid the culture-based diversity identification method. This research also provides a reference for research conducted on the microalgae diversity present in the River Nile in Cairo. This is important for continuous monitoring and protecting of the biodiversity present in the section of the River Nile surrounded by the highest populated city in Egypt. This is especially needed at this time for constant monitoring of the influence of the Ethiopian grand renaissance dam on the nutrients flow in downstream countries. Monitoring algae diversity in the river provides insight on the water quality, the impact of human activity on natural resources, and enable conservation biology.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors have no conflicts of interests, financial or otherwise, to declare. No informed consent, human or animal rights are applicable.

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