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Some Biochemical Features of Two Filamentous Algae Isolated from Lake Sapanca, Turkey

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Özet : *Sapanca Gölünden İzole Edilen İki Ipliksi Alg Türünün Bazi Biyokimyasal Özellikleri.* Bu çalışma da Sapanca gölü fitoplanktonunda yaygın ve zaman zaman dominant olarak gözlenen iki ipliksi tatlısu alg türü, *Planktothrix rubescens* D.C. ve *Mougeotia* sp., BG11 ortamında 20 µE $m^{-2}s^{-1}$ 'lık sürekli aydınlatma altında kültüre alınmış ve karbonhidrat, protein, klorofil-*a* gibi bazı biyokimyasal içerikleri ve biyomasları incelenmiştir.

İki tür arasında önemli farklar bulunmuştur. *P. rubescens*'in klorofil-*a*, hücre içi ve hücre dışı (intraselüler ve ekstraselüler) karbonhidrat ve protein içerikleri kültürün yaşlanması ile paralel olarak artarken *Mougeotia* sp.'nin sadece klorofil-*a* içeriği kültür yaşlandıkça artış göstermiştir. Öte yandan, *P. rubescens*'in biyokimyasal içeriği *Mougeotia* sp. ye göre önemli miktarda yüksek bulunmuştur: *P. rubescens*'te ortalama protein içeriği 86.81±25.72 µg ml^{-1}, klorofil-*a* içeriği 9.46±5.3 µg ml^{-1}, alg yoğunluğu (filament olarak) 26.98±14.88 x10^4 filament ml^{-1} ve karbonhidrat 170.1±46.6 µg D-Glikoz ml^{-1} (61.3±18.4 µg D-Glikoz ml^{-1} intraselüler karbonhidrat ve 109.5±34.1 µg D-Glikoz ml^{-1} ekstraselüler karbonhidrat) bulunurken *Mougeotia* sp. de protein 26.9±9.3 µg ml^{-1}, Klorofil-*a* 4.76± 1.97 µg ml^{-1}, alg yoğunluğu 8.19±4.5x10^3 filament ml^{-1} ve karbonhidrat 146±20.6 µg D-Glukoz ml^{-1} (83.4±18.8 µg D-Glikoz ml^{-1} intraselüler karbonhidrat ve 62.6±20.4 µg D-Glikoz ml^{-1} ektraselüler karbonhidrat) olarak daha düşük ortalama değerler bulunmuştur.

Anahtar Kelimeler : *Planktothrix rubescens*, *Mougeotia* sp. karbonhidrat, protein, klorofil-*a*, algal biyomas.

Abstract : Some biochemical contents (carbohydrate, protein, chlorophyll-*a*) and biomass of two filamentous freshwater algae, *Planktothrix rubescens* D.C. and *Mougeotia* sp., which are the most conspicuous algae in the phytoplankton of Lake Sapanca, were studied in continuous cultures under irradiance of 20 µE $m^{-2}s^{-1}$. Results were obtained using BG11 medium.

Considerable differences were found between two species; chlorophyll-*a* (chl-*a*), intracellular and extracellular carbohydrate (ICH and ECH), and protein contents of *P. rubescens* increased with the development of the culture, while only chl-*a* increased with the culture age of the *Mougeotia* sp. The filamentous cyanobacterium, *P. rubescens*, had a significantly higher concentration of

biochemical content than *Mougeotia* sp. Mean concentration of protein, chl-*a*, algal cells (filaments) and carbohydrate were: proteins 86.81±25.72 μg ml^{-1}, chl-*a* 9.46±5.3 μg ml^{-1}, algal density (filaments) 26.98±14.88 $X10^4$ filaments ml^{-1} and carbohydrate 170.1 ±46.6 μg D-Glukoz ml^{-1} (61.3±18.4 μg D-Glukoz ml^{-1} for ICH and 109.5±34.1 μg D-Glukoz ml^{-1}) respectively. Lower values were found for *Mougeotia* sp. culture: proteins 26.9±9.3 μg ml^{-1}; chl-*a*: 4.76±1.97 μg ml^{-1} algal density; 8.19±4.5x10^3 filaments ml^{-1} and carbohydrate: 146±20.6 μg D-Glukoz ml^{-1} (83.4±18.8 μg D-Glukoz ml^{-1} for ICH and 62.6±20.4 μg D- Glukoz ml^{-1} for ECH) and were measured.

Key Words : *Planktothrix rubescens*, *Mougeotia* sp. carbohydrate, protein, chlorophyll-*a*, algal biomass.

Introduction

There are numerous studies about the biochemical composition, and the specific nutrients of the micro algae and seaweed. (Fabregas et al. 1987; James et al. 1989; Çetingül et al. 1990; Brown et al.1993; Lourenco et al. 1997; Wong and Cheung 2000).
However, little is known regarding the filamentous green and blue-green algae.

Although the genus *Mougeotia* is one of the well known bloom forming algae in acid waters of Europe and North America (Graham et al., 1996), species of *Mougetia* are able to grow in a wide range of environments, from eutrophic to oligotrophic and also across a range pH levels, 5.5 to 9.9 either (Hillebrand, 1983; Simons, 1987). This genus is also considered metaphytic algae taking into account that the filaments are only weakly attached to the substratum by means of cellular interdigitation (Casco and Toya, 1994).

The other species, *Planktothrix rubescens* D.C. (*Oscillatoria rubescens*), is established in eutrophic lakes and characterized by a red colour due to phycoerythrin and the ability to form metalimnetic layers (Feuillade, 1994). In addition, various field studies have confirmed the hepatotoxin production of this species in lakes (Berg et al.1986; Loizzo et al.1988; Sivonen et al.1990).

The biochemical profiles of the algae, which are important for human consumption in aquaculture, for the production of chemical and for the bioconversion of solar energy (Kharatyan, 1978; Fabregas et al. 1987; Brown et al. 1993). Furthermore, it is widely accepted that the algae are good source of proteins, vitamins, carbohydrates, minerals and essential aminoacids (Fujiwara – Arasaki et al. 1984; Fleury and Lahaye, 1991; Kolb et al. 1999). Therefore, in general, the nutritional value of microalgae is related to the nutritional requirements of animals and humans.

Since 1997, a programme to reveal eutrophication problems of Lake Sapanca have been started. During the first stage of the study, limnological problems of the Lake Sapanca were investigated. It was found that *P. rubescens* and *Mougeotia* sp. were becoming dominant species in Lake Sapanca. The first severe bloom of *P. rubescens* was recorded in May 1997 and after the bloom several hundreds of fish died. Therefore, this occurrence was related to *Planktothrix* bloom. Aim of the present study was to compare the content of protein, carbohydrate, chl-*a* and the filament densities of *P. rubescens* and *Mougeotia* sp. which were grown on the same culture media.

Material and Methods

Microalgal Cultures:

Algal isolates from Lake Sapanca were screened using nutrient agar plates enriched with BG11 media (Stainer et al. 1971). After the initial inoculation, the plates were incubated at 20-25 °C under constant illumination by cool white fluorescent light at an irradiance of about 20 $\mu E\ m^{-2}s^{-1}$ for three weeks. Although several algal isolates were screened, two algal isolates were identified as *Planktothrix rubescens* D.C. (TS 9801) and *Mougeotia* sp. (TS 9802) and these species were transferred to 200 ml flasks containing BG11 medium. The light intensity was maintained continuously for 24 hour a day at 20 $\mu E\ m^{-2}s^{-1}$ irradiance at 20-25°C.

Biochemical Analysis:

For the first measurement, cultures were harvested to determine protein, carbohydrate, chlorophyll-*a* and algal cells (filaments) at the end of 15 days cultivation. Measurements were performed every five days between the 15^{th} and 25^{th} days and every three days between the 25^{th} and 37^{th} day. Protein was measured according to the Lowry method (Lowry et al., 1951) using Folin-Ciocalteau reagent (2 replicates), carbohydrate (CH) was measured using the phenol-sulphuric acid method (Dubois et al., 1956) using D+ glucose standards.

Filaments Density and Chlorophyll-*a*:

The algal filament counts were performed using a Sedgewick Rafter cell and chl-*a* was measured according to the Nusch (1980) method.

Statistical analysis

All analyses were performed in replicates and data were presented as mean values ± S.D. The mean values were analysed by the student's t-test ($p<0.05$) to determine the significance of differences between the mean values obtained from two filamentous algae.

Result and Discussion

Filaments density and algal growth

The results of this investigation revealed that the filament density of the *P. rubescens* was significantly higher ($p<0.001$) than that of the *Mougeotia* sp. under continuous light, 20 $\mu E\ m^{-2}\ s^{-1}$, during the study period. The filament density for *P. rubescens* $3.4x10^4$ filament ml^{-1} and $2.6x10^3$ filament ml^{-1} for and *Mougeotia* sp. respectively at the first counting which were obtained from 15 day-old cultures. Although between days 15 and 31, there was a 12.1 fold increase of filament density of *P. rubescens*, *Mougeotia* sp. increased only 3.65 fold. At the end of the day 37 *P. rubescens* reached to $42.62x10^4$ filament ml^{-1} while *Mougeotia* sp. was only $9.49x10^3$ filament ml^{-1} (Figure 1). Mean filament density was $26.98\pm15.4x10^4$.

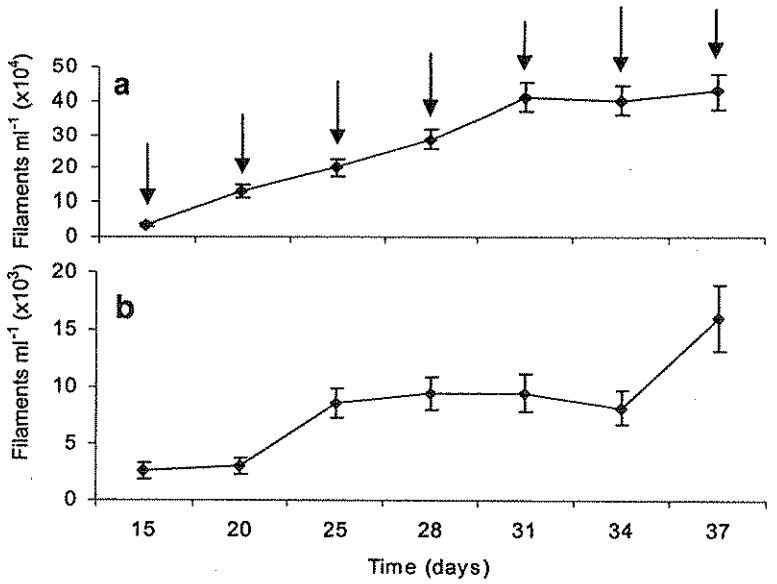

Figure 1. Growth curves of *Planktothrix rubescens* (a) and *Mougeotia* sp. (b), filaments ml^{-1}, with the age of culture. Arrows indicate the sampling time for biochemical analysis.

Table 1. Biochemical composition and filament density of *Planktothrix rubescens* and *Mougeotia* sp. Apart from the filament density, all values are on a volume basis (μg ml^{-1}). Filament density is on a numbers basis (filaments ml^{-1}). Abbreviations: CH, carbohydrate; ICH, intracellular carbohydrate; ECH, extracellular carbohydrate; chl-*a*, chlorophyll-*a*; PR/CH, protein/carbohydrate ratio.

Species	Parameters	n	mean	Std. Deviation	P
P. rubescens	CH	14	170.1	46.6	0.04
Mougeotia. sp.		14	146	20.6	
P. rubescens	ICH	14	61.	18.4	0.001
Mougeotia. sp.		14	83.4	18.8	
P. rubescens	ECH	14	109.5	34.1	0.001
Mougeotia. sp.		14	62.6	20.4	
P. rubescens	Protein	14	86.3	25.7	0.001
Mougeotia. sp.		14	26.9	9.3	
P. rubescens	Filament density	14	$26.98x10^4$	14.88	0.001
Mougeotia. sp.		14	$8.19x10^3$	4.49	
P. rubescens	Chl-*a*	14	9.46	5.3	0.001
Mougeotia. sp.		14	4.76	1.97	
P. rubescens	PR/CH	14	0.54	0.16	0.001
Mougeotia. sp.					

The chl-*a* content increased continuously in *P. rubescens* and *Mougeotia* sp. with time (Figure 2). This increase was considerably higher in *P. rubescens* than in the *Mougeotia* sp. culture. Similar chl-*a* contents were found at the first measurement (2.02 μg ml^{-1} for *P. rubescens* and 2.19 μg ml^{-1} for *Mougeotia* sp.) in 15 day old cultures, However, in the following days significant increase was observed in the *P. rubescens* culture ($p<0.001$); whereas in the 31 day old cultures of chl-*a* content increased 7.76 fold in *P. rubescens,* only 2.27 fold increase was recorded for *Mougeotia* sp. Mean chl-*a* content was measured 9.46±5.3 μg ml^{-1} in *P. rubescens* and 4.76±1.97μg ml^{-1} in *Mougeotia* sp.

A very strong positive correlation ($r= 0.92$; $p<0.01$) and a strong positive correlation ($r= 0.88$; $p<0.01$) were found between the filament densities and chl-*a* content of both species.

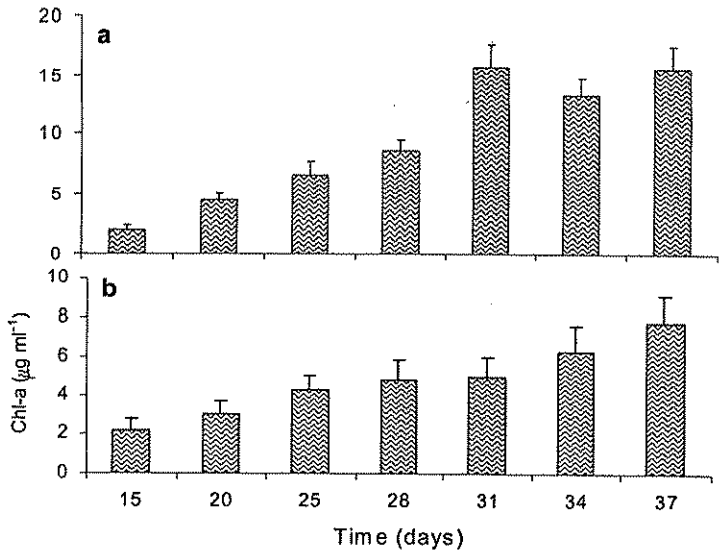

Figure 2. Changes in chlorophyll-*a* concentration over time in cultures of *Planktothrix rubescens* (a) and *Mougeotia* sp. (b).

Protein

Protein content showed a continuous increase in *P. rubescens*; This increase was very fast between days 15 to 28, while it only gradually increased between days 28-37 (Figure 3). *Mougeotia* sp. showed a similar continuous increase in content of protein during the first two weeks and it reached to 35.67 $\mu g\ ml^{-1}$ (Figure 3). However, it decreased to 25.94 $\mu g\ ml^{-1}$ in the 31 day old culture. In the following days, the amount of protein increased with the development of the culture. A strong positive correlation between *P. rubescens* filaments density and protein content ($r= 0.87$; $p<0.01$) and a strong positive correlation between *Mougeotia* filament density and protein contents ($r= 0.7$; 0.01) was found (Table 2).

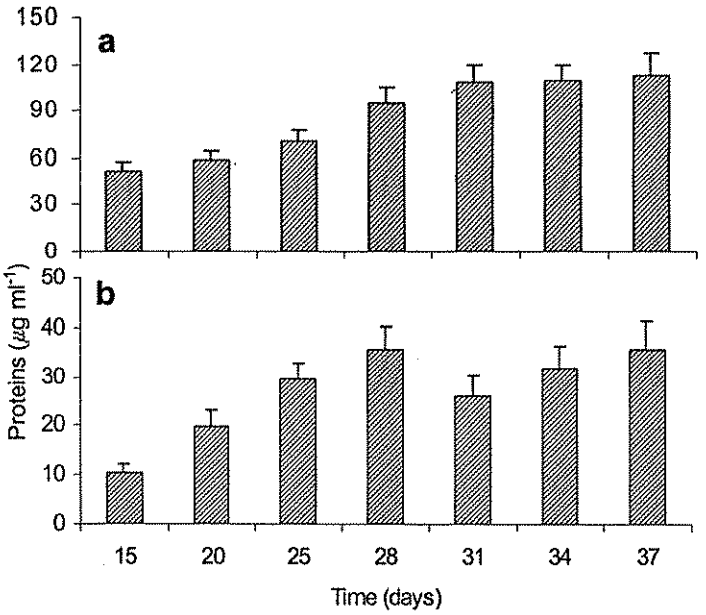

Figure 3. Changes in proteins over time in cultures of *Planktothrix rubescens* (a) and *Mougeotia* sp. (b).

Zavodnic and Juranic (1982) stated that the content of the protein in algae is related to algal development. However, Fabregas et al. (1985) concluded that changes in the protein content are not related to cellular density, because the biochemical composition of microalgal cells may change within more or less narrow limits depending on culture conditions. Comparing the mean protein contents of two filamentous species; significantly high concentrations of protein (mean 86.82±25.7 μgml^{-1}; $p<0.001$) were found in *P. rubescens*' culture (Table 1). Like filament density, content of the chl-*a* showed a very strong positive correlation with proteins ($r= 0.93$; $p<0.01$) in *P. rubescens'* culture. However, a modest positive correlation ($r= 0.63$; $p<0.01$) was found between the content of the chl-*a* and proteins in *Mougeotia* sp. culture (Table 2).

Carbohydrate

The proportion of carbohydrate (CH) in *P. rubescens* changed substantially over the experimental period. Figure (4) reveals that a rapid increase in CH content was recorded in *P. rubescens* at days 15 and 20. After a growth period of 20 days, CH content did not change considerably and it varied at 183 to 194 µg D-Glukoz ml^{-1} until the end of the study. Mean concentration of CH in *P. rubescens* was found to be 170.1±46.6 µg D-Glukoz ml^{-1}.

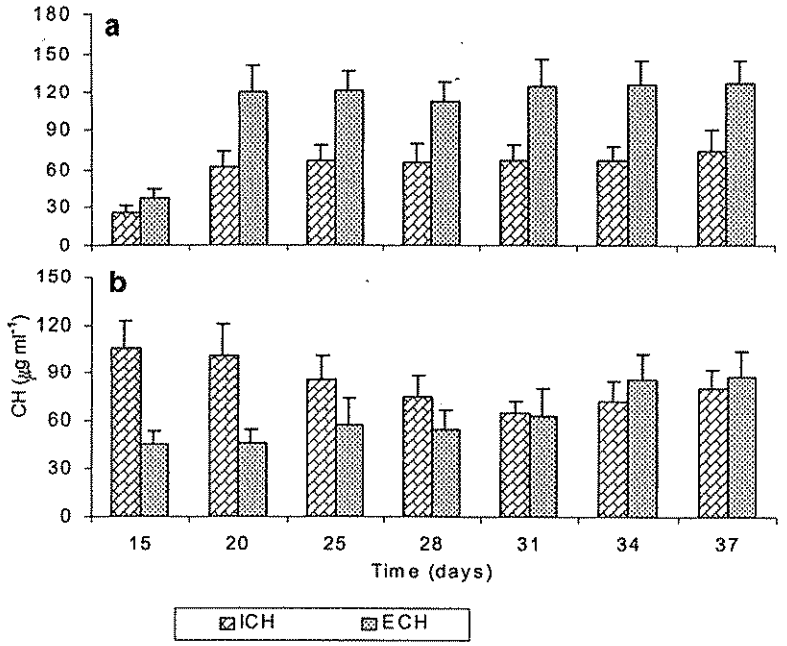

Fig. 4. Changes in intracellular (ICH) and extracellular carbohydrates (ECH) over time in *Planktothrix rubescens* (a) and *Mougeotia* sp. (b) cultures.

Content of the CH in *Mougeotia* sp. did not fluctuate that of the *P. rubescens* during the study period (Figure 4). It was indicated that the mean content of the CH level was 14.2 % lower than in *P. rubescens*. After testing of the mean values of CH-proteins, CH-filament density, and CH-chl-*a*; modest positive correlations ($r= 0.61$, $r= 0.69$ and $r= 0.63$; $p<0.01$ respectively) were found in *P. rubescens'* culture, whereas these parameters were not correlated in *Mougeotia* sp. culture (Table 2).

Table 2. Correlation coefficients (r) among the measured parameters of the *Planktothrix rubescens* and *Mougeotia* sp. (* for $p<0.05$, ** for $p<0.01$, -- not significant, n=14).

Parameters	r *Planktothrix rubescens*	*Mougeotia* sp.
Protein versus filament density	0.87^{**}	0.7^{**}
Protein versus chlorophyll-*a*	0.93^{**}	0.63^{**}
Protein versus ICH	0.61^{**}	--
Protein versus ECH	0.57^{**}	0.49^{*}
Protein versus carbohydrate	0.61^{**}	--
Carbohydrate versus filament density	0.69^{**}	--
Chlorophyll-*a* versus filament density	0.92^{**}	0.88^{**}
Chlorophyll-*a* versus ICH	0.56^{**}	--
Chlorophyll-*a* versus ECH	0.6^{**}	0.71^{**}
Chlorophyll-*a* versus carbohydrate	0.63^{**}	--
Filament density versus ICH	0.6^{**}	--
Filament density versus ECH	0.62^{**}	0.57^{**}
ICH versus ECH	0.84^{**}	--

The concentrations of the intracellular and extracellular carbohydrate (ICH and ECH) were similar, 25 and 37 μg D-Glukoz ml^{-1} respectively, at 15 days old cultures (Figure 4). In the following days, extracellular carbohydrate (ECH) showed a rapid increase in comparison with intracellular carbohydrate (ICH); whereas ECH reached 120 μg D-Glukoz ml^{-1}, ICH was decreased to 63 μg D-Glukoz ml^{-1} in 20 day old cultures. Content of the ECH and ICH did not vary significantly between the 20 and 37 day old cultures of *P. rubescens*. It was indicated that the mean value of ECH content of the *P. rubescens* was considerably higher than ICH content ($p<0.001$; Table 1). However, both ICH and ECH were positively correlated with proteins filament density and chl-*a* (Table 2).

Content of the CH in *Mougeotia* sp. did not differ from that of the *P. rubescens* and it fluctuated between 127 to 167 μg D-Glukoz ml^{-1} during the study period. When *Mougeotia* was harvested at the same growth stage, 15 day old cultures, the differences in the composition of CH was 2.44 fold higher than in *P. rubescens* ($p<0.001$). In the following days, contrary to *P. rubescens*, CH of *Mougeotia* sp. decreased gradually with time and it was measured 143 and 127 μg D-Glukoz ml^{-1} in 25 and 31 day old cultures of *Mougeotia* sp. (Figure 4). But a small increase was found in 34 and 37 days old cultures and CH measured as 158 and 167 μg D-Glukoz ml^{-1} respectively. Mean concentration of CH in *Mougeotia* sp. was found as 146±20.6 μg D-Glukoz ml^{-1}.

Content of the ICH and ECH in *Mougeotia* sp. was negatively correlated to each other ($r= -0.51$); $p<0.05$) between days 15 and 31; whereas ICH decreased from106 μg D-Glukoz ml^{-1} to 64 μg D-Glukoz ml^{-1}, ECH increased from 45 to 63 μg D-Glukoz ml^{-1}. ICH and ECH contents showed similar patterns at days 34 and 37 and their increase coincided with each other. Contrary to *P. rubescens*, the content of the ECH in *Mougeotia* culture was not correlated with proteins, filament density and chll-*a*.

However, ECH was found to be positively correlated with proteins ($r= 0.5$; $p<0.05$) with filament density ($r= 0.57$; $p<0.01$) and with chl-*a* ($r= 0.71$; $p<0.01$). Mean concentration of ICH in *Mougeotia* culture was significantly higher than ECH (Table 1).

Continuing studies on the feeding habit of some fishes in the lake showed that particularly *Mougeotia* sp. is preferred as a food (Pers. Com. Hacer Okgerman). In this study, some biochemical compositions of the *P. rubescens* and *Mougeotia* sp., which were the dominant species of Lake Sapanca, were revealed. However, it is crucial that more studies have to be done in order to do sustainable fisheries in Lake Sapanca.

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