TEMPORAL VARIATION IN THE PIGMENT COMPOSITION OF CAULERPA PROLIFERA (FORSSKÅL) LAMOUREUX MEADOWS IN THE MAR MENOR LAGOON (SE SPAIN)

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Abstract

Eighteen photosynthetic pigments (chlorophylls, carotenoids and degradation products) were separated and quantified from Caulerpa prolifera in a single-step procedure by reversed-phase high-performance liquid chromatography over the period from November 1995 to October 1996. The chlorophyll a and chlorophyll b, are typical and characteristic pigments of the Caulerpa prolifera, appeared in a quantity very similar. In several months of the year the chlorophyll b appears in greater quantity than the chlorophyll a, especially during the months of June and July, coinciding with the greater biomass of the alga, and also with a great concentration of the carotenoid siphonixin.

As regards the carotenoids, it was found that violaxanthin, siphonoxanthin and neoxanthin-like were the most common carotenoids.

Key words: Caulerpa prolifera, seaweed, chlorophylls, carotenoids.

Introduction

Caulerpa prolifera (Forsskål) Lamouroux is widely distributed coenocytic species in the Western Mediterranean Sea. It is an invasive seaweed that has successfully colonized a great part of the Mar Menor lagoon (SE Spain) which cover more than 85 - 90 % of the bottom (Terrados, 1991 and Hegazi, 1999). The spreading of Caulerpa is still in progress and it is colonizing also rocky substrates. Caulerpa meadows and other marine plants are the main primary producers in the lagoon. They form the standing crop and determine the productivity of all communities in the lagoon. Caulerpa like all other species of algae are characterized by specific sets of pigment, chlorophyll a being the most abundant, while the other photosynthetic pigments are considered as accessories (Dawes, 1981 and Hegazi, et al. 1998). Variations in Caulerpa colour are related to varying amounts of pigment (chlorophylls, carotenoids and their breakdown products), with changes in colour during the growth and reproduction being caused by the accumulation of secondary carotenoids (Burczyk, 1987). Although many methods involving column and thin-layer chromatography have been described for the separation and identification of seaweed pigments (Jeffrey, 1969, Kleining, 1969, Czeczuga, 1975, 1976, 1986, Burczyk, 1987, Palermo et al. 1991). It is difficult to separate all the
photosynthetic pigments in a single-step procedure. However, while great efforts have
been made to separate the photosynthetic pigments of phytoplankton (Mantoura and
1997). Little time has been dedicated to those of seaweeds until the work done by Hegazi
et al. (1998) has led to the wide spread use of reverse phase high performance liquid
chromatography (HPLC) techniques for the determination of seaweed pigments.

The aim of this work is to determine the concentrations of individual
photosynthetic pigments in the Caulerpa prolifera meadows using a sensitive method of
HPLC analysis in one year, for acquisition of data on the primary productivity of the
lagoon and find a relationship between colour change and the concentration of
photosynthetic pigments.

**Material and Methods**

*Caulerpa prolifera* was collected from the hyper saline coastal lagoon (Mar
Menor) SE Spain over a period of one year from November 1995 to October 1996. The
samples were done by skin and SCUBA diving. The algal thallus was gently washed by
sea water and immediately transferred carefully to the laboratory in an ice box and stored
at - 80 °C until the moment of chromatographic analysis. The algal thallus was dried
gently with absorbent Whatman filter papers for a few seconds and weighed. The thallus
was ground manually in a porcelain mortar in a cold and darkened fume cup board to
prevent photo-oxidative breakdown of the labile pigments (Jeffrey, 1961). In the present
work 100 % acetone and small amount of magnesium carbonate were added in order to
prevent the accidental formation of chlorophyll metabolites. This procedure was repeated
until the algal thallus became colorless, at which point 5 ml of ethyl ether were added and
the extract was filtered. The extract was concentrated by low pressure rotary evaporator at
25 °C. The dry extract was dissolved in 1 ml of acetone, microfiltered at 0.45 μm and 20
μl were injected into the chromatograph.

The analytical HPLC separation of seaweed pigments was carried out on a
Hewlett Packard Series 1100 chromatograph and GI315 diode-array detector. Absorbance
was registered at 430 nm (carotene detection) and 660 nm (chlorophyll detection). The
complete spectrum of the photosynthetic pigments in the 400 to 700 nm range was saved
in the computer memory for later interpretation.

The injection loop size was 20 μl. The method used consists of an elution
gradient of methanol, acetone and ammonium acetate solution (1 M) Hegazi et al. (1998).
Semi-preparative HPLC separation of the authentic standard pigments was carried out
using a Shimadzu chromatographic system (LC-6A series) equipped with SPD-M6A
photodiode array detector. The column was a Spherisorb ODS-2, using 5 μm spherical
particles (250 mm x 10 mm I.D.). The gradient program was similar to that used in the
analytical column with a flow of 4 ml/min. The pigments were collected at the outlet of
the detector, and the solvent was evaporated immediately under N₂ flow. The dry pigment
was re-dissolved in acetone, benzene, diethyl ether, ethanol, or hexane.

All the separated photosynthetic pigments were identified according to their
spectral characteristics and compared with the published data in different types of solvent
(Dawson, et al. 1969, Foppen, 1971, Mantoura and Llewellyn, 1983, Wright and Shearer,
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Van Heukelom, et al. 1992, 1994, Jeffrey et al. 1997). The HPLC peaks were identified by comparing the retention times and spectral data with those of the authentic standards. The quantifications were done according to the methods used by Hegazi (1999).

**Results and Discussion**

In *Caulerpa prolifera* (chlorophyta), eighteen separate photosynthetic pigments were detected: chlorophyllide *a*, siphonixin, neoxanthin, neoxanthin-like, violaxanthin, microxanthin, micronone, micronone-like, lutein-5,6-epoxide, siphonoxanthin, lutein, chlorophyll *b*, chlorophyll *'b*, chlorophyll *a*, chlorophyll *d*, *α*-carotene, *β*-carotene, and phycophythin *a*. Siphonoxanthin was seen to be as the most typical and distinct carotene in caulerpales. Chlorophylls *a* and *b* were the most common pigments in the green alga studied and in fact responsible for the green colour of this group of algae. The individual photosynthetic pigments of the *Caulerpa prolifera* studied had a broad spectrum polarity, ranging from the low polarity of carotenoids to the very high polarity of chlorophyllides, which are dissociated at neutral pH according to the method of Hegazi et al., 1998.

*Fig. 1: Three dimensions chromatogram of the individual photosynthetic pigments of Caulerpa prolifera.*

The procedure used permitted the analysis of chlorophylls, carotenoids and *xanthophylls* in a single-step and at a fixed temperature. Fig. 1 illustrates a well-resolved...
three dimensions chromatogram corresponding to the separation of photosynthetic pigments from the green alga *Caulerpa prolifera*. In this chromatogram the separated factor (α) and peak resolution (Rₚ) were higher than 1. This indicates the absence of overlapping between peaks, while the Rₚ values demonstrate that resolution between adjacent bands was greater than 98%.

Low values of the chlorophyll a in June and July, which gradually increased until October with 2.32 mg/g fresh weight, where the chlorophyll b oscillated between 1.40 mg/g in August and 2.33 mg/g in October. Low amounts of degradation product (chlorophyllide a and phaeophytin a) were obtained (Fig. 2.1). The carotenoids appeared with high value of siphonixin in June and September with 0.27 and 0.29 mg/g respectively. Violaxanthin showed a greater quantity in the months of winter, oscillating between 0.1 and 0.21 mg/g during the study period, followed by siphonoxanthin ranged from 0.08 – 0.17 mg/g, and the β-carotene from 0.06 – 0.12 mg/g (Fig. 2.2).

**Fig. 2.1.** Temporal variation of chlorophylls and degradation products values in mg/g fresh weight of *Caulerpa prolifera*.

**Fig. 2.2.** Temporal variation of carotenoids values in mg/g fresh weight values of *Caulerpa prolifera*.

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The breakdown of pigments and variations in algal colour were also observed in our study during the reproduction period and under severe environmental conditions in the intertidal zone of the lagoon. We are agree that all taxonomic groups of algae have a specific sets of pigments which have a different concentration from one species to another. The balance between pigments values were occurred within a few margins in each group and the variability of the concentration must be related to the quantity and quality of the light in the marine environment (Oring, 1982). Nevertheless, Nakayama et al. (1983) reported that there is a fixed relationship in brown algae between fucoxanthin and total carotenoids (between 70-90%). Our results confirmed that Caulerpa prolifera have their own photosynthetic pigments, and the variation of their concentration values depend on a multiple factors such as depth, temperature, seasonality, habitat, availability of light, etc. With all these factors we can calculate the efficiency of photosynthetic process and no fixed proportion between all pigments during the work period.

Table 1 identifies the photosynthetic pigments according to the absorption maxima (nm) of each peak in the mobile phase. These are compared with spectral data in different types of solvents. All the absorption maxima of the spectral data coincide with the previous published data (Foppen, 1971, Burczyk, 1987, Shahidi, et al., 1998 and Hegazi et al., 1998).

<table>
<thead>
<tr>
<th>No.</th>
<th>Pigment</th>
<th>Acetone</th>
<th>Benzene</th>
<th>Diethyl ether</th>
<th>Ethanol</th>
<th>Hexane</th>
<th>Eluant</th>
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<tbody>
<tr>
<td>1</td>
<td>Chlorophyll a</td>
<td>428, 616,652</td>
<td>428, 662</td>
<td>408, 432, 508, 536, 580, 608, 664</td>
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<tr>
<td>2</td>
<td>Siphonous</td>
<td>455</td>
<td>452</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>Neoxanthin</td>
<td>423, 448, 478</td>
<td>415, 438, 467</td>
<td>416, 437, 466</td>
<td>416, 440, 468</td>
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<td></td>
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<tr>
<td>4</td>
<td>Neoxanthin-like</td>
<td>423, 448, 478</td>
<td>415, 438, 467</td>
<td>416, 437, 466</td>
<td>416, 436, 464</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Violaxanthin</td>
<td>428, 454, 483</td>
<td>419, 441, 471</td>
<td>443, 472</td>
<td>416, 440, 472</td>
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<tr>
<td>6</td>
<td>Maroxanthin</td>
<td>396, 420, 447</td>
<td>396, 418, 446</td>
<td>424, 448</td>
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<tr>
<td>7</td>
<td>Mesoene</td>
<td>445</td>
<td>419, 440, 467</td>
<td>440, 468</td>
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<tr>
<td>8</td>
<td>Mesoene-like</td>
<td>445</td>
<td>419, 440, 467</td>
<td>440, 464</td>
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<tr>
<td>9</td>
<td>Lutein-5,6-epoxide</td>
<td>428, 444,483</td>
<td>424, 440, 468</td>
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<tr>
<td>10</td>
<td>Siphonocapsin</td>
<td>448</td>
<td>448, 464</td>
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<td>11</td>
<td>Laten</td>
<td>420, 445,473</td>
<td>424, 448, 472</td>
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<td>12</td>
<td>Chlorophyll b</td>
<td>454, 596,644</td>
<td>453, 593,642</td>
<td>468, 548, 596, 652</td>
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<tr>
<td>13</td>
<td>Chlorophyll b</td>
<td>454, 596,644</td>
<td>453, 592,642</td>
<td>468, 548, 596, 657</td>
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<tr>
<td>14</td>
<td>Chlorophyll a</td>
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<td>430, 615,661</td>
<td>412, 432, 332, 580, 616, 664</td>
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<tr>
<td>15</td>
<td>Chlorophyll a</td>
<td>428, 616,662</td>
<td>428, 634,664</td>
<td>412, 432, 332, 580, 616, 664</td>
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<tr>
<td>16</td>
<td>a-Carotene</td>
<td>420, 447,472</td>
<td>420, 448, 476</td>
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<tr>
<td>17</td>
<td>b-Carotene</td>
<td>425, 449,477</td>
<td>428, 452, 476</td>
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<tr>
<td>18</td>
<td>Phaeophytin a</td>
<td>410, 468,668</td>
<td>408, 503,667</td>
<td>412, 448, 472, 508, 536, 560, 658, 668</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Our results are more efficient than that of Barlow et al. (1997), who used a C8 column of greater polarity than C18, which would explain the low resolution for polar pigments and reduced retention times. They are also more efficient than those of Van.
Heukelem et al. (1994), who used more than one run with different temperatures to separate the photosynthetic pigments of phytoplankton.

Although all the steps of the procedure were carried out rapidly using fresh algae in cool, dark fume cupboards to prevent breakdown of the photosynthetic pigments, only small quantities of phaeophytin a were detected, while no phaeophytins were detected from other chloropigments or magnesium-free metabolites such as phaeophorbides. The results, therefore, do not agree with those of Zapata et al. (1987), who used dried algae in a dessicator at 4 °C for pigment extraction or Henley and Ramus (1989), who analysed pigments extracted over a period of 24 h from Ulva rotundata in N, N-dimethyl formamide (DMF) at room temperature. In our opinion, when dried algae are used or the extracts are kept at in room temperature for 24 h, the photosynthetic pigments are broken down and incorrect analytical results ensue.

The advantage of the chromatographic separation technique and quantification used by in this study compared with the other published works is that we used a sensitive method and worked over a period of one year.

Finally all the photosynthetic pigments of Caulerpa prolifera listed here have been reported by Hegazi et al. (1998) and the quantification is considered a preliminary data for further investigations for seaweed pigments.

References


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التغيرات الوظيفية في التركيب الصفني لطحلب الكولوريون بروليفيرا في بحر البحر الصغير (جنوب شرق إسبانيا)

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كلية البيولوجيا - جامعة كومبينس نهر
كلية البيولوجيا - جامعة مصر

تم فصل الأنواع الشائعة من البذور البيلوكية (الكولوريونات والكوارتوينات ومشتقاتها) من طحلب كولوريون بروليفيرا في خلية واحدة وتقدر كمية باستعمال جهاز كرومتوغرافيا السوائل ذو الكفاءة العالية في الفترة من 1965 إلى 1981. 

ظهر الكولوريون (A) والكولوريون (B) كما من أنواع الأنسج المختلفة للنوع كولوريون بروليفيرا بكمية متغيرة. وقد ظهر الكولوريون (B) كمية عالية عن الكولوريون (A) في العديد من شهور السنة وخصوصًا أثناء شهر يوني ويليوGY حيث يرتبط مع أعلى معدل للمكثة الحيوية للطحلب خلال هذه الفترة، وأيضاً مع أعلى تركيز للكاروتينات الصفراء.

وينتبه الكوارتوينات الأخرى فقد وجد أن الفيولاكساسينون و الفيولوكساسينون ونظير-
النيوكساسينون هم أكثر الكوارتوينات كمية خلال مدة الدراسة.

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