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SEASONAL FLUCTUATIONS OF THE CAROTENE (α, β) CONTENT IN FRESHWATER PHYTOPLANKTON

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SUMMARY

The total (α + β) carotene and the chlorophyll a content of fresh water phytoplancton were measured spectrophotometrically and were found to be higher during autumn and a part of the winter (100 to 200 μg/l chlorophyll a and 4 to 6 μg/l total (α and β) carotene) with maxima during December and January. From March to June the content decreased continuously from 50 μg to 20 μg (or even 10 μg/l) chlorophyll a and from 5 μg to less than 1 μg/l total carotene (α + β). The ratio of the α-carotene to the β-carotene content, fluctuated periodically. During January to May more α-carotene than β-carotene was found and during the rest of the sampling period more β- than α-carotene.

1. INTRODUCTION

Almost all algae produce α- and β-carotene (HERTZBERG & LIAAEN-JENSEN 1966a, 1966b; CZECZUGA 1972; GOODWIN 1965; STRAIN 1965; BAUERFEIND 1972), although generally only traces of α-carotene are observed in microscopic species (GOODWIN 1965, STRAIN 1965). GOODWIN (1965) and STRAIN (1965) observed that the cryptophyta yield much α-carotene, while the chlorophyta contain only α-carotene. A quantitative determination of α- and β-carotene could be of great value in studying the growth of biomass of the phytoplancton.

There do exist a multitude of methods for the extraction and separation of carotenes, but most of them are very time consuming and none permits the determination (separately) of the α- and β-carotene content.

Al_2O_3 is frequently used as an adsorbent in the column chromatography of carotenes. The activity grade of the adsorbent and the polarity of the eluant vary considerably from author to author.

HANNY et al. (1972) used Al_2O_3 desactivated with 5% H_2O as adsorbent and ether with acetone in increasing amounts as eluant. The first fraction contains phytoene and phytofluene, the second one α- and β-carotene.

CZECZUGA (1972) used activated Al_2O_3 and employs petroleum-ether for the elution of the carotenes.

HERTZBERG & LIAAEN-JENSEN (1966a, b) chromatographs on neutral Al_2O_3 (activity grade II) with petroleum ether-diethyl ether (98 :2) as solvent system (elution of β-carotene). By changing the activity grade of the Al_2O_3 (activity

grade III) and the polarity of the eluant (hexane + 2% acetone) we obtained a more rapid elution of the α- and β-carotene, but still, both can not be separated from each other by this method.

We recently (ANDRE & VERCRUYSSE 1975) developed a method that enables a determination of the α- and β-carotene content (by calculation) using the visible light spectrum of a solution containing these both carotenes.
Using the combined method (extraction-chromatography and calculation) we investigated upon the fluctuation of the α-carotene, the β-carotene and the chlorophyll a content in the total algal species from pondwater samples.

2. MATERIAL AND METHODS

A. *Sampling:*

Samples from the pond (Heverlee park) were taken every 14 days. Five liters of water were taken from the surface at 4 different points (2, 3, 4 and 5). Three liters were used for the determination of the carotene (α- and β-) content and 0,6 liter for the analysis of the chlorophylls.

B. *Filtration and extraction*

a. Filtration

For the determination of the carotenes, the sample is filtered on five filters (Schleicher and Schull no 595–∅5.5 cm) covered with a thin layer of $MgCO_3$ as filtering aid.
For the analysis of the chlorophylls, one filter (0.6 l of sample) is sufficient.
$MgCO_3$ can be omitted by using millipore filters-Solvinert URWP 4700 (pore size 1.5 μ,m ∅47 mm).

b. Extraction and saponification of the carotenes

Except for some minor modifications, we used the procedure described by HERZBERG & LIAAEN-JENSEN (1966a).

In order to minimize the presence of water in the final extract we exchanged the ether used by Hertzberg with hexane in our procedure. The extraction is illustrated in *scheme 1.*

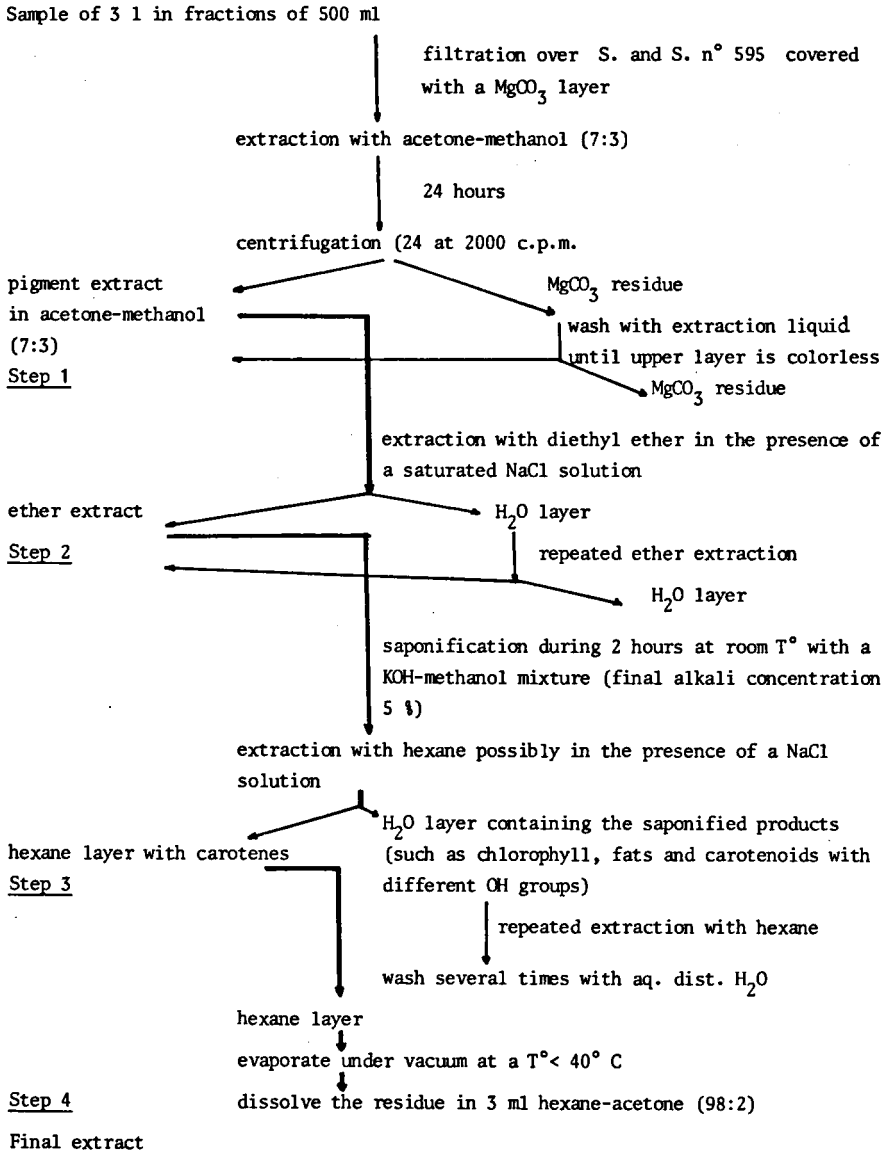
c. Extraction and determination of the chlorophylls

The extraction of the chlorophylls is based on a procedure described by the SCOR-UNESCO WORKING GROUP 17 (1966). After filtration we allow the filter covered with the $MgCO_3$ and the phytoplancton, to extract for 24 hours in 10 ml of an acetone – H_2O solution (90 : 10).

After centrifugation for 5 minutes at 2,000 r.p.m., we measure the chlorophyll content as described by the Working Group.

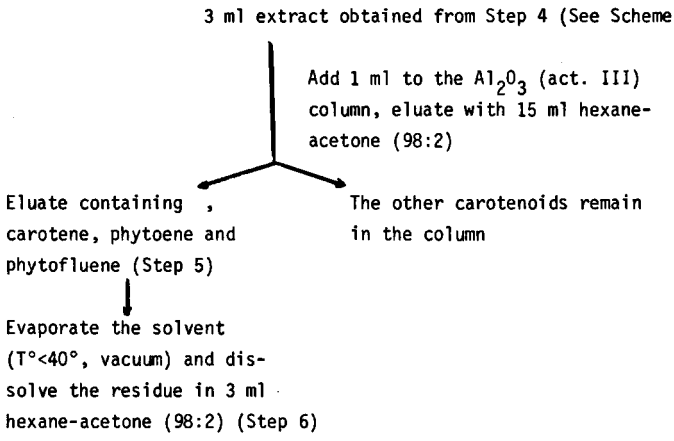
C. *Column chromatography*

We used a column (column : 30 cm length and 0,6 cm ∅) with Al_2O_3 (neutral-Woelm activity grade III) as adsorbent and hexane-acetone (98 : 2) as eluent.



Scheme 1. The extraction and saponification.

A good separation and a recovery of more than 95% (α- or β-carotene) has been obtained.
The procedure is given in *scheme 2.*



Scheme 2. Elution of the Al_2O_3 column.

D. *Spectrophotometric analysis*

The spectra of the extracts are recorded between 550 nm to 370 nm on a Perkin Elmer double-beam spectrophotometer, model 124, equipped with a Perkin-Elmer recorder, model 56.

We use as standards α-carotene (Sigma type V CO 251) and β-carotene (Sigma type IV CO 126).

E. *Used Formulas*

- Chlorophyll a (μg/ml extract) = $11.62\ A_{663} - 2.16\ A_{645} - 0.10\ A_{630}$ (Scor-Unesco Working Group 17 (1966).
- α-carotene (10^{-3} molar) = $A_{444} \times 0.07330 - A_{449} \times 0.06893$ (ANDRE & VERCRUYSSE (1975).
- β-carotene (10^{-3} molar) = $A_{449} \times 0.07330 - A_{444} \times 0.07002$ (ANDRE & VERCRUYSSE (1975).

3. RESULTS AND DISCUSSION

Since the samples from the 4 stations have comparable pigment contents and variations; we will use only the results from station 2 for discussion.

A. The observations made on the spectra recorded from the extracts containing . α- and β-carotene (in mixture) can be summarized as follows:

a) all the spectra show two maxima and a minimum.

b) the λ max. of these spectra fluctuate between the range of 449 nm (λ max standard β-carotene) and 444 nm (β max standard -carotene), they never exceed these limits *(fig. 1)*.

c) the same observations can be made for the λ 2nd max and the λ min.

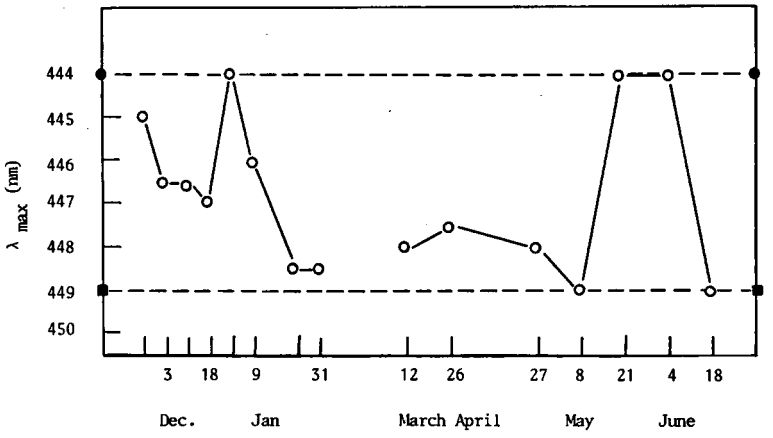


Fig. 1. λ_{max} fluctuations of the samples taken at station 2.
λ_{max}: ●- - -●, standard solution α-carotene, ■- - - -■, standard solution β-carotene, ○——○, samples.

B. Discussion of the results

a) ratio α/β-carotene content

The observations a and b suggest the presence of α- and β-carotene (together) in the analysed extract. This is proved by using the procedure described by STRAIN & SHERMA (1969). So, since no other carotenoids are present in this extract, we can perfectly use the recorded spectrum for calculation of the α- and β-carotene content.

Observation b suggests a changing ratio of the α-/β-carotene content. The calculation of both contents shows this clearly *(fig. 2)*. These changes seem to follow a seasonal pattern. Indeed, more α- than β-carotene was found in the period between 27/11/72 and 9/11/73 and after 21/5/73. The inverse was noticed during the period 9/1/73 to 8/5/73.

b) pigment content (carotene, chlorophyll a)

The sum of the α- and β-carotene content (= total carotene content) is compared with the chlorophyll a content *(fig. 2)*. The ratio total carotene content chlorophyll a content remains almost constant (±0.03), only during the last two months it rises to values of 0.10 to 0.20. The reason for this increase is unknown. It could be a natural occurring phenomenon but it could also be caused by the decreased accurary of the determination at such low pigment concentration.

Comparing the chlorophyll a, the α- and the β-carotene contents *(fig. 2)* we observe a period of high pigment concentration from 27/11/72 to 26/3/73 with a marked fall during two weeks and two months. This period coincided with periods of frosty weather. The pigment concentration rises immediately when the weather became more mild. A continuous decrease in pigment concentra-

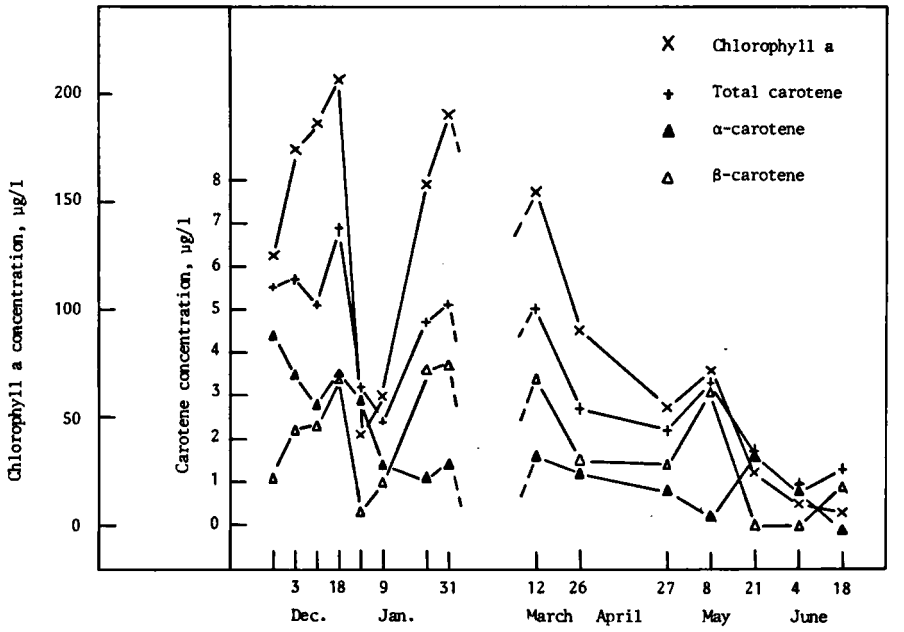


Fig. 2. The chlorophyll a, total carotene ($\alpha + \beta$), α-carotene and β-carotene concentrations of the samples taken at station 2 as a function of time.

tion is noted after 26/3/73 and very low values were found over the summer period (20 μg/l and sometimes 10 μg/l chlorophyll a; less than 1 μg/l carotenes). Interferences of different origin (for example films or ice floating on the water) can influence strongly the pigment content of the microorganisms (for example: sudden pigment content falls can occur).

It would be of interest to investigate upon the correlation between the growth of the microorganisms present in the pondwater and the pigment content and composition.

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