EFFECT OF LIGHT ON GROWTH AND PIGMENT BIOSYNTHESIS IN SOME FRESH WATER GREEN AND BLUE-GREEN ALGAE .

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ABSTRACT

Low and moderate light flux densities promote pigment biosynthesis leading to an increase in growth parameters in both green and blue green algal species used in this investigation. On the other hand, higher light irradiances induced photodestruction of chlorophyll but not of carotenoids. Short light wavelengths (blue, green and yellow) proved to be stimulatory to pigment synthesis (either for photosynthetic or accessory pigments) and consequently resulted in an increase in the fresh, dry weights and velocity of growth. Longer wavelengths (red light) were less stimulatory for both growth and pigment production except for ***Chlorella*** *(green alga) and* ***Aphanothece*** *(Cyanobacterium). One may ask, what are the pigment(s) which control these metabolic responses?*

INTRODUCTION

Light is a driving force of photosynthesis, as such, its quantity, its spectral composition and their fluctuations within the culture, control biomass production rate and may cause variations in the photosynthetic responses, growth rates, and cell metabolism of algae (Senger, 1987; Sukenik *et al*., 1989). Algae have evolved various pigments for the purpose of light absorption, these include chlorophylls, which strongly absorb blue and red lights, carotenoids, that absorb blue and green lights and phycobilins with maximum absorption in the green, yellow and orange regions of the spectrum.

Algae have considerable flexibility in responding to various environmental factors by altering their pigment composition and or spectral response. The best example of such adaptational strategy is that of chl a-biliprotein system of cyanobacteria and red algae. Phycobiliproteins in these algal groups function as photosynthetic accessory light harvesting pigments, because they absorb light energy in the green region, which chlorophyll does not absorb.

Light photon flux density determines in which way the chromatic adaptation is directed, which is more probable at low than at high light intensities. Temperature do not react separately but plant respond to combination of both effects. Intense light associated with higher temperatures may cause the destruction of chlorophylls, stimulation of chlorophyllase activity, and may not exert any effect on photosynthetically inactive carotenoids. On the contrary low light flux densities promote pigment production in green , and blue-green algae .

Light quality, as light flux density , is an important parameter in determining photo metabolic responses . Each light colour, as it is absorbed by specific

photoreceptor, it induce certain metabolic responses for example blue light as it is absorbed by the photoreceptor cryptochrome, it may stimulate linear and bidimentional growth, carotenogenesis and may induce changes in enzyme activities. On the other hand, red light when it is absorbed by the phytochrome, may enchance chlorophyll production and inhibit both linear and bidimentional growth (Hopkins, 1995).

The aim of this investigation is to study the role exerted by light on the growth and pigment content of some fresh water green and blue-green algae isolated from Ain Helwan (Egypt) thermal spring , taking in to consideration , the seasonal changes, hoping to answer the question , why certain algal species predominate in specific season and nearly or completely disappear in another one ??

MATERIALS AND METHODS

I - Establishment of algal cultures :

The algal species used in this study were previously seasonally isolated from the water samples of the thermal spring of Ain Helwan during year 1999.

Unialgal species were separated by isolation and repeated subculturing on Bold's solid media, then purification was carried out by using a mixture of antibiotics , according to Stein (1973), and finally identifications of algal species were performed with the aid of Desikachary 1959 , Bourrelly 1970 , and Prescott 1978 .

Unialgal cultures were maintained incubated under continuous illumination of light intensity of 10μmol / m^2 / s at 20 ± 1°C for two weeks until use in experiments.

II – Experimental conditions:

i. Effect of light quantity (Photon flux density):

In 150 ml conical flasks , 45 ml of Bold's liquid nutritive media was mixed with 5 ml of concentrated algal suspensions (after centrifugation of algal culture at 3000 rpm for 15 min.) incubations at 20 ± 1°C under continuous illumination of light flux densities of 40 , 25 , 19 , 15 , 9 and 6 μmol / m^2 / s for 10 days (measurements were carried out by a radiometer U.D.T. type 40x United Detector Technology Inc.,USA) .

ii. Effect of light quality (monochromatic light):

The same steps were proceeded as in studying the effect of photon flux densities , concerning the volumes of nutritive media and algal suspension, the incubation temperature and duration of experiment . The coloured lights used were the blue (445 – 453 nm), green (496 – 504 nm) , yellow (575.5 – 584.5 nm) and red light (696 – 705 nm) which were transmitted by 5 cm x 5 cm glass filters (manufactured by Oriel Stamfor, conn.)and the white light of the control was given by colourless white glass [the light source was a halogen lamp (Osram HWL 250 watt)] .

The monochromatic light and the white light of control have isoquantic flux density of 14 μmol / m^2 / s. This was adjusted by controlling the distance between the flasks , the filters and the source of light .

The algal species used in this investigation are :

1. *Ankistrodesmus falcatus* (Corda) Ralfs _____ Abbrev. _____ Ank.
2. *Scenedesmus obliquus* (Turp.) Kuetzing _____ Abbrev. _____ Sc.

3. *Chlamydomonas debaryana* Gorosch. Abbrev. Chlam.
4. *Chlorella vulgaris* Beye. Abbrev. Chlo.
5. *Microcystis aeruginosa* Kütz. Abbrev. Mic.
6. *Aphanothece caldariorum* Richter Abbrev. Aph.

III – Extraction and estimation of pigments:

▪Chlorophylls and carotenoids (chl a., chl b. and carot.):

Extractions were carried out according to Metzner *et al.* (1965). Spectrophotometric absorbance at 633 , 644 , 663 nm were recorded and estimation of pigments were performed by substitution in the following equation :

Chl a (µg / ml) = 10.3 A_{663} – 0.918 A_{644}

Chl b (µg / ml) = 19.9 A_{644} – 3.87 A_{633}

Carotenoids (µg /ml) = 4.2 $A_{452.5}$ – (0.0246 chl a + 0.426 chl b)

▪Phycobilins : (Phycocyanin and Phycoerythin):

Extraction were carried out according to O'Carra and O'h'Eocha (1976) , and estimation of pigments were performed by spectrophotometric absorbance at 550 , 663 nm and substitution in the following equation :

Phycocyanin = A_{550} x15.15 x total vol.of extract / taken vol. x 1/1000

Phycoerythrin =A_{663} x 12.4 x total vol.of extract / taken vol. x 1/1000

IV – Growth measurements :

Growth was recorded by the following parameters:

Fresh weight (Fwt µg/ml): using previously weighed (Watman no. 1) filter paper and the starting inoculum (W1), the final content of each experiment was filtered then air dried and reweighed the filter paper + alga (W2), the fresh weight correspond to the difference between the two weights (W1 - W2) and then divided by the culture volume.

Dry weight (Dwt µg/ml): the same procedure as in case of Fwt but the sample, instead of being air dried it was oven dried at 60 – 70 °C for 48 hours or till constant weight.

Velocity of growth (Vgr µg/day) = Dry weight (Dwt µg) / Duration of exp. (Days) = µg/day.

RESULTS

Figs. 1,2,3 and 4 demonstrate the effect of light flux densities on pigment production and growth of green and blue green algae. The results illustrate the stimulatory effects of moderate and lower light intensities (19 – 6 µmol / m^2 / s) on both principal photosynthetic and accessory pigments (Fig. 1,2).

Growth parameters were enhanced by the same light irradiances as pigments except for Ank. and the cyanobacterial species which were stimulated by wider range of photon flux densities (25 – 6 µmol / m^2 / s.) and it seems to have certain tolerance to higher irradiances (Fig. 3,4).

Figs. 5,6,7 and 8 Illustrate the effect of monochromatic light on pigment synthesis and growth of both green and cyanobacterial species. Yellow light was the most

stimulatory in case of Sc., Ank. and Chlam., while maximum production of pigments in Chlor. were performed under the effect of red light (Fig. 5).

Production of cyanobacterial principal photosynthetic pigments were enhanced by blue, green and red lights in case of Mic. and by blue, and yellow lights, in Aph., while the maximum production of the accessory phycobilin pigments were achieved by red, yellow lights, and followed by other light colours (Fig. 6).

Growth parameters (Fwt, Dwt and Vgr) reached their maximum values due to stimulation of yellow light in case of Ank. while in case of Sc. and *Chlo.*, they aquire comparable growth values under all the light colours used. Chlam. has maximum growth values under the effect of blue light followed by the green and red (Fig. 7).

In cyanobacterial species growth (Fwt, Dwt and Vgr) of Mic. was stimulated by green, and yellow lights followed to a lesser extent, by blue and red lights while in case of Aph. the red light is the most controlling light factor followed by the yellow, blue and green lights (Fig. 8) .

DISCUSSION

Light is the most important photomorphogenic factor for algae , either as light quality, quantity, and duration.

Deeling with the effect of different light intensities, two types of adaptive reactions can be distinguished; the most usual is the *Chlorella* type which is characterized by an inverse relationships between the light intensity to which the algae are exposed and their chl a content. Algae belonging to the *Cyclotella* type on the other hand show an inverse correlation between the activities and/or concentrations of photosynthetic enzymes and light intensity. The time required by *Cyclotella sp.* and *Chlorella vulgaris* to adapt to a new light intensity is less than 30 hours (Jorgensen, 1969).

The obtained results in this investigation demonstrated that, growth and pigment production of the green algal species used, were stimulated by low to moderate photon flux densities. These results were in accordance with Cook (1963) who reported that *Euglena sp.* synthesize chl a and chl b at low photon flux densities, and with Tan *et al.* (1993) who demonstrated that *Trentepohlia odorata* increased its carotenoid content at relatively lower light flux densities than *Dunaliella bardawil* and they concluded that *T. odorata* could be considered as a potential source of carotenoids. Concomitant with the previous results, studies on three species of Chlorophyceae, Brown and Richardson (1968) recorded that, the ratio chl b / chl a increased as a result of exposure to low flux densities (resembling shade plants). In this context, dark-grown *Chlamydomonas* cells can synthesize chlorophyll from the protochlorophyll after 10 seconds of preillumination (Matsuda *et al.*, 1971).

High light intensities may inhibit respiration of the actively photosynthesizing cells. It may also cause decrease in chl a concentrations leading to an incease in the ratio carot. / chl a and production of the red carotenoid, astraxanthin, in *Chlorococcum wimmeri* (Brown *et al.*, 1967). Also, the high light acclimated strains of *Scenedesmus obliquus* and *Chlorella sp.* when grown under high photon flux densities, may produce great amount of photosynthetically inactive (and protective) carotenoids, which can occur also under other extreme environmental coditions (Halldal, 1970; Grobbelaar *et al.*, 1996). In addition, photodestruction of chlorophyll may occur in *Chlorella vulgaris* during prolonged exposure to high light intensities (Kok, 1956). On the contrary, Matthern *et al.*, 1969 demonstrated that certain *Chlorella* strains in dense turbulent

suspensions tolerate light intensities as high as 300 µmol / m^2 / s, which is equivalent to about 30 times as full sunlight. Cyanobacteria are characterized by its complementary chromatic adaptation and nitrogen-fixation (in the heterocystous species), which were propably more controlled by low photon flux densities than high .

The obtained results illustrated the tolerance of cyanobacterial species to high flux densities and it can grow relatively well under all light irradiances used (6 – 40 µmol /m^2/s), while the production of principal photosynthetic and accessory pigments were stimulated by moderate and even by lower photon flux densities. These results coincide with the findings of Brody and Brody-seymour (1962) who reported that pigment production in the blue-green algal species *Phormidium*, *Gloeocapsa* and the red alga *Porphyridium*, were enhanced by relatively low light flux densities. With increasing irradiances, pigment bleaching increased rapidly and the phycoerythrin content in *Porphyridium cruentum* was more influenced by variations in light intensities than chl a causing changes in the ratio of the two pigments. In this context, moderate light intensity (30 µmol /m^2 /s) and optimal growth temperature (30-40 °C) produce maximal chlorophyll concentrations in *Anacystis nidulans* (Halldal and French, 1958). While, Powels (1984); Jensen and Knutsen (1993); Kebede and Ahlgren (1996), reported that an increase in carot. / chl a ratio, growth, inhibition and even damage of *Spirulina platensis* were produced in the laboratory and outdoor mass culturing as a result of exposure to high light flux densities. On the other hand, *Oscillatoria rubescens* takes one to two weeks to adapt to a light intensity change (from 4-14 µmol /m^2 /s) and sometimes, in fact, there is no evidence of adaptation. This fits in with ecological finding (Meffert, 1971) that planktonic species of *Oscillatoria* are usually found in the deeper layers of the euphotic zone and are easily injured by intense illumination. Also, the preference of *Cryptomonas sp.* for greater depths may also be a reflection to its preference for low light intensities (Stewart, 1974). Moreover, Karentz *et al.*, (1991) and Karsten *et al.*, (1999) studied the arctic endemic red alga *Devaleraea ramentacca* , they reported that , this alga seemed to be well adapted to high radiation conditions to protect the photosynthetic apparatus and all estimates suggested that significant protection from UV damage was performed by the synthesis and accumulation of Mycosporine-like amino acids (MAAs) which function as intracellular photon screening agent.

Strong solar radiation can cause photoinhibition and photodamage of the D1 protein of photosystem II (Krause, 1988). Dynamic photoinhibition is a protective mechanism converting excess radiation energy into the harmless dissipation of heat by the so called xanthophyll cycle (Demmig – Adams and Adams 1992).

Concerning the effect of different light wavelengths, the obtained results revealed that yellow light exerted a stimulatory effect on the production of chlorophyll a and b of green algae especially in case of *Scenedesmus obliquus* , *Ankistrodesmus falcatus*, *Chlamydomonas debaryana*, while blue light controlled carotenoid synthesis. On the other hand the photosynthetic pigments (chl a, chl b and carotenoids) in *Chlorella* were especially produced under the effect of red light followed by green and blue ones. This may be due to the fact that *Chlorella* can adapt to grow under unusual light colours (red). Generally growth of the green algal species was maintained relatively well under all light colours used and comparable values in growth parameters were recorded.

Our results coincid with those of Jacques, (1968); Larpent *et al.* (1973) and Ducher, (1987) who reported that stimulation of pigment synthesis by green-yellow lights in algae was similar to the effect of the same wavelengths in higher plants. This

also agree with [Czeczuga, (1977, 1985); Czeczuga *et al.* (1986)] who reported that unicellular aquatic green algae grown under different light filters showed highest concentration of photosynthetically active pigments under blue- green lights, while in three multicellular *Chara spp.* (*C. fragilis*, *C. vulgaris* and *C. delicatula*) chlorophylls and carotenoid contents were highest under green-yellow light and lower values with blue and red lights. Also, Dagar *et al.* (1980) and Czeczuga, (1985) reported similar results with the aquatic moss *Riccia discolor* and in the unicellular green algae growning on tree bark. This is probably a manifestation of chromatic adaptation to light conditions prevalent in a given environment, in addition *Chara spp.* as well as the green algal species under investigation were grown in shallow waters which were penetrated not only by short rays (blue and green) but also by some of the longer rays as the yellow.

In cyanobacteria, carotenoids were produced under blue and green lights , while chl a, was stimulated by blue, green and red in case of Mic. and by blue and yellow in case of Aph., the accessory pigments were stimulated by the red (Mic.) and yellow (Aph.). The highest values of growth parameters (Fwt, Dwt and Vgr) in *Microcystis aeruginosa* were produced under the effect of green and yellow lights, while in case of *Aphanothece caldariorum* red light is the most stimulatory followed by yellow, green and blue lights which induced comparable growth values.

Our previous results showed great similarity with those of Yocum and Blinks, (1958) who reported that the photosynthetic efficiency of chl a in *Porphyridium cruentum* (red alga) was very high at low intensity of blue or red light, while in green light, absorbed by phycoerythrin, chl a efficiency is low and pigment ratio changes with alteration in photosynthetic spectral response; this adaptability occurred only in lower red algae. Blue or green light is the most favourable for phycoerythrin production in *Tolypothrix tenuis* (Fujita and Hattori, 1960), while red light is the least favourable. The opposite was true for phycocyanin formation and the same results were obtained in *Porphyridium cruentum.*

We can conclud that not all green algal species had similar responses to environmental factors (as light), inspite of the fact that they all have the same photosynthetic pigments. This may be due to the induction of certain alteration in pigment composition leading to modifications in growth parameters (metabolic responses). This agrees fairly well with *Chlorella* which can tolerate long term cultivation in red light which may be due to increase in long wavelength forms of chl a (Oquist, 1969).

In cyanobacteria, monochromatic light, controls the ratio of phycocyanin and phycoerythrin , taking in consideration that red light enhances phycocyanin production, while green light stimulates phycoerythrin synthesis.

Ain Helwan is a very shallow thermal spring (26-30 °C). It may receive not only variable seasonal light flux densities, but also variable light wavelengths. It may receive, short light wavelengths (blue and green) in addition to longer ones (yellow and red). Its algal flora vary in its response to the seasonal values of light flux densities, this fits in with the ecological finding that unicellular green algae grow well in autumn and winter, while their growth were inhibited by the high light irradiance in summer.

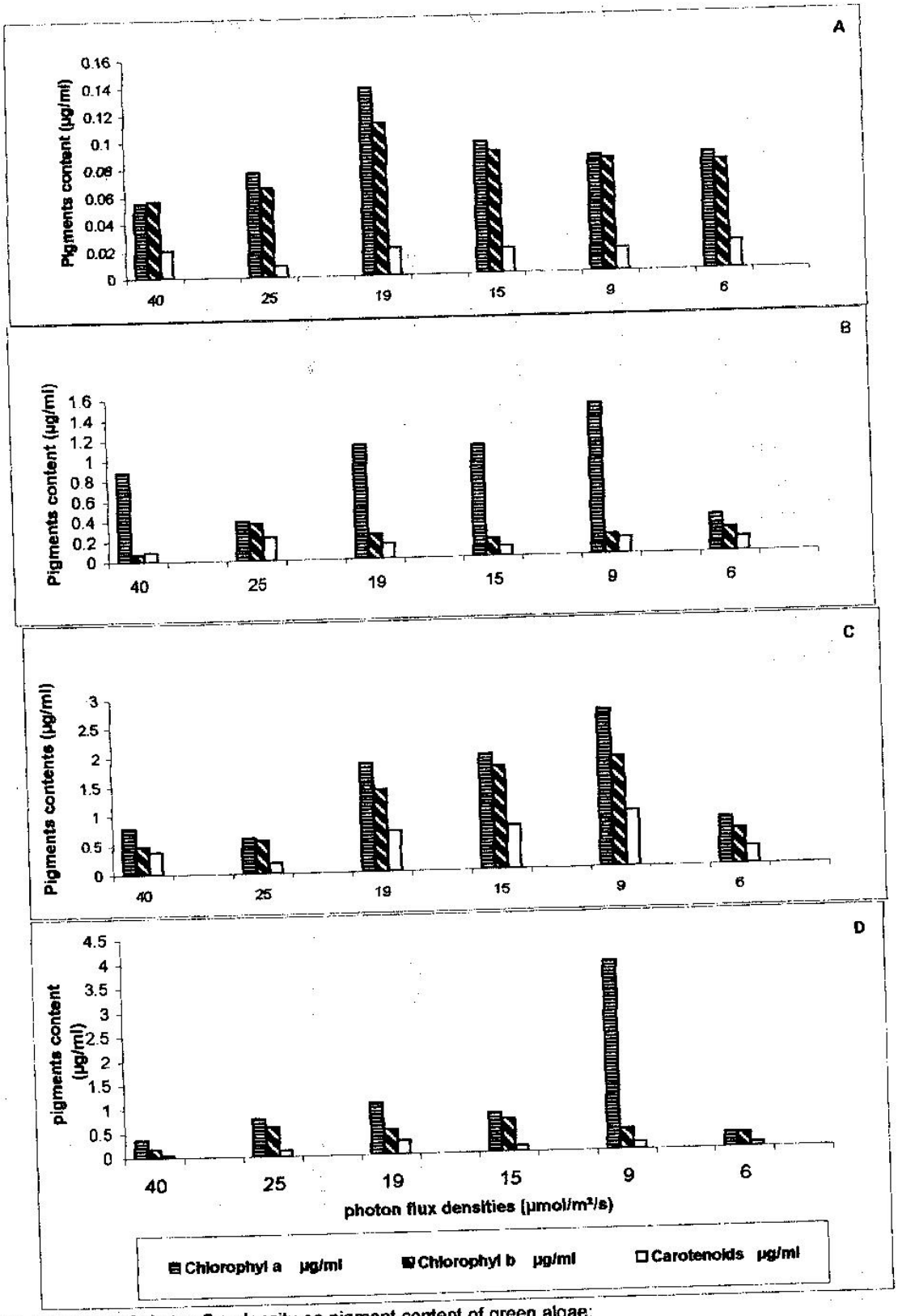

Fig (1): Effect of photon flux density on pigment content of green algae:
A- *Scenedesmus obliquus*;
B-*Ankistrodesmus falcatus;*
C-*Chlamydomonas debaryana;*
D-*Chlorella vulgaris.*

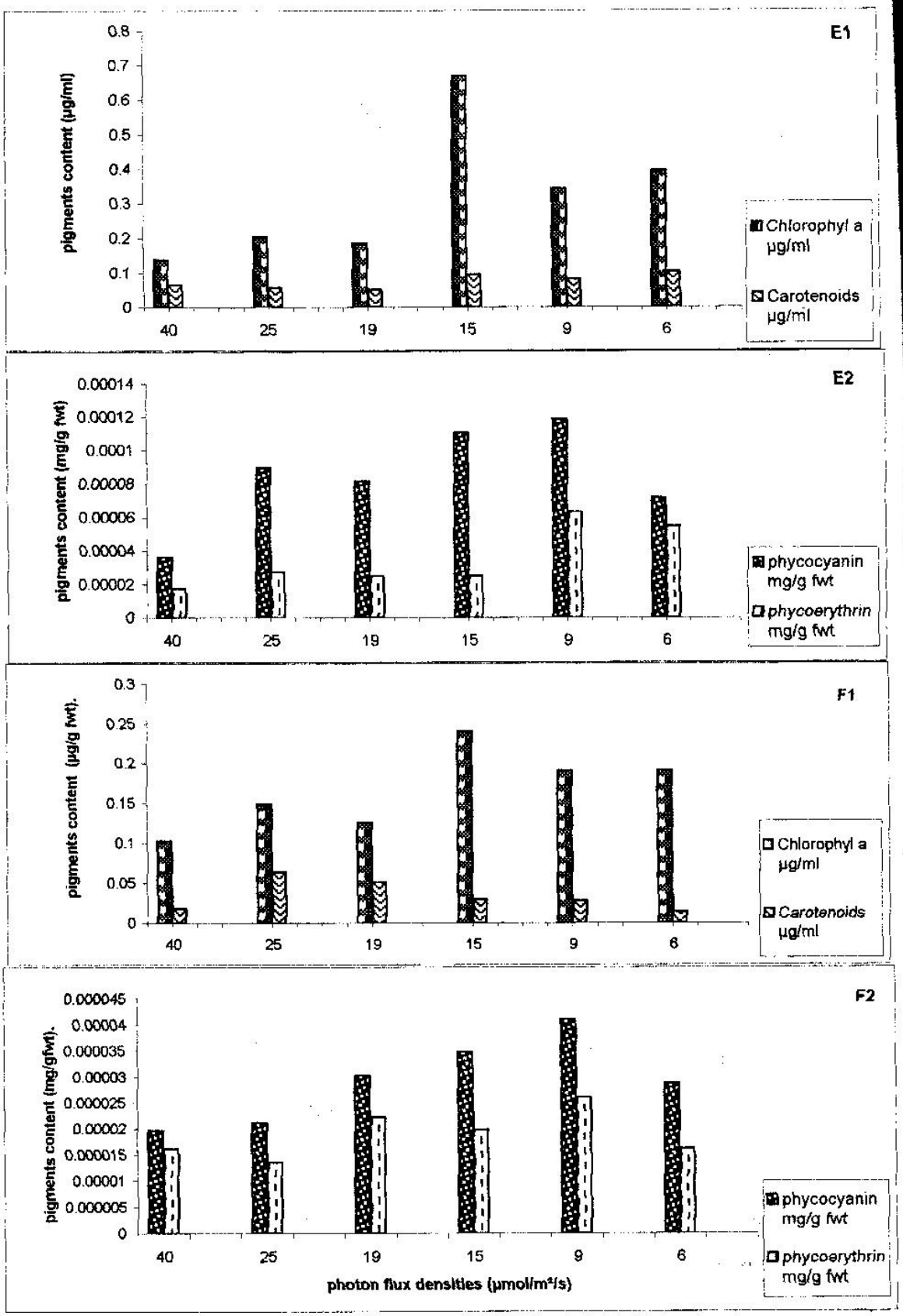

Fig (2): Effect of photon flux density on pigments content of blue-green algae:
E1-E2-*Microcystis aeruginosa*;
F1-F2-*Aphanothece caldariorum*.

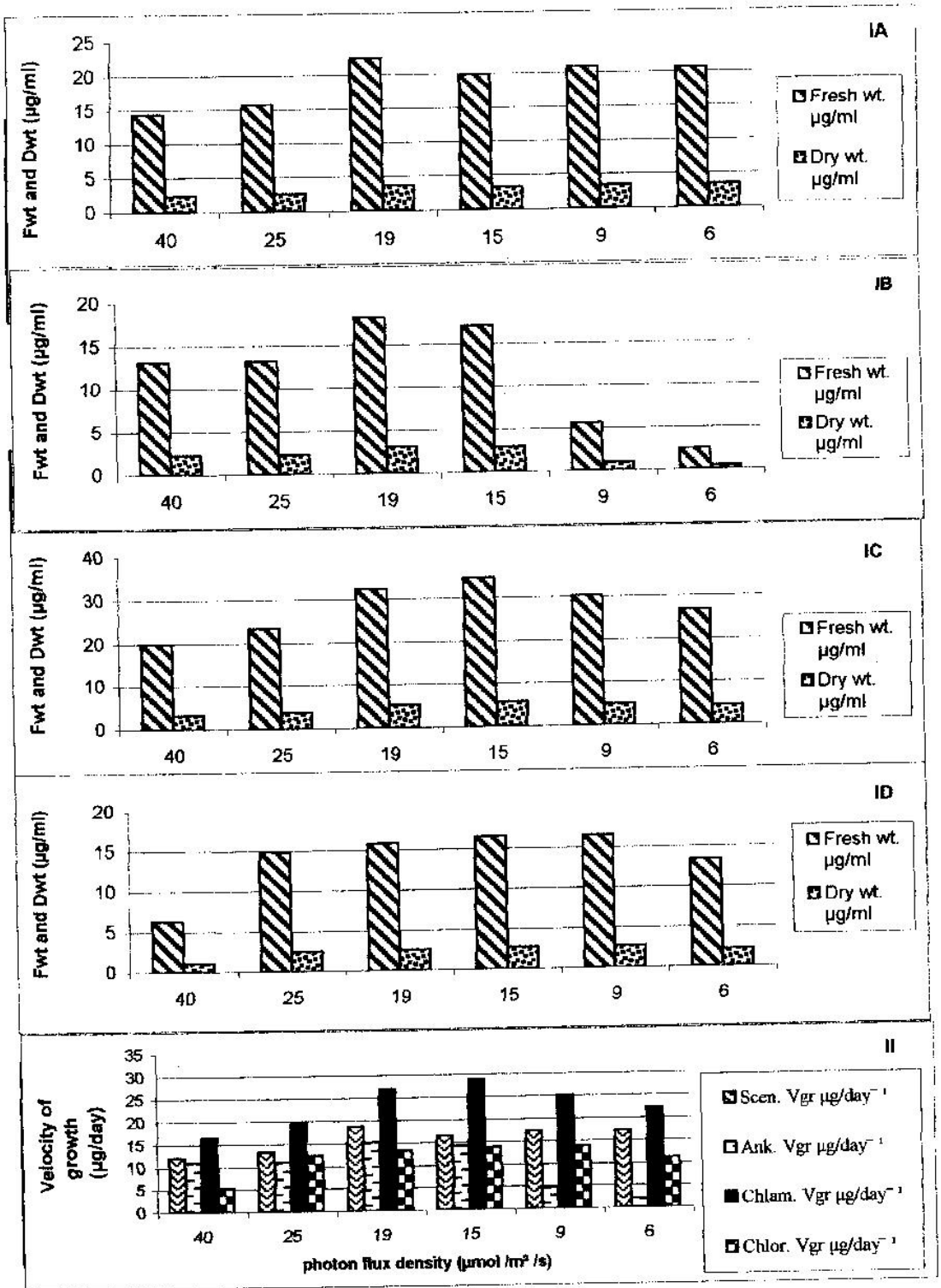

Fig (3): Effect of photon flux density on green algae:
I-*Fresh weights and Dry weights* (µg/ml) of :
IA-Scenedesmus obliquus ; IB-*Ankistrodesmus falcatus* ;
IC-*Chlamydomonas debaryana* ; ID-*Chlorella vulgaris.*
II-Velocity of growth (µg/day)

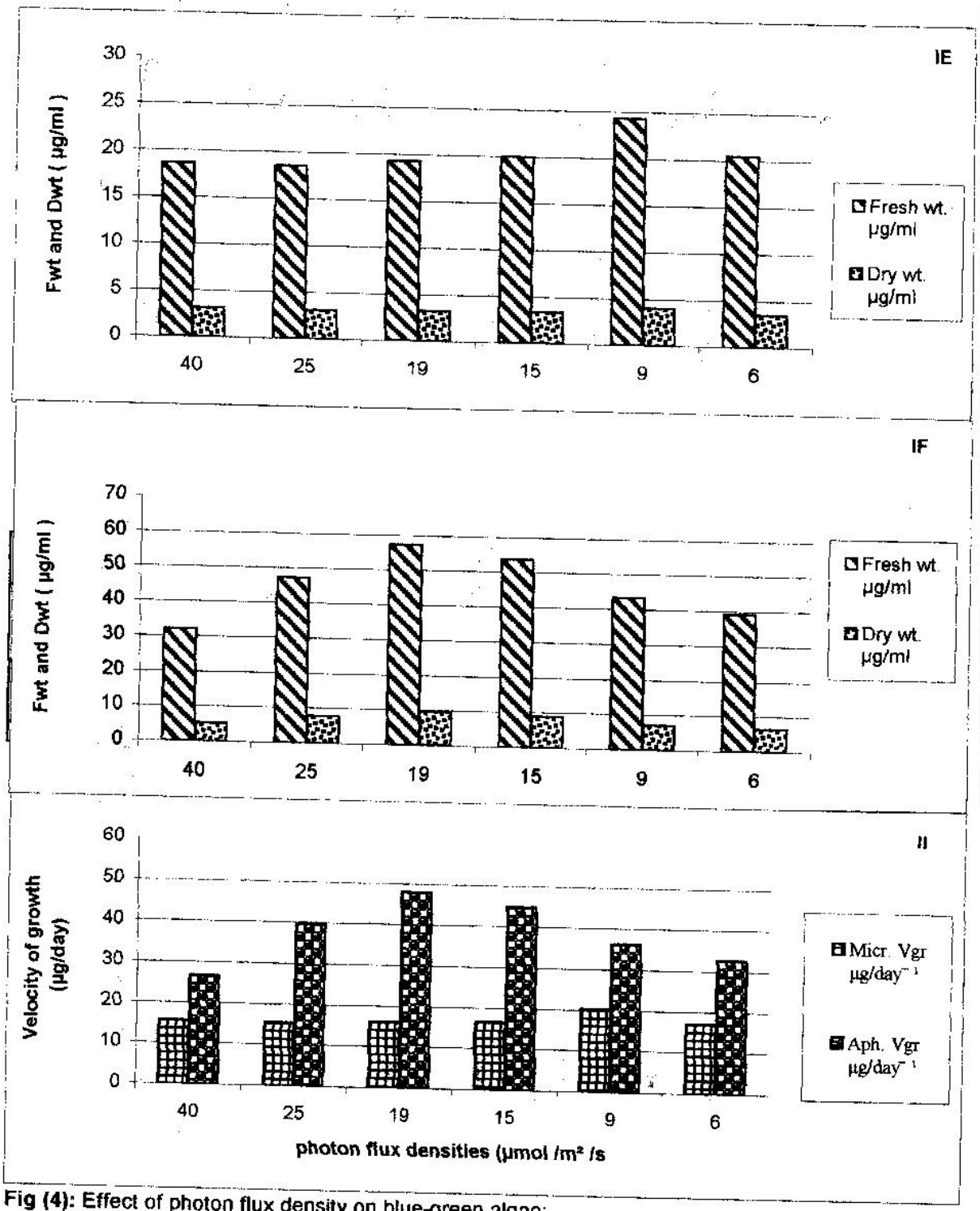

Fig (4): Effect of photon flux density on blue-green algae:
I-Fresh weights and Dry weights (µg/ml) of:
IE-*Microcystis aeruginosa;* IF-*Aphanothece caldariorum.*
II-Velocity of growth (µg/day).

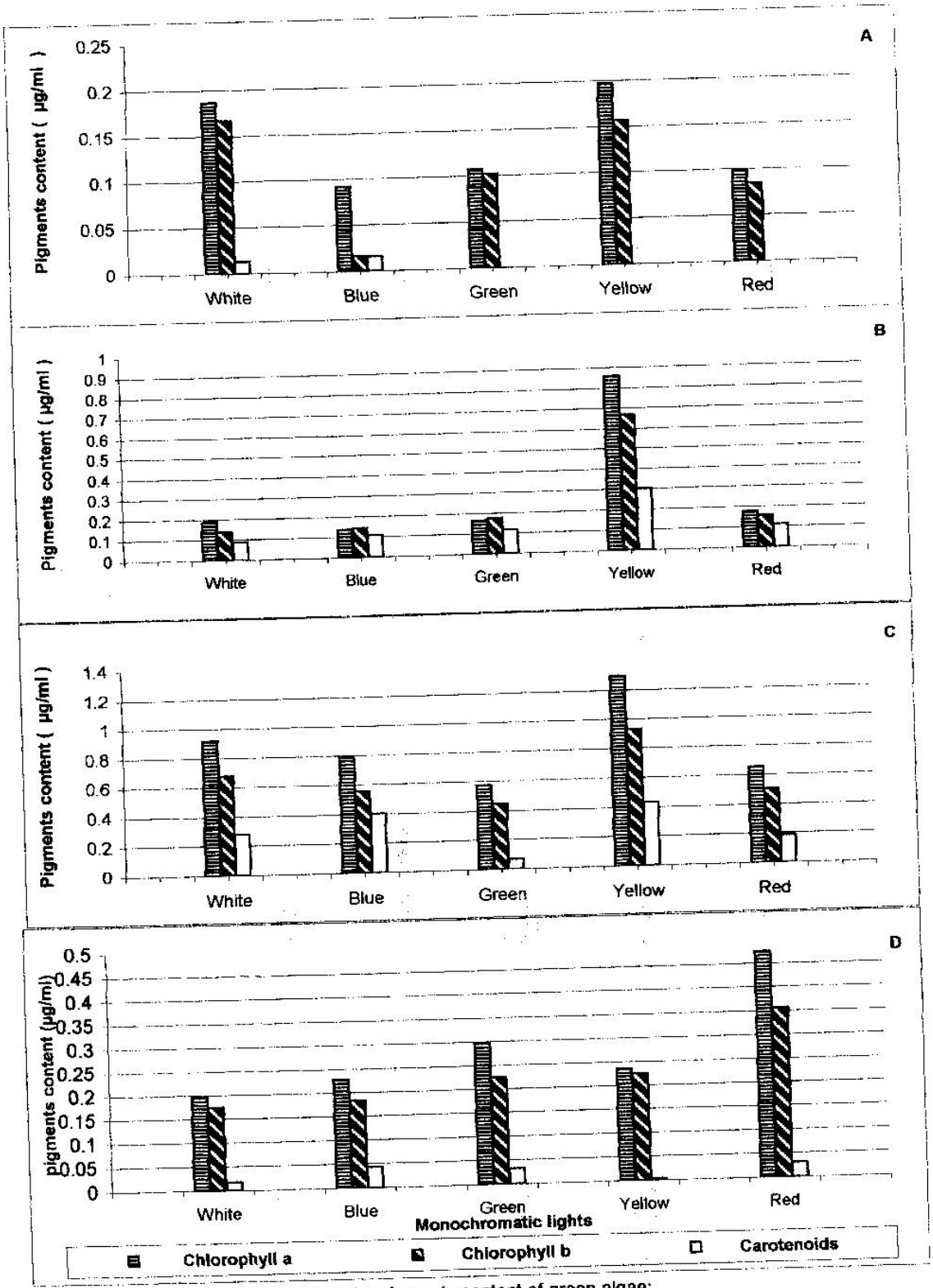

Fig (5): Effect of monochromatic lights on the pigments content of green algae:
A- *Scenedesmus obliquus* ;
B-*Ankistrodesmus falcatus* ;
C-*Chlamydomonas debaryana* ;
D-*Chlorella vulgaris* .

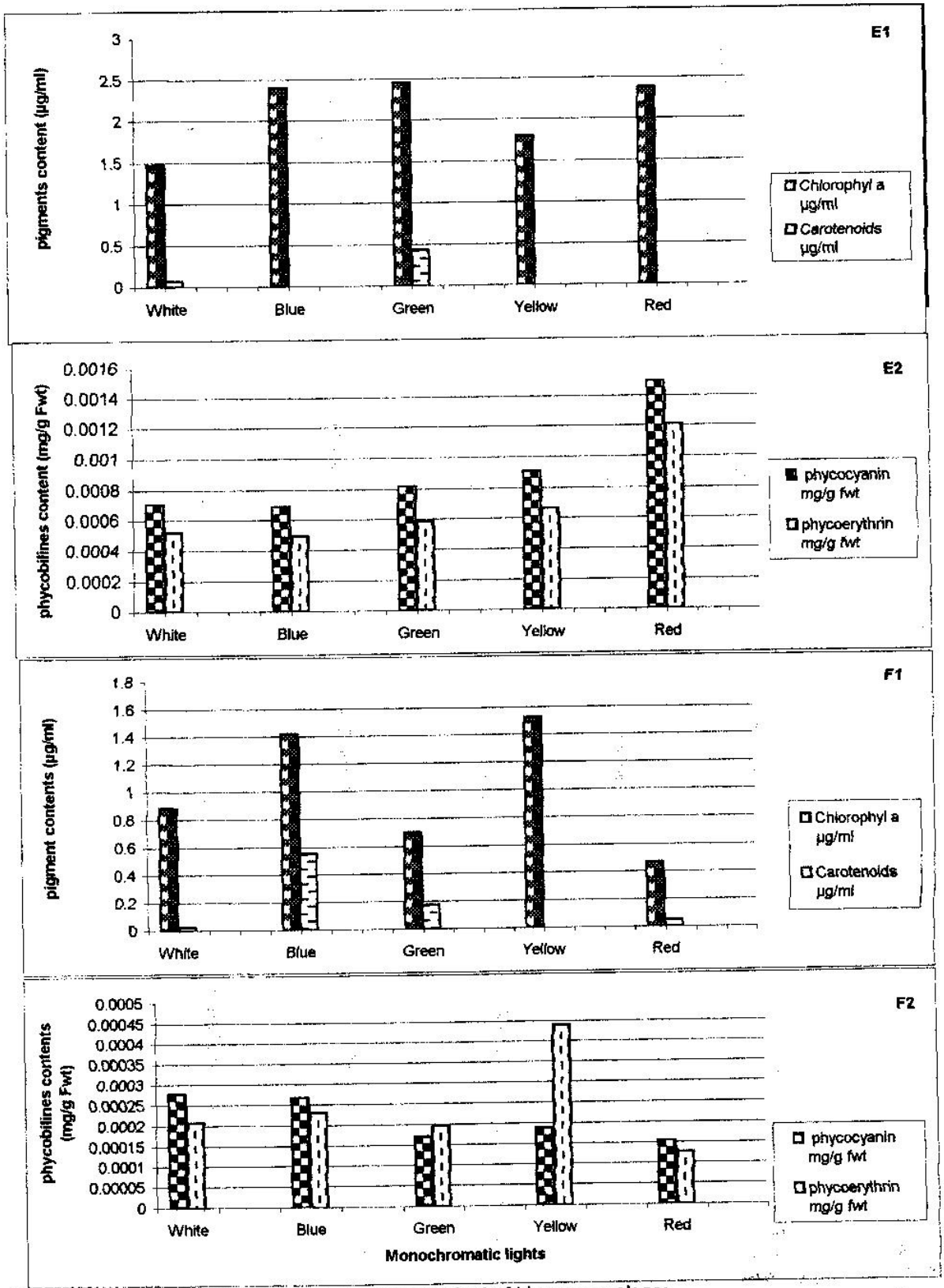

Fig (6): Effect of monochromatic light on pigments content of blue-green algae:
E1-E2-*Microcystis aeruginosa;*
F1-F2-*Aphanothece caldariorum.*

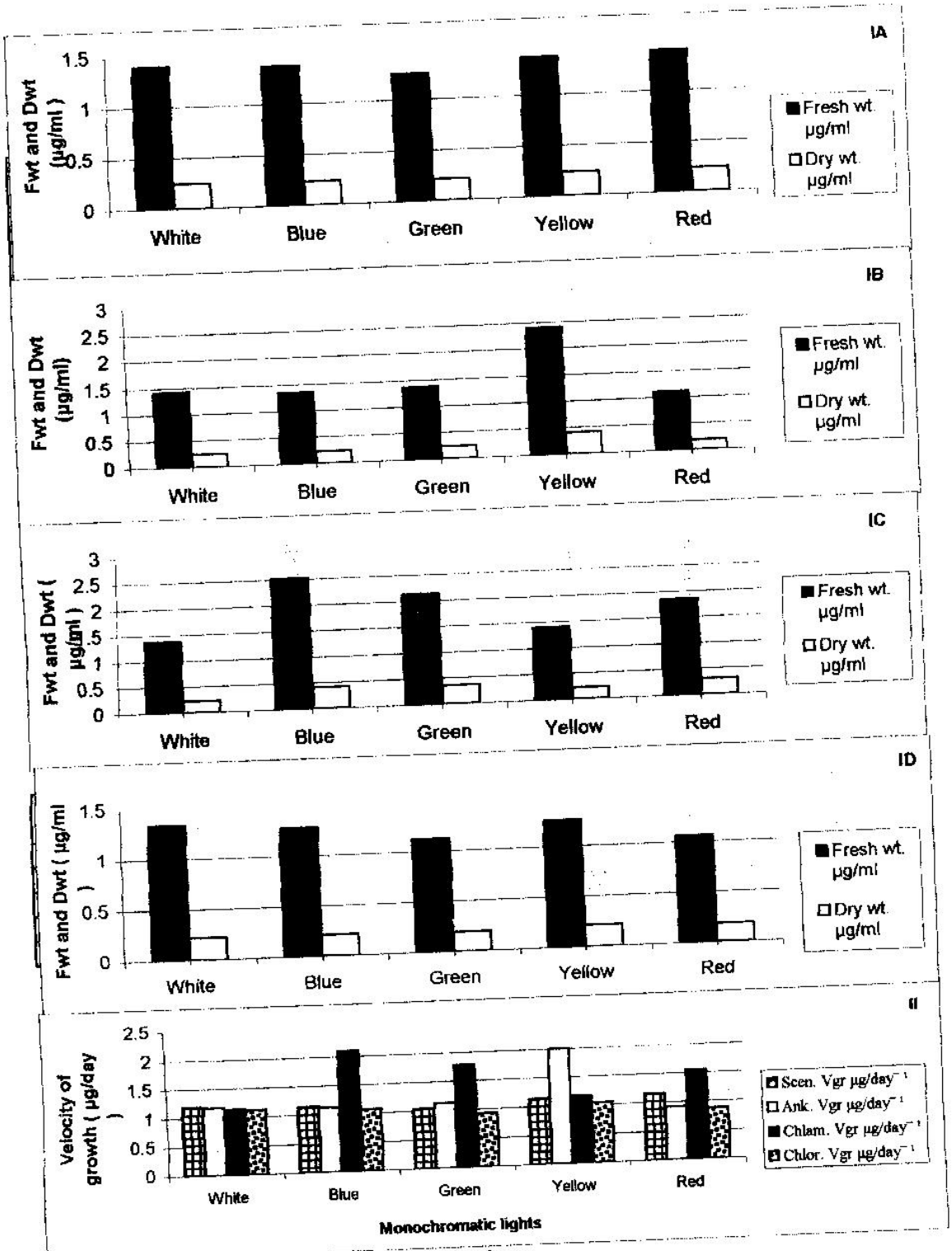

Fig (7): Effect of monochromatic light on green algae:
I-Fresh weight and Dry weight (µg/ml) of:
IA-Scenedesmus obliquus; IB-Ankistrodesmus falcatus;
IC-Chlamydomonas debaryana ; ID-Chlorella vulgaris .
II-Velocity of growth (µg/day).

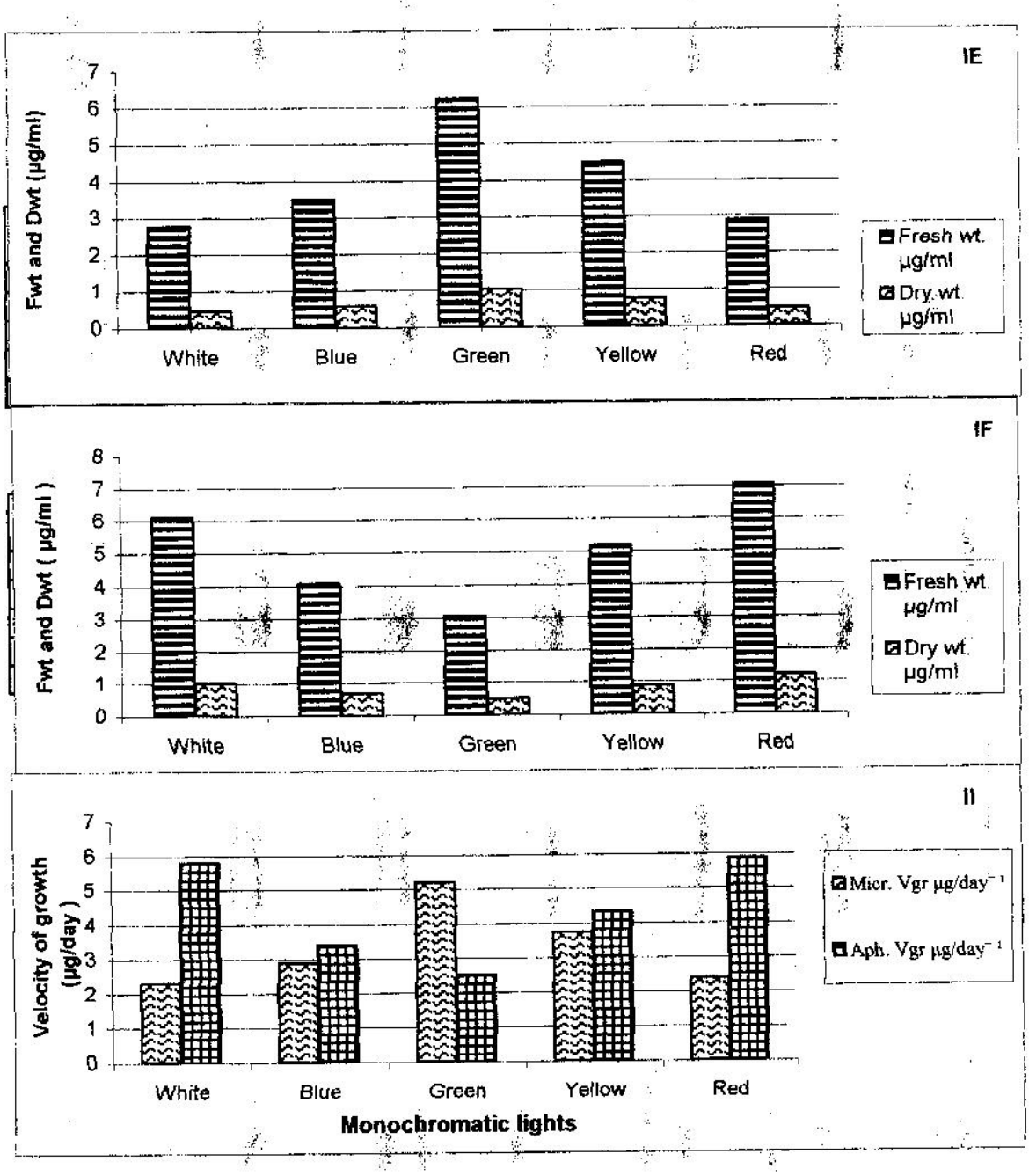

Fig (8): Effect of monochromatic light on blue-green algae:
I-Fresh weight and Dry weight (µg/ml) of:
IE-*Microcystis aeruginosa*; IF-*Aphanothece caldariorum*.
II-Velocity of growth (µg/day).

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تأثير الضوء على نمو و تكوين الأصباغ لبعض طحالب الماء العذب الخضراء و الخضراء المزرقة

للدكتورة

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يعتبر الضوء من حيث شدته و نوعيته من اكثر العوامل المؤثرة على نمو الطحالب التي تعيش في جميع البيئات المائية .

تم دراسة شده الضوء (6 – 40 µmol / m² / s) وأطياف الضوء المرئي كالأزرق ، الأخضر ، الأصفر والأحمر على إنتاج الأصباغ الأساسية في عملية البناء الضوئي والأصباغ المساعدة الفيكوبيلينية ، وتأثيرها على النمو (وزن طري ، وزن جاف ومعدل النمو) لبعض طحالب الماء العذب الخضراء والخضراء المزرقة .

أوضحت النتائج التي تم الحصول عليها أن إنتاج الأصباغ ومعدلات النمو تزيد بتأثير شدة الضوء المنخفضة و المتوسطة (6 – 19 µmol / m² / s.) على كل من الطحالب الخضراء والخضراء المزرقة المستخدم في هذه الدراسة ، بينما شدة الضوء المرتفعة (25 – 40 µmol / m² / s.) تسبب الهدم الضوئي للكلوروفيل وإنتاج كاروتينات غير نشطة بنائيا .

أما من حيث تأثير الضوء الملون ، فقد وجد أن الأطياف الضوئية قصيرة الطول الموجي كالأزرق و الأخضر و الأصفر تشجع إنتاج الأصباغ و زيادة النمو بينما الأطياف الضوئية طويلة الطول الموجي كالأحمر فقد ثبت عدم فاعليتها مع جميع الطحالب المستخدمة باستثناء طحلبي ***Chlorella*** (الأخضر) و ***Aphanothece*** (الأخضر المزرق) حيث ظهرت فاعليتها في تشجيع إنتاج الأصباغ و في زيادة النمو.

وقد نتساءل عن نوعية المستقبلات الضوئية المتحكمة في هذه الاستجابات المورفوجينية. الأمر الذي يحتاج لدراسات تالية .