EFFECT OF ULTRAVIOLET RADIATION (UVR) ON THE TROPICAL MICROALGA *Chlorella vulgaris*

[1]Wong C.Y.*, [2]Teoh M.L., [3]Phang S.M. and [4]Chu W.L.

[1]National Antarctic Research Centre, University of Malaya, 50603 Kuala Lumpur, Malaysia; [2,3] Institute of Ocean and Earth Sciences, University of Malaya 50603 Kuala Lumpur, Malaysia; [4] International Medical University, Plaza Komanwel, Bukit Jalil 57000, Kuala Lumpur, Malaysia.
*chiew_yen@um.edu.my (corresponding author)

ABSTRACT The effect of ultraviolet radiation (UVR) on the growth, lipid content and fatty acid profile of the tropical microalga *Chlorella vulgaris* UMACC 001 was investigated under laboratory and natural UVR conditions. The *Chlorella* was exposed to 10 days of PAR+UVA+UVB (1.17 Wm^{-2}), PAR+UVA (8.54 Wm^{-2}) and PAR alone (42 $\mu molm^{-2}s^{-1}$) in the laboratory study. The natural UVR study was carried out by exposing the cultures to the natural environment on the roof-top of the Institute of Graduate Studies Building, University of Malaya for 54 hours. The average levels of UVA radiation, UVB radiation and PAR irradiance over the exposure period ranged from 3.66 to 27.95 Wm^{-2}, 1.61 to 16.50 Wm^{-2} and 282 to 1480 $\mu mol\ m^{-2}s^{-1}$, respectively. UVA radiation did not affect the growth of *Chlorella vulgaris* UMACC 001 in both the laboratory and natural UVR studies. In contrast, growth was adversely affected by UVB radiation in the laboratory study. UVB radiation is known to decrease the stability of D1 protein (the herbicide binding protein of chloroplasts) of the photosystem II reaction centre (PSII), affect rubisco activity, pigment composition as well as generate superoxide dismutase, all of which would reduce photosynthesis and growth. No significant difference was observed in the lipid content of the cultures exposed to UVR compared to PAR alone in both laboratory and roof-top (natural UVR) cultures. *Chlorella vulgaris* UMACC 001 exhibited different response in fatty acid profiles under laboratory condition compared to roof-top (natural UVR) cultures. In the laboratory condition, more saturated fatty acids (SFA) were produced in the cultures exposed to laboratory-produced UVB radiation compared to PAR alone, while more polyunsaturated fatty acids (PUFAs) were produced in the cultures exposed to natural UVB radiation compared to PAR alone.

Abstrak Kesan radiasi ultraungu ke atas pertumbuhan, kandungan lemak dan profil asid lemak bagi mikroalga tropika *Chlorella vulgaris* UMACC 001 telah disiasat dalam keadaan makmal dan semulajadi. *Chlorella* tersebut didedah kepada PAR+UVA+UVB (1.17 Wm^{-2}), PAR+UVA (8.54 Wm^{-2}) dan PAR persendirian (42 $mol\ m^{-2}s^{-1}$) selama 10 hari dalam kajian makmal. Kajian semulajadi radiasi ultraungu dijalankan dengan mendedahkan kultur tersebut kepada persekitaran semulajadi di atas bumbung bangunan Institut Penggajian Pascasiswazah, Universiti Malaya selama 54 jam. Paras purata radiasi UVA, UVB dan PAR dalam tempoh pendedahan radiasi ultraungu semulajadi adalah 3.66 hingga 27.95 Wm^{-2}, 1.61 hingga 16.50 Wm^{-2} dan 282 hingga 1480 $\mu mol\ m^{-2}s^{-1}$, masing-masing. Tiada kesan radiasi UVA ke atas tumbesaran *Chlorella vulgaris* UMACC 001 dalam kedua-dua eksperimen makmal dan semulajadi. Sebaliknya tumbesaran mikroalga tersebut menurun dengan ketara apabila didedahkan kepada radiasi UVB di dalam makmal. Diketahui bahawa radiasi UVB mengurangkan kestabilan protein D1 dalam fotosistem II (PSII), mempengaruhi aktiviti rubisco, komposisi pigment dan menghasilkan radikal superoxide yang mana semua akan mengurangkan fotosintesis dan tumbesaran. Tiada perbezaan ketara diperhatikan dalam kandungan lemak bagi kultur yang didedahkan kepada radiasi ultraungu berbanding dengan PAR persendirian dalam kedua-dua kajian makmal dan semulajadi. *Chlorella vulgaris* UMACC 001 menunjukkan tindak balas yang berbeza dalam profil asid lemak apabila kultur tersebut didedahkan kepada radiasi ultraungu dalam keadaan makmal dan semulajadi. Dalam keadaan makmal, lebih banyak asid lemak tepu (SFA) dihasilkan dalam kultur yang didedahkan kepada radiasi UVB berbanding dengan PAR persendirian, tetapi lebih banyak asid lemak poli tidak tepu (PUFAs) dihasilkan dalam kultur yang didedahkan kepada radiasi ultraungu-B semulajadi berbanding dengan PAR persendirian di atas bumbung (radiasi ultraungu semulajadi).

INTRODUCTION

One of the seriously debated environmental issues among scientists is the depletion of the ozone layer which causes an increase of UVB radiation reaching the Earth's surface. The ozone layer is vital to organisms on Earth as it protects organisms from direct ultraviolet radiation (UVR). The amount of ozone that shields the Earth's surface from UVR has been reported to have decreased by an average of 3% globally over the last decade and the ozone hole increased steadily [1,2]. Ozone depletion is expected to increase and spread over a broader range of altitudes and latitudes throughout most of the current century [3]. It is predicted to increase in severity and duration in the coming decades and continue until at least 2020 [4,5] although the growth in CFC levels is decreasing in response to the Montreal Protocol [6]. The most severe depletion is predicted to occur during 2010 - 2019 [5,7].

UVA (320-400nm) and UVB (280-320) radiation are the two major forms of UVR well known to have deleterious effects on many organisms on the Earth. The increase of UVR has been reported to affect growth, photosynthesis, biochemical composition, nutrient uptake, reproduction and many other physiological processes as well as causes damage to DNA of microalgae which populate terrestrial and aquatic ecosystems [8-13]. Furthermore, UVR may cause nonspecific damage by generating highly reactive oxidants such as singlet O_2, superoxide (O_2^-), hydroxyl radicals and hydrogen peroxide [14]. These highly reactive oxidants may lead to pigment bleaching and the destruction of the cellular components [15]. The nutritional value of algae may be affected by UVR stress as shown by the decreased levels of omega-3 fatty acids in *Pavlova lutheri* and *Isochrysis galbana* [16] and diatoms [10, 11]. There are increasing concerns about the deleterious effects of UVR on algae because any changes to the size and composition of algae communities will directly affect food production from the marine ecosystem [17,18].

Studies showed that UVB radiation induced impairment of photosynthesis by decreasing the stability of D1 protein of the PSII reaction centre, triggering increased degradation of this protein [19,20], and contribute to a lower photosynthetic efficiency [21,22]. The net loss of D1 pools due to increased UVB radiation has been studied in temperate and tropical natural phytoplankton assemblages [23]. Besides that, UVR may affect rubisco activity [24], pigment composition [25], carbon fixation [26], maximum quantum yield (F_v/F_m) and also the electron transport rate (ERTmax) [27-30] or induce structural alterations of thylakoid membranes [31] as well as reduced expression of genes involved in photosynthesis [32] thus resulting in the inhibition of photosynthesis and growth in algae. The PSII of the cold ocean diatoms *Pseudonitzcshia seriata* and *Nitzchia* sp. was affected when exposed to UVB radiation, with the decrease of F_v/F_m ratio by 27% compared to non-UVB exposed cultures [29]. A 10 to 25% loss in photosynthetic activity was observed in *Isochrysis galbana* and *Dicrateria inornata* grown under UVB radiation [33].

Microalgae have developed several strategies to cope with potentially harmful ambient UVR. One defense strategy is by avoidance, through migration to deeper water columns [34]. Another form of protection is achieved by synthesising or accumulating a series of photoprotective compounds such as mycosporine-like amino acids (MAAs) or antioxidant compounds that directly or indirectly absorb the energy of the solar radiation [14,35,36]. Fatty acids especially saturated fatty acid (SFA) could be an adaptation strategy in stress response because this fatty acid actually serves as an energy source for stress adaptation. It was found that the percentage of SFA in microalgae from Antarctic, tropical and temperate regions increased in response to UVB stress [10,11].

Microalgae are important primary producers which dominate terrestrial and aquatic ecosystems. They form the basis of the food web and account for up to 60% of the total global oxygen production and about 50% of the global carbon dioxide fixation [33,37]. The microalga *Chlorella* is one of the best-studied phototrophic eukaryotes. *Chlorella* is a small coccoid green alga with cosmopolitan occurrence. In recent years, the use of *Chlorella* in life science research has received increasing attention. This chlorophyte has potential applications as dietary supplements in human nutrition which are able to provide additional physiological and pharmacological benefits for human health [38]. *Chlorella* strains are also being used for a variety

of applications in biotechnology such as production of antioxidative compounds [39], waste water treatment [40] and as feedstock for biofuel production [41].

The aim of the present study is to compare the effect of UVR on the growth, lipid content and fatty acid profiles of tropical *Chlorella vulgaris* UMACC 001 grown under laboratory and field conditions. The findings will be useful in providing reference data for future assessment of possible changes in environmental conditions especially increasing levels of UVR on tropical microalgae.

MATERIAL AND METHODS

Algae culture
Chlorella vulgaris UMACC 001 used in the present study was obtained from the University of Malaya Algae Culture Collection (UMACC) [42] (Figure 1). It was isolated from a pond at the experimental farm of the University of Malaya. The cultures were grown in Bold's Basal Medium [BBM; 43] and maintained in a controlled-environment incubator at 28°C, with cool white fluorescent lamps (42μmol $m^{-2}s^{-1}$) on a 12:12h light-dark cycle.
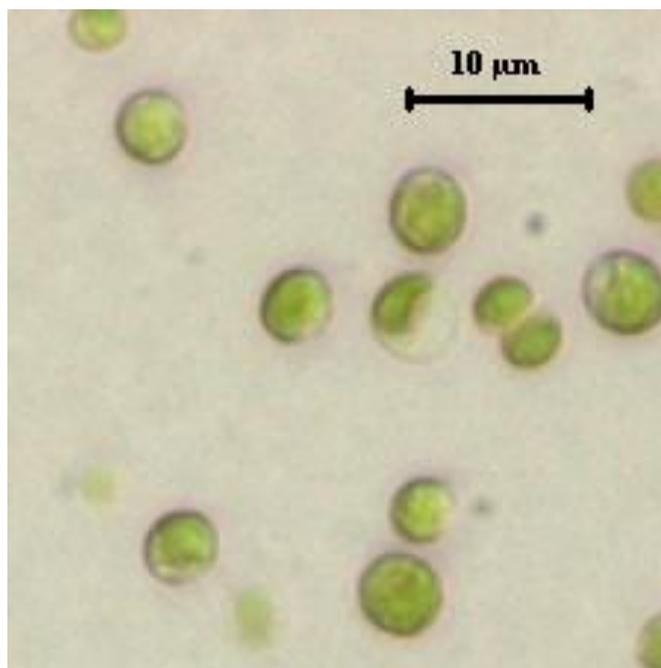

Figure 1: *Chlorella vulgaris* UMACC 001

Experimental design
For the laboratory study, the culture was exposed to three light treatments: PAR+UVA+UVB, PAR+UVA and PAR alone in the incubator set at 28°C with a 12h:12h light dark cycle for 10 days. The cultures were irradiated with a combination of three types of lamps: two tubes of day light fluorescent lamps providing 42 μmol $m^{-2}s^{-1}$ of photosynthetically active radiation (PAR), one tube of UVB lamp (38W, Phillip) providing irradiance of 1.17 Wm^{-2} and two tubes of UVA lamps (18W, Phillip) providing irradiance of 8.54 Wm^{-2}. For the natural UVR study, the cultures were exposed to the natural environment on the roof-top of the Institute of Graduate Studies Building, University of Malaya for 54h. An inoculum size of 20% standardized

at an optical density at 620nm (OD_{620nm}) of 0.2 from exponential phase cultures was used. The cultures were grown in triplicate in beakers and Whirl-Pack^R bags for laboratory and field study, respectively. Various cut-off filters were used to obtain the different UVR treatments. For the PAR alone, the culture was covered with polycarbonate sheet to eliminate UVA and UVB radiation. To obtain the PAR+UVA treatment, Mylar sheet was used to cut off the UVB radiation. The culture receiving PAR+UVA+UVB was covered with Whirl-Pack^R bag to allow light spectrum above 280nm to pass through.

Meteorological Data
Selected meteorological parameters were

monitored during the natural UVR experiment on the roof-top of the Institute of Graduate Studies, University of Malaya. Temperature and UVR were measured using thermometer and UV radiometer, respectively. The PAR was measured as irradiance using Pan Lux meter and was converted to mol $m^{-2}s^{-1}$ following equation below [44]:

$$10,000 \text{ Lux} = 0.1345 \text{ mol } m^{-2}s^{-1}$$

Growth studies

Growth was monitored by measuring the OD_{620nm} and determining the chlorophyll *a* concentration. Chlorophyll *a* was determined by spectrophotometry after extraction of the filtered samples (glass-fibre filters, 0.45μm) in acetone [45]. Specific growth rate (μ) (d^{-1}) was determined using the following formula:

$$\mu = \frac{Ln\ N_1 - Ln\ N_2}{t_1 - t_2}$$

Where, N_1 and N_2 represent the chl-*a* concentrations at times t_1 and t_2, respectively, within the whole experiment period. The cells were harvested at the end of the experiment by filtration for dry weight determination (100°C,

24h) and extraction of lipids.

Lipid extraction and fatty acid analysis

Lipids were extracted in methanol-chloroform-water (2:1:0.8) before being determined by the gravimetric method [46]. The lipids were transesterified in 1% H_2SO_4 in methanol and the fatty acid methyl esters were analysed by gas chromatography [47].

Statistical analysis

One-way analysis of variance (ANOVA) was used to determined whether there was any significant difference between the treatments used at P>0.05 followed by comparison of means using Newman-Keuls Multiple Range test. All statistical analyses were performed using the statistical software Statistical Version 6.0.

RESULTS

The intensities of UVA radiation, UVB radiation and PAR from 25th to 27th January 2006 recorded in the roof-top (natural) UVR study ranged from 3.66 to 27.95 Wm^{-2}, 1.61 to 16.50 Wm^{-2} and 282 to 1480 μmol $m^{-2}s^{-1}$, respectively (Figure 2). The water temperature during the roof-top (natural) UVR experiment was difficult to control and the temperatures ranged from 26.6 to 39.6°C. On the other hand, the air temperature ranged from 31.8 to 41.5°C (Figure 3).
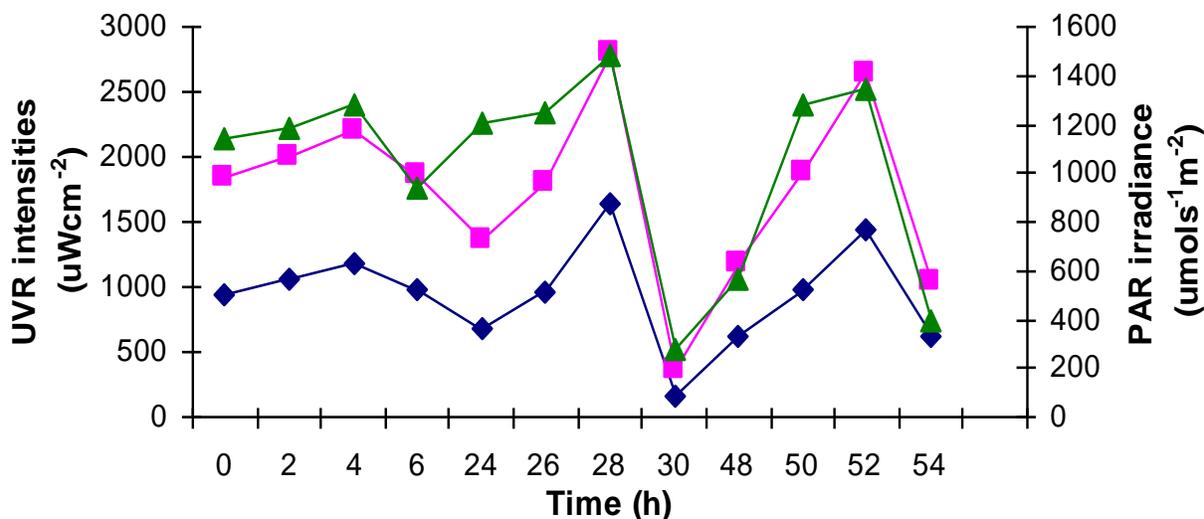

Figure 2: UVR and PAR irradiance measured during the roof-top (natural UVR) study at Institute of Graduate Studies Building, University of Malaya . ♦ UVB radiation; ■ UVA radiation; ▲ PAR
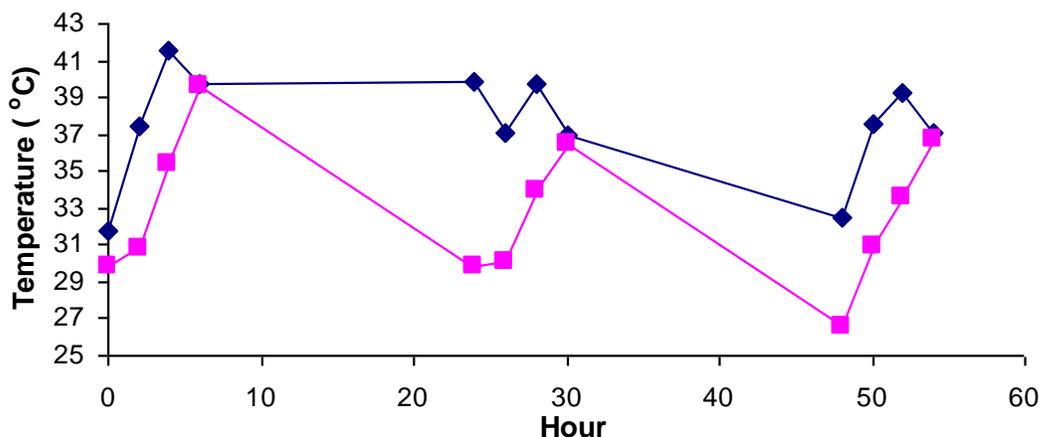
Temperature

Figure 3 : Variations of temperature during the roof top (natural UVR) study at Institute of Graduate Studies Building, University of Malaya. ♦ air temperature; ■ water temperature

Similar growth trends were observed in *Chorella vulgaris* UMACC 001 subjected to PAR+UVA and PAR alone in the laboratory study as shown in Figure 4. The μ value for *Chlorella vulgaris* UMACC 001 exposed to PAR+UVA did not differ significantly (P>0.05) compared to PAR alone. In contrast, the cultures exposed to PAR+UVA+UVB did not grow well compared to PAR alone as indicated by their lower μ value compared to PAR alone (P<0.05) (Figure 5). Similarly, the final chlorophyll *a* concentration at day 10 of the culture exposed to PAR+UVA+UVB (0.31 mgL^{-1}) was very low compared to the culture exposed to PAR alone (1.19 mgL^{-1}). However, there was no significant difference in the chlorophyll *a* concentration for the cultures subjected to different UVR treatments at day 2 (P>0.05). Similar result for chlorophyll *a* concentration was observed in the cultures exposed to the natural UVR at day 2. In addition, there was no significant difference in the μ values of the cultures exposed to PAR+UVA+UVB, PAR+UVA and PAR alone in the natural environment (P>0.05).

Lipid content decreased in cells exposed to PAR+UVA compared to PAR alone while there was no significant difference in cells exposed to PAR+UVA+UVB compared to PAR alone in the laboratory study (Figure 6). On the other hand, the lipid content decreased in cells exposed to PAR+UVA+UVB compared to PAR alone in the roof-top (natural UVR) study. However, the effect of UVB radiation was not significant compared to PAR alone. There was also no significant difference (P>0.05) in lipid content for the cultures exposed to

PAR+UVA and PAR alone in the natural environment.

Fatty acid profiles of *Chlorella vulgaris* UMACC 001 in the laboratory and roof-top (natural UVR) studies are shown in Table 1. Saturated Fatty Acids (SFA) were dominant in the control in both laboratory and roof-top (natural UVR) studies. However, its content decreased when the cultures were exposed to PAR+UVA and increased under PAR+UVA+UVB exposure in laboratory condition. The reverse trend was found in the content of polyunsaturated fatty acids (PUFA). Different response was observed for the cultures exposed to natural UVR in the roof-top study. The content of SFA was slightly lower in the cultures exposed to PAR+UVA and PAR+UVA+UVB compared to PAR alone. In contrast, the contents of MUFA and PUFA were significantly increased (P<0.05) when the cultures were exposed to natural PAR+UVA and PAR+UVA+UVB compared to PAR alone.

DISCUSSION

The levels of UVR decrease with increasing latitude, being highest in the tropics and lowest in the polar region [48]. It is because of the high solar angle and a relatively low amount of stratospheric ozone near the equator [48,49]. The intensities of UVA radiation, UVB radiation and PAR irradiance measured during the roof-top (natural UVR) study in January 2006 were 3.66 to 27.95 Wm^{-2}, 1.61 to 16.50 Wm^{-2} and 282 to 1480 μmol m^{-2}s^{-1}, respectively. The measurements conducted across Argentina showed
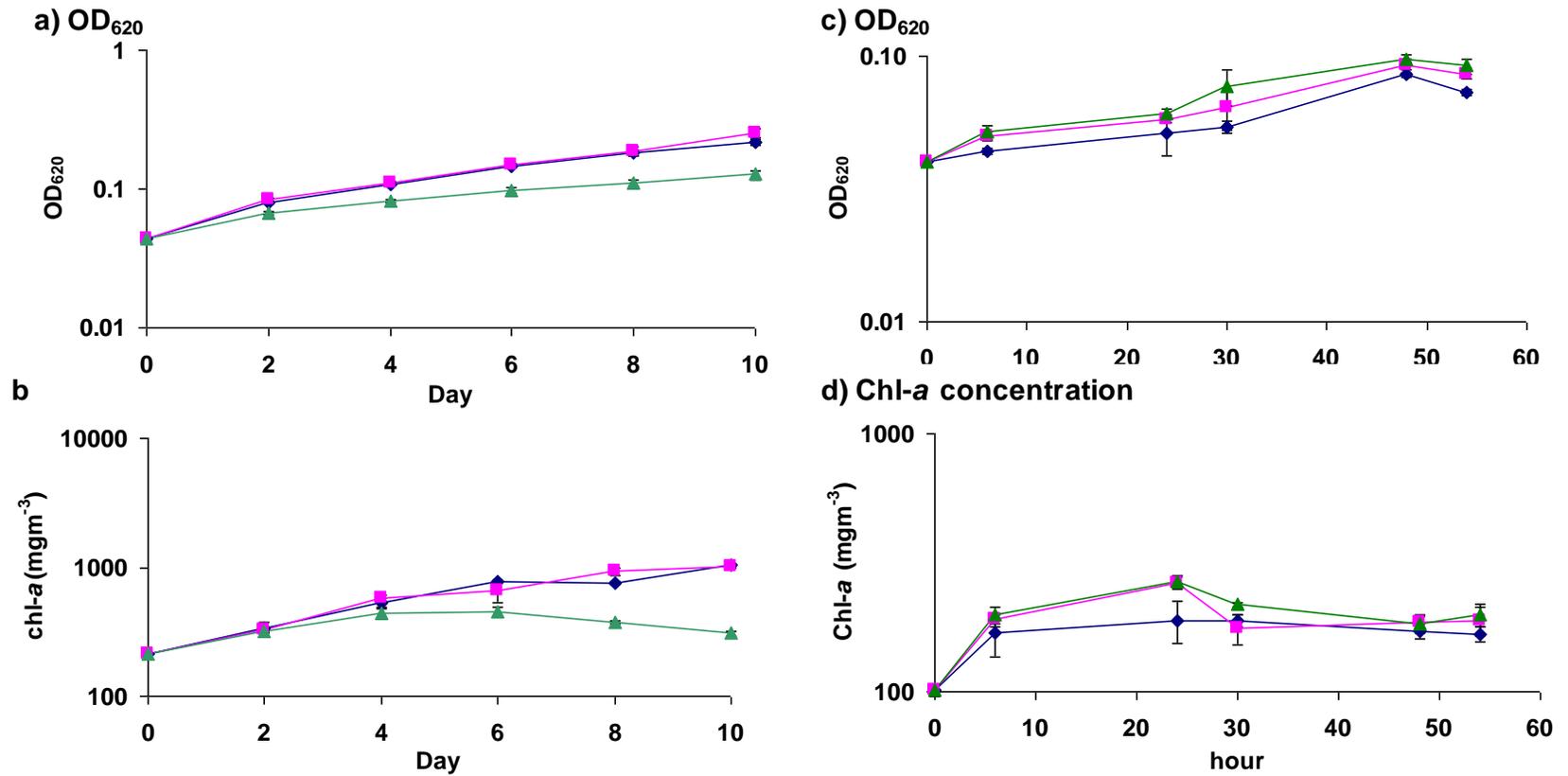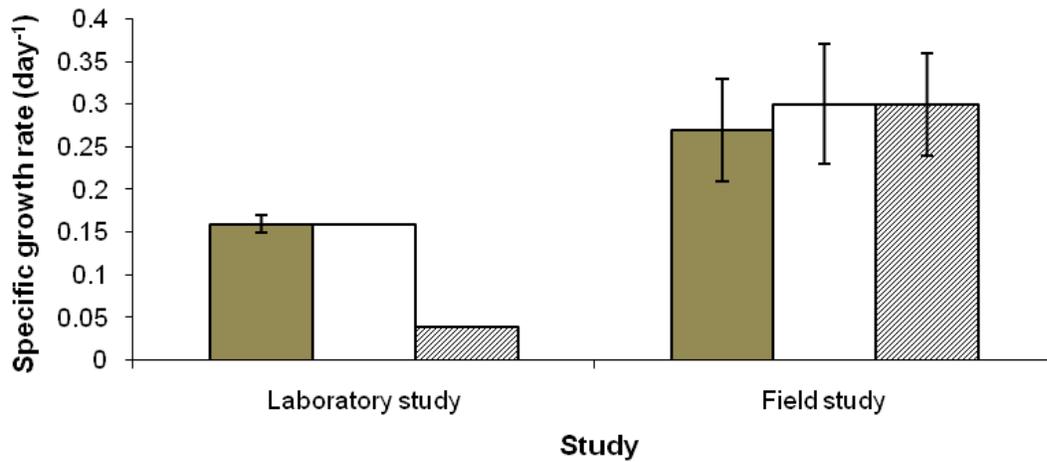
Figure 4: Semi-logarithmic growth curves based on chl-*a* concentration of *Chlorella vulgaris* UMACC 001 exposed to UVR and PAR alone under laboratory (a, b) and roof-top (natural UVR) (c, d) conditions. Vertical bars denote standard deviations from triplicate samples. ♦ PAR alone; ■ PAR+UVA; ▲ PAR+UVA+UVB

a) Specific growth rate
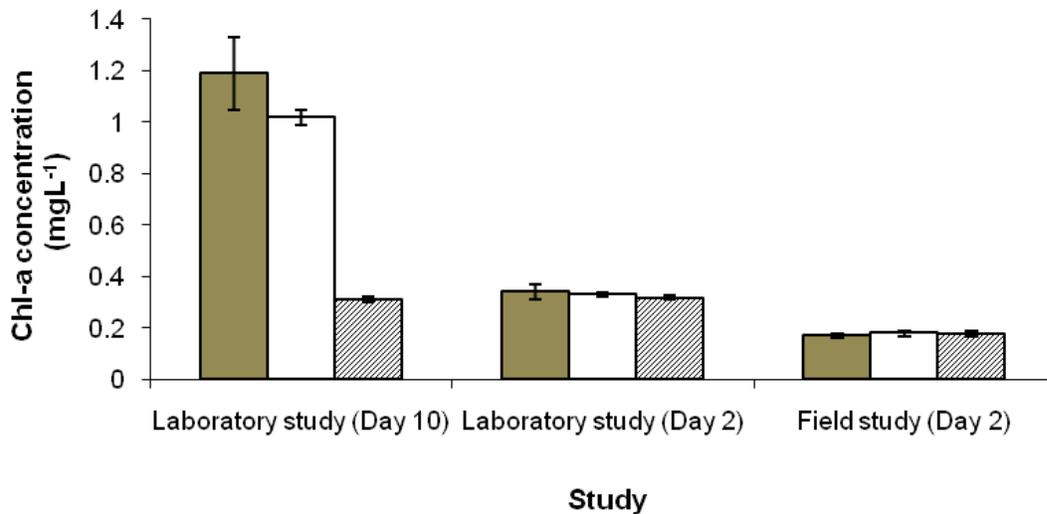

b) Chl-*a* concentration

Figure 5: Specific growth rates (a) and chl-*a* concentration (b) of *Chlorella vulgaris* UMACC 001 exposed to UVR and PAR alone under laboratory and roof-top (natural UVR) conditions. Vertical bars denote standard deviations from triplicate samples. ▨ PAR alone; ▢ PAR+UVA; ▨ PAR+UVA+UVB

that the noontime levels of UVB radiation decreased from 4.46 Wm^{-2} at Jujuy (tropics) to 3.99 Wm^{-2} at Buenos Aires (temperate) and to 2.61 Wm^{-2} at Ushuaia (sub-Antarctic) [50]. Whereas under clear skies at temperate to equatorial latitudes, total UVB radiation maybe as high as 7 to 8 Wm^{-2} and UVA radiation as high as 45 to 50 Wm^{-2}, respectively [25]. On the other hand, the water temperature during the

field experiment was difficult to control and there was great fluctuation in air temperature. This was due to the unstable weather during the field experiment.

Chlorella vulgaris UMACC 001 showed similar response to UVA radiation under both laboratory and roof-top (natural UVR) studies. The present study

Table 1: Fatty acid profiles of *Chlorella vulgaris* UMACC 001 exposed to UVR and PAR alone in laboratory and roof-top (natural UVR) condition.

Fatty acid	Laboratory study			Field study		
	PAR	PAR+UVA	PAR+UVA+UVB	PAR	PAR+UVA	PAR+UVA+UVB
Saturated fatty acids						
14:0	2.0	2.0	3.2	4.4	5.4	4.3
16:0	27.3	26.0	33.0	28.9	26.7	27.4
18:0	25.5	18.4	25.8	45.9	29.9	31.3
Total	54.8	46.4	62.0	79.2	62.0	63.0
Monounsaturated fatty acids						
16:1	5.3	5.8	3.7	2.3	3.4	3.9
18:1	2.6	2.9	4.9	1.0	3.3	2.6
Total	7.9	8.7	8.6	3.3	6.7	6.5
Polyunsaturated fatty acids						
16:2	3.4	4.3	1.1	0.3	1.1	1.8
16:3	8.0	9.3	6.8	3.8	7.0	6.6
18:2	8.4	10.6	5.0	4.8	7.8	7.4
18:3	17.5	20.7	16.5	8.6	15.3	14.7
Total	37.3	44.9	29.4	17.5	31.3	30.5

Data as mean, percentage of total fatty acids, *n*=3

showed that there was no negative effect of UVA radiation on growth. Positive effects of UVA radiation have been extensively reported. For example, Wong et al [10,11] reported that there was no adverse effect of UVA radiation on the growth of Antarctic, tropical and temperate microalgae. UVA radiation has been proved to favor photosynthesis in some selected phytoplankton [34, 35]. In addition, the presence of UVA radiation has resulted in higher biomass production in *Arthrospira platensis* compared to those grown under PAR alone [53].
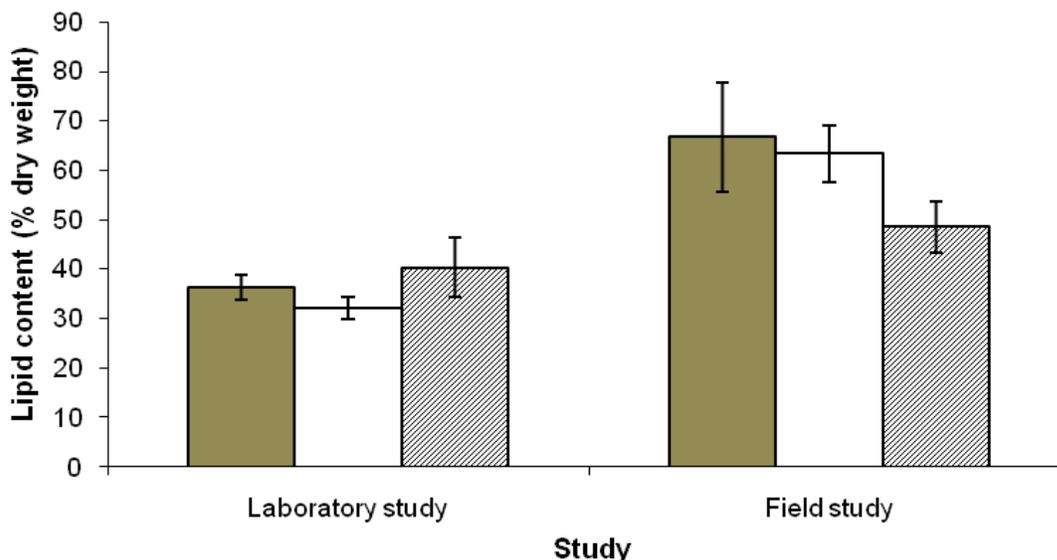
Lipid content

Figure 6: Lipid content (% dry weight) of *Chlorella vulgaris* UMACC 001 exposed to UVR and PAR alone in laboratory and roof-top (natural UVR) condition. Vertical bars denote standard deviations from triplicate samples. ▨ PAR alone; ☐ PAR+UVA; ▨ PAR+UVA+UVB

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On the other hand, the growth of *Chlorella vulgaris* UMACC 001 was adversely affected by UVB radiation in the laboratory study as shown by the significant decrease of μ value and final chlorophyll *a* concentration at day 10 compared to PAR alone. However, there was no adverse effect of UVB radiation in the roof-top (natural UVR) study. It could be explained that the duration of the experiment (two days) was too short for UVB radiation to impose significant effect on the growth of this microalga, as there was also no significant effect of UVB radiation on chlorophyll *a* concentration at day 2 in the laboratory study. However, a reduction of chlorophyll *a* was observed in *Chlorella fusca* after being exposed to UVB fluences of 1230 Jm^{-1}day^{-1} for 5h/day for 2 days [54]. The difference in response compared to the present

study could be due the different UVB radiation intensity used.

The adverse effect of UVB radiation on growth has been reported in Antarctic *Chlorella* UMACC 237 and temperate *Chlorella vulgaris* UMACC 248 [10,11]. In addition, Malanga and Puntarulo [55] also reported that growth expressed as chlorophyll content, declined significantly with increased UVB dose in *Chlorella vulgaris*. The reduction in the concentration of chlorophyll *a,* which functions as a photosynthetic pigment observed after 10 days of UVB radiation exposure suggest that UVB radiation may reduce the photosynthetic capacity of this microalga. According to Buma et al [56] and Estevez et al [14], the adverse effect of UVB could be due to its influence on photosynthesis and the generation of oxygen radicals which may damage molecules such as proteins and DNA.

The present study showed that there was no significant effect of UVB radiation on lipid content in *Chlorella vulgaris* UMACC 001 in both laboratory and roof-top (natural UVR) studies although the reduction of lipid content was observed in the cultures exposed to UVB radiation in roof-top (natural UVR) study. An increase in lipid content has been reported in Antarctic *Chlorella* UMACC 237 after exposure to UVB radiation compared to PAR

alone [10]. However, decrease in lipid content has been observed in Antarctic microalgae such as *Klebsormidium* UMACC 227 and *Navicula* UMACC 231 exposed to 5.15 Wm^{-2} of UVB radiation [10] and *Odontella weissflogii* [57]. The decrease of lipid content under UVB exposure may indicate the degradation of complex lipid. The different response of this tropical *Chlorella vulgaris* UMACC 001 in the present study compared to other studies suggest that *Chlorella vulgaris* UMACC 001 might be using different adaptive mechanisms involving molecules other than lipids to cope with the UVB stress.

Chlorella vulgaris UMACC 001 exhibited different response in terms of fatty acid profiles when exposed UVB radiation in the laboratory and roof-top (natural UVR) studies. There was an increase in the SFA at the expense of PUFA when the microalga was exposed to UVB radiation in the laboratory study and it was in agreement with most of the past studies. For example, the percentage of PUFA in Antarctic *Chlorella* [10] and *Chaetoceros simplex* [57] decreased while SFA increased significantly when exposed to a high intensity of UVB radiation. SFA has been reported to serve as energy source for stress adaptation while PUFAs are vital constituents of the cell wall and are essential for chlorophyll membrane development as well as important dietary function in the food web [58,59]. Decrease in the proportion of PUFA within the cell wall membranes of phytoplankton can reduce membrane permeability and therefore weaken the ability of phytoplankton to assimilate nutrients important for growth [60,61].

On the other hand, a reverse trend of fatty acid profile was found when the *Chlorella vulgaris* UMACC 001 was exposed to natural UVB radiation in roof-top (natural UVR) study where there was a significant increase in PUFA at the expense of SFA. The response of this microalga to UVB radiation in the roof-top (natural UVR) study suggested that biosynthesis of PUFA might be enhanced and SFA formation was suppressed when exposed to solar UVB radiation for two days. This agrees with the findings of Liang et al [28] who showed that the exposure of UVR radiation for two days resulted in an increase of PUFA at the expense of SFA in both *Phaeodactylum tricornutum* and *Chaetoceros muelleri*. The difference observed in fatty acid profiles of *Chlorella vulgaris* UMACC 001 in the laboratory and roof-top (natural UVR) studies could be related to the different UVR exposure time because the laboratory was studied for ten days while the roof-top (natural UVR) study lasted only two days. The increases of MUFA and PUFA in the two-days roof-top (natural UVR) study indicate that these fatty acids might serve as an immediate adaptive mechanism to short-term UVB exposure.

In conclusion, laboratory-produced UVA and natural UVA radiation (roof-top) has no effect on the growth of *Chlorella vulgaris* UMACC 001 based on chl-*a* concentration. In contrast, the growth of this microalga was adversely affected by UVB radiation exposure for 10 days under laboratory culture condition. The biochemical properties especially the fatty acid profiles of the microalga changed from laboratory culture to roof-top (natural UVR) culture condition. The response of *Chlorella vulgaris* UMACC 001 to UVR was dependent on culture location (laboratory, roof-top) and duration of UVR exposure. As microalgae form the basis in many food-webs, any change in fatty acid composition may affect the nutritional value of the microalgae for organisms at higher trophic levels in the ecosystem.

ACKNOWLEDGEMENTS

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