Algal Research 83 (2024) 103736

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

UV-B light (radiation) affects the metabolism of pigments and fatty acids in green algae *Edaphochlorella mirabilis* and *Klebsormidium flaccidum in vitro*

Giulia Santunione [a], Francesca Masino [a,b], Giuseppe Montevecchi [a,b], Elisabetta Sgarbi [a,b,*]

[a] *Department of Life Sciences, University of Modena and Reggio Emilia, Via G. Amendola 2 (Padiglione Besta), 42122 Reggio Emilia, Italy*
[b] *BIOGEST – SITEIA Interdepartmental Center, University of Modena and Reggio Emilia, Piazzale Europa 1A, 42124 Reggio Emilia, Italy*

ARTICLE INFO

Keywords:
Algae
Carotenoids
Pigments
Fatty acids
UV-B stress

ABSTRACT

Algae offer a rich source of bioactive compounds suitable for food products and bioenergy. Environmental challenges such as nutrient scarcity, extreme pH and temperature, high light intensity, and UV radiation usually trigger algae to produce excess of lipids, antioxidants, and other bioprotective molecules as part of their adaptations for survival. Algal cultivation provides proteins, lipids, carbohydrates, vitamins, antioxidants, and trace elements. This study focused on understanding how UV-B irradiance, as an abiotic stressor, can influence the growth and metabolite production of two green algal species, *Edaphochlorella mirabilis* (Chlorophyta) and *Klebsormidium flaccidum* (Charophyta). Using a temporary immersion system bioreactor for *in-vitro* algal growth, the results showed no significant difference in biomass for both algal species after the exposure to UV-B rays. However, the assessment of malondialdehyde levels revealed a significantly higher tendency towards lipid peroxidation in treated *E. mirabilis* (+ 90 %) compared to control. Conversely, *K. flaccidum* did not display significant differences, thereby highlighting a more advanced adaptive capacity against UV-B radiation. Overall, both algal species treated with UV-B showed increased pigment accumulation. *K. flaccidum* exhibited an average pigment increase of over 53 %, while *E. mirabilis* showed a lower increase, over 30 % on average. The notable rise in antioxidant compounds (lutein, β-carotene, and chlorophyll *a*) in UV-B exposed *K. flaccidum* samples also suggested a more suitable adaptive strategy to mitigate oxidative stress in Charophyta. In *K. flaccidum*, the increase in polyunsaturated fatty acids can be associated with increased production of antioxidant compounds. Conversely, *E. mirabilis* appeared to protect itself by decreasing polyunsaturated fatty acids in favor of saturated ones. In both algal species, the increase in secondary metabolites under UV stress highlighted potential as a novel food source for human consumption, deserving further investigation.

1. Introduction

A wide range of bioactive molecules and nutrients including proteins, lipids, carbohydrates, vitamins, and trace nutrients are present in algae [1–3]. In recent years, algae have drawn significant attention due to their potential applications in chemical, pharmaceutical, nutraceutical, and food production industries [4–7]. Large-scale cultivation of algae, thanks to their photoautotrophic nature, presents several advantages, such as ease of implementation, climate neutrality, and carbon sequestration capacity, thus making it a promising climate change mitigation strategy [3,8,9]. Furthermore, microalgae exhibit rapid growth rates, doubling in size every 1–2 days, as well as possessing wide phylogenetic diversity, which could lead to a wider array of valuable metabolites that could be used in various biotechnological applications [6,7,10]. Algae also exhibit great adaptability to abiotic stressors, which allow them to thrive in harsh environmental conditions. In addition, they can produce a variety of biomolecules, including carotenoids, phenolic compounds, and polyunsaturated fatty acids (PUFAs), which possess protective and antioxidative properties.

Consequently, the study of algal physiology and growth has attracted increasing interest [11–14]. The demand for algal bioactive molecules is increasing and the global market value for algal biomass has reached billions of dollars. PUFAs and carotenoids, renowned for their antioxidant properties, are particularly sought after in food and nutraceutical industries [2,11,15–17]. Additionally, extraction of antioxidant substances from algae provides the building blocks to produce plant-based

---

* Corresponding author at: Department of Life Sciences, University of Modena and Reggio Emilia, Via G. Amendola 2 (Padiglione Besta), 42122 Reggio Emilia, Italy.

E-mail address: elisabetta.sgarbi@unimore.it (E. Sgarbi).

https://doi.org/10.1016/j.algal.2024.103736
Received 19 February 2024; Received in revised form 1 October 2024; Accepted 2 October 2024

compounds, thus catering for the growing demand for non-animal-derived products.

Algal pigments are grouped into chlorophylls, carotenoids, and phycobiliproteins. Different types of chlorophylls are present in different algal species (chlorophyll *a*, *b*, *c*, *d*, and *f*); despite these pigments all being green, a slight variability in hue can be observed depending on their different molecular structures [18,19]. They can be used as colorants and antioxidants in food and dietary supplements. Carotenoids have a physiological photoprotective role and accumulate after exposure to specific environmental stress, thus playing a specific role in scavenging the reactive oxygen species (ROS) and dissipating excessive energy [20,21]. Over 400 different carotenoid molecules have been identified in phototrophic organisms; among these, β-carotene and astaxanthin are widely commercialized, followed by lutein, zeaxanthin, and lycopene [17,22].

Certain PUFAs derived from algae are considered essential and therefore, have a noteworthy role in animal and human nutrition. Once ingested, PUFAs can be converted into bioactive compounds, which are important for human health: eicosanoids regulate various physiological processes such as inflammation, blood clotting, and blood pressure; resolvins, protectins, and maresins, have been found to have potent anti-inflammatory properties and promote resolution of inflammation and immune regulation; endocannabinoids and lipid compounds affect mood, appetite, pain perception, and cognitive functions by acting on cannabinoid receptors [3,23–25]. Moreover, algal oils are rich in omega-6 and omega-3 essential fatty acids, including docosahexaenoic acid (DHA), which can be used in cosmetic products, such as skin protection formulations [26,27]. Unsaturated fatty acids extracted from microalgae have also been considered as potential candidates for biodiesel production [28].

The growth of algae and their primary and secondary metabolite accumulation can be influenced by cultivation conditions and possible exposure to environmental stress, such as changes in light intensity, temperature, salinity levels, nutrients availability, pH, and exposure to UV-radiation [1,13,15]. UV-B radiation can alter physiological and biochemical algal activities depending on the algal species, UV-B intensity, and exposure time. Recently, a UV-B photoreceptor already known in plants (*Arabidopsis thaliana*) has also been identified in green algae [29].

For all the above reasons, this study took into consideration the metabolic responses to UV-B light exposure of two green algal species: *Edaphochlorella mirabilis* (Chlorophyta Division) and *Klebsormidium flaccidum* (Charophyta Division). UV-B light was applied through an *in vitro* culture photobioreactor system. Both these freshwater algae can grow in terrestrial environments. *Edaphochlorella mirabilis* is currently accepted as a “*Chlorella*” species but a recent study suggested its affiliation to *Prasiola* clade within core Trebouxiophyceans [30]. *Edaphochlorella mirabilis* specifically has spherical cells with a mean diameter of 5 μm and its reproduction occurs *via* autosporulation. Despite literature not providing information on cell structure and ultrastructure, many studies have described the cell organization and cell wall composition of genus *Chlorella*. Generally, Trebouxiophyceans cells are characterized by a multilayered cell wall structure, mainly composed of polysaccharides with a single cup-shaped parietal chloroplast and a pyrenoid matrix crossed by a single double-layered thylakoid [31,32].

Charophyta are characterized by a very simple thallus, either unicellular or filamentous, and are phylogenetically very close to terrestrial plants [33]. This character is physiologically translated into the presence of structures that allow them to cope with abiotic stresses [34]. Among these, morphological and ultra-structural observations of *K. flaccidum* species describe the presence of a thick and multilayered cross-wall, a lobed plate-shaped chloroplast with smooth, undulating or variously dissected edges and several polymorphic mitochondria, which are located around the nucleus and along the chloroplast lobes. Cells have a central pyrenoid surrounded by several or many starch grains. The nucleus is located opposite the pyrenoid [34]. The typical organization of the filamentous thallus in multi-layered aggregates can ensure high protection [35]. Recent studies have shown that *Klebsormidium* possesses genes similar to those found in terrestrial plants, which thanks to their adaptation to terrestrial environments, offer protection against the harmful effects of intense light. These findings align with the theory suggesting the gradual colonization of land by descendants of streptophyte algae [36,37].

This study aimed at evaluating the effectiveness of UV-stress treatment in enhancing the mass production of biomolecules of algal origin [38]. In particular, UV-B exposure was used to assess the production of a stress marker, malondialdehyde (MDA), which is derived from the oxidation of polyunsaturated fatty acids, as well as the production of protective metabolites, such as pigments and fatty acids [1,15,35].

2. Materials and methods

2.1. *Algal species*

The two algae species used in this study were *Edaphochlorella mirabilis* and *Klebsormidium flaccidum*. *E. mirabilis* (V.M. Andreyeva) Darienko et al. [69] is a unicellular green alga belonging to the Regnum Plantae - Viridiplantae - Chlorophyta Division, Trebouxiophyceae Class [70]. *K. flaccidum* (Kützing) Silva et al. [71], is a pluricellular green alga with a filamentous thallus, belonging to the Regnum Plantae - Viridiplantae - Charophyta Division Klebsormidiophyceae Class [70], widely studied for its tolerance to UV light [39].

The algal strains involved in the research were provided by the Algal Collection University Federico II (ACUF). The details of the source of species collection are reported in Table 1.

2.2. *In vitro culture system*

The two algal species were separately grown in a temporary immersion system (TIS) photobioreactor (Plantform), which was slightly modified in this study (Fig. 1) [72]. TIS photobioreactors, currently used for plant micropropagation [40], are characterized by a growth vessel made of transparent polycarbonate (180 × 160 × 150 mm). Each Plantform was filled with 500 mL of sterile Bold's Basal Medium (Phyto Tech Lab, USA, 0.705 g/L) and buffered at pH 6.6. The growth medium inside each photobioreactor was inoculated with 25 mL of sterile algal culture. In each bioreactor, the starting cell concentration was analyzed using a Sedgewick rafter chamber: in both species the value was within a range of 150 ± 50 cell/mL.

Each vessel was connected to a pumping system, in which a PTFE filter allowed a change of sterile air in the bioreactor. In particular, a timer automatically activated the pump for 10 min, 6 times per day. The experimental protocol required the hermetic plastic photobioreactor lid to be replaced with a layer of transparent film in order to allow the UV-B rays to pass through the bioreactor, thus reaching the algal culture. Each preparatory step was performed under a vertical laminar flow hood (Thermo Scientific).

Table 1
Algal taxonomy and source of collection provided by National Center for Biotechnology Information (GenBank by NCBI: https://www.ncbi.nlm.nih.gov/genbank/).

Genus and Species	Phylum	Culture collection strain	GenBank Accession number
E mirabilis	Chlorophyta	http://www.acuf.net/ Strain n° 038 SAG:38.88	GenBank:KM462865
K. flaccidum	Charophyta	http://www.acuf.net/ Strain n° 065	–

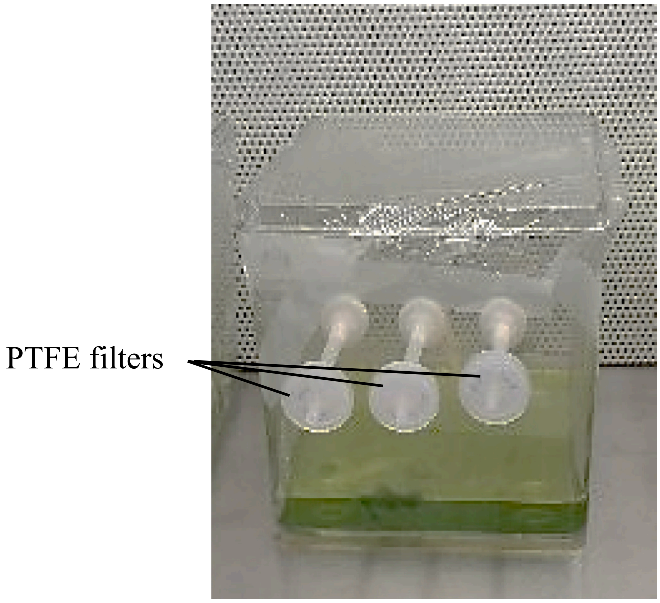

Fig. 1. Temporary immersion system photobioreactor filled with 500 mL of Bold's Basal Medium and inoculated with *K. flaccidum* during the last week of growth.

The experimental plan consisted of independent trials repeated three times. Each trial involved the setting up of four photobioreactors for each algal species (4 × 2 = 8). Two photobioreactors were exposed to the treatment (UV-B rays), whilst the other two photobioreactors were not exposed and therefore used as controls (CTRL).

All photobioreactors were placed inside a growth chamber (FrigorBox, Reggio Emilia, Italy), kept at 24 ± 1 °C and equipped with one LED lamp for each shelf providing a light intensity of 18,000 lx, measured using a Luxmeter (HD8366, Eldes Instruments, Sesto San Giovanni, Italy). The photoperiod was set at 14 h/day. The amount of photosynthetically active radiation (PAR) was measured and maintained at 100 ± 10 μmolm^{-2} s^{-1} on each shelf of the incubator. The UV-B rays were applied as treatment to the photobioreactors 2 h per day, for two weeks using a UV-B lamp (TL-20 W/01, Philips) with narrow waveband of between 305 and 315 nm, which peaks at 311 nm. The control photobioreactors were exposed exclusively to LED light. Each trial lasted two weeks.

2.3. Biomass growth

Over the course of all the experimental trials, algal growth was monitored by measuring the biomass dry weight. An aliquot of 30 mL of the liquid growth medium was collected on cellulose ester filter membranes (0.22 μm pores), then dried with a gentle stream of pure N_2 and, finally, weighed. Results were expressed as $g_{\text{dry biomass}}$/L. After culture medium collection, no medium solution was added to the bioreactor to not influence the algal cell concentration. During the experimental trial, a total of three medium samplings were taken, for a total withdrawal of 90 mL before the final sampling on the 15th day of trial. Measurements were carried out two times a week, and a growth curve was plotted using values from t0 (1st day of trial) up to t4 (15th day). At the end of the growth period, the remaining amount of liquid medium was entirely filtered under vacuum using a Büchner funnel, and the algal biomass was collected on cellulose ester filter membranes for further analysis.

2.4. Metabolites extraction analyses

The effects of exposure to UV-B ray on the algal samples were analyzed through the quantification of malondialdehyde (MDA), a useful chemical marker of lipid peroxidation. Moreover, a thorough determination of pigments and fatty acids was carried out.

2.4.1. MDA extraction and analyses

The MDA extraction from algal biomass was performed according to the protocol described by Hodges et al. [41]. An amount of 0.2 g of algal biomass fresh weight (FW) was homogenized together with inert sand in a 2 mL ethanol:water solution 80:100 (*v*/v). Then, 2 mL of an acidic solution of trichloroacetic acid at 20.0 % (*w/v*) and thiobarbituric acid at 0.5 % (w/v) were added to each sample. Samples were then vigorously mixed, heated at 95 °C in a block heater for 25 min, then cooled down, and finally centrifuged at 3000*g* for 10 min. Absorbance was read with a UV–Vis spectrophotometer (V-730, Jasco Europe Srl, Cremella, Italy) at 532 nm and corrected for nonspecific turbidity by subtracting the absorbance at 600 nm. MDA equivalents were calculated according to Heath & Packer [42].

2.4.2. Total lipid extraction and analyses and fatty acids' profile

Lipid extraction from algal biomass was carried out following the Folch method and adjusted with subsequent modifications [43]. This protocol included an extraction with a 1 mL chloroform:methanol 2:1 (v/v) solution and saturated NaCl solution, which facilitates the separation of the samples into two distinct phases: a polar phase (methanol and a watery upper layer) and a lower lipophilic phase ($CHCl_3$). The latter contains the extracted lipid fraction.

The lipophilic phase was transferred into another tube and a second extraction cycle was performed on the solid residue. Then, the extracts pooled together were then dried using a gentle stream of pure N_2. The total amount of lipid was weighed (expressed as $mg_{\text{total lipid}}/g_{\text{biomass}}$ FW), then 1 mL of hexane was added to the sample to redissolve lipid material, hence 20 μL of nonadecanoic acid methyl ester (1 % w/v hexane) was added and used as an internal standard.

Triglycerides were trans-esterified by adding a water-free alkaline solution (KOH 2 M in MeOH) [44]. After vigorous mixing and centrifugation (3000 *g*, 10 min), fatty acid methyl esters (FAME) were separated. The FAME fraction (1 μL) of each sample was withdrawn from the upper phase of the tube and injected into a gas chromatograph (8860 GC System, Agilent Technologies Italia SpA, Milan, Italy) coupled with a mass spectrometer (5977B GC/MSD, Agilent Technologies), and equipped with a Crossbond® acid-deactivated Carbowax® polyethylene glycol capillary column (Stabilwax®-DA, Restek, Bellefonte, USA, 30-m length, 0.25 mm i.d., 0.25 μm f.t.).

Injections were performed *via* a split/splitless injection port, operating in splitless mode at 230 °C. The carrier gas was ultrapure helium (with a constant flow rate of 1 mL/min). The temperature of the GC oven was set at 90 °C, held for 1 min and then ramped at 10 °C/min to 180 °C. Subsequently, the temperature was ramped at 50 °C/min to 230 °C and held for 8 min. The total chromatographic run time was 20 min.

Electron ionization (EI) was used to obtain molecular fragmentation. The data were obtained in full-scan mode and the mass to charge ratio (m/z) was recorded between 33 and 350 at 70 eV. Chromatograms were captured using "Enhanced Chem Station" software (GA170AA version A.03.00). Peaks were identified using three strategies: i) comparison of peak retention times with those of the Supelco 37 Component FAME Mix (Supelco, Merck); ii) scanning using deconvolution software (Agilent); iii) comparison of the peak mass spectra with those present in the system libraries dedicated to FAs (Famedb23.l and Famedbwax.l; Agilent Technologies). Quantification was performed using the internal standard method (nonadecanoic acid methyl ester, 1 % *w/v* hexane). Each fatty acid was expressed as $mg_{\text{fatty acid}}/g_{\text{biomass}}$ FW.

2.4.3. Pigments extraction and analyses

Pigments were extracted from fresh algal biomass using the solvent-based method outlined by Masino et al. [45], with adaptations made to suit the specific characteristics of the samples. Briefly, an aliquot of 20 mg of algal biomass was suspended into 1 mL of absolute ethanol, then shaken for 30 min, and centrifuged for 10 min at 4000*g*. The extraction cycle was repeated on the residue further two times, using the same volume but different solvents (absolute acetone in the second cycle and hexane in the third cycle). The pooled supernatants were concentrated to dryness using a gentle stream of pure N_2. The residue was finally redissolved and made up to 1 mL with acetone. The extract was then subjected to pigment determination.

All pigments contained in the samples were quantified using an HPLC system (PU 4180, Jasco Europe Srl, Cremella, Italy), equipped with a 250 × 4.6 mm, 5 μm particle size C_{30} column for carotenoids (YMC Europe, Dinslaken, Germany), placed in a column oven set at 25 °C. The solvent system consisted of phase A, MeOH/MTBE/H_2O (6:90:4) (*v*/v/v); and phase B, MeOH/MTBE/H_2O (81:15:4) (v/v/v). The elution (75 min run) was carried out according to the following gradient: 0 % B (0.0 min), 66.6 % B (60.0 min), 0 % B (75.0 min), with a flow rate of 1.0 mL/min. Samples (20 μL) were injected through an autosampler (AS-4050 A, Jasco) and chromatograms were captured using a UV/Vis detector (4070, Jasco) set at 450 nm [46].

Identification was performed through the comparison of the peak retention times with those obtained by injecting pure standards solutions of β-carotene (β-car), lutein (Lut), and chlorophylls (Chl) *a* and *b*. Analytes were quantified using calibration curves built through five concentration levels of β-car, Lut¸ Chl *a*, and Chl *b* (Table 2). The concentration of each substance was expressed as μg/mg of fresh algal biomass (FW).

2.4.4. Statistical analysis

All the data were subjected to statistical analyses (one-way ANOVA and t-student test) to compare repeated data sets using PAST3 software (freeware data analyzer app and calculator developed by Oyvind Hammer for Windows, 2017).

3. Results and discussion

The two algal species, *E. mirabilis* and *K. flaccidum*, were selected in the present study for their high growth rate and high speed of adaptation to external environmental factors, which make them a useful model for experimental applications [1].

3.1. Algal biomass growth results

Fig. 2 shows that there were no significant differences (*p-value* > 0.05) in algal biomass collected over the course of the experiment between control and treated samples. Algal biomass produced on average at the end of two-weeks trial growth was 0.29 ± 0.11 g/L and 0.33 ± 0.06 g/L in control samples (*E. mirabilis* and *K. flaccidum*, respectively); and 0.22 ± 0.02 g/L and 0.29 ± 0.09 g/L in treated samples (*E. mirabilis* and *K. flaccidum*, respectively).

The growth curves had similar trends: both algal species showed a slight increase over the course of the first three days, which corresponded to the “lag phase”. Then, biomass production increased more rapidly, until the end of the 10th day in the *E. mirabilis* culture and the 7th day in the *K. flaccidum* culture (Fig. 2). This common behavior could be attributed to the tendency of both species to aggregate rather than disperse evenly throughout the culture medium. Consequently, biomass production for both algal species under both cultivation conditions (control and treated) remained lower than the growth rates reported in other studies [47,48], where the peak biomass was reached after 15 days of abiotic stress exposure and was between 2 and 4 times higher.

Table 2
Concentration range of each standard substance, max absorbance wavelengths, straight line equations, and coefficients of determination (R^2).

	Concentration range (μg/mL)	Absorbance wavelength (λ)	Equation	R^2
β-Car	6.9–46	453	y = 56,709× + 49,581	0.9989
Chl *a*	10–150	430	y = 17,687× + 243,353	0.8899
Chl *b*	10–150	462	y = 11,139× + 46,625	0.9981
Lut	10–150	446	y = 1203.6× + 3294.4	0.9969

These outcomes highlighted that the UV-B rays did not significantly inhibit the growth of algae in culture. As above described, the ability of algae to deploy various resilience mechanisms and rapid regulatory processes, such as a ‘photoprotective network’, indicates their adaptive capacity to overcome potential UV stress and other challenges. This capability allows algae to maintain normal biological functions, as thoroughly described by Rastogi et al. [49]. In particular, certain algal species have developed various tolerance mechanisms against environmental UV stress [50].

The resilience is related to the activation of multiple photo and dark repair mechanisms, antioxidant systems and biosynthesis of UV-photoprotectants such as mycosporine-like amino acids, carotenoids, and polyamines [49].

3.2. MDA results

MDA is a secondary metabolite produced through an oxidative phenomenon involving polyunsaturated fatty acids. For this reason, it is considered a useful chemical marker to assess the lipid peroxidation process.

In the present investigation, higher MDA levels emerged only in treated samples of *E. mirabilis* ($p \leq 0.05$), where the production of MDA was 90 % higher (0.27 ± 0.07 μmol/g and 0.14 ± 0.03 μmol/g, respectively) than in the control samples. In treated samples of *K. flaccidum*, MDA production increased by 34 % compared to controls. However, this difference was not statistically significant (0.20 ± 0.06 μmol/g and 0.15 ± 0.03 μmol/g, respectively) (Fig. 3).

The higher values of MDA found in algal biomass of *E. mirabilis* exposed to UV-B indicated increased lipid peroxidation, which is a clear phenomenon of stress in the living cells. On the contrary, the lower increase of MDA in treated *K. flaccidum* samples suggested a peculiar ability to activate an effective protective mechanism against UV-B stress. Based on the obtained data, *K. flaccidum* confirmed its status as an algal species resistant to harsh environmental conditions, as it can grow even in terrestrial habitats exposed to high-intensity sunlight [36,51].

3.3. Lipid determination and fatty acids' profile

The exposure to UV-B radiation did not considerably affect the total amount of lipids in both algal species. In *E. mirabilis*, the lipid content was on average 191.7 ± 78.0 $mg_{total\ lipid}/g_{biomass}$ FW in the control samples and 160.5 ± 67.8 $mg_{total\ lipid}/g_{biomass}$ FW in the treated samples, while *K. flaccidum* produced 116.0 ± 38.7 and 147.3 ± 95.3 $mg_{total\ lipid}/g_{biomass}$ FW in the control and treated samples, respectively. The t-student test did not highlight any significant difference (*p-value* > 0.05). Considering that samples used for metabolite extraction (fresh biomass) contained 50 % water (determined by calculating the average dry matter content of several algal biomass samples after drying them in an oven at 70 °C for 2 h), the total amount of fatty acids achieved was similar to that obtained in other studies, where the FA production remained within a wide range of between 10 and 250 mg/g DW [14,24]. Some algal species specifically grown for oil production, such as *Monoraphidium contortum*, *Chlorolobion braunii*, and *Neochloris oleoabundans* could accumulate even higher amounts of lipids in cells, up to 40 to 70 % of the biomass [44,52].

A thorough evaluation of the fatty acid profile (Fig. 4a and b) revealed some interesting findings. Palmitic acid ($C_{16:0}$) was the most abundant fatty acid in both algal species. This finding is coherent with other study outcomes, where $C_{16:0}$ was the most represented fatty acid

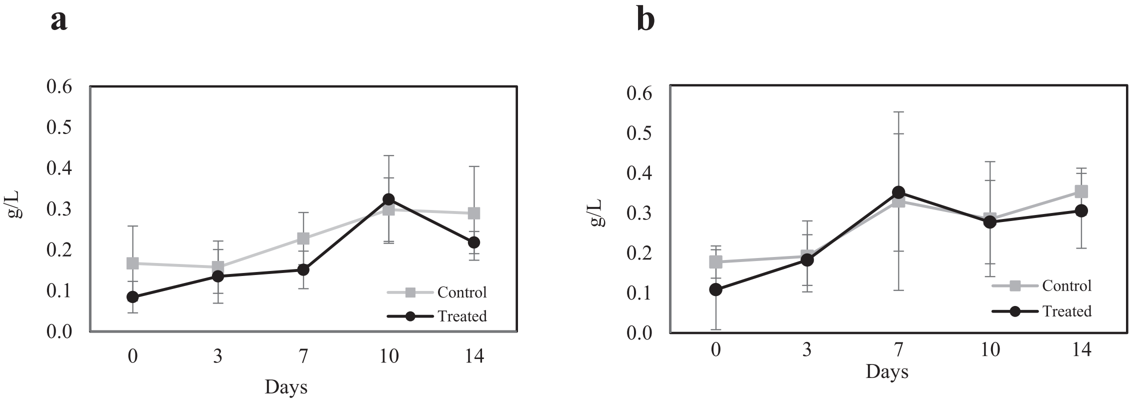

Fig. 2. Growth curves of biomass produced by *E. mirabilis* (a) and *K. flaccidum* (b). Data were collected on the sampling days 0, 3, 7, 14, which correspond to t0, t1, t2, and t3. The data shown are the mean values of three completed repeated trials ($n = 6$). Standard deviations are shown using error bars.

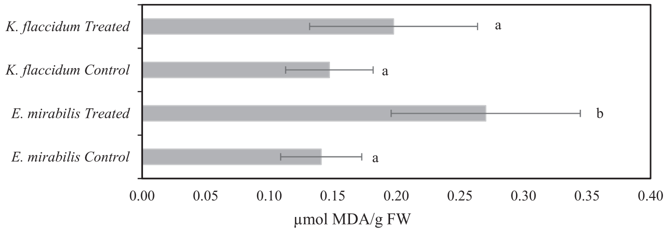

Fig. 3. Malondialdehyde (MDA) production (µmol/g FW) in *E. mirabilis* and *K. flaccidum* after two weeks of growth. The data shown are the mean values of three completed repeated trials ($n = 6$). Standard deviations are shown using error bars. Significant differences (*p-value* ≤ 0.05) are shown with different letters.

[14]. Indeed, the biosynthesis of palmitic acid ($C_{16:0}$) is one of the most common fatty acid synthesis pathways described in plants and microalgae and supported by enzymatic systems. Further chain elongations and desaturations usually occur as part of the n-3 and n-6 pathways to produce more complex polyunsaturated fatty acids [53]. The control samples of *E. mirabilis* produced 65.0 ± 23.0 mg/g FW of $C_{16:0}$, while treated samples produced 46.8 ± 8.7 mg/g FW. In contrast, control samples of *K. flaccidum* produced 44.8 ± 16.6 mg/g FW of $C_{16:0}$, with treated samples producing 57.0 ± 31.7 mg/g FW. In this case, the treatment did not significantly influence palmitic acid production, which remained similar to previous studies [25,53].

Among the unsaturated fatty acids, α-linolenic acid ($C_{18:3\alpha}$) and linoleic acid ($C_{18:2}$) were the most abundant. Notably, the amount of $C_{18:3\alpha}$ in treated *K. flaccidum* was significantly higher than in the control samples (*p-value* ≤ 0.05), showing concentrations of 33.0 ± 17.4 mg/$g_{biomass}$ FW in treated samples and 14.0 ± 3.7 mg/$g_{biomass}$ FW in control samples. *E. mirabilis* also exhibited a significant increase (*p-value* ≤ 0.05) in eicosapentaenoic acid (EPA) ($C_{20:5}$) in treated samples compared to control samples. However, the absolute amount of EPA was fairly low: 0.60 ± 0.1 mg/g $_{biomass}$ FW in control samples and 1.2 ± 0.5 mg/$g_{biomass}$ FW in treated samples. Although some freshwater algae can synthesize PUFAs, they are generally less efficient in producing EPA compared to their marine counterparts [54,55]. These results align with previous findings, suggesting that these algal species mainly contain $C_{16:0}$ and $C_{18:0}$ fatty acids, with a number of double bonds from 0 to 4 [15,53,56].

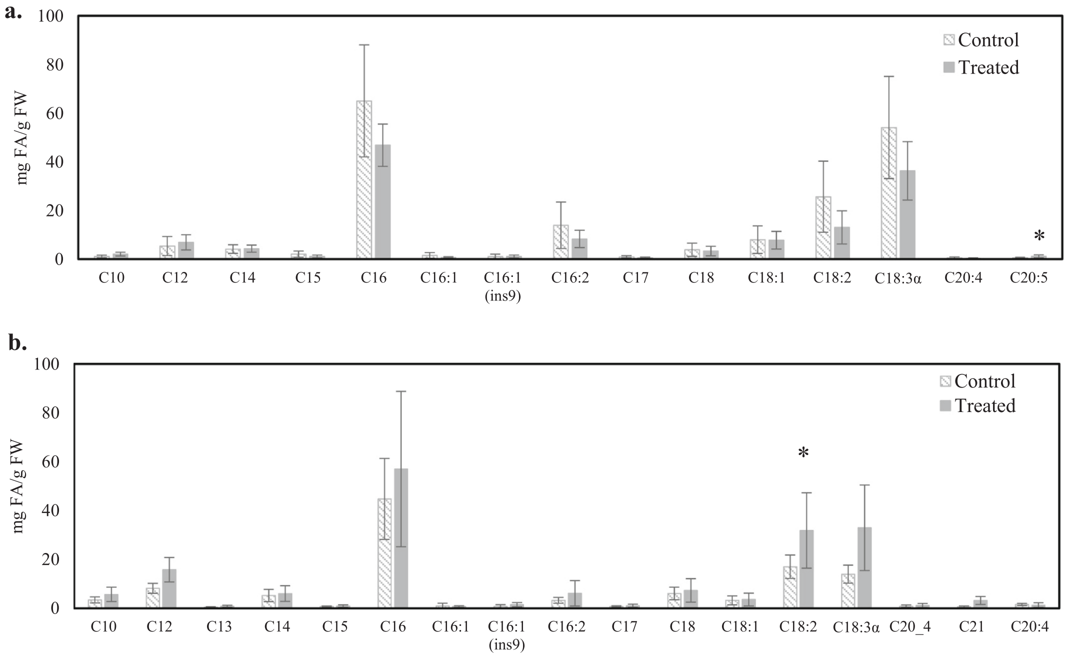

Fig. 4. Fatty acid profile found in *E. mirabilis* (a) and *K. flaccidum* (b) species. Data are expressed as mg of fatty acid/g of fresh biomass (FW). The data shown are the mean values of three completed repeated trials ($n = 6$). Standard deviations are shown using error bars. * $p \leq 0.05$.

The results showed a decreasing trend of polyunsaturated fatty acids in *E. mirabilis* samples exposed to UV-B radiation, although ANOVA did not show significant differences due to a wide dispersion of the data around the mean values. This shift may be attributed to the susceptibility of polyunsaturated lipids to peroxidation under such conditions. The notable loss of polyunsaturated fatty acids could result directly from the effects of UV-B radiation. As described by Kumar et al. [57], PUFA synthesis requires a significant amount of ATP. However, under UV light stress, ATP is needed by various cellular mechanisms to protect against intracellular oxidative stress caused by UV light. This observation aligns with the parallel increase in MDA values, especially prominent in *E. mirabilis* [15].

On the contrary, *K. flaccidum* showed a different~~ee~~ behavior. In fact, the amount of the main polyunsaturated acids increased in treated samples compared with controls. This could be due to the stronger resilience of *K. flaccidum* to the radiation stressor, probably linked to an increased production of antioxidant substances.

The different results achieved by the two different algal species could be also explained through the production of the so-called mycosporine-like amino acids (MAAs) by genus *Klebsormidium*, rather than the *Edaphoclorella* one. MAAs are photochemically stable molecules and act as passive shielding biomolecules by dissipating the energy of the previously absorbed UV rays as harmless heat, thereby avoiding any photochemical reaction [36].

The presence of EPA ($C_{20:5}$) in *E. mirabilis* has a great nutritional value, as it is an essential fatty acid in human nutrition belonging to the omega-3 (ω3) series. Cohort studies have reported that EPA intake is associated with lower rates of all-cause mortality and adverse cardiac outcomes [58]. In a broader context, it can be asserted that ω3 fatty acids have the potential to positively impact human health, particularly related to cardiovascular function.

The significant increase of EPA in *E. mirabilis* when exposed to UV-B radiation could explain the interest shown by food companies in obtaining ω3 fatty acids from a plant-based source. This could reduce the reliance on fish products as the primary source of ω3 fatty acids in the human diet. However, the absolute production of EPA by *E. mirabilis* in response to UV-B stress was very low compared to the control samples, representing only 0.6 % of the total lipid production by this species.

When algae experience stress induced by intense solar radiation, temperature variations, salinity changes, chemical contaminants or nutrient deficiencies, their lipid metabolism undergoes adaptive changes to protect cells from stress-induced damage. These adaptations often involve modifying the membrane lipid composition to maintain fluidity and stability. For instance, increasing the amount of unsaturated lipids enhances membrane fluidity [3]. Paliwal et al. [13] described how specific limiting stress conditions (including light intensity and UV radiation) may induce microalgae to accumulate structural lipids, phospholipids and/or starch, which serve as an energy source and strengthen the cell membrane and enhance its structural integrity under stress [59,60]. This observation can explain the increased levels of an important PUFA ($C_{18:3\alpha}$, α-linoleic acid) in *K. flaccidum* after the application of UV-B rays in this study. However, during high UV-B ray exposure, unsaturated fatty acids are more susceptible to oxidative degradation, in particular when organisms lack UV-shielding molecules, leading to detrimental effects on the cell membranes, DNA, proteins, and other metabolic alterations [27]. This typically leads to the decrease of monounsaturated and polyunsaturated fatty acids following the increase of irradiance and light duration, as observed on *E. mirabilis* response and as already supported by other Authors [61,62].

3.4. Determination of pigments

The algal species studied showed lutein as the most abundant xanthophyll, as already described in previous works [49,63]. In *E. mirabilis*, there was a significant reduction in lutein in treated samples (*p-value* ≤ 0.05), indeed it decreased from 50.2 ± 5.3 μg/g $_{biomass}$ FW in control samples to 32.6 ± 3.2 μg/mg $_{biomass}$ FW in treated ones. Conversely, in *K. flaccidum*, this value significantly increased after exposure to UV-B rays (*p-value* ≤ 0.05) (Fig. 5). The production of lutein averaged 30.2 ± 8.2 μg/mg $_{biomass}$ FW in treated *K. flaccidum* samples, compared to 13.2 ± 4.1 μg/mg $_{biomass}$ FW in control samples. The content of chlorophyll *a* significantly increased in both algal species grown under UV-B exposition (*p-value* ≤ 0.05) (Fig. 5). The mean values of Chl *a* in treated and control samples of *E. mirabilis* were 58.7 ± 10.5 μg/mg $_{biomass}$ FW and 33.1 ± 4.6 μg/mg $_{biomass}$ FW, respectively; while the mean values of Chl *a* in treated and control samples of *K. flaccidum* were 26.7 ± 5.9 μg/mg $_{biomass}$ FW and 12.6 ± 8.2 μg/mg $_{biomass}$ FW, respectively.

The amount of Chl *b* obtained in the present study aligns with the results of previous investigations [64]. The mean values of Chl *b* in treated and control samples of *E. mirabilis* were 16.7 ± 10.8 μg/mg $_{biomass}$ FW and 11.0 ± 4.3 μg/mg $_{biomass}$ FW, respectively, whereas the mean values of Chl *b* in treated and control samples of *K. flaccidum* were 9.0 ± 4.7 μg/mg $_{biomass}$ FW and 3.8 ± 2.5 μg/mg $_{biomass}$ FW, respectively. The observed differences in both cases were not statistically significant (*p-value* > 0.05).

Carotenoids are a group of pigments known for their protective role against oxidative stress. Their increased production is a strong indicator of the microalgae's capacity to employ preventive strategies to limit UV-B damage [1]. The results found in the present work indicated that β-carotene accumulated after the exposure to UV-B radiation reaching a concentration of 21.3 ± 5.3 μg/mg $_{biomass}$ FW in *K. flaccidum*, while in control samples it was 10.6 ± 7.0 μg/mg $_{biomass}$ FW. By contrast, in *E. mirabilis*, there was no significant difference in concentrations of β-carotene in the treated and control samples that were 21.4 ± 11.0 μg/mg $_{biomass}$ FW and 14.8 ± 1.7 μg/mg $_{biomass}$ FW respectively.

The increase of Chl *a* in both algal species is explained by previous findings: the increased levels obtained by *E. mirabilis* could be correlated with the higher initial content of lutein, known as a powerful antioxidant xanthophyll, which could work as a UV-stress screen, as proposed by Figueroa et al. [65]. In general, a higher increase of Chl *a* content after short-term exposure to UV-B flux (not >2 h/day) has already been described as a response in microalgae [1,65]. Notably, UV-B radiation induces an over excitation of Chl *a*, which can potentially transfer energy to oxygen, thus leading to the production of reactive oxygen species (ROS) [66]. The increased levels of ROS in microalgae induce an antioxidant defense system consisting of enzymatic and non-enzymatic protection to mitigate organelle damage instigated by ROS: examples of non-enzymatic production, are antioxidant compounds, which play important roles in cellular metabolism, helping protect cells from oxidative stress [15]. The antioxidant compounds are represented by photoprotective pigments and compounds known for their filtering, quenching, and scavenging roles in preventing prevent cell damage: β-carotene and lutein, which significantly increase in *K. flaccidum,* are all included in these types of molecules [67].

The outcomes of this study suggest that, while in *E. mirabilis* the production of ROS induces a higher level of cell damage (identified by the increase of MDA and decrease of lutein), *K. flaccidum* is capable to activate a stronger defense system. The metabolites production described through this study in *K. flaccidum* is consistent with the evolutionary history of Klebsormidiophyceae, which has been identified among the “basal Charophytes” genera. Becker and Marin [68] have suggested that *Charophyta* represents the algal ancestor of land plants, since this taxon started to colonize moderately moist habitats in the proximity of water, thus promoting a gradual transition to dry lands. For this reason, Klebsormidiophyceae are considered ideal model organisms for studying stress tolerance mechanisms connected with transition to land, one of the most important events in plant evolution and the earth's history [35]. Then, the combined effect of accumulation of carotenoids and PUFAs after UV-B exposure highlights the tolerance of *K. flaccidum* to stressed environmental conditions, confirmed also by the fact that there was not a significant variation of MDA production. Genetic

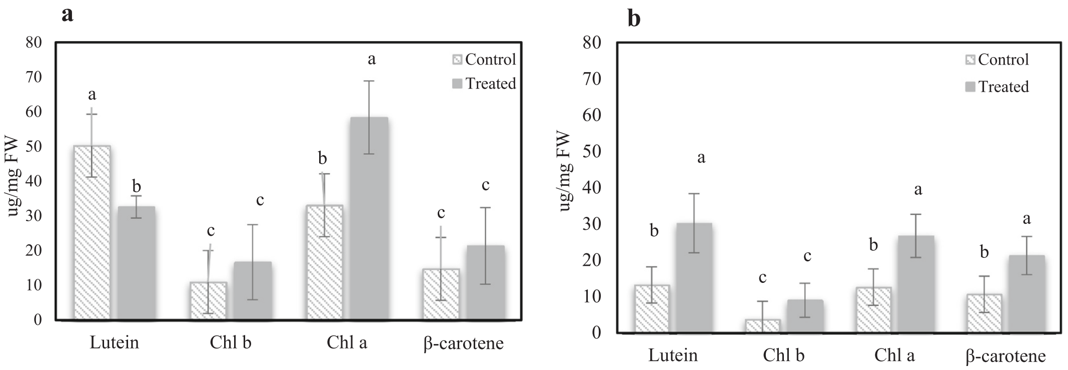

Fig. 5. Average total pigment contents in control and treated samples of *E. mirabilis* (a) and *K. flaccidum* (b). The data shown are the mean values of three completed repeated trials ($n = 6$). Standard deviations are shown using error bars. Significant differences ($p\text{-}value \leq 0.05$) are shown with different letters.

evidence suggests that various plant protective mechanisms (such as accumulation of UV-screening compounds) originated in Charophyta green algae [35].

4. Conclusions

UV-B radiation applied as abiotic stress on the growth of *E. mirabilis* and *K. flaccidum* showed significant effects on their metabolisms. Growth data show that exposure to UV-B rays did not inhibit algal culture growth. However, MDA determination revealed oxidative processes in *E. mirabilis*, with a 90 % increase in treated samples compared to the control. Conversely, treated samples of *K. flaccidum* activated various adaptation strategies to minimize oxidative stress, showing no significant differences between control and treated samples. Exposure to UV-B rays can stimulate the production of certain metabolites in both algal species, helping counteract the negative effects on cell physiology. This indicates their ability to respond and adapt to UV-B radiation.

Notably, *K. flaccidum* showed a higher increase in total pigment content (+53 %) compared to *E. mirabilis* (+30 %), indicating a greater adaptive capacity.

The increase in PUFAs in *K. flaccidum* is associated with the presence of more effective cell defense strategies and the enhanced production of antioxidant substances, while *E. mirabilis* seems to protect itself by increasing Chl *a* production and by reducing polyunsaturated fatty acids.

The secondary metabolites found in the present study, such as carotenoids (lutein and β-carotene), and polyunsaturated fatty acids (EPA, α-linoleic acid), have potential applications in both pharmaceutical and food industries due to their antioxidant function.

CRediT authorship contribution statement

Giulia Santunione: Writing – original draft, Investigation, Formal analysis, Data curation. **Francesca Masino:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Giuseppe Montevecchi:** Writing – review & editing, Software, Methodology, Investigation, Conceptualization. **Elisabetta Sgarbi:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This research project, titled: “Risposta a stress abiotici e produzione di metaboliti ad azione bioattiva in microalghe”, was funded with the “Fondo di Ateneo per la Ricerca 2019” – University of Modena and Reggio Emilia, Department of Life Sciences (FAR 2019).

In addition, we would like to acknowledge the English-language reviewing work done by Dr. Elizabeth Zanazzo.

References

[1] R. Singh, A.K. Upadhyay, D.V. Singh, J.S. Singh, D.P. Singh, Photosynthetic performance, nutrient status and lipid yield of microalgae *Chlorella vulgaris* and *Chlorococcum humicola* under UV-B exposure, Current Research in Biotechnology 1 (2019) 65–77, https://doi.org/10.1016/j.crbiot.2019.10.001.

[2] D.Y.Y. Tang, K.S. Khoo, K.W. Chew, Y. Tao, S.H. Ho, P.L. Show, Potential utilization of bioproducts from microalgae for the quality enhancement of natural products, Bioresour. Technol. 304 (January) (2020) 122997, https://doi.org/10.1016/j.biortech.2020.122997.

[3] A. Udayan, M. Arumugam, A. Pandey, Nutraceuticals from algae and cyanobacteria, Algal Green Chemistry: Recent Progress in Biotechnology 65–89 (2017), https://doi.org/10.1016/B978-0-444-63784-0.00004-7.

[4] Â.P. Matos, The impact of microalgae in food science and technology, JAOCS, Journal of the American Oil Chemists’ Society 94 (11) (2017) 1333–1350, https://doi.org/10.1007/s11746-017-3050-7.

[5] G. Montevecchi, G. Santunione, F. Licciardello, Ö. Köker, F. Masino, A. Antonelli, Enrichment of wheat flour with Spirulina. Evaluation of thermal damage to essential amino acids during bread preparation, Food Res. Int. 157 (May) (2022), https://doi.org/10.1016/j.foodres.2022.111357.

[6] P. Santhakumaran, S.M. Ayyappan, J.G. Ray, Nutraceutical applications of twenty-five species of rapid-growing green-microalgae as indicated by their antibacterial, antioxidant and mineral content, Algal Research 47 (February) (2020) 101878, https://doi.org/10.1016/j.algal.2020.101878.

[7] P. Santhakumaran, S.K. Kookal, L. Mathew, J.G. Ray, Experimental evaluation of the culture parameters for optimum yield of lipids and other nutraceutically valuable compounds in *Chloroidium saccharophillum* (Kruger) comb. Nov, Renew. Energy 147 (2020) 1082–1097, https://doi.org/10.1016/j.renene.2019.09.071.

[8] Y. Chisti, Microalgae biotechnology: A brief introduction, in: Handbook of Microalgae-Based Processes and Products, Elsevier Inc., 2020, https://doi.org/10.1016/b978-0-12-818536-0.00001-4.

[9] M.P. Sudhakar, S. Viswanaathan, Algae as a sustainable and renewable bioresource for bio-fuel production, in: New and Future Developments in Microbial Biotechnology and Bioengineering: Microbial Biotechnology in Agro-environmental Sustainability, Elsevier B.V, 2019, https://doi.org/10.1016/B978-0-444-64191-5.00006-7.

[10] M. Blasio, S. Balzano, Fatty acids derivatives from eukaryotic microalgae, pathways and potential applications, Front. Microbiol. 12 (September) (2021) 1–21, https://doi.org/10.3389/fmicb.2021.718933.

[11] K. Goiris, K. Muylaert, I. Fraeye, I. Foubert, J. De Brabanter, L. De Cooman, Antioxidant potential of microalgae in relation to their phenolic and carotenoid content, J. Appl. Phycol. 24 (6) (2012) 1477–1486, https://doi.org/10.1007/s10811-012-9804-6.

[12] K. Goiris, W. Van Colen, I. Wilches, F. León-Tamariz, L. De Cooman, K. Muylaert, Impact of nutrient stress on antioxidant production in three species of microalgae, Algal Res. 7 (2015) 51–57, https://doi.org/10.1016/j.algal.2014.12.002.

[13] C. Paliwal, M. Mitra, K. Bhayani, S.V.V. Bharadwaj, T. Ghosh, S. Dubey, S. Mishra, Abiotic stresses as tools for metabolites in microalgae, Bioresour. Technol. 244 (2017) 1216–1226, https://doi.org/10.1016/j.biortech.2017.05.058.

[14] P. Santhakumaran, S.K. Kookal, L. Mathew, J.G. Ray, Bioprospecting of three rapid-growing freshwater green algae, promising biomass for biodiesel production,

Bioenergy Res. 12 (3) (2019) 680–693, https://doi.org/10.1007/s12155-019-09990-9.

[15] M.R. Gauthier, G.N.A. Senhorinho, J.A. Scott, Microalgae under environmental stress as a source of antioxidants, Algal Res. 52 (March) (2020) 102104, https://doi.org/10.1016/j.algal.2020.102104.

[16] S.-J. Heo, S.-H. Cha, K.-W. Lee, Y.-J. Jeon, Antioxidant activities of red algae from Jeju Island, Algae 21 (1) (2006) 149–156, https://doi.org/10.4490/algae.2006.21.1.149.

[17] R. Sathasivam, R. Radhakrishnan, A. Hashem, Abd_Allah, E. F., Microalgae metabolites: a rich source for food and medicine, Saudi Journal of Biological Sciences 26 (4) (2019) 709–722, https://doi.org/10.1016/j.sjbs.2017.11.003.

[18] E. Alou-Font, S. Roy, S. Agustí, M. Gosselin, Cell viability, pigments and photosynthetic performance of Arctic phytoplankton in contrasting ice-covered and open-water conditions during the spring-summer transition, Mar. Ecol. Prog. Ser. 543 (February 2017) (2016) 89–106, https://doi.org/10.3354/meps11562.

[19] M. Chen, M. Schliep, R.D. Willows, Z.L. Cai, B.A. Neilan, H. Scheer, A red-shifted chlorophyll, Science 329 (5997) (2010) 1318–1319, https://doi.org/10.1126/science.1191127.

[20] B. Demmig-Adams, W.W. Adams, Food and photosynthesis: antioxidants in photosynthesis and human nutrition, Science 298 (5601) (2002) 2149–2153, https://doi.org/10.1126/science.1078002.

[21] C. Grewe, C. Griehl, Time-and media-dependent secondary carotenoid accumulation in *Haematococcus pluvialis*, Biotechnol. J. 3 (9–10) (2008) 1232–1244, https://doi.org/10.1002/biot.200800067.

[22] P. Spolaore, C. Joannis-Cassan, E. Duran, A. Isambert, Commercial applications of microalgae, J. Biosci. Bioeng. 101 (2) (2006) 87–96, https://doi.org/10.1263/jbb.101.87.

[23] F. Bona, A. Capuzzo, M. Franchino, M.E. Maffei, Semicontinuous nitrogen limitation as convenient operation strategy to maximize fatty acid production in *Neochloris oleoabundans*, Algal Research 5 (1) (2014) 1–6, https://doi.org/10.1016/j.algal.2014.03.007.

[24] J. Liu, P. Vanormelingen, W. Vyverman, Fatty acid profiles of four filamentous green algae under varying culture conditions, Bioresour. Technol. 200 (2016) 1080–1084, https://doi.org/10.1016/j.biortech.2015.11.001.

[25] J. Liu, P. Vanormelingen, W. Vyverman, Fatty acid profiles of four filamentous green algae under varying culture conditions, Bioresour. Technol. 200 (2016) 1080–1084, https://doi.org/10.1016/j.biortech.2015.11.001.

[26] S. Kondo, K. Hori, Y. Sasaki-Sekimoto, A. Kobayashi, T. Kato, N. Yuno-Ohta, T. Nobusawa, K. Ohtaka, M. Shimojima, H. Ohta, Primitive extracellular lipid components on the surface of the charophytic alga *Klebsormidium flaccidum* and their possible biosynthetic pathways as deduced from the genome sequence, Front. Plant Sci. 7 (June) (2016) 1–15, https://doi.org/10.3389/fpls.2016.00952.

[27] A. Stasiewicz, T. Conde, A. Gęgotek, M.R. Domingues, P. Domingues, E. Skrzydlewska, Prevention of UVB induced metabolic changes in epidermal cells by lipid extract from microalgae *Nannochloropsis oceanica*, Int. J. Mol. Sci. 24 (14) (2023), https://doi.org/10.3390/ijms241411302.

[28] P. Santhakumaran, S.K. Kookal, J.G. Ray, Biomass yield and biochemical profile of fourteen species of fast-growing green algae from eutrophic bloomed freshwaters of Kerala, South India, Biomass and Bioenergy 119 (September) (2018) 155–165, https://doi.org/10.1016/j.biombioe.2018.09.021.

[29] T. Huarancca Reyes, L. Mariotti, C. Chiellini, L. Guglielminetti, G.G. Fonseca, UV-B irradiation effect on microalgae performance in the remediation of effluent derived from the cigarette butt cleaning process, Plants 11 (18) (2022), https://doi.org/10.3390/plants11182356.

[30] C. Lemieux, C. Otis, M. Turmel, Chloroplast phylogenomic analysis resolves deep-level relationships within the green algal class Trebouxiophyceae, BMC Evol. Biol. 14 (1) (2014) 1–15, https://doi.org/10.1186/s12862-014-0211-2.

[31] P.H. Baudelet, G. Ricochon, M. Linder, L. Muniglia, A new insight into cell walls of Chlorophyta, Algal Research 25 (April) (2017) 333–371, https://doi.org/10.1016/j.algal.2017.04.008.

[32] S. Weber, P.M. Grande, L.M. Blank, H. Klose, Insights into cell wall disintegration of *Chlorella vulgaris*, PLoS ONE 17 (1 January 2022) (2022) 1–14, https://doi.org/10.1371/journal.pone.0262500.

[33] J.A. Raven, M. Giordano, Algae, Curr. Biol. 24 (13) (2014) 590–595, https://doi.org/10.1016/j.cub.2014.05.039.

[34] T. Mikhailyuk, A. Holzinger, A. Massalski, U. Karsten, Morphology and ultrastructure of *Interfilum* and *Klebsormidium* (Klebsormidiales, Streptophyta) with special reference to cell division and thallus formation, Eur. J. Phycol. 49 (4) (2014) 395–412, https://doi.org/10.1080/09670262.2014.949308.

[35] A. Holzinger, M. Pichrtová, J. Moore, Abiotic Stress Tolerance of Charophyte Green Algae: New Challenges for Omics Techniques 7(May), 2016, pp. 1–17, https://doi.org/10.3389/fpls.2016.00678.

[36] A. Hartmann, K. Glaser, A. Holzinger, M. Ganzera, U. Karsten, Klebsormidin a and B, two new UV-sunscreen compounds in green microalgal interfilum and *Klebsormidium* species (Streptophyta) from terrestrial habitats, Front. Microbiol. 11 (March) (2020) 1–12, https://doi.org/10.3389/fmicb.2020.00499.

[37] M. Stoyneva-Gärtner, B. Uzunov, G. Gärtner, M. Radkova, I. Atanassov, R. Atanasova, C. Borisova, P. Draganova, P. Stoykova, Review on the biotechnological and nanotechnological potential of the streptophyte genus *Klebsormidium* with pilot data on its phycoprospecting and polyphasic identification in Bulgaria, Biotechnol. Biotechnol. Equip. 33 (1) (2019) 559–578, https://doi.org/10.1080/13102818.2019.1593887.

[38] M.L. Wells, P. Potin, J.S. Craigie, J.A. Raven, S.S. Merchant, K.E. Helliwell, A. G. Smith, M.E. Camire, S.H. Brawley, Algae as nutritional and functional food sources : revisiting our understanding, 2017, pp. 949–982, https://doi.org/10.1007/s10811-016-0974-5.

[39] C.K. Karsten, U., Effects of UV radiation in optimum quantum yield and sunscreen contents in memebers of the genera *Interfilum, Klebsormidium, Hormidiella* and *Entransia* (Klebsormidiophyceae, Streptophyta), Eur. J. Phycol. 50 (3) (2015) 279–287.

[40] E. Gatti, E. Sgarbi, E.A. Ozudogru, M. Lambardi, The effect of PlantformTM bioreactor on micropropagation of *Quercus robur* in comparison to a conventional in vitro culture system on gelled medium, and assessment of the microenvironment influence on leaf structure, Plant Biosyst. 151 (6) (2017) 1129–1136, https://doi.org/10.1080/11263504.2017.1340356.

[41] D.M. Hodges, J.M. DeLong, C.F. Forney, R.K. Prange, Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds, Planta 207 (4) (1999) 604–611, https://doi.org/10.1007/s004250050524.

[42] R.L. Heath, L. Packer, Photoperoxidation in isolated chloroplasts, Arch. Biochem. Biophys. 125 (1) (1968) 189–198, https://doi.org/10.1016/0003-9861(68)90654-1.

[43] M. Axelsson, F. Gentili, A single-step method for rapid extraction of total lipids from green microalgae, PloS One 9 (2) (2014) 17–20, https://doi.org/10.1371/journal.pone.0089643.

[44] M. Altunoz, G. Montevecchi, F. Masino, L. Zanasi, A. Antonelli, Biodiesel properties of *Neochloris oleoabundans* grown in sludge waste, Clean. Eng. Technol. 5 (2021) 100295, https://doi.org/10.1016/j.clet.2021.100295.

[45] F. Masino, A. Ulrici, A. Antonelli, Extraction and quantification of main pigments in pesto sauces, Eur. Food Res. Technol. 226 (3) (2008) 569–575, https://doi.org/10.1007/s00217-007-0572-5.

[46] A.S. Fernandes, F.C. Petry, A.Z. Mercadante, E. Jacob-Lopes, L.Q. Zepka, HPLC-PDA-MS/MS as a strategy to characterize and quantify natural pigments from microalgae, Curr. Res. Food Sci. 3 (2020) 100–112, https://doi.org/10.1016/j.crfs.2020.03.009.

[47] W. Ding, P. Zhao, J. Peng, Y. Zhao, J.W. Xu, T. Li, R.J. Reiter, H. Ma, X. Yu, Melatonin enhances astaxanthin accumulation in the green microalga *Haematococcus pluvialis* by mechanisms possibly related to abiotic stress tolerance, Algal Research 33 (April) (2018) 256–265, https://doi.org/10.1016/j.algal.2018.05.021.

[48] A. Ljubic, E.T. Thulesen, C. Jacobsen, J. Jakobsen, UVB exposure stimulates production of vitamin D3 in selected microalgae, Algal Research 59 (August) (2021) 102472, https://doi.org/10.1016/j.algal.2021.102472.

[49] R.P. Rastogi, D. Madamwar, H. Nakamoto, A. Incharoensakdi, Resilience and self-regulation processes of microalgae under UV radiation stress, J. Photochem. Photobiol. C Photchem. Rev. 43 (xxxx) (2020), https://doi.org/10.1016/j.jphotochemrev.2019.100322.

[50] B. Wang, T. Ye, C. Li, X. Li, L. Chen, G. Wang, Cell damage repair mechanism in a desert green algae *Chlorella* sp. against UV-B radiation, Ecotoxicol. Environ. Saf. 242 (July) (2022) 113916, https://doi.org/10.1016/j.ecoenv.2022.113916.

[51] K. Hori, F. Maruyama, T. Fujisawa, T. Togashi, N. Yamamoto, M. Seo, S. Sato, T. Yamada, H. Mori, N. Tajima, T. Moriyama, M. Ikeuchi, M. Watanabe, H. Wada, K. Kobayashi, M. Saito, T. Masuda, Y. Sasaki-Sekimoto, K. Mashiguchi, H. Ohta, *Klebsormidium flaccidum* genome reveals primary factors for plant terrestrial adaptation, Nat. Commun. 5 (May) (2014) 2–6, https://doi.org/10.1038/ncomms4978.

[52] T. Joseph, J.G. Ray, Experimental assessment of lipid yield and phycoremediation potential of five indigenous microalgae under various nutrient regimes, Bioenergy Res. 0123456789 (2024), https://doi.org/10.1007/s12155-024-10722-x.

[53] M. Remize, Y. Brunel, J.L. Silva, J. Berthon, E. Filaire, Microalgae n-3 PUFAs Production and Use in Food and Feed Industries, 2021, pp. 1–29.

[54] G. Ahlgren, I.B. Gustafsson, M. Boberg, Fatty acid content and chemical composition of freshwater microalgae, J. Phycol. 28 (1) (1992) 37–50, https://doi.org/10.1111/j.0022-3646.1992.00037.x.

[55] I.A. Guschina, J.L. Harwood, Lipids and lipid metabolism in eukaryotic algae, Prog. Lipid Res. 45 (2) (2006) 160–186, https://doi.org/10.1016/j.plipres.2006.01.001.

[56] C. Niemi, S. Lage, F.G. Gentili, Comparisons of analysis of fatty acid methyl ester (FAME) of microalgae by chromatographic techniques, Algal Res. 39 (November 2018) (2019) 101449, https://doi.org/10.1016/j.algal.2019.101449.

[57] V. Kumar, M. Nanda, S. Kumar, P.K. Chauhan, The effects of ultraviolet radiation on growth, biomass, lipid accumulation and biodiesel properties of microalgae, Energy Sources, Part A 40 (7) (2018) 787–793, https://doi.org/10.1080/15567036.2018.1463310.

[58] C. Wang, W.S. Harris, M. Chung, A.H. Lichtenstein, E.M. Balk, B. Kupelnick, H. S. Jordan, J. Lau, N-3 fatty acids from fish or fish-oil supplements, but not α-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review, Am. J. Clin. Nutr. 84 (1) (2006) 5–17, https://doi.org/10.1093/ajcn/84.1.5.

[59] D.G. Kim, S.B. Hur, Growth and fatty acid composition of three heterotrophic *Chlorella* species, Algae 28 (1) (2013) 101–109, https://doi.org/10.4490/algae.2013.28.1.101.

[60] Y. Li-Beisson, J.J. Thelen, E. Fedosejevs, J.L. Harwood, The lipid biochemistry of eukaryotic algae, Prog. Lipid Res. 74 (January) (2019) 31–68, https://doi.org/10.1016/j.plipres.2019.01.003.

[61] M. Hultberg, H.L. Jönsson, K.J. Bergstrand, A.S. Carlsson, Impact of light quality on biomass production and fatty acid content in the microalga *Chlorella vulgaris*, Bioresour. Technol. 159 (2014) 465–467, https://doi.org/10.1016/j.biortech.2014.03.092.

[62] J. Seyfabadi, Z. Ramezanpour, Z.A. Khoeyi, Protein, fatty acid, and pigment content of *Chlorella vulgaris* under different light regimes, J. Appl. Phycol. 23 (4) (2011) 721–726, https://doi.org/10.1007/s10811-010-9569-8.

[63] R. Shegokar, K. Mitri, Carotenoid lutein: a promising candidate for pharmaceutical and nutraceutical applications, Journal of Dietary Supplements 9 (3) (2012) 183–210, https://doi.org/10.3109/19390211.2012.708716.

[64] S. Takaichi, Carotenoids in algae: distributions, biosyntheses and functions, Mar. Drugs 9 (6) (2011) 1101–1118, https://doi.org/10.3390/md9061101.

[65] F.L. Figueroa, N. Korbee, P. Carrillo, J.M. Medina-Sánchez, M. Mata, J. Bonomi, P. M. Sánchez-Castillo, The effects of UV radiation on photosynthesis estimated as chlorophyll fluorescence in *Zygnemopsis decussata* (Chlorophyta) growing in a high mountain lake (Sierra Nevada, southern Spain), J. Limnol. 68 (2) (2009) 206–216, https://doi.org/10.3274/JL09-68-2-05.

[66] K.J.M. Mulders, P.P. Lamers, M. D. E. W. R. H., Phototrophic pigments production with microalgae, J. Phycol. 2 (2014) 229–242, https://doi.org/10.1111/jpy.12173.

[67] Hiroko Takahashi, Yuri Kusama, Xinxiang Li, Shinichi Takaichi, Y. N., Overexpression of Orange carotenoid protein protects the repair of photosystem II under strong light in Synechocystis sp. PCC 6803, Plant Cell Physiology 60 (2) (2019) 367–375.

[68] B. Becker, B. Marin, Streptophyte algae and the origin of embryophytes, Ann. Bot. 103 (7) (2009) 999–1004, https://doi.org/10.1093/aob/mcp044.

[69] T. Darienko, L. Gustavs, T. Pröschold, Species concept and nomenclatural changes within the genera Elliptochloris and Pseudochlorella (Trebouxiophyceae) based on an integrative approach, J. Phycol. 52 (6) (2016) 1125–1145, https://doi.org/10.1111/jpy.12481.

[70] M.D. Guiry, G.M. Guiry, AlgaeBase, World-wide electronic publication, National University of Ireland, 2021.

[71] P.C. Silva, K.R. Mattox, W.H. Blackwell Jr, The generic name *Hormidium* as applied to green algae, Taxon 21 (1972) 639–645.

[72] M. Welander, J. Persson, H. Asp, L.H. Zhu, Evaluation of a new vessel system based on temporary immersion system for micropropagation, Sci. Hortic. 179 (2014) 227–232, https://doi.org/10.1016/j.scienta.2014.09.035.