

Article

Cyanostatic Potential of an Oleaginous *Chlorella vulgaris* Strain Against the Bloom-Forming Cyanobacterium *Microcystis aeruginosa*

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Abstract

Interactions between cyanobacteria and eukaryotic algae are traditionally viewed as favoring cyanobacteria, which are often the dominant competitors in freshwater ecosystems. However, increasing evidence suggests that eukaryotic algae also possess effective chemical defenses capable of suppressing cyanobacterial proliferation. In this study, the anti-cyanobacterial potential of a methanolic biomass extract of the green microalga *Chlorella vulgaris* against the bloom-forming cyanobacterium *Microcystis aeruginosa* was investigated. Optical density measurements revealed the inhibition of cyanobacterial growth, with extract concentrations of 0.4–1.6 mg mL^{−1} significantly reducing biomass accumulation. The lowest concentration (0.2 mg mL^{−1}) stimulated the accumulation of phycobiliproteins, whereas concentrations of 0.8–1.6 mg mL^{−1} significantly decreased phycobiliprotein content, indicating progressive physiological impairment. The results suggest that the highest extract concentration applied may also cause alteration in phosphate acquisition. Lipid profiling of the *Chlorella* biomass showed that 97% of the extractable lipids consisted of methanol-soluble fatty acids, with C18 fatty acids predominating among the 20 identified compounds. These metabolites are reported to possess allelopathic activity and therefore represent plausible contributors to the observed inhibition. These findings support that anti-cyanobacterial activity may represent a widespread ecological trait of *Chlorella* and potentially other green microalgae, providing a promising foundation for environmentally friendly cyanobacterial bloom management.

Keywords: *Microcystis aeruginosa*; *Chlorella vulgaris*; cyanostatic effects; fatty acids



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1. Introduction

Today, cyanobacterial blooms pose serious environmental, public health, and economic problems worldwide. This phenomenon is often associated with the development of oxygen-depleted conditions, toxin production, and habitat transformation, all of which threaten the balance of aquatic ecosystems, drinking water supplies, fisheries, and recreational water use [1,2]. Due to the limitations of traditional bloom control methods (physical

methods, non-selective algacides), there is increasing interest in alternative, naturally derived interventions, particularly those with selective effects. Among these, allelopathy stands out; such interventions may be capable of influencing the structure of algal communities over the long term without causing significant harm to non-target species [3,4].

During the competition for resources, different species have evolved diverse physiological and biochemical strategies that provide them with advantages over their competitors. Competition between species is not based solely on the efficient use of resources, i.e., exploitative competition. In some cases, a competitive advantage may also be achieved through direct physiological effects, a phenomenon described in the literature as interference competition [5,6]. Allelopathy also falls into this category, in which a given species releases (secondary) metabolites (allelochemicals) into the environment that directly influence the growth, reproduction, or physiological processes of another species, regardless of resource availability [4,7]. Allelochemicals released into the environment may act directly or indirectly, and several compounds with inhibitory effects often exhibit stimulatory effects at low concentrations [3].

Although field studies provide a realistic picture of how allelochemicals influence community structure and species dynamics under natural environmental conditions, field observations present several significant challenges. Community dynamics are influenced not only by allelochemicals but also by other environmental factors, such as competition for nutrients, predation, fluctuations in pH and temperature, and water movements such as flow or turbulence [4,8]. These various effects make it difficult to determine precisely to what extent the observed phenomena are actually attributable to allelopathy and to what extent they are due to other mechanisms, such as simple nutrient depletion or physical stress [4]. In contrast to the complexity of natural ecosystems, laboratory settings allow for the exclusion of confounding factors such as nutrient limitation, fluctuations in light intensity, or water movement, thereby enabling a more precise examination of the direct effects of individual compounds [9,10]. One common method is co-culturing based on direct contact, in which various interactions, including competition and potential allelopathic effects, are examined during the co-cultivation of two species, such as a eukaryotic alga and a cyanobacterium [11]. This approach is suitable for identifying mechanisms arising from direct contact; however, distinguishing between competition and true allelopathy often presents difficulties [4]. To overcome this problem, filtrate or exudate assays are also used, in which dissolved metabolites secreted by the donor organism are tested on recipient species without direct contact [4,12]. This method allows for a more accurate assessment of purely chemical-based effects. Furthermore, the isolation and testing of individual allelochemicals in purified form also plays a key role in understanding the biological activity of these compounds [13].

Numerous studies examining interactions between eukaryotic algae and cyanobacteria have reported that cyanobacteria often possess a competitive advantage over eukaryotic algae [12,14–20]. At the same time, there are studies highlight that eukaryotic algae also produce metabolites affecting the growth and physiological processes of cyanobacteria. Vardi [21] reported the mutual allelopathy between the dinoflagellate *Peridinium gatunense* and a *Microcystis* species. Co-cultivation experiments by Gantar et al. [22] also confirmed allelopathic interactions between cyanobacteria and green algae. Harel et al. [23] observed that in the presence of the cyanobacterium *Microcystis aeruginosa*, the green alga *Scenedesmus huji* releases biologically active substances into its environment that inhibit the growth of the cyanobacterium and cause serious phycobilin leakage. Experiments with spent media or cellular extracts also provide further evidence [23] or may prove that species otherwise showing no inhibitory properties release compounds with allelopathic effects into their environment during cell lysis.

Currently there is relatively little direct evidence available regarding the potential anti-cyanobacterial effects of species belonging to the genus *Chlorella* (particularly *Chlorella vulgaris* and *Chlorella sorokiniana*), but some research suggests that under certain conditions, they may be able to inhibit the growth or competitive advantage of toxic cyanobacteria, such as *Microcystis aeruginosa* [24]. According to the literature, the extracts alter the permeability of the cell membrane, disrupt ion flow, and increase the production of reactive oxygen species (ROS), which can lead to cellular damage [2]. Bioactive compounds have been identified in several species of the genus *Chlorella* (including long-chain fatty acids, carotenoids, and numerous other compounds [25,26]). Free fatty acids from *Chlorella vulgaris* were reported to have a cytotoxic effect on the plasma membrane on *Anabaena* P-9 [27]. According to many studies, the synthesis of these bioactive metabolites in numerous microalgae increases as cell growth slows down, specifically during the late exponential and stationary growth phases [28–30].

The aim of the present study was to investigate the potential anti-cyanobacterial activity of the biomass extract derived from stationary culture of the green alga *Chlorella vulgaris*, using *Microcystis aeruginosa* as the model cyanobacterium. The objectives were to monitor the (i) growth and (ii) quantitative and qualitative changes in characteristic cyanobacterial pigments (phycobilins, i.e., the physiological state of the cyanobacterial cultures) and (iii) to observe any changes in nutrient uptake of the cyanobacterium to confirm or rule out the role of nutrients in the observed phenomena. Furthermore, (iv) the lipophilic fraction of the *Chlorella* biomass was partly identified, and it was checked how many of these molecules remain in the biomass after an extraction aimed primarily to isolate small polar molecules (<1500 Da; methanolic extraction; [31]) to assess the extent to which these metabolites may play a role in the observed phenomena.

2. Materials and Methods

2.1. Strains, Culturing Conditions and Preparation of the *Chlorella vulgaris* Extract

Microcystis aeruginosa (Chroococcales), the derivative of the strain BGSD-243 (Department of Botany, University of Debrecen), was used in the experiment. The strain was maintained in the algae culture collection of the Department of Hydrobiology at the University of Debrecen in Allen's Medium [32] at 24 °C, with a photoperiod of 14 h of light (40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and 10 h of darkness.

The *Chlorella vulgaris* strain used for the experiment was isolated from an aquarium of red-eared sliders (*Trachemys scripta elegans*) in 2011, and maintained since then in the algae culture collection of the Department of Hydrobiology at the University of Debrecen in Jaworski's Medium [33], under a photoperiod of 14 h of light (40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and 10 h of darkness, at 24 °C. The algal biomass of stationary-phase cultures (three-month-old standing cultures) was collected by centrifugation (15,317 $\times g$, 10 min, 24 °C; Multifuge X4R Pro, Thermo Electron LED GmbH, Osterode am Harz, Germany), and was freeze-dried (lyophilized; Christ Alpha 1–2 LD plus, Osterode am Harz, Germany). A total of 0.5 g of *C. vulgaris* lyophilized biomass was resuspended in 80% methanol and extracted overnight. After extraction, the supernatant was separated by centrifugation and the solvent was completely removed by vacuum centrifugation (RVC 2 CD plus, Osterode am Harz, Germany). The obtained dry material was resuspended in 1 mL of Allen's Medium at the start of the experiment, after which the *Microcystis* cultures were treated with this green algal extract.

2.2. Experimental Design and Sampling

Microcystis aeruginosa cultures were set in 100 mL Erlenmeyer flasks with a final volume of 50 mL under shaking conditions (90 rpm, Wids SHO-2D orbital shaker, Witeg

Labortechnik GmbH, Wertheim, Germany) and the above-detailed culturing environment. The duration of the experiment was 14 days. *Chlorella vulgaris* extract (prepared as described above) was added to the treated cultures, to reach extract concentrations corresponding to 0.2, 0.4, 0.8, and 1.6 mg mL⁻¹ of dry *Chlorella* biomass.

To monitor growth and to analyze phycobilin, nitrate, and phosphate content, samples were collected at the start of the experiment (day 0), and then on days 2, 4, 7, 9, 11, and 14. After sampling, the volume loss resulting from sampling or evaporation was replenished in each case with sterile distilled water to the original 50 mL, thereby ensuring a constant volume while eliminating the replenishment of nutrients. Another 1 mL sample was taken to measure other nitrogen and phosphorus forms on days 0 and 14.

2.3. Measurement of Growth and Pigment Content of *M. aeruginosa*

To estimate cell density, optical density (OD) was measured at 800 nm in a 1 mL sample (Hach Lange DR 6000 UV/VIS spectrophotometer, Hach Lange GmbH, Düsseldorf, Germany). This indirect method correlates well with biomass quantity and allows for the easy monitoring of growth dynamics [34].

After measuring optical density, the samples were centrifuged (16,200× *g*, 5 min, Heraeus Fresco 17, Heraeus Group, Hanau, Germany), and the cell-free supernatant was removed. Pigment content was measured from the cells collected by centrifugation, and changes in the levels of the major phycobiliproteins—phycocyanin, allophycocyanin, and phycoerythrin—were monitored. For extraction, 500 µL of phosphate buffer solution (a mixture of NaH₂PO₄ and Na₂HPO₄ in distilled water, pH ~7) was added to the samples, and then they were placed in a freezer for 24 h. The samples were subsequently thawed in a refrigerator (~8 °C), and after another 24 h freeze, they were thawed again to enhance the efficiency of the extraction [35].

2.4. Measurement of Nitrogen and Phosphorous Forms

Changes in the initial nitrate and phosphate content of the culturing medium were monitored spectrophotometrically in accordance with Hungarian Standards [36,37]; measurements were performed on cell-free supernatants obtained by centrifugation after optical density measurement, with the sample and reagent quantities proportionally reduced compared to specified in the standards. Samples taken on days 0 and 14 were used to measure ammonium content [38] possibly added with the extract. The additional samples collected on days 0 and 14 were subjected to alkaline potassium persulfate digestion using a mixture of NaOH and K₂S₂O₈ [39] to measure the total nitrogen and phosphorous content, enabling to follow the quantitative and qualitative changes in the other nitrogen and phosphorus forms possibly introduced with the extract.

2.5. Extraction and Measurement of Pigment and Lipid Content of *Chlorella vulgaris*

To calculate the pigment content of the original *C. vulgaris* biomass and the prepared *C. vulgaris* extract, 0.025 g of dry material from the original biomass and from the biomass after extraction was mixed in 1 mL of DMSO (Velp Scientifica ESP, Usmate Velate, Italy) for 30 min at 24 °C in the dark, and then the samples were centrifuged (16.2× *g*, 5 min, Heraeus Fresco 17, Hanau, Germany). After removing the supernatant, the pellet was resuspended in 1 mL of fresh DMSO and the extraction was repeated; this cycle was performed four times [40]. To avoid pigment degradation, sample processing was performed with minimal light exposure. Chlorophyll-a, chlorophyll-b, and total carotenoid content were measured spectrophotometrically from the DMSO extracts [41].

The main carotenoid components (lutein, astaxanthin-ester-like compounds, and β-carotene) were separated by thin-layer chromatography (TLC) and their quantities were given relative to dry biomass weight. For TLC analysis, the pigments were transferred from

DMSO to ethyl acetate based on the method of Du et al. [42,43] with minor modifications. A 20 µL aliquot of DMSO-free ethyl acetate phase was transferred to TLC plates (silica gel 60 GF254, 10 × 20 cm, Merck, Darmstadt, Germany). Carotenoid standards (lutein, astaxanthin ester mixture, and β-carotene; Merck, Darmstadt, Germany) were used to identify and estimate the quantities of individual carotenoids. In each case, 1.0 mg of pure pigment standard was dissolved in 2.0 mL of DMSO. From each of the three standard solutions, 50 µL was transferred to Eppendorf tubes and diluted to 400 µL with DMSO. Similarly to the samples, the standards were transferred from DMSO to ethyl acetate, and then 2.5, 5.0, 7.5, and 10.0 µL of the DMSO-free ethyl acetate phase were transferred to TLC plates. The TLC analysis was performed based on the recommendations of Hynstova et al. [44], with minor modifications. The samples were transferred to the plate in the form of bands, each 5.0 mm in width. The first and last bands were placed 15.0 mm from the edges of the plate and 20.0 mm from the bottom of the plate. The mobile phase was a mixture of petroleum ether:isopropanol:water 100:10:0.25 v/v, and the solvent front was 60.0 mm.

After running, the TLC plates were dried at room temperature, then illuminated on the glass surface of a 3M 9700 overhead projector (3M Visual System Division, Austin, TX, USA) and photographed with a digital camera (Nikon D3200, AF-S DX NIKKOR 18–105 mm VR lens, Nikon Corporation, Tokyo, Japan), and the images were analyzed using GelAnalyzer 23.1 software [45].

Lipids were extracted according to the description in Bácsi et al. [46], where 1 mL of chloroform–methanol mixture (2:1 by volume) was added to 0.025 g of dry, homogenized samples. The mixture was stirred in the dark for 30 min at 24 °C (Velp Scientifica ESP, VELP Scientifica Srl, Usmate Velate, Italy), then centrifuged, and the clear supernatant was collected in Packard cuvettes. This procedure was repeated four times. The 4 mL of supernatant collected in this way was poured into pre-weighed round-bottom flasks and evaporated at 40 °C using a vacuum evaporator (Rotadest Type 2118; KUTESZ Kft., Budapest, Hungary). The flasks were weighed again (Ohaus Adventurer™ Pro, Ohaus Corporation, Parsippany, NJ, USA), and after correcting the weight data with the calculated pigment contents, the results were expressed in µg mg^{−1} relative to the unit of dry weight.

The fatty acids were specified at the Biotechnology Institute of Bay Zoltán Applied Research Nonprofit Ltd. (Szeged, Hungary) using gas chromatography (GC-MS) with the fatty acid methyl ester (FAME) method from 0.1 g of lyophilized stationary *C. vulgaris* biomass. During the process, the biomass was treated in the presence of antioxidants in a methanol–chloroform–hydrochloric acid system at 90 °C, during which compounds containing carboxyl groups are converted into methyl esters. The equilibrium was broken by adding water, and the FAME compounds present in the chloroform phase were identified by internal standard calibration using the GC-MS method. The reference material was Sigma-Aldrich Supelco FAME mix CRM47885 (Merck KGaA, Darmstadt, Germany, and its subsidiaries), which contained the 37 most biologically important fatty acid methyl esters in known concentrations. The identified fatty acids were grouped according to chain length and saturation based on Agostoni and Bruzzese [47].

2.6. Statistical Analysis

The experiments were done in triplicate, and the replicates were independent biological cultures. The statistical analysis of the data was carried out using Jamovi statistical analysis software (jamovi 2.7.37), which is based on the R programming language. For the analyses of OD800, phycobilin, and nutrient (nitrogen and phosphorous) data, a linear mixed-effects model was applied. Treatment, Time, and their interaction were included as fixed effects. Individual culture was treated as the repeated experimental unit; parameters

were estimated using restricted maximum likelihood (REML). When the Treatment $\times$ Time interaction was significant, pairwise comparisons among treatments within each sampling day were performed using estimated marginal means with Bonferroni adjustment for multiple comparisons. Statistical significance was accepted at $p < 0.05$.

The data of the lipid-soluble components of the stationary *Chlorella* biomass before (SB) and after extraction (SB-E) were evaluated by an independent-samples *t*-test.

3. Results

3.1. Growth and Phycobilin Content Changes in *M. aeruginosa* Cultures

The data of optical density measured at 800 nm and phycobilin content of *M. aeruginosa* cultures are summarized in Table S1. The linear mixed-effects model revealed significant effects of Treatment and Time and a significant Treatment $\times$ Time interaction in the case of OD800, indicating that the temporal response differed among treatments (Table S2). The optical density values of all treated cultures were significantly lower than those of the control culture from day 9 (Figure 1; Table S2; $p < 0.05$). A lower degree of inhibition was observed in the 0.2 mg mL⁻¹ treatment compared to higher extract concentrations, but the differences were not significant. No concentration-dependent differences were detected in the growth-inhibitory effect of the higher concentrations (0.4–1.6 mg mL⁻¹; Figure 1).

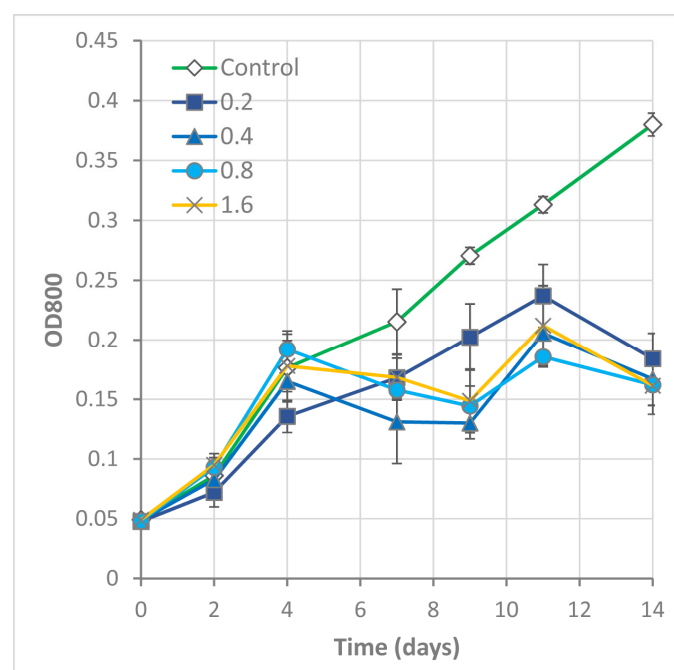


Figure 1. Growth of *Microcystis aeruginosa* control and *Chlorella vulgaris* extract-treated cultures based on optical density measured at 800 nm. 0.2–1.6: the applied *Chlorella* extract concentrations in mg mL⁻¹. Mean values and standard deviations are plotted ($n = 3$). Significant differences are summarized in Table S1.

The linear mixed-effects model revealed significant effects of Treatment and Time and a significant Treatment $\times$ Time interaction in the case of phycobilins (total phycobilin, phycocyanin, allophycocyanin, and phycoerythrin content), indicating that the temporal response differed among treatments (Tables S3–S6). *M. aeruginosa* cultures treated with the lowest *Chlorella* extract concentration (0.2 mg mL⁻¹) had higher phycobiliprotein content than the control, while those treated with 0.8 and 1.6 mg mL⁻¹ had lower contents (Figure 2). Significant differences between the total phycobiliprotein content of the treated cultures were detected on days 7–14 (Table S3). In all cases, phycocyanin was the dominant pigment,

followed by allophycocyanin, with phycoerythrin present in the smallest amount. These ratios were not affected by the treatments (Figure 2). Extract concentration-dependent changes were observed from day 7 in the case of phycocyanin and allophycocyanin, and mainly from day 9 in the case of phycoerythrin (Tables S4–S6). A significant pigment content increase was observed in the culture treated with 0.8 mg mL^{-1} *Chlorella* extract from day 11 to day 14; this regeneration was less pronounced in the culture treated with the highest (1.6 mg mL^{-1}) *Chlorella* extract concentration (Tables S3–S6).

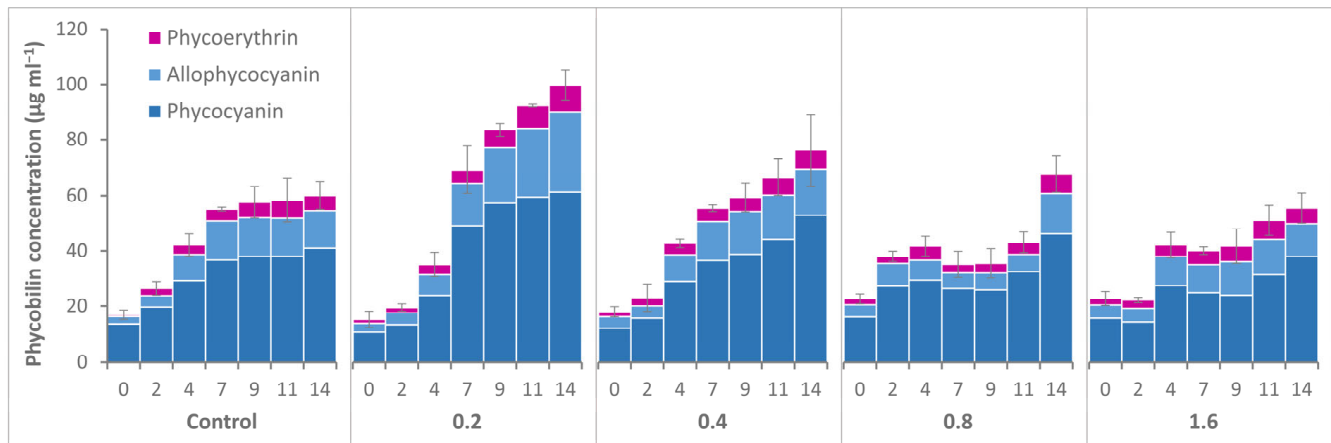


Figure 2. Phycobiliprotein content in control and *Chlorella vulgaris* extract-treated *Microcystis aeruginosa* cultures. 0.2–1.6: the applied *Chlorella* extract concentrations in mg mL^{-1} . 0–14: sampling days. Pigment mean values and standard deviations for total phycobiliproteins are shown ($n = 3$). Significant differences are summarized in Table S2.

3.2. Nitrogen and Phosphorus Content Changes in *M. aeruginosa* Cultures

The nitrogen source in Allen's Medium is nitrate. The data of daily nitrate content changes of *M. aeruginosa* cultures are summarized in Table S1. The linear mixed-effects model revealed significant effects of Time and a significant Treatment $\times$ Time interaction in the case of nitrate concentration in the cultures (Table S7). Within a given treatment, a significant decrease was first observed from day 4 (control) and from days 7–9 (treatments; Figure 3a; Table S7). There was no significant difference in the nitrate content of the cultures measured on a given day during the exposure period (Table S7), suggesting that the treatment had no effect on nitrate uptake.

The phosphorus source in Allen's Medium is dissolved inorganic orthophosphate. The data of daily phosphate content changes of *M. aeruginosa* cultures are summarized in Table S1. The linear mixed-effects model revealed significant effects of Treatment and Time and a significant Treatment $\times$ Time interaction in the case of phosphate content (Table S8). As the concentration of *Chlorella* extract increased, less and less phosphate was consumed by day 14 (Figure 3b). Phosphate uptake was more intense than nitrate uptake: significantly less phosphate was present in the cultures compared to previous days already on days 2–7 (Table S8). Although higher phosphate levels were detected in the culture with the highest extract concentration of 1.6 mg mL^{-1} than in the other cultures, a significant difference was observed only in comparison with the 0.2 mg mL^{-1} treated culture on days 7–11 (Table S8), suggesting that within the studied concentration range, the *Chlorella* extract did not affect phosphate uptake significantly.

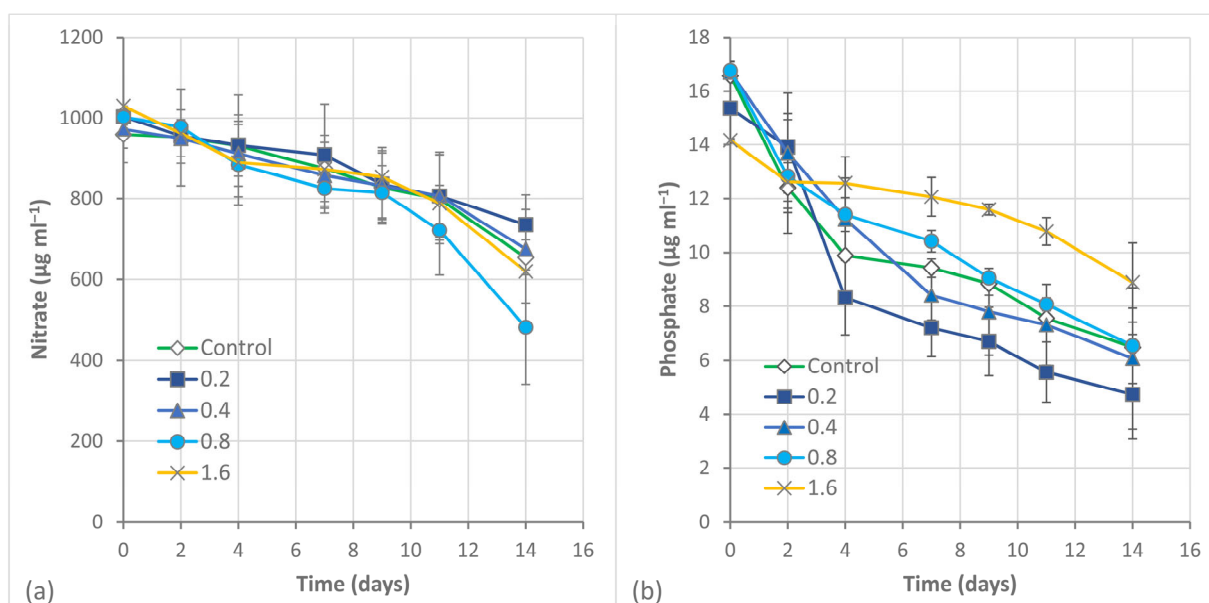


Figure 3. Nitrate (a) and phosphate (b) concentrations in control and *Chlorella vulgaris* extract-treated *Microcystis aeruginosa* cultures. 0.2–1.6: the applied *Chlorella* extract concentrations in mg mL^{-1} . Mean values and standard deviations are shown ($n = 3$). Significant differences are summarized in Table S3.

Nitrogen and phosphorus forms were also added to the treated cultures with the green algae extract, as a large number of nitrogen- and phosphorus-containing organic compounds could be extracted during the extraction process. The data of nitrogen and phosphorous content of *M. aeruginosa* cultures are summarized in Table S9. Although the amount of Other-N (presumably organic N) added to the treatments increased more or less proportionally with the increase in extract concentration, none of the nitrogen forms showed significant differences among the cultures on day 0 (Tables S10–S14). The amount of Other-N decreased by day 14 in all cultures, but ammonium and nitrite appeared in detectable amounts alongside them (Figure 4; Tables S10–S14). It should be noted that there was no significant difference in the amount of any nitrogen form between the control and treated cultures on day 14 (except the ammonium-N content in 0.2 mg mL^{-1} treated culture; Tables S10–S14).

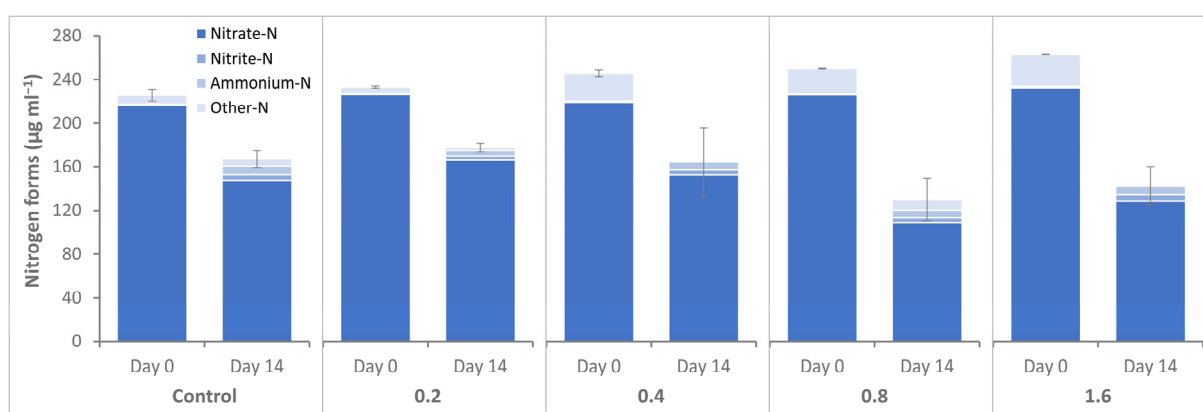


Figure 4. Nitrogen content of control and *Chlorella vulgaris* extract-treated *Microcystis aeruginosa* cultures at the time of inoculation (day 0) and at the end of the exposure period (day 14). 0.2–1.6: the applied *Chlorella* extract concentrations in mg mL^{-1} . Nitrogen form mean values and standard deviations for total nitrogen content are shown ($n = 3$). Significant differences are summarized in Table S4.

A significant amount of other phosphorus compounds (most likely phosphorylated organic compounds) are present after inoculation even in the control culture (Figure 5, Other-P). The concentration of both phosphorus forms decreased significantly by day 14, except for the 0.2 and 1.6 mg mL⁻¹ treatments, where the decrease in Other-P was not significant (Table S16). The Total-P content of the 1.6 mg mL⁻¹ treated culture was significantly higher than that of the control and the other treated cultures on day 14 (except 0.2 mg mL⁻¹ treatment; Table S17).

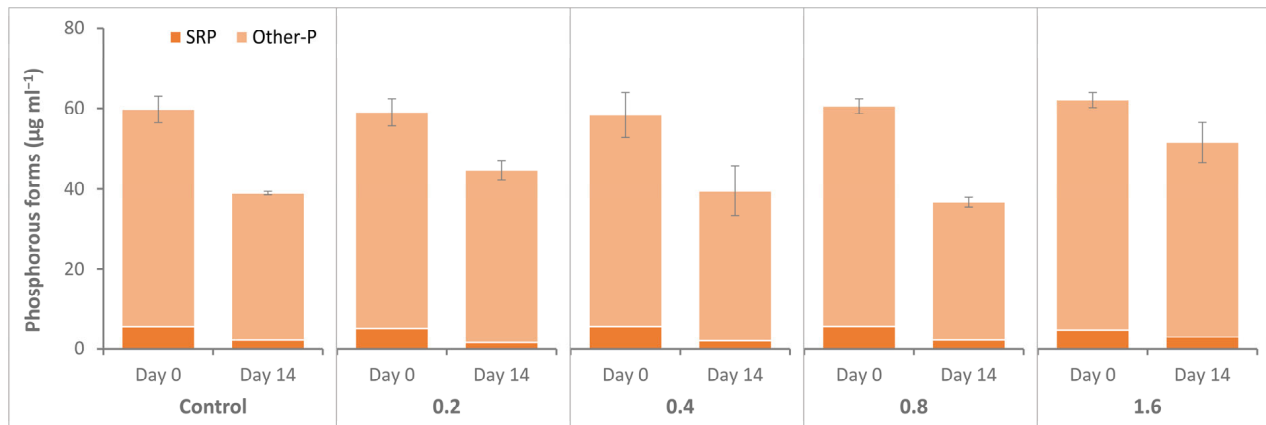


Figure 5. Phosphorous content of control and *Chlorella vulgaris* extract-treated *Microcystis aeruginosa* cultures at the time of inoculation (day 0) and at the end of the exposure period (day 14). 0.2–1.6: the applied *Chlorella* extract concentrations in mg mL⁻¹. Phosphorous form mean values and standard deviations for total phosphorous content are shown ($n = 3$). Significant differences are summarized in Table S5.

3.3. Pigment and Lipid Content of *Chlorella vulgaris* Biomass

The data of lipid-soluble components of the stationary *C. vulgaris* biomass before and after extraction are summarized in Table S18. Carotenoids were the dominant group among the main pigments, while chlorophylls were detected in substantially lower amounts (Figure 6a). During the preparation of the *Chlorella* extract, a solution rich in bioactive molecules smaller than 1500 Daltons and free of macromolecules (proteins, nucleic acids, and membrane components) was obtained by the extraction of the stationary-phase *Chlorella* biomass with 80% methanol. The purpose of analyzing the biomass remaining after extraction was to determine how much lipid-soluble material was transferred into the 80% methanolic extract (based on how much remained in the biomass). After methanolic extraction, 83% of chlorophyll *a*, 86% of chlorophyll *b*, and 68% of total carotenoids remained in the biomass (Figure 6a); i.e., 17% of chlorophyll *a*, 14% of chlorophyll *b* and 32% of total carotenoids were extracted with methanol during the preparation of the *Chlorella* extract for the treatment. This means that the presence of nonpolar metabolites including carotenoids up to 30% can be expected in the methanolic phase after extract preparation.

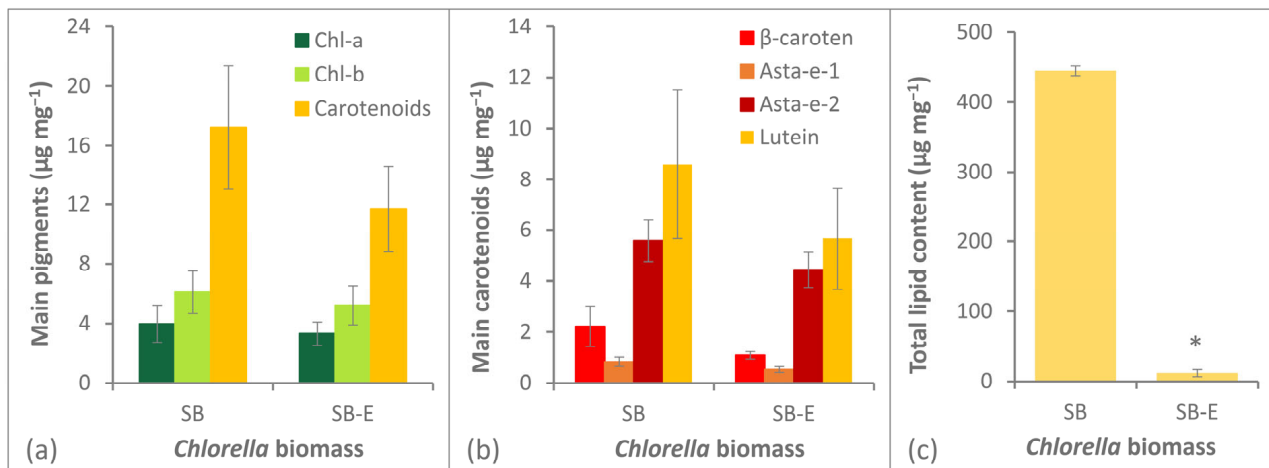


Figure 6. Pigment and lipid content of *Chlorella vulgaris* biomass collected from a stationary culture. (a) Main pigments; (b) identified carotenoids; (c) total lipid content. SB: original stationary biomass; SB-E: stationary biomass after methanolic extraction. Mean values and standard deviations are shown ($n = 3$). The asterisk shows significant differences between SB and SB-E ($p < 0.05$; statistical analysis results are summarized in Tables S19–S26).

The carotenoid content of the *Chlorella* biomasses was further analyzed by thin-layer chromatography. Although TLC is primarily a qualitative method, it also allows for relatively precise quantitative analysis, as the pigment concentrations can be estimated using calibration curves [48]. In addition to β -carotene, two astaxanthin ester-like metabolites and a significant amount of lutein were detected in stationary *C. vulgaris* biomass (Figure 6b). Compared to the quantities measured in the original stationary biomass, 49% of the β -carotene content, 63% of the astaxanthin ester-like metabolite 1 content, 80% of the astaxanthin ester-like metabolite 2 content, and approximately 66% of the lutein content remained in the biomass after the preparation of the *Chlorella* extract. In other words, the identified carotenoids were extracted at an average rate of $30 \pm 10\%$ during the 80% methanolic extraction.

The stationary *Chlorella* biomass can be characterized with a relatively high total lipid content ($\sim 445 \mu\text{g mg}^{-1}$ dry mass; Figure 6c). Only 3% of total lipid content remained in the *Chlorella* biomass after extraction with 80% methanol (Figure 6c), meaning that 97% of total lipid content of *Chlorella* biomass was present in the extract. During fatty acid analysis, 20 fatty acids were identified. The identified fatty acids were classified into groups based on Agostoni and Bruzesse (carbon atom number and degree of unsaturation; [47]). The most dominant group was long-chain monounsaturated fatty acids, which accounted for nearly 39% of the total fatty acid content (Table 1). The second most dominant group was long-chain polyunsaturated fatty acids, with linoleic acid and linolenic acid being the most significant. They accounted for 36% of the fatty acid content in stationary *C. vulgaris* biomass (Table 1).

Table 1. Fatty acids isolated from stationary *Chlorella vulgaris* biomass. The yellow highlighting indicates the dominant fatty acids.

Fatty Acid	<i>Chlorella vulgaris</i> Biomass		Fatty Acid Group [42]
	$\mu\text{g mg}^{-1}$	% of Total Fatty Acids	
C12:0 laurylic acid	0.06	0.02	MCSFA ¹
C14:0 myristic acid	1.65	0.53	LCSFA ²
C15:0 pentadecanoic acid	0.19	0.06	
C16:0 palmitic acid	60.86	19.57	
C17:0 heptadecanoic acid	0.48	0.15	
C18:0 stearic acid	10.44	3.36	
C20:0 arachidic acid	1.79	0.58	VLCSFA ³
C22:0 docosanoic acid	1.46	0.47	
C24:0 lignoceric acid	0.37	0.12	
C16:1 palmitoleic acid	1.09	0.35	LCMUFA ⁴
C18:1n9c oleic acid	117.83	37.88	
C20:1n9c gadoleic acid	2.17	0.70	VLCMUFA ⁵
C22:1n9 docosenoic acid	0.18	0.06	
C24:1 nervonic acid	0	0	
C18:2n6c linoleic acid	64.33	20.68	LCPUFA ⁶
C18:3n6 gamma-linoleic acid	2.57	0.83	
C18:3nc3 alfa-linoleic acid	45.34	14.58	
C20:2n6 cisz-11,14-eikozadienic acid	0.11	0.04	VLCPUFA ⁷
C20:3n6 cisz-8,14,17-eikozatrienoic acid	0.07	0.02	
C20:4n6 arachidonic acid	0.06	0.02	

¹ MCSFA: Medium-Chain Saturated Fatty Acid; ² LCSFA: Long-Chain Saturated Fatty Acid; ³ VLCSFA: Very-Long-Chain Saturated Fatty Acid; ⁴ LCMUFA: Long-Chain Monounsaturated Fatty Acid; ⁵ VLCMUFA: Very-Long-Chain Monounsaturated Fatty Acid; ⁶ LCPUFA: Long-Chain Polyunsaturated Fatty Acid; ⁷ VLCPUFA: Very-Long-Chain Polyunsaturated Fatty Acid.

4. Discussion

4.1. Growth and Phycobilin Content of *M. aeruginosa* Cultures

The results showed growth inhibition of *M. aeruginosa* cultures treated with *C. vulgaris* extract, although there were no clear concentration-dependent effects, which may make the interpretation of the results a bit more difficult. Temporal changes in microbial abundance during ecotoxicological assays are not necessarily monotonic. Following an initial decline, microbial populations may exhibit partial recovery or transient increases due to physiological acclimation, compensatory growth, hormetic responses, or intrinsic oscillatory population dynamics. Consequently, fluctuations in cell abundance during toxicity experiments should not automatically be interpreted as experimental artifacts but may reflect inherent biological responses of microbial populations [49–51].

Although species of the genus *Chlorella*, including *C. vulgaris*, have been extensively studied, to our knowledge, there is very little data in the literature regarding their anti-cyanobacterial effects. One of the best-known metabolites isolated from *Chlorella* species, including *Chlorella vulgaris*, is chlorellin. In addition to its inhibitory effect on both Gram-negative and Gram-positive bacteria [52], this fatty acid mixture also inhibits the growth of another green alga, *Pseudokirchneriella subcapitata* [53]. The antibacterial [54,55] and antifungal [56,57] effects of various *C. vulgaris* extracts are well-known. However, to our knowledge, a direct anti-cyanobacterial effect has been reported for only one *C. vulgaris* strain: a cyclic peptide named protoamid was described, which proved to have an in-

hibitory effect on the filamentous cyanobacterium *Cylindrospermopsis raciborskii*; however, no inhibition was detected in the case of the unicellular (colonial) *Microcystis*, as well as the filamentous *Aphanizomenon* and *Anabaena* species [58]. Our results show that the studied *M. aeruginosa* strain is also not highly sensitive to the *C. vulgaris* extract: based on the optical density measured at 800 nm, increased concentration-dependent inhibition was not observable; it might only be expected at extract concentrations higher than the applied range. Nevertheless, a clear cyanostatic effect was observed in the treated cultures.

The concentration of phycobiliproteins (particularly phycocyanin and allophycocyanin) accurately reflects the physiological state of cyanobacteria, as they are among the most sensitive components of their photosynthetic apparatus [59]. Numerous allelopathic compounds and other algal-inhibiting substances cause damage to phycobilisomes, as well as a decrease in phycobiliproteins or their leakage from the cell; therefore, these pigments are frequently used as biomarkers in toxicity studies [23,24,27]. Although higher phycobilin content was observed in the *M. aeruginosa* culture treated with *Chlorella* extract concentrations of 0.2 and 0.4 mg mL⁻¹, this apparent stimulation of growth was not supported by optical density measurements correlating with cell density. One possible explanation could be that, when cell division is inhibited, ongoing metabolic processes continue to consume available nutrients [60]. Consequently, a portion of the nitrogen may be incorporated into pyrrole-ring pigments, as indicated by changes in pigment composition observed in *Haematococcus* cultures treated with non-steroidal anti-inflammatory drugs [43]. Proving this hypothesis requires further, targeted studies; however, it seems that the growth-inhibiting *C. vulgaris* extract suppressed phycobiliprotein synthesis or caused pigment leakage only in higher concentrations (0.8 and 1.6 mg mL⁻¹) and temporarily.

4.2. Nitrogen and Phosphorus Content in *M. aeruginosa* Cultures

By analyzing the nutrient content, it was sought to study whether the nutrient content of the *Chlorella* extract or the presence of allelopathic metabolites causes a change in cyanobacterial nutrient uptake, which may also underlie the change in growth. The daily changes in the nitrate content of the cultures and the lack of significant differences between the cultures on given days suggest that the *Chlorella* extract did not significantly affect nitrogen metabolism of the cyanobacterium. Increasing nitrogen content with the higher concentration of the added *Chlorella* extract was observed only for total nitrogen, and only in this case were there significant differences between the individual treatments on day 0. It should also be noted that for the nitrogen forms labeled as Other-N, significantly higher amounts were detected in treatment 1.6 mg mL⁻¹ compared to the control and treatment 0.2 mg mL⁻¹. All of this indicates that *Chlorella* extract contributes measurably to the nitrogen content of the cultures only at higher concentrations (1.6 mg mL⁻¹ in the present study). The reduced inorganic nitrogen forms detected on day 14 most likely appeared as a result of the release from disintegrating cyanobacterial cells and the associated enzyme activities.

Given the absence of significant differences on day 0 in the amounts of phosphorous forms, and the higher phosphorus content observed in the 1.6 mg mL⁻¹ treated culture on subsequent days and at the end of the experiment, it seems that *Chlorella* extract may have an effect on phosphate uptake at this concentration. Physiological adaptation to changes in external phosphate concentration involves two energy-dependent processes: the transport of phosphate across the cell membrane and the conversion of absorbed phosphate into ATP in equilibrium with polyphosphates. The energetic and kinetic properties of the phosphate transport system and ATP synthase are coordinated in such a way that energy dissipation is minimized under prevailing growth conditions [61]. Further, more targeted studies are needed to determine which processes could be modified at the applied *Chlorella* extract

concentrations. The significant decrease in Other-P levels may be due to the well-known high extracellular phosphatase activity of cyanobacteria [62].

4.3. Lipid-Soluble Components of *Chlorella vulgaris* Biomass

The synthesis of bioactive (storage or secondary) metabolites of numerous micro- and macroalgae increases as cell growth slows down, specifically during the late exponential and stationary growth phases, since during these phases the cells allocate more resources to stress responses and the production of compounds that fulfill ecological functions rather than to biomass accumulation [28–30]. The *Chlorella* strain used for the experiments accumulates lipids and carotenoids during the stationary phase, which is why the stationary-phase biomass was chosen to prepare the extract for the experiments. The results of pigment and lipid analyses of the stationary *Chlorella* biomass demonstrated that the identified carotenoids were extracted at an average rate of $30 \pm 10\%$ during the 80% methanolic extraction, while 97% of total lipid content of *Chlorella* biomass was present in the extract. The most dominant fatty acid groups were long-chain monounsaturated and long-chain polyunsaturated fatty acids. This composition explains why a significant portion of the fatty acid content of the original biomass could be extracted: unsaturated fatty acids are more soluble in methanol than saturated fatty acids [63,64]. It may also partially explain the extract's cyanostatic activity: one of the earliest metabolites isolated from *Chlorella* species, including *Chlorella vulgaris*, is chlorellin, whose antibacterial effects have also been known for a long time, and this fatty acid mixture also inhibits the growth of the green alga, *Pseudokirchneriella subcapitata* [53], as discussed in Section 4.1. *Chlorella* biomass contains numerous bioactive metabolites, including carotenoids, glycolipids and unsaturated fatty acids, several of which have been reported to exhibit allelopathic or algicidal activity. In particular, free unsaturated fatty acids such as linoleic, α -linolenic and oleic acids can impair membrane integrity, induce oxidative stress and inhibit photosynthesis in competing algal species. Although carotenoids primarily function as photoprotective antioxidants, their oxidative cleavage products may also contribute to allelopathic interactions [25–27,52–58]. Consequently, the allelopathic activity of *Chlorella* biomass is generally considered to result from the combined action of multiple classes of bioactive metabolites. Further, more targeted studies are needed to determine whether any of the more polar, more water-soluble components of the *Chlorella* extract may have contributed to the observed cyanostatic effects.

5. Conclusions

Based on the results, the *C. vulgaris* extract caused moderate inhibition of *Microcystis* growth; however, no concentration-dependent inhibition was observed within the applied *Chlorella* extract concentration range. Pigment levels increased in some cases, which at first glance might have appeared to be a stimulatory effect, but OD values reflecting cell density did not support this assumption. It is more likely that pigment production increased as a result of residual metabolic activity next to growth inhibition. Regarding nutrients, nitrate uptake did not change significantly in response to the treatments, while inhibition of phosphate uptake was suggested at the highest (1.6 mg mL^{-1}) extract concentration. Based on the degradation and transformation of nitrogen forms and organic phosphates, it can be assumed that the extract may have altered not only growth but also certain metabolic processes; however, further studies are needed to clarify the mechanisms involved.

Overall, it can be stated that the *Chlorella vulgaris* extract exerted a moderate inhibitory effect on *Microcystis aeruginosa* cultures and also influenced certain physiological parameters, such as pigment content and phosphate uptake. These results support the hypothesis that metabolites derived from eukaryotic algae—as potential environmentally friendly

biological control agents—may offer a promising alternative for managing harmful algal blooms, particularly if their selectivity and mechanism of action are further elucidated.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/phycology6030088/s1>. Table S1. Data for OD800, total phycobilins, phycocyanin, allophycocyanin, phycoerythrin, nitrate and phosphate measured in *Microcystis aeruginosa* control and *Chlorella vulgaris* extract-treated cultures. Table S2. Statistical analyses results for OD800. Table S3. Statistical analyses results for total phycobilins. Table S4. Statistical analyses results for phycocyanin. Table S5. Statistical analyses results for allophycocyanin. Table S6. Statistical analyses results for phycoerythrin. Table S7. Statistical analyses results for nitrate. Table S8. Statistical analyses results for phosphate. Table S9. Data for ammonium-N, nitrite-N, nitrate-N, other-N, total-N, soluble reactive P (SRP), other-P and total-P measured in *Microcystis aeruginosa* control and *Chlorella vulgaris* extract-treated cultures. Table S10. Statistical analyses results for ammonium-N. Table S11. Statistical analyses results for nitrite-N. Table S12. Statistical analyses results for nitrate-N. Table S13. Statistical analyses results for other-N. Table S14. Statistical analyses results for total-N. Table S15. Statistical analyses results for SRP. Table S16. Statistical analyses results for other-P. Table S17. Statistical analyses results for total-P. Table S18. Pigment and lipid content of *Chlorella vulgaris* biomass collected from a stationary culture. Table S19. Statistical Analyses Results for Chlorophyll-a. Table S20. Statistical Analyses Results for Chlorophyll-b. Table S21. Statistical Analyses Results for Total Carotenoids. Table S22. Statistical Analyses Results for β -carotene. Table S23. Statistical Analyses Results for Astaxanthin-ester-like Compound 1. Table S24. Statistical Analyses Results for Astaxanthin-ester-like Compound 2. Table S25. Statistical Analyses Results for Lutein. Table S26. Statistical Analyses Results for Total Lipids [65–68].

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References

1. Paerl, H.W.; Fulton, R.S., 3rd; Moisander, P.H.; Dyble, J. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *Sci. World J.* **2001**, *1*, 76–113. [CrossRef] [PubMed]
2. Tan, K.; Huang, Z.; Ji, R.; Qiu, Y.; Wang, Z.; Liu, J. A review of allelopathy on microalgae. *Microbiology* **2019**, *165*, 587–592. [CrossRef] [PubMed]
3. Rice, E.L. *Allelopathy*, 2nd ed.; Academic Press: New York, NY, USA, 1984.
4. Legrand, C.; Rengefors, K.; Fistarol, G.O.; Granéli, E. Allelopathy in phytoplankton—Biochemical, ecological and evolutionary aspects. *Phycologia* **2003**, *42*, 406–419. [CrossRef]
5. Paine, R.T. Benthic macroalgal competition: Complications and consequences. *J. Phycol.* **1990**, *26*, 12–17. [CrossRef]
6. Maggi, E.; Bulleri, F.; Bertocci, I.; Benedetti-Cecchi, L. Competitive ability of macroalgal canopies overwhelms the effects of variable regimes of disturbance. *Mar. Ecol. Prog. Ser.* **2012**, *465*, 99–109. [CrossRef]

7. Wolfe, G.V. The chemical defense ecology of marine unicellular plankton: Constraints, mechanisms, and impacts. *Biol. Bull.* **2000**, *198*, 225–244. [CrossRef] [PubMed]
8. Craig, M.; Holmes, C.F.B. Freshwater hepatotoxins: Microcystin and nodularin, mechanisms of toxicity and effects on health. In *Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection*, 1st ed.; Botana, L.M., Ed.; CRC Press: New York, NY, USA, 2000; pp. 643–671. [CrossRef]
9. Gross, E.M. Allelopathy of aquatic autotrophs. *Crit. Rev. Plant Sci.* **2003**, *22*, 313–339. [CrossRef]
10. Semmouri, I.; Janssen, C.R.; Asselman, J. Allelopathy in macroalgae: Ecological principles, research opportunities and pitfalls reviewed. *J. Appl. Phycol.* **2024**, *36*, 441–458. [CrossRef]
11. Fistarol, G.O.; Legrand, C.; Graneli, E. Allelopathic effect of *Prymnesium parvum* on a natural plankton community. *Mar. Ecol. Prog. Ser.* **2003**, *255*, 115–125. [CrossRef]
12. Suikkanen, S.; Fistarol, G.O.; Granéli, E. Allelopathic effects of the Baltic cyanobacteria *Nodularia spumdigena*, *Aphanizomenon flos-aquae* and *Anabaena lemmermannii* on algal monocultures. *J. Exp. Mar. Biol. Ecol.* **2004**, *308*, 85–101. [CrossRef]
13. Gross, E.M.; Wolk, C.P.; Jüttner, F. Fisherellin, a new allelochemical from the freshwater cyanobacterium *Fischerella muscicola*. *J. Phycol.* **1991**, *27*, 686–692. [CrossRef]
14. Lam, C.W.V.; Silvester, W.B. Growth interactions among blue-green (*Anabaena Oscillarioides*, *Microcystis aeruginosa*) and green (*Chlorella* sp.) algae. *Hydrobiologia* **1979**, *63*, 135–143. [CrossRef]
15. Schagerl, M.; Unterrieder, I.; Angeler, D.G. Allelopathy among Cyanoprokaryota and other algae originating from Lake Neusiedlersee (Austria). *Int. Rev. Hydrobiol.* **2002**, *87*, 365–374. [CrossRef]
16. Sukenik, A.; Eshkol, R.; Livne, A.; Hadas, O.; Rom, M.; Tchernov, D.; Vardi, A.; Kaplan, A. Inhibition of growth and photosynthesis of the dinoflagellate *Peridinium gatunense* by *Microcystis* sp. (cyanobacteria): A novel allelopathic mechanism. *Limnol. Oceanogr.* **2002**, *47*, 1656–1663. [CrossRef]
17. Žak, A.; Kosakowska, A. Allelopathic Influence of cyanobacteria *Microcystis aeruginosa* on green algae *Chlorella vulgaris*. In *Insights on Environmental Changes*; Zielinski, T., Pazdro, K., Dragan-Górska, A., Weydmann, A., Eds.; Springer: Cham, Switzerland, 2014; pp. 141–150. [CrossRef]
18. Bittencourt-Oliveira, M.C.; Chia, M.A.; de Oliveira, H.S.B.; Araújo, M.K.C.; Molica, R.J.R.; Dias, C.T.S. Allelopathic interactions between microcystin-producing and non-microcystin-producing cyanobacteria and green microalgae: Implications for microcystins production. *J. Appl. Phycol.* **2015**, *27*, 275–284. [CrossRef]
19. Hernández-Zamora, M.; Santiago-Martínez, E.; Martínez-Jerónimo, F. Toxigenic *Microcystis aeruginosa* (Cyanobacteria) affects the population growth of two common green microalgae: Evidence of other allelopathic metabolites different to cyanotoxins. *J. Phycol.* **2021**, *57*, 1530–1541. [CrossRef] [PubMed]
20. Teneva, I.; Velikova, V.; Belkinova, D.; Moten, D.; Dzhambazov, B. Allelopathic potential of the cyanotoxins microcystin-LR and cylindrospermopsin on green algae. *Plants* **2023**, *12*, 1403. [CrossRef] [PubMed]
21. Vardi, A.; Schatz, D.; Beerli, K.; Motro, U.; Sukenik, A.; Levine, A.; Kaplan, A. Dinoflagellate-cyanobacterium communication may determine the composition of phytoplankton assemblage in a mesotrophic lake. *Curr. Biol.* **2002**, *12*, 1767–1772. [CrossRef] [PubMed]
22. Gantar, M.; Berry, J.P.; Thomas, S.; Wang, M.; Perez, R.; Rein, K.S. Allelopathic activity among *Cyanobacteria* and microalgae isolated from Florida freshwater habitats. *FEMS Microbiol. Ecol.* **2008**, *64*, 55–64. [CrossRef] [PubMed]
23. Harel, M.; Weiss, G.; Lieman-Hurwitz, J.; Gun, J.; Lev, O.; Lebendiker, M.; Temper, V.; Block, C.; Sukenik, A.; Zohary, T.; et al. Interactions between *Scenedesmus* and *Microcystis* may be used to clarify the role of secondary metabolites. *Environ. Microbiol. Rep.* **2012**, *5*, 97–104. [CrossRef] [PubMed]
24. Hong, Y.; Zou, D.; Xiao, Q.; Zhang, Z.; Hu, H. Interactive relationships of *Microcystis aeruginosa* and three species of *Chlorella* under the condition of coexistence. In *Proceedings of the International Conference on Environmental Engineering and Applications (ICEEA)*, Singapore, 10–12 September 2010; pp. 250–254. [CrossRef]
25. LOTUS: Natural Products Online. Available online: <https://lotus.naturalproducts.net/search/simple/Chlorella> (accessed on 11 May 2026).
26. KNApSACk Core System. Available online: https://www.knapsackfamily.com/knapsack_core/result.php?sname=all&word=Chlorella (accessed on 11 May 2026).
27. Wu, Y.T.; Chiang, Y.R.; Huang, W.Y.; Jane, W.N. Cytotoxic effects of free fatty acids on phytoplankton algae and cyanobacteria. *Aquat. Toxicol.* **2006**, *80*, 338–345. [CrossRef] [PubMed]
28. Michalak, I.; Chojnacka, K. Algae as production systems of bioactive compounds. *Eng. Life Sci.* **2015**, *15*, 160–176. [CrossRef]
29. de Morais, M.G.; Vaz Bda, S.; de Morais, E.G.; Costa, J.A. Biologically active metabolites synthesized by microalgae. *BioMed Res. Int.* **2015**, *2015*, 835761. [CrossRef] [PubMed]
30. Park, Y.H.; Han, S.-I.; Oh, B.; Kim, H.S.; Jeon, M.S.; Kim, S.; Choi, Y.-E. Microalgal secondary metabolite productions as a component of biorefinery: A review. *Bioresour. Technol.* **2022**, *344*, 126206. [CrossRef] [PubMed]

31. Creydt, M.; Arndt, M.; Hudzik, D.; Fischer, M. Plant metabolomics: Evaluation of different extraction parameters for nontargeted UPLC-ESI-QTOF-mass spectrometry at the example of white *Asparagus officinalis*. *J. Agric. Food Chem.* **2018**, *66*, 12876–12877. [CrossRef] [PubMed]
32. Allen, M.M. Simple conditions for growth of unicellular blue-green algae on plates (1, 2). *J. Phycol.* **1968**, *4*, 1–4. [CrossRef] [PubMed]
33. CCAP Media Recipes b. Available online: <https://www.ccap.ac.uk/media/documents/JM.pdf> (accessed on 5 February 2021).
34. Nielsen, S.L.; Hansen, B.W. Evaluation of the robustness of optical density as a tool for estimation of biomass in microalgal cultivation: The effects of growth conditions and physiological state. *Aquac. Res.* **2019**, *50*, 2698–2706. [CrossRef]
35. Hotos, G.N.; Antoniadis, T.I. *An Illustrated Procedure of Phycocyanin Allophycocyanin and Phycoerythrin Measurement in Cyanobacterial Cultures*; University of Patras: Patras, Greece, 2022. [CrossRef]
36. MSZ 1484-13:2009; Water Quality. Part 13: Determination of Nitrate and Nitrite. Content by Spectrophotometric Method. Hungarian Standards Institution: Budapest, Hungary, 2009.
37. MSZ EN ISO 6878:2004; Water Quality. Determination of Phosphorus. Ammonium Molybdate Spectrometric Method. Hungarian Standards Institution: Budapest, Hungary, 2004.
38. MSZ ISO 7150-1:1992; Water Quality. Determination of Ammonium. Part 1: Manual Spectrophotometric Method. Hungarian Standards Institution: Budapest, Hungary, 1993.
39. Patton, C.J.; Kryskalla, J.R. *Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Evaluation of Alkaline Persulfate Digestion as an Alternative to Kjeldahl Digestion for Determination of Total and Dissolved Nitrogen and Phosphorus in Water*; 2003-4174; U.S. Geological Survey: Denver, CO, USA, 2003. [CrossRef]
40. Aquasearch. *Analysis of Total Astaxanthin in Algae meal Prepared from Haematococcus pluvialis*; Technical Report TR.1002.001; Aquasearch: Kona, Hawaii, 1999.
41. Sumanta, N.; Haque, C.I.; Jaishee, N.; Suprakash, R. Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. *Res. J. Chem. Sci.* **2014**, *4*, 63–69.
42. Du, X.; Dong, C.; Wang, K.; Jiang, Z.; Chen, Y.; Yang, Y.; Chen, F.; Ni, H. Separation and purification of astaxanthin from *Phaffia rhodozyma* by preparative high-speed counter-current chromatography. *J. Chromatogr. B* **2016**, *1029–1030*, 191–197. [CrossRef] [PubMed]
43. Bácsi, I.; Deli, J.; Gonda, S.; Mészáros, I.; Veréb, G.; Dobronoki, D.; Nagy, S.A.; B-Béres, V.; Vasas, G. Non-steroidal anti-inflammatory drugs initiate morphological changes but inhibit carotenoid accumulation in *Haematococcus pluvialis*. *Algal Res.* **2018**, *31*, 1–13. [CrossRef]
44. Hynstova, V.; Sterbova, D.; Klejdus, B.; Hedbavny, J.; Huska, D.; Adam, V. Separation, identification and quantification of carotenoids and chlorophylls in dietary supplements containing *Chlorella vulgaris* and *Spirulina platensis* using High Performance Thin Layer Chromatography. *J. Pharm. Biomed. Anal.* **2018**, *148*, 108–118. [CrossRef] [PubMed]
45. Lázár, I., Jr.; Lázár, I. GelAnalyzer 23.1. Available online: <http://www.gelanalyzer.com/?i=1> (accessed on 25 May 2026).
46. Bácsi, I.; Tóthfalusi, F.; Márton, K.; B-Béres, V.; Gonda, S. The effects of photobioreactor type on biomass and lipid production of the green microalga *Monoraphidium pusillum* in laboratory scale. *Appl. Sci.* **2022**, *12*, 2196. [CrossRef]
47. Agostoni, C.; Bruzzese, M.G. Fatty acids: Their biochemical and functional classification. *Pediatr. Medica Chir. Med. Surg. Pediatr.* **1992**, *14*, 473–479. [PubMed]
48. Bácsi, I.; Figler, A.; Simon, E.; Yaqoob, M.M.; Márton, K.; B-Béres, V. Salinity tolerance and desalination properties of a *Haematococcus lacustris* strain from eastern Hungary. *Front. Microbiol.* **2024**, *15*, 1332642. [CrossRef] [PubMed]
49. Benjamin, R.B.; Brown, R.; Fraizer, M.; Joab, B.M.; Casey, R.E.; Klaine, S.J. Algal growth rate fluctuations observed under uniform ambient test conditions using static and semicontinuous assay techniques. *Environ. Toxicol. Chem.* **1998**, *17*, 460–467. [CrossRef]
50. Ackermann, M. Functional heterogeneity in microorganisms. *Nat. Rev. Microbiol.* **2015**, *13*, 497–508. [CrossRef] [PubMed]
51. Nguyen, J.; Lara-Gutiérrez, J.; Stocker, R. Environmental fluctuations and their effects on microbial communities, populations and individuals. *FEMS Microbiol. Rev.* **2021**, *45*, fuaa068. [CrossRef] [PubMed]
52. Pratt, R.; Daniels, T.C.; Eiler, J.J.; Gunnison, J.B.; Kumler, W.D.; Oneto, J.F.; Strait, L.A.; Spoehr, H.A.; Hardin, G.J.; Milner, H.W.; et al. Chlorellin, an antibacterial substance from *Chlorella*. *Science* **1944**, *99*, 351–352. [CrossRef] [PubMed]
53. DellaGreca, M.; Zarrelli, A.; Fergola, P.; Cerasuolo, M.; Pollio, A.; Pinto, G. Fatty acids released by *Chlorella vulgaris* and their role in interference with *Pseudokirchneriella subcapitata*: Experiments and modelling. *J. Chem. Ecol.* **2010**, *36*, 339–349. [CrossRef]
54. Rojas, V.; Rivas, L.; Cárdenas, C.; Guzmán, F. Cyanobacteria and eukaryotic microalgae as emerging sources of antibacterial peptides. *Molecules* **2020**, *25*, 5804. [CrossRef] [PubMed]
55. Shaima, A.F.; Yasin, N.H.M.; Ibrahim, N.; Takriff, M.S.; Gunasekaran, D.; Ismaeel, M.Y.Y. Unveiling antimicrobial activity of microalgae *Chlorella sorokiniana* (UKM2), *Chlorella* sp. (UKM8) and *Scenedesmus* sp. (UKM9). *Saudi J. Biol. Sci.* **2022**, *29*, 1043–1052. [CrossRef] [PubMed]
56. Bacellar Mendes, L.B.; Vermelho, A.B. Allelopathy as a potential strategy to improve microalgae cultivation. *Biotechnol. Biofuels* **2013**, *6*, 152. [CrossRef] [PubMed]

57. Zielinski, D.; Fraczyk, J.; Debowski, M.; Zielinski, M.; Kaminski, Z.J.; Kregiel, D.; Jacob, C.; Kolesinska, B. Biological activity of hydrophilic extract of *Chlorella vulgaris* grown on post-fermentation leachate from a biogas plant supplied with stillage and maize silage. *Molecules* **2020**, *25*, 1790. [[CrossRef](#)] [[PubMed](#)]
58. Chaïb, S.; Pistevos, J.C.A.; Bertrand, C.; Bonnard, I. Allelopathy and allelochemicals from microalgae: An innovative source for bio-herbicidal compounds and biocontrol research. *Algal Res.* **2021**, *54*, 102213. [[CrossRef](#)]
59. Joshua, S.; Mullineaux, C.W. Phycobilisome diffusion is required for light-state transitions in cyanobacteria. *Plant Physiol.* **2004**, *135*, 2112–2119. [[CrossRef](#)] [[PubMed](#)]
60. Meng, X.; Yang, J.; Xu, X.; Zhang, L.; Nie, Q.; Xian, M. Biodiesel production from oleaginous microorganisms. *Renew. Energy* **2009**, *34*, 1–5. [[CrossRef](#)]
61. Falkner, G.; Falkner, R. The Complex Regulation of the phosphate uptake system of cyanobacteria. In *Bioenergetic Process of Cyanobacteria*, 1st ed.; Peschek, G.A., Obinger, C., Renger, G., Eds.; Springer: Dordrecht, The Netherlands, 2011; pp. 109–130. [[CrossRef](#)]
62. Wan, L.; Song, C.; Zhou, Y.; Cao, X. Cyanobacterial extracellular alkaline phosphatase: Detection and ecological function. *J. Oceanol. Limnol.* **2002**, *40*, 1840–1854. [[CrossRef](#)]
63. Japir, A.A.W.; Salimon, J.; Derawi, D.; Yahaya, B.H.; Jamil, M.S.M.; Yusop, M.R. Optimization of methanol crystallization for highly efficient separation of palmitic acid from palm fatty acid mixture using response surface methodology. *Grasas Aceites* **2017**, *68*, e224. [[CrossRef](#)]
64. Japir, A.A.W.; Salimon, J.; Derawi, D.; Yahaya, B.H.; Bahadi, M.; Al-Shuja'a, S.; Yusop, M.R. A highly efficient separation and physicochemical characteristics of saturated fatty acids from crude palm oil fatty acids mixture using methanol crystallisation method. *Oilseeds Fats Crops Lipids* **2018**, *25*, a203. [[CrossRef](#)]
65. The Jamovi Project. *Jamovi, Version 2.7 [Computer Software]*; The Jamovi Project: Sydney, Australia, 2025. Available online: <https://www.jamovi.org> (accessed on 25 May 2026).
66. R Core Team. *R: A Language and Environment for Statistical Computing, Version 4.5 [Computer Software]*; R Foundation for Statistical Computing: Vienna, Austria, 2025. Available online: <https://cran.r-project.org> (accessed on 25 May 2026).
67. Kerby, D.S. The simple difference formula: An approach to teaching nonparametric correlation. *Compr. Psychol.* **2014**, *3*, 11.IT.3.1. [[CrossRef](#)]
68. Fox, J.; Weisberg, S.; Price, B. *Car: Companion to Applied Regression, Version 3.1-3 [R Package]*; 2024. Available online: <https://cran.r-project.org/package=car> (accessed on 25 May 2026).

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