

Low-dose copper and blue light increases the yield of value-added biomolecules in *Kirchneriella contorta* (Chlorophyceae)

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Abstract

Microalgae are a natural source of valuable compounds with a wide range of applications. Given their physiological plasticity, strategies aiming at increasing the content of biomolecules have been proposed. In this study, low-dose copper and blue/red lights were combined and used as a manipulative strategy to induce biomolecule accumulation in *Kirchneriella contorta* (Schmidle). Cultures were exposed to a 1.6×10^{-9} to 1.7×10^{-8} mol L⁻¹ free copper (Cu²⁺) concentration range under white light for 48 h. Afterwards, the white light was replaced with blue or red lights for up to 96 h. We evaluated population growth through growth rates, photosynthesis through pulse amplitude modulated fluorescence (PAM) and biomolecules by quantifying chlorophyll *a*, carotenoids, carbohydrates and proteins. The results showed that at 3.8×10^{-9} mol L⁻¹ Cu²⁺ and blue light, 2x more carotenoids, 4x more carbohydrates and 3x extra proteins were accumulated in the cells compared to the control. Among the light colors, the blue light most significantly interfered with the alga metabolism compared to the other colors. Although growth rates and quantum yields were not affected, non-photochemical quenching (NPQ) increased under blue light, showing that its higher energy compared to red and white lights may have impacted the photosynthetic apparatus. This study contributes to the biotechnology of microalgae by proposing a manipulative strategy that triggered the build-up of biomolecules in *K. contorta*.

1. Introduction

Microalgae are primary producers in aquatic environments and changes in their biochemical composition or population numbers can impact higher trophic levels. In addition, microalgae produce a wide variety of compounds such as proteins, antioxidants, anti-inflammatories, carbohydrates, omega-3 fatty acids, and others that can be used in the biotechnology industry (Borowitzka, 2016).

Industrial production relies on high biomass and a favorable cost/benefit ratio, but so far this has been achieved for just a few species considering the diversity of microalgae. Examples of commercial strains include *Dunaliella salina*, *Chlorella* sp., *Haematococcus pluvialis* and *Spirulina platensis*; the latter is Cyanobacteria. Strategies to increase the biomass yield of microalgae and/or biomolecules per unit of biomass can be a way to increase the number of species that can be cultivated for commercial purposes. To this end, the biochemical manipulation of algal cultures has been the subject of extensive studies (Abiusi et al., 2014; Chia et al., 2017; da Fontoura Prates et al., 2020; McGee et al., 2020). Nitrogen or phosphorus depletion in combination with cadmium or copper has been shown to magnify biomolecule accumulation in *Chlorella vulgaris* (Chia et al., 2017; 2013). However, Lombardi and Wangersky (1991) showed that nutrient deprivation can cause cell membrane fragility, which can become a problem. This is usually overlooked but can lead to the loss of the compounds of interest during cell harvesting due to membrane disruption.

As a micronutrient, copper plays an important role in microalgae physiology, affecting biological functions through its depletion or excess (Lombardi and Maldonado, 2011). It is not uncommon to have copper as a stimulating factor for biomolecule accumulation in microalgae; it depends on the exposure

concentration of the metal. Investigating the effects of copper (4.7×10^{-7} to 4.0×10^{-6} mol L⁻¹) on the physiology of *Chlorolobium braunii*, Baracho et al. (2019) showed increased proteins, carbohydrates, and lipids, and Silva et al. (2018) showed higher carbohydrates and proteins in *Scenedesmus quadricauda*. Therefore, as a micronutrient, copper can be proposed as a manipulating agent.

Light quality is another manipulating agent that has been widely investigated (Müller et al., 2001; Keeling, 2013). Light is characterized by its emission wavelength (or its color) and has been shown to affect the production of biomolecules (Yuan et al., 2020) and growth rates in microalgae (Keeling, 2013), thus stimulating specific metabolic routes. Changes in the composition of microalgae photoreceptors (Müller et al., 2001), smaller cells and higher growth rates have been shown to occur in cells exposed to red light (Keeling, 2013). Under blue light, effects in gene expression have been reported (Ruyters, 1984). Yuan et al. (2020) showed that the red light led to a greater accumulation of carbohydrates while the blue light of lipids, in addition to both favoring growth rates.

The aim of achieving an increase in biomolecules in microalgae by mild stress caused by exposure to a low dose of toxicant is attractive because this can be achieved by preserving or slightly altering the population growth rates (Agathokleous and Guo, 2022). However, such a stimulus is less intense regarding the accumulation of biomolecules (Silva et al., 2018) than stress induced by, for example, the depletion of major nutrients (Chia et al., 2013). This means that in the first situation, a combination of manipulating agents may be needed to magnify the accumulation of biomolecules. Here, we combined the effects of copper at a low dose with a red or blue light aiming at an amplification of biomolecules in a freshwater Chlorophyceae. We showed that the combination of low-dose copper and blue light resulted in higher yields of biomolecules in *Kirchneriella contorta* compared to the red light and the controls.

2. Material and methods

2.1 Cultures, growth rates and photosynthesis

The green microalgae, *Kirchneriella contorta* (SisGen AD7D518), was obtained from the Freshwater Microalgae Culture Collection of the Botany Department, Federal University of São Carlos (São Paulo, Brazil), where it is kept in WC culture medium (Guillard and Lorenzen, 1972). The Collection is registered in the World Data Center for Microorganisms under number 835.

The microalga was grown in laboratory-controlled conditions. Sterile WC culture medium with initial pH 7.0, at $25 \pm 1^\circ\text{C}$ and 12:12 h light:dark cycle were used. The light intensity used was 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and had been previously verified by means of rapid light curves and I_k determination. Cultures were monitored daily for population density (cells mL⁻¹) and maximum photosynthetic quantum yield (Φ_M). Cell counts were done on a MUSE flow cytometer (Millipore, USA), and Φ_M using a PHYTO-PAM, Heinz Walz (Germany). Growth rates (d⁻¹) were determined by means of linear regression plotting the natural logarithm of cell mL⁻¹ vs experimental time (days) from 0–48 h; the line slope represents the specific growth rate for exponentially growing cells. Cell biovolume (μm^3) was determined in 30 cells

following Hillebrand et al. (1999) at 48 h and 96 h using an optical microscope (Nikon Eclipse, Japan) connected to a computer.

Photosynthesis related determinations were done using pulse amplitude modulated fluorescence following the methodology described in (Lombardi and Maldonado, 2011). Effective quantum yield (Φ'_M), photochemical (qP) and non-photochemical (NPQ) quenchings were determined at 48 h and 96 h with 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ light pulses applied every 20 s for 10 min. The equations used for calculations of these parameters are presented below.

$$\Phi_M = (F_M - F_0) / F_M \quad (1)$$

$$\Phi'_M = (F'_M - F'_S) / F'_M \quad (2)$$

$$qP = (F'_M - F'_S) / (F'_M - F'_0) \quad (3)$$

$$NPQ = [F_M - F'_M] / F'_M \quad (4)$$

2.2 Manipulation strategy

The manipulation strategy we propose entails two steps. First, the cells are exposed to low dose copper and then to different light colors. We began by defining the range of copper concentrations that would not influence growth rate or that would do it minimally. Thus, in three experimental replicates and using 96-well microplates, we performed a copper screening test whereby the cells were exposed to 3×10^{-8} – $3 \times 10^{-5} \text{ mol L}^{-1}$ nominal copper concentration range for 120 h. The microplates were covered with Parafilm®, incubated under white light from below and population growth was monitored by measuring absorbance at 684 nm (Epoch™ Microplate Spectrophotometer, U.S.A.). A commercial copper standard solution (AAS/ICP, 1000 mgL^{-1} , 38996 Sigma-Aldrich, USA) was used for copper addition into culture medium 24 h before microalgae inoculation ($10^4 \text{ cells mL}^{-1}$ from exponential culture). Controls contained the usual nominal copper concentration of WC culture medium ($3 \times 10^{-8} \text{ mol L}^{-1}$). From this copper screening test, the nominal copper concentration range that would not negatively affect growth rates was defined as 3×10^{-8} to $3 \times 10^{-6} \text{ mol L}^{-1}$, equivalent to determined free copper ions from 1.6×10^{-9} to $1.7 \times 10^{-8} \text{ mol L}^{-1}$. Therefore, this copper concentration range was used to proceed with the manipulation process.

The manipulation experiment began with cells exposed to 1.6×10^{-9} to $1.7 \times 10^{-8} \text{ mol L}^{-1}$ copper range (Table 1) and white LED for 48 h, after which they were exposed to red and blue lights for another 48 h. Thus, the total manipulation process lasted 96 h. The emission spectra of the lights were registered using an Ocean Optics UBS 2000 model spectroradiometer equipped with a BIF-600-UV-VIS optical fiber and OIbase32 collection software (Dunedin, FL). The spectra obtained confirm the different wavelengths with peaks at white 455/566 nm, blue 445 nm and red 652 nm, as shown in Fig. 1S (supplemental material).

Cultures were performed in 250 mL polystyrene tissue culture flasks kept vertically, thus with 3 cm optical path, illuminated by the side and internal light intensity was kept $\sim 200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. The light:dark cycle was 12:12 h and cultures were bubbled with filtered air (0.22 μm ; Chromafil Xtra PUDF 20/25, Germany). They were inoculated with 5×10^4 cells mL^{-1} from exponentially growing cells whose physiological condition was confirmed by maximum quantum yield (Φ_M) ~ 0.70 (Lombardi and Maldonado, 2011).

Biomolecules, e.g., total carbohydrates and proteins, chlorophylls *a* and *b*, and total carotenoids were determined in 96 h cultures. Total carbohydrates were determined following Albalasmeh et al. (2013) and total proteins according to Slocombe et al. (2013). The concentrations of chlorophylls *a* and *b*, and total carotenoids were determined according to Wellburn (1994). For these analyses, culture samples were centrifuged in a refrigerated centrifuge (Thermo Scientific, Sorvall Legend XTR, USA) for 20 min at 4400 rpm. The supernatant was discarded, and the pellet stored at -22°C until analysis, which occurred within 15 days of sampling.

2.3 Determination of free copper ions (Cu^{2+})

Free copper ions were determined according to Lombardi et al. (2007). A copper ion selective electrode (ISE-Cu, Thermo Scientific Orion 9429BN) was used as working electrode and a Thermo Scientific Orion 900200 double junction electrode (D/J safe flow) as the reference electrode. For the calibration of the ISE system, copper buffers were used to extend the detection limit down to $10^{-10} \text{ mol L}^{-1}$. The metallic buffer comprised sodium borate (Sigma-Aldrich, Germany), sodium nitrate (Sigma-Aldrich, Germany), copper standard (38996 Sigma-Aldrich, USA) and nitrilotriacetic acid (Sigma-Aldrich, Germany). All measurements were performed in a filtered air cabinet and under controlled temperature ($24 \pm 1^\circ\text{C}$), and a calibration curve for ISE-Cu system was performed at pH 5. It was made from serial dilution of a commercial monoelementary copper standard AAS/ICP (1000 mg L^{-1} , 38996 Sigma-Aldrich, USA). Table 1 shows nominal copper and free copper ions concentrations (mol L^{-1}) used for the manipulation experiment.

Table 1
Nominal copper, and free copper ion concentrations (mol L⁻¹). The values were determined by Cu-ISE in WC culture medium before cell inoculation. *Control treatment. The CV for Cu determination was within 5%.

Nominal Cu	Free Cu ²⁺
*3x10 ⁻⁸	1.6x10 ⁻⁹
4x10 ⁻⁸	2.2x10 ⁻⁹
1x10 ⁻⁷	3.8x10 ⁻⁹
1x10 ⁻⁶	1.4x10 ⁻⁸
3x10 ⁻⁶	1.7x10 ⁻⁸

2.4 Data analysis

Statistical analyses of algal growth rates obtained for the copper screening test and maximum growth rates under the white light (0–48 h) were done using One-way ANOVA (Minitab, version 17 for Windows), while Two-Way ANOVA and Tukey's Test were used to compare data obtained at 96 h. Normality and homoscedasticity assumptions were verified before analysis using the Shapiro-Wilk test and Levene test. Graphical representations were made using IgorPro 6.0.5 (WaveMetrics, USA) and Graph Pad Prism 6 programs.

3. Results

The higher end of copper concentration in the screening test resulted in negative effect on growth curves (Fig. 2S). Thus, we defined the cell-supportable copper concentration range for this species to be within 1.6x10⁻⁹ to 1.7x10⁻⁸ mol L⁻¹ and it was used for the biochemical manipulation experiment.

Figure 1 shows the growth curves for the biochemical manipulation experiment. Growth rates are shown in Table 2.

Table 2

Growth rate at 48 and 96 hours in different concentrations of free copper and light. The numbers in parentheses represent the standard deviation from the mean ($n = 3$). Letters in parentheses represent statistical evaluation (same letter indicates no significant difference, ANOVA, $p > 0.05$).

Free Cu ²⁺	0–48 h white	48–96 h white	48–96 h red	48–96 h blue
1.6×10^{-9}	0,973 (0.003) (a)	0,373 (0.035) (b)	0,444 (0.026) (ab)	0,546 (0.052) (ab)
2.2×10^{-9}	0,861 (0.013) (b)	0,441 (0.086) (ab)	0,462 (0.059) (ab)	0,458 (0.033) (ab)
3.8×10^{-9}	0,942 (0.004) (a)	0,468 (0.061) (ab)	0,490 (0.056) (ab)	0,613 (0.061) (a)
1.4×10^{-8}	0,942 (0.030) (a)	0,415 (0.038) (b)	0,436 (0.074) (ab)	0,440 (0.035) (ab)
1.7×10^{-8}	0,973 (0.041) (a)	0,387 (0.046) (b)	0,365 (0.042) (b)	0,379 (0.025) (b)

Under white light, growth rate was not significantly different among copper treatments ($\sim 0.96 \text{ d}^{-1}$), except for $2.2 \times 10^{-9} \text{ mol L}^{-1} \text{ Cu}^{2+}$, which was lower (0.86 d^{-1}). Cell viability provided by the Muse Cell Analyzer® was similar among treatments, remaining above 95%, regardless of light color or copper concentration (data not shown). Different from literature results, we showed that, in general, blue or red lights did not affect the growth rates of *Kirchneriella contorta*.

Figure 2 reports biomass (Fig. 2a), and the photosynthetic quenchings, qP and NPQ (Fig. 2b).

Figure 2a shows that cultures exposed to $3.8 \times 10^{-9} \text{ mol L}^{-1}$ under red or blue lights had 43% higher biomass (ANOVA, $p < 0.05$) than white light under the same copper concentration or the respective light color control. Regarding photosynthetic quenchings (Fig. 2b), the photochemical quenching was similar among treatments, but NPQ differed. It was systematically higher under the blue light (ANOVA $p < 0.05$). No statistical difference was obtained for maximum (0.72–0.74) and operational quantum yields (0.52–0.56) (Fig. 3S – supplemental material).

Figure 3 shows the specific productivities of biomolecules in *K. contorta*: carbohydrates (Fig. 3a), proteins (Fig. 3b), chlorophyll *a* (Fig. 3c) and total carotenoids (Fig. 3d).

In general, higher biomolecules were obtained in cells exposed to the combination of blue light and $3.8 \times 10^{-9} \text{ mol L}^{-1} \text{ Cu}^{2+}$. Compared to the control, the treatments, which combined $3.8 \times 10^{-9} \text{ mol L}^{-1} \text{ Cu}^{2+}$ and red or blue lights, accumulated 4x more carbohydrates. The effect of light colors in biomolecule accumulation is further corroborated by the $\sim 3\text{x}$ higher carbohydrates in cells exposed to blue light in treatment without extra copper. The productivity of proteins (Fig. 3b) in *K. contorta* was affected by copper and light colors. Although red and blue lights resulted in $\sim 1.7\text{x}$ higher proteins in treatments without extra copper, it was the combination of blue light and $3.8 \times 10^{-9} \text{ mol L}^{-1} \text{ Cu}^{2+}$ that increased proteins the most. It is noticeable that exposure to $3.8 \times 10^{-9} \text{ mol L}^{-1} \text{ Cu}^{2+}$ caused twice as many proteins

than the white light control. Further magnification (3x higher) on the protein content was obtained by the combination with the blue light. Considering the pigments, the chlorophyll *a* data (Fig. 3c) followed a similar behavior to proteins, with the highest value (~ 1.5 x higher) in $3.8 \times 10^{-9} \text{ mol L}^{-1}$ free copper and the blue light. The total carotenoids (Fig. 3d) were highest under the blue light and $3.8 \times 10^{-9} \text{ mol L}^{-1} \text{ Cu}^{2+}$, a situation where it doubled in contrast to the white light control.

4. Discussion

The similarity in growth rates for the copper and white light treatments were expected as a carefully selected copper concentration range based on the screening test was used. According to Davies and Bennett (1985), the copper concentrations we used are considered environmentally significant, thus commonly found in natural aquatic environments and the cause of a lack of influence on *K. contorta* growth. Different from our results, the literature has shown that the blue light can lead to increased growth rates in microalgae. Teo et al. (2014) showed higher growth rates for *Nannochloropsis sp.* and *Tetraselmis sp.* exposed to the blue light compared to white light controls. According to the authors, the blue light has greater penetration in the culture when compared to the white light, thus improving cell division and microalgae growth rates. In the present study, it is possible that the short optical path together with low density cultures that we used may have made the blue light effect unnoticeable, as both the white and red lights have penetrated thoroughly into the culture flasks. Furthermore, the light intensity inside the cultures were the same regardless of its color.

The usually higher biomass in cultures exposed to $3.8 \times 10^{-9} \text{ mol L}^{-1}$ and red/blue light means that in this research, *K. contorta* was stimulated by the combined effect. Literature data show that light color can affect microalgae biomass yield, but this did not happen in the present study. Abiusi et al. (2014) showed a 2.5-fold increase in *Tetraselmis suecica* biomass under red compared to the white light. They proposed that the red light is better absorbed and used by the photosynthetic pigment, chlorophyll *a*, than the white light. Baba et al. (2012) showed a higher growth rate (and biomass) in *Botryococcus braunii* cultures under a red light compared to white and blue lights and attributed it to higher chlorophyll *a* content under the red light.

Related to photosynthesis, our results are in agreement with Zhong et al. (2018). They showed no variation in maximum quantum yield, but higher NPQ under the blue light for *Chlorella vulgaris* and *Chlorella pyrenoidosa*. Additionally, under low copper we obtained a gradual NPQ increase for red (2-fold) and blue lights (2.4-fold), but constant and high NPQ at the highest copper concentration, suggesting that the light reactions of photosynthesis were affected at the highest copper value. It is known that NPQ, a heat related dissipation process, is a means of protecting photosynthetic microalgae against photodamage by high light (Baer et al., 2016). Therefore, its increase indicates that photoprotection mechanisms were triggered under both blue and red lights. This means that cells in $1.7 \times 10^{-8} \text{ mol L}^{-1}$ free copper could have been facing oxidative stress.

To understand the effects of light colors in microalgae cultures, several aspects should be considered. First, cell density and optical path of the culture flask can have a significant effect as the wavelengths of red and blue lights and the energy they carry per photon are significantly different. The red light (~ 650 nm) is less energetic than the blue one (~ 440 nm), affecting the extension with which it penetrates in the cultures. The energy of blue light is calculated to be 1.44x higher per photon than the red light, thus able to induce higher NPQ (Fu et al., 2013). Higher penetration of blue light and its higher energy per photon can lead to higher NPQ and carotenoids as a means of protection mechanism (Costa et al., 2013; Katsuda et al., 2004). In this research, because we used cultures with low density and flasks with short optical path, the effects of different colors of light on the NPQ may have been highlighted, as seen in Fig. 2b. Although we assured the same light intensity regardless of its color inside the culture flasks, the higher NPQ under the blue light can be related to the higher energy carried by their photons than the white and red lights.

Considering biomolecules, the literature results show a great variation on the effects of light colors on carbohydrates in microalgae. Our results are in line with those of Schulze et al. (2016), who showed that *Nannochloropsis oculata* produced more carbohydrates under red and blue lights, while *Tetraselmis chuii* under a red light. However, Abiusi et al. (2014) showed no effect of red/ blue lights on carbohydrate content in *Nannochloropsis* sp, but higher levels were detected in *Tetraselmis suecica* under white/blue lights. A possible reason for such differences might be the adaptations and evolutionary histories of each species, in addition to culture densities associated to its optical path. McGee et al. (2020) evaluated *Rhodella* sp. and *Phaeothamnion* sp. under green, blue, red and white lights, and observed that blue light led to twice as many carbohydrates. According to the authors, the shorter wavelength of blue light provides greater energy for biomass and biomolecule production.

The results we obtained for proteins agree with the literature, where increased proteins in other Chlorophyceae exposed to copper ions are shown. Silva et al. (2018) exposed *Scenedesmus quadricauda* to copper and showed protein increase. Shakya et al. (2022) investigated sub-lethal copper concentrations in *Chlorella* sp and showed modulation of proteins involved in energy production pathways, such as photosynthesis, carbon fixation, glycolysis, and oxidative phosphorylation. The authors propose that protein biosynthesis may play a role in copper exposure, protecting the cells against toxicity. Studying *Chlorella ellipsoidea* exposed to white, green, red and blue lights, Baidya et al. (2021) showed that it was the blue light that resulted in the highest protein increase and concluded that this light color was the best for the production of nutritionally rich biomass. According to Payer et al (1969), this may be related to an increase in the efficiency of CO₂ fixation and its incorporation into proteins under blue light.

The results we obtained for chlorophyll *a* showed that copper was more important in determining their concentration than light colors. This is compatible with Baracho et al. (2019) and Silva et al. (2018) that showed higher levels of chlorophyll *a* in green microalgae exposed to copper. The authors attributed the results to a protection mechanism against copper toxicity. Considering light colors without extra copper, different from our results, the literature shows that exposure of microalgae to blue light can increase

chlorophyll a. Vadiveloo et al. (2015) showed it in *Nannochloropsis* sp., Rebolledo-Oyarce et al. (2019) in *Dunaliella tertiolecta* (14% higher Chl *a*) and Baidya et al. (2021) in *Chlorella ellipsoidea*. Thus, intracellular effects, which may include modulation of photosynthesis and cell division processes that led to higher biomolecules and biomass, supports the combined effect of blue light and 3.8×10^{-9} mol L⁻¹ copper in *K. contorta*.

Considering carotenoid accumulation in microalgae, our results agree with the literature, which shows that specific light colors can increase carotenoid production in microalgae, but this can be species-specific. Suyono et al. (2015) obtained 8x higher carotenoids in *Haematococcus pluvialis* upon exposure to blue light. Different from the present results, Han et al. (2019) investigated *Dunaliella salina*, and showed that the red light increased carotenoid production by 13%. Fu et al. (2013) combined red and blue lights and reported 2.3x higher carotenoid biosynthesis in *Dunaliella salina*. According to Chory (2010), the variation in the composition of photoreceptors is crucial for microalgae adaptation to the natural variation of light in the environment and can be expected to occur under different light colors. Therefore, the mechanism of pigment production in green microalgae can be favored using different colors of light and, as we demonstrated, the potential of increasing photosynthetic pigments such as carotenoids can be magnified by combining the blue light and low dose copper exposure.

Finally, our suggested explanation for the combined effect of the blue light and low copper (3.8×10^{-9} mol L⁻¹), which increased carbohydrates, proteins, and carotenoids in *K. contorta*, is that at low doses the metal caused minor oxidative stress in the cells, making them more sensitive to the blue light. As a response, the cells have accumulated biomolecules.

5. Conclusions

This study showed that exposure of *K. contorta* to copper at low doses under the white light was enough to induce effects in the cells, but blue/red lights without extra copper were not as effective. The combination of blue light and 3.8×10^{-9} mol L⁻¹ free copper magnified biomolecules in the cells. Approximately 4x higher carbohydrates, 3x higher proteins and 2x higher carotenoids were detected. We showed that the photosynthetic apparatus was more sensitive to changes in light colors than to increasing copper at sublethal levels. This research demonstrated the viability of a manipulation strategy by combining the copper and blue light to magnify biomolecules in green microalgae.

Declarations

Competing interests

The authors declare that there is no conflict of interest in this study.

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Authors' contributions

All authors contributed to the conception and design of the study. The material was prepared by Leonardo Murilo Aoyagi and Douglas Henrique Baracho. Data analysis was carried out by Leonardo Murilo Aoyagi, Douglas Henrique Baracho and Ana Teresa Lombardi. Supervision was carried out by Douglas Henrique Baracho and Ana Teresa Lombardi. The first draft of the manuscript was written by Leonardo Murilo Aoyagi and all authors have commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

Data will be made available on request.

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Figures

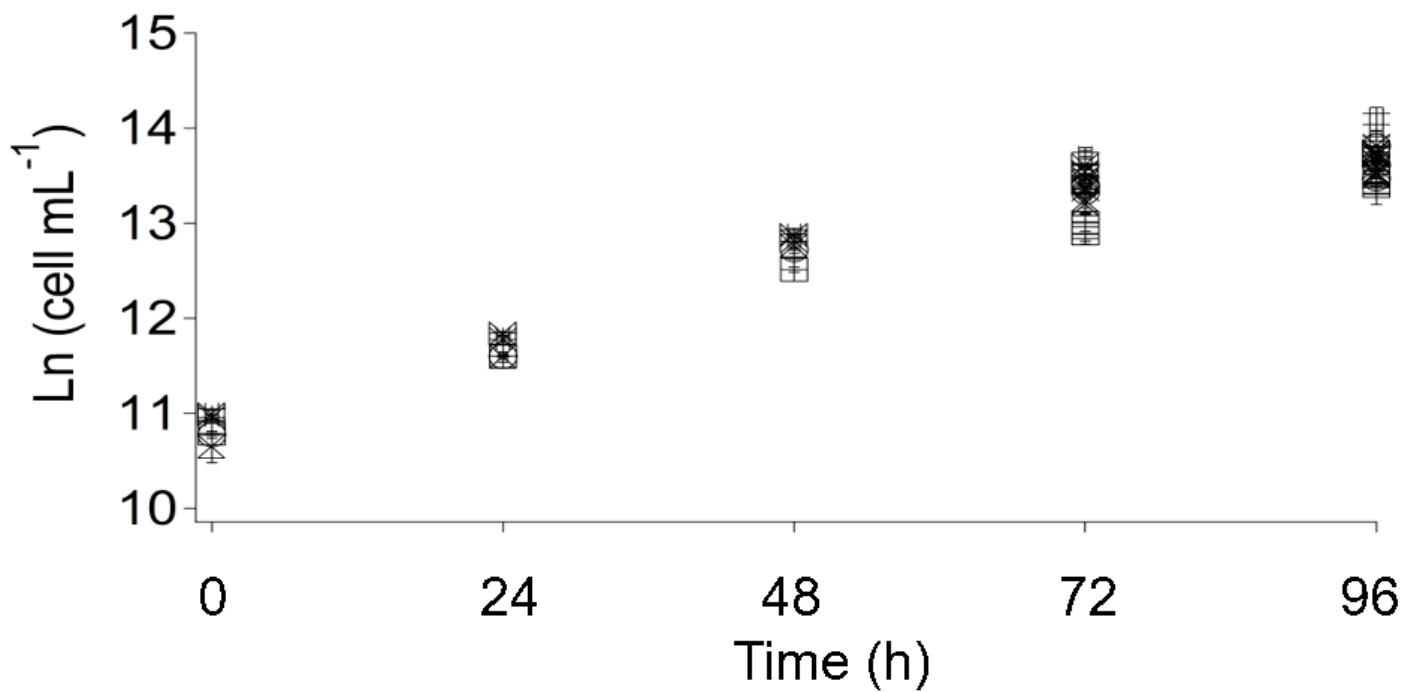


Figure 1

Growth curves of *Kirchneriella contorta* exposed to selected copper concentrations during the manipulation process.

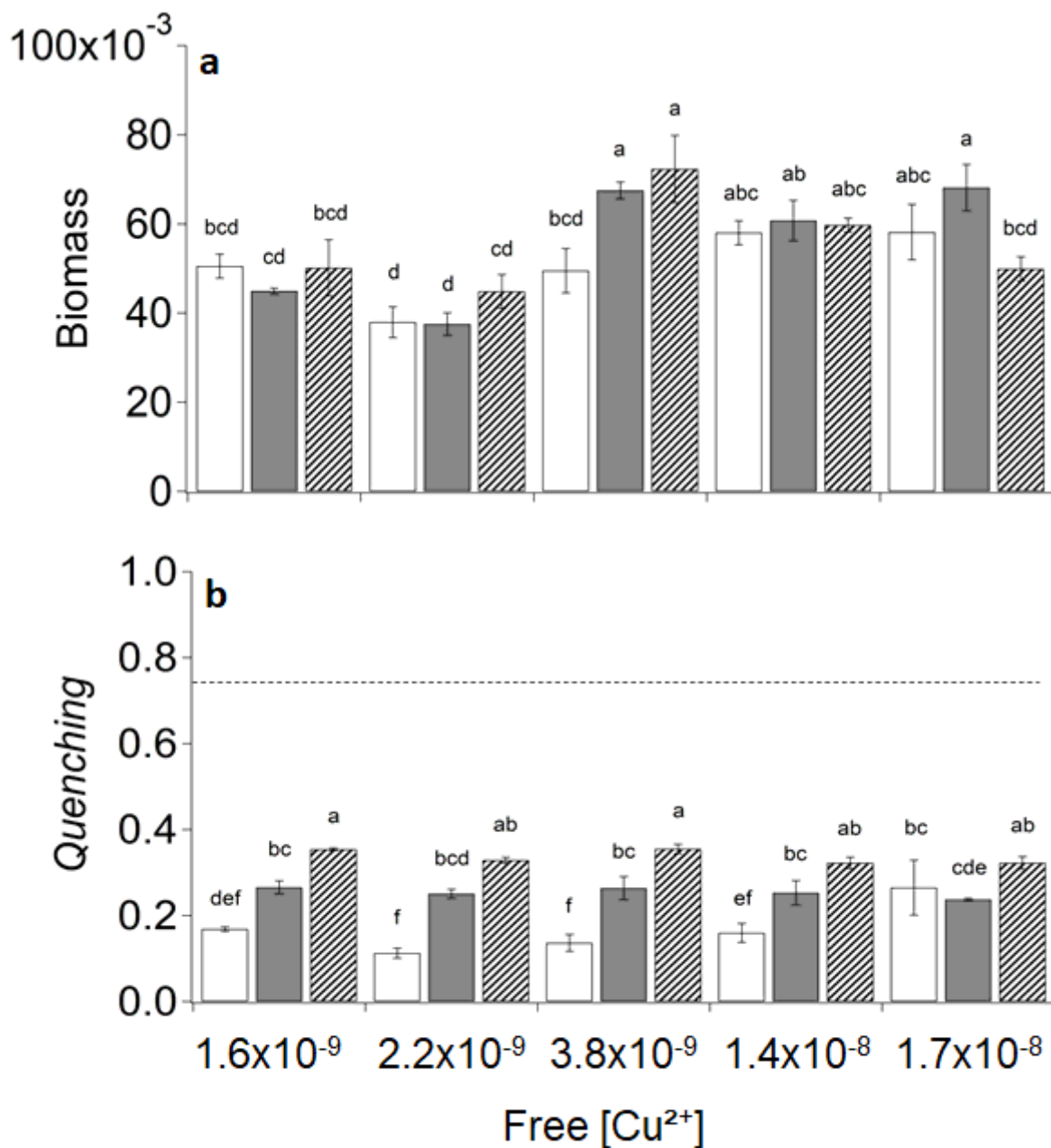


Figure 2

Variables obtained in the manipulation experiment of *K. contorta* at 96 h. (a) dry biomass (mg L⁻¹) and (b) fluorescence *quenchings*, where qP is represented by the dashed line and NPQ by the bars. White bars refer to control, grey to red light and hatched to blue light. Error bars represent the standard deviation from the mean (n=3). Letters above bars represent statistical evaluation (same letter indicates no significant difference, ANOVA, p>0.05).

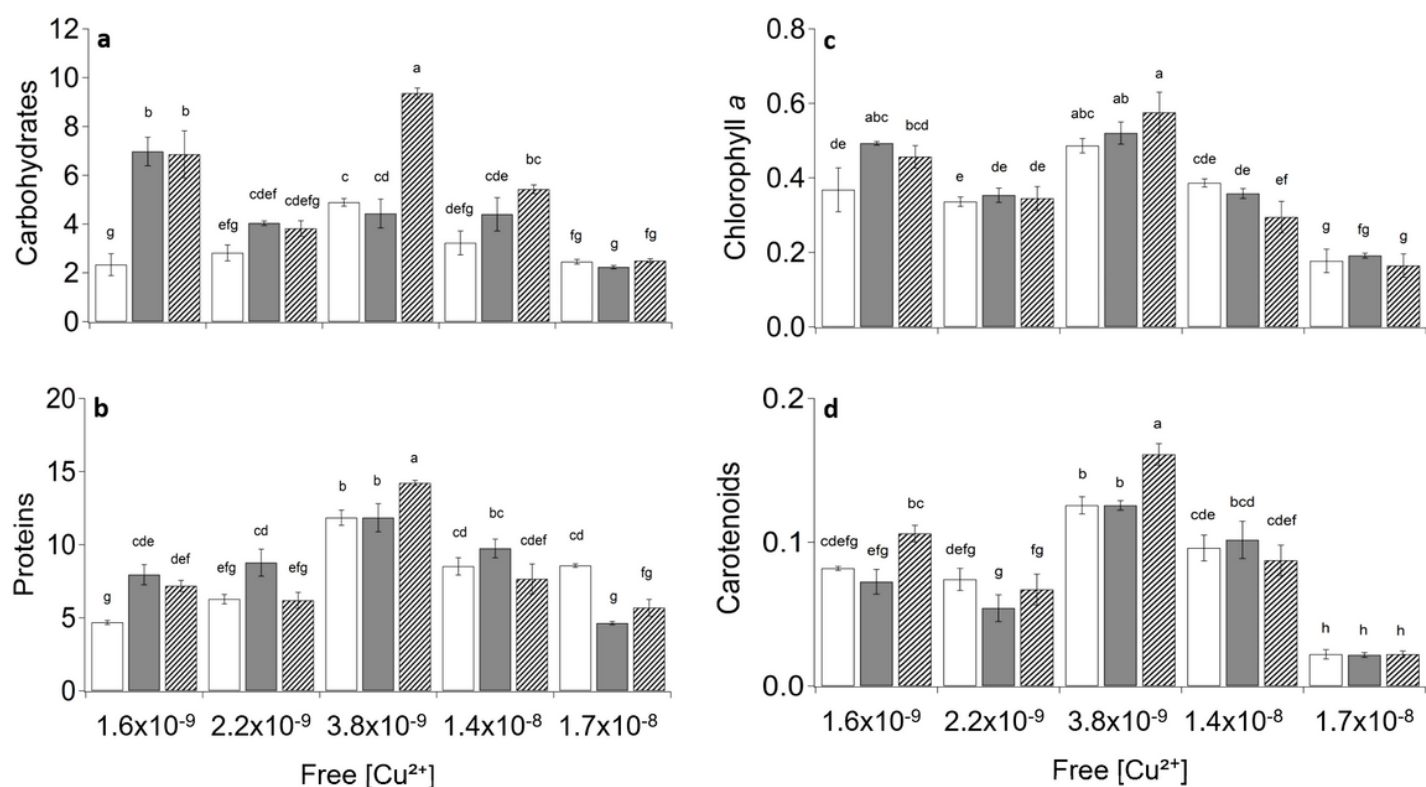


Figure 3

Biomolecule productivity obtained in the manipulation experiment of *K. contorta* at 96 h. Total proteins (a), carbohydrates (b), chlorophyll *a* (c), and carotenoids (d) ($\mu\text{g mL d}^{-1}$). White bars refer to control, grey to red light and hatched to blue light. Error bars represent the standard deviation from the mean ($n=3$). Letters above bars represent statistical evaluation (same letter indicates no significant difference, ANOVA, $p>0.05$).

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