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Original Article

The herbicide tebuthiuron and temperature increase related to climate change can impair the photosynthesis of *Oedogonium* sp. (Chlorophyta)

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ABSTRACT

Freshwater habitats are among the most degraded environments, with organisms living in multi-stressor conditions. We tested the photosynthetic performance of *Oedogonium* sp., a freshwater green alga, after exposure to an herbicide combined with temperature increases related to climate change. Treatments were designed by combining nominal concentrations (0.00 or control, 0.05, 0.6 and 1.2 mg/L) of tebuthiuron with temperature increases projected by the Intergovernmental Panel on Climate Change for the scenarios RCP 4.5 (+2.3 ºC) and RCP 8.5 (+3.4 ºC). Treatment concentrations were determined based on i) the maximum concentration allowed by the US Environmental Protection Agency in water bodies, ii) the recommended application dosage by the manufacturer and iii) a worst-case scenario. Chlorophyll *a* fluorescence analysis showed that tebuthiuron concentrations of 0.6 mg/L or higher, regardless of temperature, negatively affected the photosynthetic performance of the alga, with reduced quantum photosynthetic yield associated with increased non-regulated, non-photochemical energy loss. Oxygen evolution curve analyses revealed a significant drop in the photosynthetic rate of *Oedogonium* sp. under both RCP scenarios in comparison to the scenario without temperature increase, with decreases ranging from 13% to 70% among treatments. Despite the clear negative effects of exposure to both stressors individually, no combined effect was observed.

Keywords: Chlorophyll *a* Fluorescence; IPCC; Sugarcane crops; Green algae; Chlorophyta

Introduction

The large array of industrially developed chemical compounds that end up in diverse ecosystems and the associated human activities that promote habitat destruction constitute a multi-stressor setting for biodiversity as a whole (Smith *et al.* 2015; Sabater *et al.* 2019). Among the most degraded natural environments in this sense are freshwater ecosystems (Romero *et al.* 2018).

Such stressful conditions in freshwater ecosystems affect important physiological processes, including photosynthesis, which not only endangers the well-being of primary producers but also generates impacts across multiple levels of organization, possibly generating trophic cascade effects (Woodward *et al.* 2010). Although freshwater

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trophic webs receive a great amount of energy from external sources (allochthonous energy), primary producers (autochthonous energy), particularly benthic algae (Branco *et al.* 2017), have a crucial role in the environment and can sustain many freshwater communities (Lau *et al.* 2008; Neres-Lima *et al.* 2017).

Green macroalgae represent one of the larger groups of primary producers, especially in high-irradiance lotic environments (Branco *et al.* 2017; Peres *et al.* 2017). Among these, the genus *Oedogonium* Link ex Hirn stands out for its worldwide distribution, its biotechnological potential, and its reported use in bioremediation (Lawton *et al.* 2014; Adegoke *et al.* 2018; Roberts *et al.* 2018; Tófoli *et al.* 2023).

There has been a significant increase in the registration of new pesticides in Brazil in the past few years, resulting in greater risks of contamination of freshwater environments, especially those close to agricultural areas (Brovini *et al.* 2021). Brazil is one of the largest producers of sugarcane in the world (CONAB 2020), and after the ban of the practice of burning the remaining sugarcane straw on crop fields before planting, farmers have increased the volume of herbicides applied in fields in order for the chemicals to pass through the straw layer and reach the soil (Toniêto *et al.* 2016).

One commonly used herbicide in sugarcane farming in Brazil is tebuthiuron (1-(5-tert-Butyl-1,3,4-thiadiazol-2-yl)-1,3-dimethylurea). This herbicide belongs to the phenylurea class of pesticides, which are compounds that inhibit electron transport in photosystem II (PSII), preventing photosynthesis by early weed shoots after absorption by the root (Liu 2010). Tebuthiuron acts on algae by inhibiting the same target pathway of PSII, negatively affecting the photosynthesis of free-living and symbiotic species of marine microalgae and possibly decreasing their growth rate (Magnusson *et al.* 2010; Thomas *et al.* 2020b; Marzonie *et al.* 2021). Tebuthiuron has a low degradation rate, high water solubility, and high leaching potential (Grott *et al.* 2021). The World Health Organization classifies tebuthiuron as moderately toxic (World Health Organization 2019), while international authorities such as the European Union have limited its usage and classified it as very toxic, especially to aquatic life (European Chemicals Agency 2021).

Along with exposure to anthropogenic chemical compounds, organisms must withstand other chemical, physical, and biological stresses that affect ecosystems (López-Valcárcel *et al.* 2023), including those related to climate change (Romero *et al.* 2018). Climate change affects freshwater organisms mainly by altering water temperature and rainfall patterns (Engelman *et al.* 2008), making these environments, which may already be heavily degraded, highly susceptible to biodiversity loss (Dudgeon 2019).

In its 5th Assessment Report, the Intergovernmental Panel on Climate Change (IPCC 2013) proposed four Representative Concentration Pathways (RCPs): RCP 2.6, in which strong mitigation policies would take place; RCP 4.5 and RCP 6.0, as intermediate scenarios in which greenhouse gas emissions would stabilize at their current level without many efforts to constrain emissions; and RCP 8.5, in which global greenhouse gas emissions would continue to increase. The predictions of all four RCPs indicate a global mean temperature increase of at least 2 ºC in the following decades.

Temperature is highly relevant to biodiversity, being a strong driver of carbon flow in some freshwater environments (Belle *et al.* 2018) and capable of changing habitat suitability (Marotzke *et al.* 2017), with influences on the life history, interaction and persistence of individuals within populations (Knouft & Ficklin 2017). Considering the relevance of global warming, its impacts on biodiversity, and the importance of benthic algal communities, the concomitant stressful action of anthropogenic substances such as herbicides in the natural environment demands that the photosynthetic performance of algal species subjected to such multi-stressor conditions be analyzed. These effects could ultimately cause significant changes not only to the performance of a single species but also to the functioning of the ecosystem as a whole, especially in those environments where these organisms are responsible for a large part of primary production.

Thus, we aimed to test the effects of simultaneous exposure to different tebuthiuron concentrations and temperature increases related to climate change scenarios on *Oedogonium* sp., a globally distributed algal species. Since the photosynthetic process of *Oedogonium* sp. is similar to that of the target organisms of tebuthiuron, we hypothesized that the alga will be negatively affected by the herbicide. In addition, we considered that temperature may not be a significant factor, as this genus is globally distributed and commonly found in environments with higher solar irradiance. However, due to the energy spent by thermoregulation processes, a temperature increase could exacerbate the negative responses of the alga to the herbicide.

Material and methods

Sampling and preparation of algal samples

Specimens of *Oedogonium* sp. Link ex Hirn were sampled within a 20-meter section of the Pari River, located in the Cervo River microbasin in the western region of the state of São Paulo, Brazil (22°38’33”S, 50°12’14”W), and taken to the laboratory in transparent vials containing river water. Samples were cleaned manually using a stereoscopic microscope, jets of distilled water, hard bristle brushes and forceps to remove sediment, possible epiphytes and invertebrates. Sixty 150 mg (± 10 mg) samples were prepared by weighing on an analytical scale (n = 5 for each multi-stressor treatment).

Samples were acclimated for 24 hours under experimental conditions in 150 ml Erlenmeyer flasks containing 100 ml of Bold's basal medium (BBM) (Watanabe 2005). For this, samples were randomized inside bio-oxygen demand (BOD) incubators (Nova Ética, model 411 / FDP355) and kept under constant irradiance (140 μmol.m-2s-1), with a 12 h / 12 h photoperiod (light / dark cycle) and constant temperature determined for each experimental treatment.

After acclimation, the medium was replaced with new medium containing the active ingredient of tebuthiuron, in accordance with each multi-stressor treatment. Photosynthetic performance was analyzed after seven days. The medium of all samples was renewed on the third and fifth day to avoid nutrient depletion and to keep a constant nominal concentration of the active ingredient, following Oliveira *et al.* (2016) and adopting the recommendations of the 221-chemistry test guide of the Organization for Economic Cooperation and Development (OECD 2006).

Determination of temperature scenarios

The control temperature was determined by calculating the mean of measured temperatures (MMT) of the streams in the Cervo River microbasin, where the *Oedogonium* sp. samples were collected. Although the highest stream temperatures are typically in the summer, the seasonality of freshwater algae in tropical regions is mostly related to the rainfall regime (Branco & Pereira 2002), with larger populations occurring in winter (rainy season). The highest stream temperatures during winter are at 16 h. Therefore, temperature measurements were taken between 15 h and 17 h during winter (June 2019) using a multiparameter probe (HORIBA U-50), with a resulting mean of 21.6 (± 0.7) ºC (Table S1).

Experimental temperatures were calculated as described by Vilas Boas and Branco (2022) and considering two of the scenarios of the Intergovernmental Panel on Climate Change (IPCC): RCP 4.5 and RCP 8.5. In general terms, scenario RCP 4.5 projects the stabilization of emissions into the atmosphere, while scenario RCP 8.5, projects an increase in emissions (IPCC 2013). Thus, experimental temperatures were obtained by adding the maximum projected values (Collins *et al.* 2013) of 2.3 ºC (RCP 4.5) and 4.4 ºC (RCP 8.5) to MMT. Thus, while MMT (21.6 ºC) was used as a control, the experimental scenarios RCP 4.5 and RCP 8.5 had experimental temperatures of 23.9 ºC and 26 ºC, respectively.

Determination of nominal concentrations of tebuthiuron

The tested nominal concentrations of tebuthiuron followed those proposed by Vilas Boas and Branco (2022): 0.00 mg/L (C), 0.05 mg/L (T1), 0.6 mg/L (T2) and 1.2 mg/L (T3). Treatment T1 corresponds to the maximum concentration allowed by the United States Environmental Protection Agency in water bodies: 0.05 mg of active ingredient per liter (U.S. Environmental Protection Agency 1988). Treatment T2 corresponds to the recommended dosage for application of tebuthiuron for sugarcane crops on clay soils (according to the Combine®500 SC manufacturer): 0.6 mg/L. Finally, treatment T3 corresponds to a 'worst case scenario', with twice the recommended dosage for application on clay soils (1.2 mg/L). The control (C) did not receive an addition of herbicide.

Regarding the concentrations of tebuthiuron found in the environment, the nominal concentrations used in this study are considered high in comparison to tebuthiuron concentrations found in groundwater and in surface waters in agricultural regions in Brazil. Some studies regarding recharge areas of the Guarani aquifer in the State of Sao Paulo report tebuthiuron concentrations ranging from 0.03 to 0.08 μg/L (Gomes & Spadotto 2001), while reports of rivers within basins that present agricultural activity report concentrations up to 6.44 μg/L (Barizon *et al.* 2022). However, the tested nominal concentrations are realistic considering surface waters next to application areas and depending on time elapsed since application. The concentration for T1 (0.05 mg/L) has been reported in surface waters in wetlands close to tebuthiuron application sites in Australia 293 days after application. In addition, the concentration used for T3 (1.2 mg/L) is similar to concentrations found in these surface waters three days after tebuthiuron application (Dam *et al.* 2004; Grott *et al.* 2021). Smaller concentrations are expected in the long-term following a single application, however, herbicides are routinely applied in sugarcane crops in Brazil (Bordonal *et al.* 2018).

Treatment concentrations were prepared using a 6 mg/L stock solution of tebuthiuron. Based on the recommendation of Riedl and Altenburger (2007) and King *et al.* (2022), that exposure concentrations be measured for compounds with log K_{ow} > 3 and that tebuthiuron is nonvolatile, highly soluble in water (2.500 mg/L, Grott *et al.* 2021) and has a low octanol-water coefficient (log K_{ow} 1.8, Grott *et al.* 2021), we assumed that adsorption/binding to test flask walls was very unlikely and, therefore, the nominal concentrations of tebuthiuron used in the experiments were considered accurate.

Multi-stressor treatments

Combinations of the two stressors – tebuthiuron and temperature – were as follows: MMT plus the two temperature scenarios combined with three tebuthiuron concentrations, in addition to the control without herbicide, totaling 12 multi-stressor treatments. The 12 multi-stressor treatments were coded by the following acronyms: MMT Control; MMT T1, MMT T2 and MMT T3; RCP 4.5 Control, RCP 4.5 T1; RCP 4.5 T2 and RCP 4.5 T3; and RCP 8.5 Control, RCP 8.5 T1, RCP 8.5 T2 and, RCP 8.5 T3.

Experimental analyses

The photosynthetic performance of *Oedogonium* sp. was evaluated through chlorophyll *a* fluorescence and dissolved oxygen evolution (Necchi Júnior & Zucchi 2001; Branco *et al.* 2017; Vilas Boas *et al.* 2019; Vilas Boas & Branco 2022). Specimens were analyzed after seven days of exposure to the multi-stressor treatments.

The following photosynthetic chlorophyll *a* parameters were measured using a Diving-PAM fluorometer (Walz, Effeltrich, Germany): i) Y(II) - effective quantum yield of photosystem II; ii) Y(NPQ) - quantum yield of regulated non-photochemical energy loss in photosystem II; and iii) Y(NO) - quantum yield of non-regulated, non-photochemical energy loss in photosystem II. Measurements were taken through the “Induction Curve” function (Schreiber *et al.* 1995), with 12 pulses of saturating light (2,000 μmol photons.$m^2.s^{-1}$) lasting 0.8 s applied at 15 s intervals at samples after a 30 min period of acclimatization in the dark (Vilas Boas *et al.* 2019; Vilas Boas & Branco 2022).

Dissolved oxygen was measured with an oxygen meter equipped with a self-stirring probe (brand YSI, model 5100). Based on the variation of the initial and final dissolved oxygen concentrations after one hour of incubation in their respective treatments (Littler & Arnold 1985; Thomas 1988), net photosynthetic rate and dark respiration rate were calculated in clear and dark glass bottles, respectively. Light bottles were periodically repositioned to guarantee equal light intensities to every sample.

Formulas for the calculations were as follows: NP = [(F) - (I)] * V / IT / DW and DR = [(I) - (F)] * V / IT / DW, where NP is net photosynthetic rate; DR is dark respiration rate; (F) is final concentration of dissolved oxygen after the incubation period; (I) is initial concentration of dissolved oxygen before the incubation period; V is volume (liters) of medium in the bottle; IT is incubation time; and DW is dry weight.

Statistical Analyses

Statistical tests were performed using the statistical software IBM© SPSS© Statistics (IBM 2019). Potential differences in photosynthetic parameters (both from chlorophyll *a* fluorescence and from dissolved oxygen evolution) among treatments and control group were identified using two-way ANOVA tests, followed by Tukey’s honestly significant difference (HSD) post-hoc test (Tukey 1949). Photosynthetic parameters were used as dependent variables, while the factors of multi-stressor treatments (tebuthiuron concentrations and temperature scenarios) were used as independent variables.

Results

Different factors affected different photosynthetic parameters (Table 1). Chlorophyll *a* fluorescence was affected by nominal tebuthiuron concentration, while the parameters obtained by the oxygen evolution technique were affected by temperature scenario. There was no evidence of an interaction between factors.

Photosynthetic yield (Y(II)) was lower ($p < 0.001$, $F (3,42) = 11.29$) for treatments with nominal tebuthiuron concentrations of 0.6 mg/L or higher in comparison to the control (-77%, -66% and -60% for T2 in MMT, RCP 4.5 and RCP 8.5, respectively; -86%, -83% and -81% for T3 in MMT, RCP 4.5 and RCP 8.5, respectively) (Fig. 1). At the same time, Y(NO) values were higher ($p < 0.001$, $F (3,42) = 10.98$) for T2 (+79%, +27% and +26% for MMT, RCP 4.5 and RCP 8.5) and T3 (+104%, +34% and +53% for MMT, RCP 4.5 and RCP 8.5) compared to the control (Fig. 2). There were no significant differences among treatments for Y(NPQ) (Fig. 3).

Net photosynthetic rate (NPR) decreased ($p < 0.001$, $F (3,48) = 23.09$) as temperature increased, showing an inverse relationship. MMT had the highest NPR, with RCP 4.5 (-54% for control, -63% for T1, -70% for T2 and -57% for T3) and RCP 8.5 (-42% for control, -36% for T1, -44% for T2 and -13% for T3) being lower (Fig. 4). Dark respiration rate was lower ($p < 0.001$, $F (3,48) = 52.15$) for RCP 8.5 than for MMT (-82% for Control, -81% for T1, -89% for T2 and -93% for T3) (Fig. 5).

Discussion

Exposure to nominal tebuthiuron concentrations of 0.6 mg/L or higher negatively affected the photosynthetic performance of *Oedogonium* sp., as evidenced by the chlorophyll *a* fluorescence results. The lower values of photosynthetic yield (Y(II)) for T2 and T3 suggest that the capacity of light energy conversion of *Oedogonium* sp. is severely diminished in such concentrations (Tait *et al.* 2017). At the same time, higher Y(NO) values indicate a release of excess energy through paths that could induce damage to the photosynthetic apparatus of this filamentous green algae via photooxidative stress (Klughammer & Schreiber 2008).

The photooxidative stress in plants and algae is caused by the singlet oxygen species $^1O_2$ (Krieger-Liszkay 2005). After charge recombination in PSII occurs, either due to photoinhibition or due to the action of pesticides that bind to photosystem II (PSII), there is a formation of a chlorophyll triplet state, which, in the presence of O_2, can react to form $^1O_2$ (Fufezan *et al.* 2002). $^1O_2$ is very reactive and rapidly reacts with target molecules, causing cellular damage that ultimately can lead to cell death (Triantaphylidès *et al.* 2008).

Table 1. Effects of tebuthiuron concentrations and temperature related to IPCC scenarios on photosynthetic parameters (YII, Y(NO), Y(NPQ), Net Photosynthetic Rate (NPR), and Dark Respiration Rate (DRR)) of *Oedogonium* sp. The data are presented as mean (+ standard deviation). Bold *p*-values indicate statistically significant effects (N = 5; $p < 0.05$) and different letters after tebuthiuron concentrations and/or IPCC scenarios indicate significant differences on Tukey's HSD test.

Photosynthetic Parameter	[Tebuthiuron] (mg/L)	IPCC Scenarios			ANOVA	F-values	p-values
		MMT - control	RCP 4.5	RCP 8.5			
		(21.6º)	(23.9º)	(2.0º)			
YII	0,0 - control^{a}	0.294 (±0.08)	0.317 (±0.16)	0.205 (±0.15)	[Tebuthiuron]	11.290	<0.001***
	0.05^{a}	0.179 (±0.11)	0.239 (±0.16)	0.210 (±0.15)	IPCC Scenario	0.747	0.480
	0.6^{b}	0.067 (±0.07)	0.106 (±0.13)	0.082 (±0.05)	Interaction	0.300	0.934
	1.2^{b}	0.042 (±0.03)	0.054 (±0.04)	0.039 (±0.03)			
		MMT - control	RCP 4.5	RCP 8.5			
		(21.6º)	(23.9º)	(2.0º)			
YNO	0,0 - control^{a}	0.354 (±0.15)	0.534 (±0.18)	0.476 (±0.25)	[Tebuthiuron]	10.979	<0.001***
	0.05^{a}	0.411 (±0.26)	0.413 (±0.08)	0.479 (±0.14)	IPCC Scenario	0.663	0.520
	0.6^{b}	0.633 (±0.13)	0.678 (±0.10)	0.600 (±0.09)	Interaction	0.529	0.783
	1.2^{b}	0.722 (±0.11)	0.717 (±0.13)	0.729 (±0.05)			
		MMT - control	RCP 4.5	RCP 8.5			
		(21.6º)	(23.9º)	(2.0º)			
YNPQ	0,0 - control	0.352 (±0.12)	0.149 (±0.18)	0.319 (±0.25)	[Tebuthiuron]	2.064	0.120
	0.05	0.410 (±0.16)	0.348 (±0.08)	0.310 (±0.14)	IPCC Scenario	2.167	0.127
	0.6	0.300 (±0.11)	0.215 (±0.10)	0.318 (±0.09)	Interaction	0.782	0.589
	1.2	0.236 (±0.13)	0.229 (±0.13)	0.232 (±0.05)			
		MMT - control	RCP 4.5	RCP 8.5			
		(21.6º)^{a}	(23.9º)^{b}	(2.0º)^{b}			
NPR	0,0 - control	5.61 (±2.47)	2.56 (±1.51)	3.24 (±0.62)	[Tebuthiuron]	0.184	0.907
	0.05	5.39 (±1.90)	2.01 (±0.74)	3.44 (±1.16)	IPCC Scenario	23.088	<0.001***
	0.6	5,89 (±2.69)	1.74 (±0.43)	3.30 (±1.29)	Interaction	0.544	0.772
	1.2	4.44 (±1.67)	1.89 (±0.62)	3.85 (±1.31)			
		MMT - control	RCP 4.5	RCP 8.5			
		(21.6º)^{a}	(23.9º)^{a}	(2.0º)^{b}			
DRR	0,0 - control	8.00 (±3.33)	7.10 (±3.21)	1.41 (±0.23)	[Tebuthiuron]	0.752	0.527
	0.05	7.36 (±2.88)	6.51 (±3.15)	1.37 (±0.39)	IPCC Scenario	52.148	<0.001***
	0.6	8.91 (±2.50)	5.61 (±1.23)	1.01 (±0.37)	Interaction	0.583	0.742
	1.2	6.28 (±1.79)	6.34 (±1.96)	0.45 (±1.18)			

A study using the diatom *Chaetoceros muelleri* produced similar results regarding the reduction of photosynthetic quantum yield after exposure to lower tebuthiuron concentrations (Thomas *et al*. 2020a). In that case, the authors noted that the effects of tebuthiuron were a lowering of photosynthetic yield and a hampering of electron flow in photosystem II, which not only inhibited the photosynthesis of the diatom but also produced damage to the photosystem, which, in turn, decreased the algae's growth rate. Since *C. muelleri* is a unicellular species, the negative effects (i.e., cell death due to photooxidative stress) caused by tebuthiuron are more pronounced and dramatic than for a filamentous species like *Oedogonium* sp. This herbicide also showed large reductions in quantum photosynthetic yield of the coral endosymbiotic algae *Cladocopium goreaui*, and the marine microalgae *Rhodomonas salina*, highlighting its sub-lethal toxicity by reducing the photosynthetic capacity of these algae (Thomas *et al*. 2020b; Marzonie *et al*. 2021).

While exposure to tebuthiuron produced significant negative effects on the chlorophyll *a* fluorescence parameters tested here, with potential damage due to photooxidative stress, no differences were detected by the dissolved oxygen analyses. The lack of significant differences in oxygen concentrations might be due to the mode of action of this herbicide. Like other PSII inhibitor herbicides, tebuthiuron acts by blocking electron flow in photosynthesis, diminishing

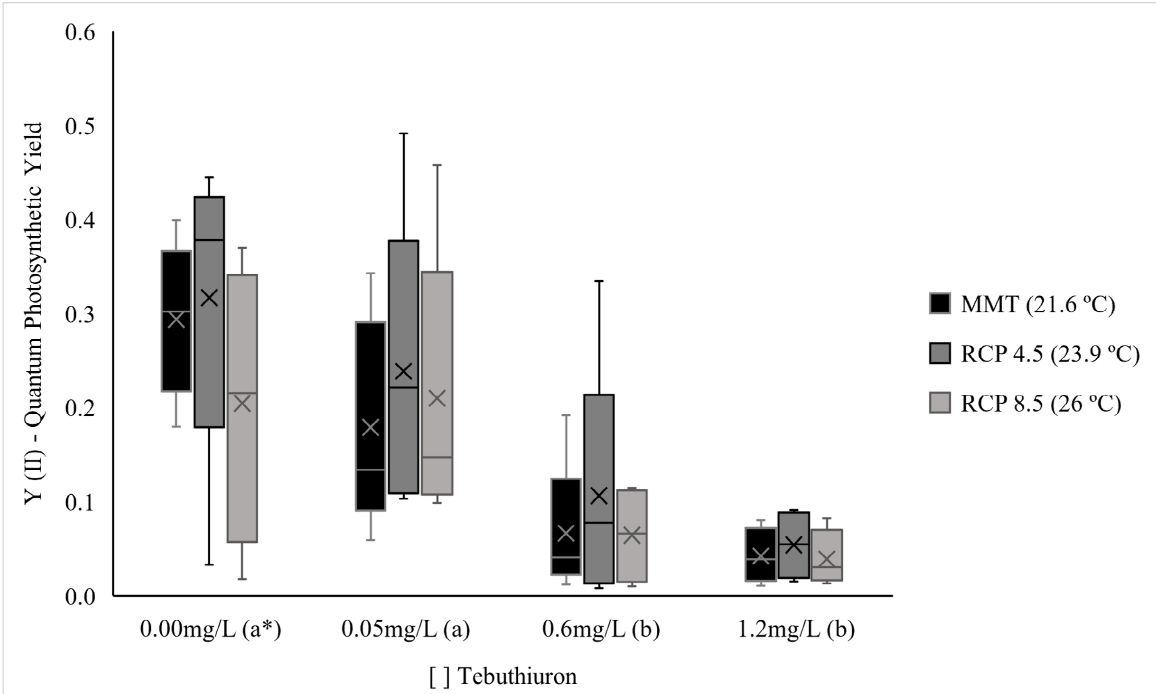

Figure 1. Y(II) (effective quantum yield of photosystem II): mean values (n = 5) for *Oedogonium* sp. after seven days of exposure to different tebuthiuron concentrations and temperature increase scenarios. MMT = mean of measured temperatures. *different letters indicate significant differences according to two-way ANOVA followed by Tukey's post-hoc test. The line represents the median value, the x represents the mean value, and the box represents the lower (25%) and upper (75%) quartiles. Minimum and maximum values are indicated by the whiskers.

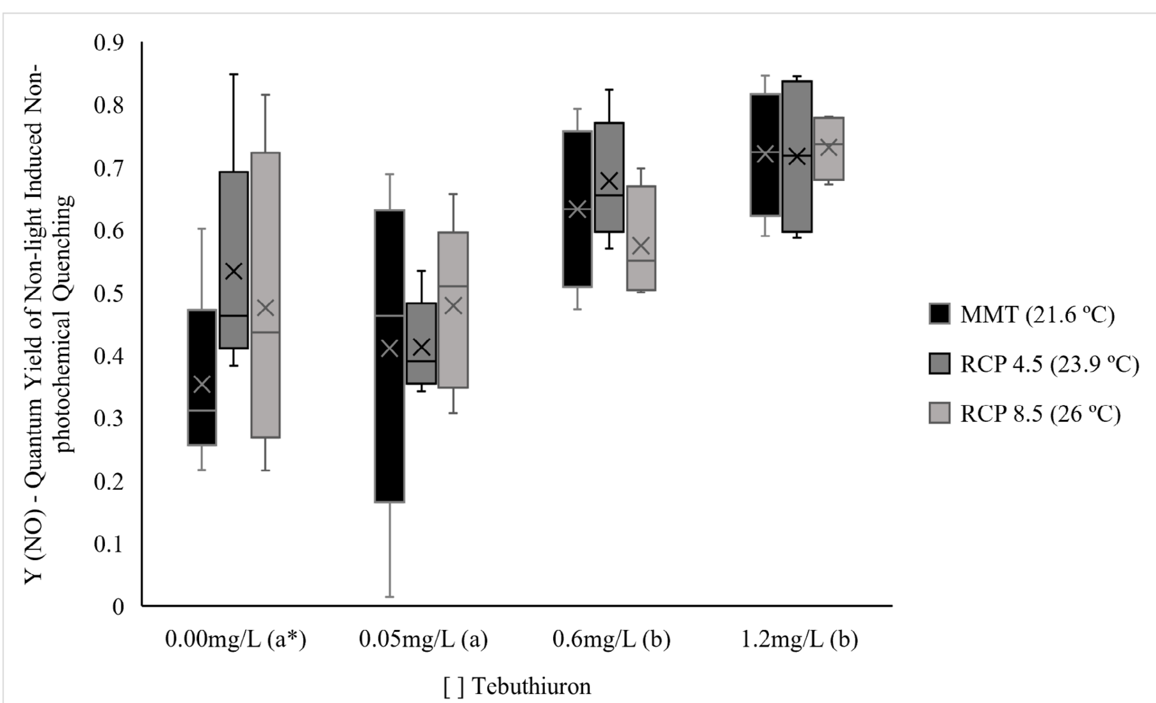

Figure 2. Y(NO) (non-regulated non-photochemical energy loss in photosystem II): mean values (n = 5) for *Oedogonium* sp. after seven days of exposure to different tebuthiuron concentrations and temperature increase scenarios. MMT = mean of measured temperatures. *different letters indicate significant differences according to two-way ANOVA followed by Tukey's post-hoc test. The line represents the median value, the x represents the mean value, and the box represents the lower (25%) and upper (75%) quartiles. Minimum and maximum values are indicated by the whiskers.

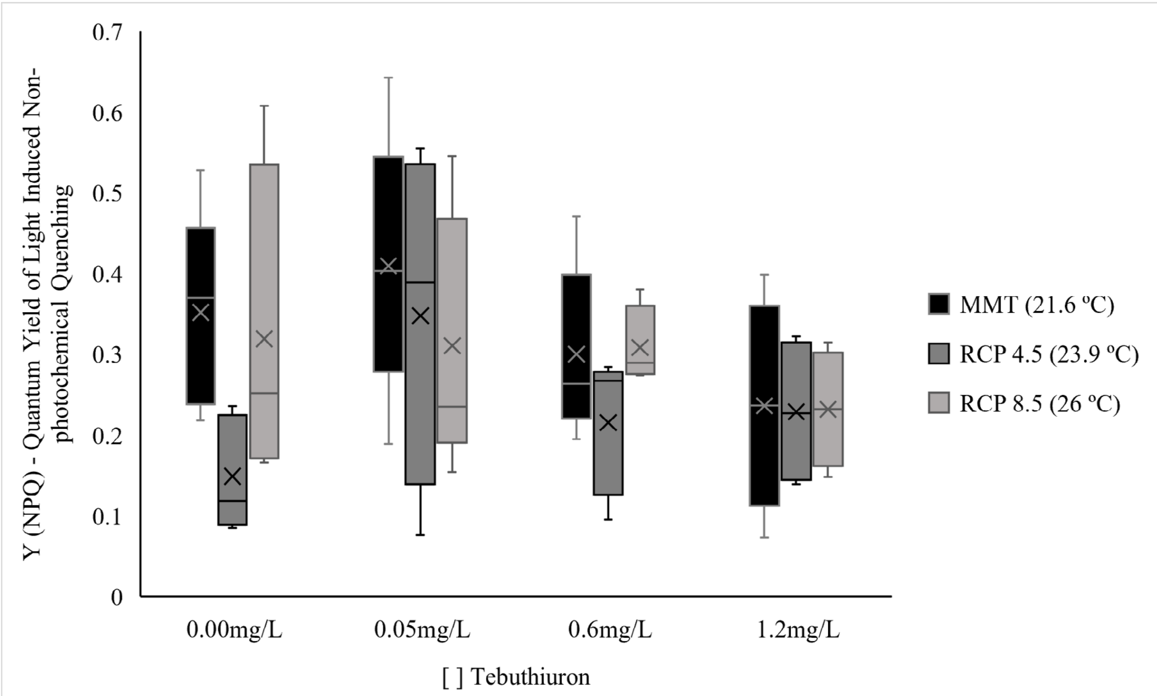

Figure 3. Y(NPQ) (regulated non-photochemical energy loss in photosystem II): mean values (n = 5) for *Oedogonium* sp. after seven days of exposure to different tebuthiuron concentrations and temperature increase scenarios. MMT = mean of measured temperatures. *different letters indicate significant differences according to two-way ANOVA followed by Tukey's post-hoc test. The line represents the median value, the x represents the mean value, and the box represents the lower (25%) and upper (75%) quartiles. Minimum and maximum values are indicated by the whiskers.

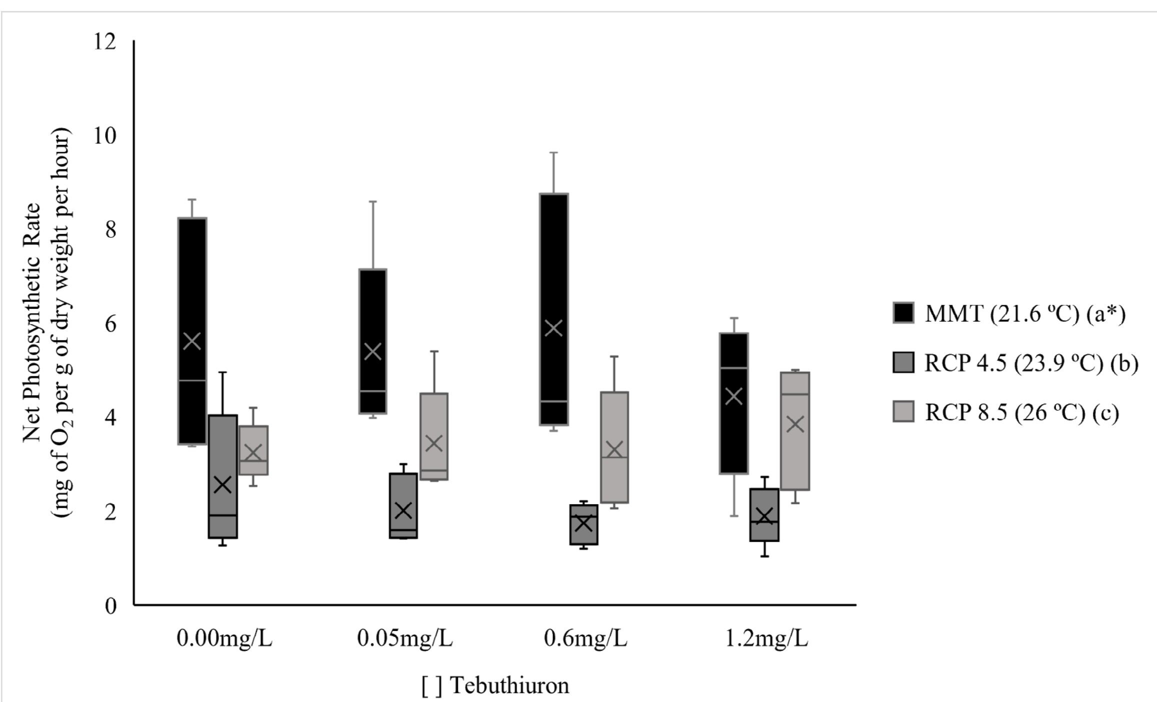

Figure 4. NPR (net photosynthetic rate): mean values (n = 5) for *Oedogonium* sp. after seven days of exposure to different tebuthiuron concentrations and temperature increase scenarios. MMT = mean of measured temperatures. *different letters indicate significant differences according to two-way ANOVA followed by Tukey's post-hoc test. The line represents the median value, the x represents the mean value, and the box represents the lower (25%) and upper (75%) quartiles. Minimum and maximum values are indicated by the whiskers.

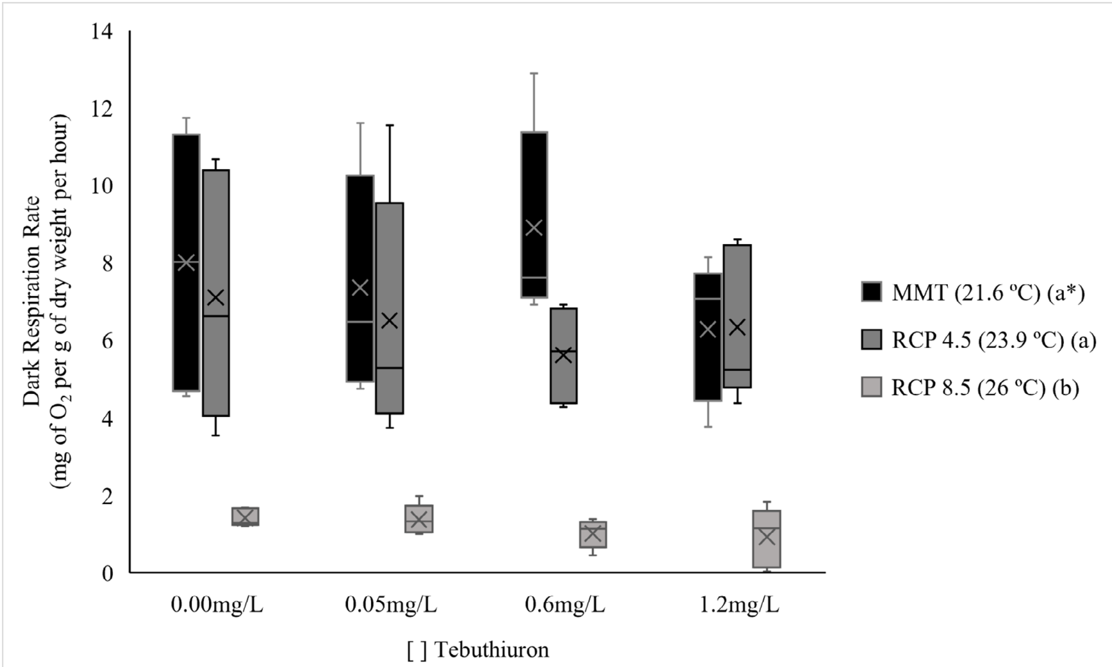

Figure 5. DRR (dark respiration rate): mean values (n = 5) for *Oedogonium* sp. after seven days of exposure to different tebuthiuron concentrations and temperature increase scenarios. MMT = mean of measured temperatures. *different letters indicate significant differences according to two-way ANOVA followed by Tukey's post-hoc test. The line represents the median value, the x represents the mean value, and the box represents the lower (25%) and upper (75%) quartiles. Minimum and maximum values are indicated by the whiskers.

the production of ATP and NADPH and ultimately hampering carbon assimilation by the organism (Duke & Dayan 2018). These so-called "dark reactions" occur in the Calvin cycle after oxidization. Therefore, while the release of O_2 is still occurring in the culture medium, the algae cannot complete its carboxylation cycle (Bukhov 2004).

The temperature increases predicted by the IPCC scenarios did not affect the measured chlorophyll *a* fluorescence parameters and, contrary to our hypothesis and some results reported in the literature for other organisms (e.g., Grott *et al*. 2021), did not show any interaction with nominal tebuthiuron concentration. However, a significant effect of this factor was observed for the net photosynthetic rate of *Oedogonium* sp. The net photosynthetic rate of *Oedogonium* sp. dropped significantly under both temperature scenarios, confirming that meeting the predictions of the IPCC scenarios RCP 4.5 and RCP 8.5 may negatively affect the primary productivity of this organism. Considering that species of *Oedogonium* are relevant primary producers in tropical lotic ecosystems, this impact could potentially influence trophic webs that are energetically supported by these filamentous green algae. In general, species of *Oedogonium* have been reported as possessing high growth rates under a wide range of temperatures, a characteristic that promotes them as potential candidates for biomass applications. However, temperature changes still significantly affect their growth rate (Lawton *et al*. 2014).

In conclusion, the results presented here partially confirm our hypotheses. Even though *Oedogonium* sp. is not the target species of tebuthiuron, due to the similarity between its photosynthesis pathway and that of seed plants, exposure to nominal tebuthiuron concentrations of 0.6 mg/L or higher significantly hampers its photosynthetic performance. In addition, contrary to our expectations, the increased temperature predicted by IPCC climate change scenarios, even the less severe scenario of RCP 4.5, negatively affected the net photosynthetic rate of this algae. Although no synergy or interaction was observed between exposure to tebuthiuron and increased twemperature, the impact on different measured photosynthetic parameters suggests that multi-stressor scenarios, like the one investigated here, constitute a stressful environment for the species, with effects on its photosynthesis and possibly ultimately other trophic levels.

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Author contribution

Both authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Lucas K. Vilas Boas. Supervision, project administration, and funding acquisition were performed by Ciro C.Z. Branco. The first draft of the manuscript was written by Lucas K. Vilas Boas; Ciro C.Z. Branco commented on, reviewed and edited previous versions of the manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

Availability of data and material

The authors declare that all data and material support the published claims and comply with field standards. The data that support the findings of this study are available from the corresponding author (CCZB) upon reasonable request.

Supplementary Material

The following online material is available for this article:

Table S1 – Temperature values (ºC) and GPS coordinates of 10 streams in the Cervo river microbasin.

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