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Light intensity affects tolerance of pyrene in *Chlorella vulgaris* and *Scenedesmus acutus*

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Abstract

The impact of light intensity on the toxicity of pyrene, a 4-ring polycyclic aromatic hydrocarbon (PAH), was studied in *Chlorella vulgaris* and *Scenedesmus acutus*. Both species were cultured under low light, LL [50–60 µmol(photon) m^{-2} s^{-1}], and high light, HL [100–110 µmol(photon) m^{-2} s^{-1}] conditions to study the effects of pyrene (PYR) toxicity on growth parameters, the content of biomolecules, chlorophyll content, and photosynthetic efficiency. In the presence of PYR, *S. acutus* could grow well in LL and HL intensity. On the other hand, *C. vulgaris* showed a drastic decrease in growth and photosynthesis during HL conditions due to PYR toxicity. Regulation of nonphotochemical and photochemical quenching was responsible for the survival of *S. acutus* under PYR toxicity in LL and HL conditions. Thus, *S. acutus* seems to be a more promising candidate for pyrene degradation under varying light conditions.

Keywords: *Chlorella vulgaris*; light intensity; photosynthesis; pyrene; *Scenedesmus acutus*.

Introduction

Microalgae play a crucial role as primary producers in aquatic habitats by providing food and bioenergy sources for all organisms as well as by powering food webs and biogeochemical cycles (Baidya *et al.* 2021). Algae are small with a comparably large surface area, due to which their exposure to toxic water-borne contaminants increases. The concentration of polycyclic aromatic hydrocarbons (PAHs), one of the persistent organic contaminants in environmental systems, such as rivers, marine sediments, drinking water supplies, groundwater, and coastal estuaries, is at an alarming level (Olayinka *et al.* 2018). Toxic effects of PAHs on freshwater algae concerning growth, photosynthesis, and respiration were reported (Aksmann *et al.* 2011, Tomar and Jajoo 2021). However, inhibition of photosynthesis is more important as it results in reduced growth resulting in lesser biomass

Highlights

- *Chlorella vulgaris* is more sensitive to PYR in high light than in low light intensity
- *Scenedesmus acutus* regulates $Y_{(NPQ)}$ and $Y_{(NO)}$ to protect PSII from pyrene toxicity
- *Scenedesmus acutus* is more suitable for the removal of pyrene under varying light conditions

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Abbreviations: C – control; Chl – chlorophyll; F_0 – minimal fluorescence; F_m – maximum fluorescence; F_v/F_0 – efficiency of the water-splitting complex; F_v/F_m – maximal quantum yield of PSII photochemistry; HL – high light; LL – low light; PAHs – polycyclic aromatic hydrocarbons; PYR – pyrene; SP – saturation pulse; $Y_{(II)}$ – quantum yield of PSII; $Y_{(NO)}$ – yield of nonregulated energy dissipation; $Y_{(NPQ)}$ – yield of regulated energy dissipation.

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yield. Moreover, light intensity plays an important role in algal photosynthesis, therefore, tolerance of algae to toxic substances is also expected to change with varying light intensities.

Light is a key parameter in microalgae cultivation. Light intensity has been reported to influence microalgae productivity and nutrient removal efficiency (Abu-Ghosh *et al*. 2016, Binnal and Babu 2017, González-Camejo *et al.* 2019). The growth of microalgae is proportional to the activity of PSI and PSII; they are both sensitive to light conditions (Nama *et al.* 2015). If the light intensity value is below or exceeds the optimum, performance of PSI and PSII is altered (Nama *et al.* 2015). The effects of light intensity, photoperiods, and light wavelength on algal growth have been extensively reported (Yan *et al.* 2013, Gris *et al.* 2014, González-Camejo *et al*. 2019). However, the effect of light intensity on the removal of organic pollutants by microalgae cultivation has not been studied in detail.

The Chlorophyta microalgae, *Chlorella vulgaris* (*C. vulgaris*) and *Scenedesmus acutus* (*S. acutus*), are unicellular photosynthetic eukaryotes found in many aquatic habitats. Both are sensitive to physicochemical changes and pollution in the surrounding environment, therefore, they are frequently used as model organisms for phytotoxicological studies. Despite large numbers of toxicity studies of pollutants such as metals and herbicides, the impact of PAHs on algal growth with different environmental factors remains poorly understood. Previous results suggest that different light intensities have a significant influence on regulating growth and photosynthesis in algae (Kim *et al*. 2013, Xu *et al*. 2016).

In the present study, pyrene (PYR) was chosen as a representative PAH since it is one of the most toxic PAHs and is listed as a priority pollutant by USEPA. Pyrene is a high-molecular-mass 4-ring PAH with higher water solubility (Juhasz and Naidu 2000). PYR harms the natural development of phytoplankton communities and algal growth (Petersen *et al*. 2008). It is thought to be a potent photosensitizer that causes intracellular oxidative stress and obstruction of the photosynthetic electron transport chain (Häder *et al*. 2015). This study compares the ecotoxicological effects of PYR on growth, pigment content, and photosynthesis of two algal species, *C. vulgaris* and *S. acutus*. The overall goal of the present work is to find a more suitable algal species that can tolerate PYR toxicity under varying light (low and high) conditions.

Materials and methods

Algal species and culture conditions: Freshwater microalgal species *C. vulgaris* was procured from Phycospectrum Environmental Research Centre, Chennai, India, and *S. acutus* from National Chemical Laboratory (NCL), Pune, India. Cells were grown in BG11 media (*M1958*, *Himedia*, India). Mother cultures were illuminated with white light having the intensity of 60 µmol(photon) m^{-2} s^{-1} at 25°C and a diurnal cycle of 16 h light and 8 h dark in the algal culture room. After 7–10 d when the exponential growth phase arrived, cells were centrifuged at 2,500 × *g* for 5 min and harvested for further experiments.

Experimental setup: For each treatment, three conical flasks (250 mL, Erlenmeyer flasks), containing 150 mL of culture medium were used. Algal mass was inoculated to each conical flask so that an initial optical density of 0.1 at 680 nm was observed. Optical density was measured by UV–VIS spectrophotometer (*Evolution 201*, *Thermo Scientific*, USA). After inoculation, 75 µL of stock solution (PYR in acetone) was added to 150 mL of media to obtain the effective concentration of 5 mg L^{-1}. The flasks without any addition of PYR were used as control. The growing conditions involved two different light intensities, low light (LL) [50–60 µmol(photon) m^{-2} s^{-1}] and high light (HL) [100–110 µmol(photon) m^{-2} s^{-1}] for both species. After 7 d of incubation, algal samples of all treatments were retrieved for various experiments.

Algal growth and biomass: Microalgal growth was monitored regularly at an interval of 24 h by measuring optical density (OD) at 680 nm using a UV–VIS spectrophotometer. Dry biomass was estimated after drying 100 mL of culture in a hot air oven at 80°C for 4–6 h. The growth rate [d^{-1}] was calculated using the following equation: $GR\ [d^{-1}] = (\ln N_2 - \ln N_0)/(t_2 - t_0)$, where N_2 is the OD at time t_2 and N_0 is the OD at time t_0 (day 0).

Lipid, carbohydrate, and protein content: Lipid content was estimated by sulpho-phospho-vanillin (SPV) colorimetric method as described in Mishra *et al*. (2014). In brief, phospho-vanillin reagent was prepared by initially dissolving 0.6 g of vanillin in 10 mL of absolute ethanol: 90 mL of deionized water and stirred continuously. Subsequently, 400 mL of concentrated phosphoric acid was added to the mixture, and the resulting reagent was stored in the dark until use. Around 40 mL of algal cell culture was centrifuged at 5,000 × *g* and the pellet was suspended in 100 µL of water. Then, 2 mL of concentrated (98%) sulfuric acid was added to the sample and heated for 10 min at 100°C, and then cooled for 5 min in ice bath. Then, 5 mL of freshly prepared phospho-vanillin reagent was added, and the sample was incubated for 15 min at 37°C incubator shaker at 200 rpm. Absorbance reading at 530 nm was taken in order to quantify the lipid within the sample.

Carbohydrate content was estimated by phenol–sulfuric acid method as described in Laurens *et al*. (2012). Protein estimation was done according to Slocombe *et al*. (2013) with some changes. In brief, protein extraction was done by 6% TCA (trichloroacetic acid) and kept at room temperature overnight. Protein content in each sample was estimated with Folin–Ciocalteu phenol reagent and absorbance of each sample was read at 600 nm (*Evolution 201*, *Thermo Scientific*, USA).

Chlorophyll (Chl) content: Pigment content was determined using the following method: from all treatments, 5 mL of culture was taken and centrifuged at 2,500 × *g* for 5 min. The supernatant was discarded and 5 mL of 99.9% methanol was added to the pellet, mixed

properly, and incubated at 90°C for 5 min. The culture was centrifuged at 9,000 × *g* for 5 min and the supernatant was used for pigment estimation (*Evolution 201*, *Thermo Scientific*, USA). Calculations were done as mentioned in Dere *et al*. (1998).

Chl *a* fluorescence: Measurements of the quantum yields of energy conversion in PSII were carried out through saturation pulse technique, using a pulse amplitude modulator (*Dual PAM-100*, *Heinz Walz*, Effeltrich, Germany) system in intact algal cell culture. The algal sample was dark adapted for 30 min at 23 ± 2°C before measurements, and then 3 mL of cell culture was taken in a cuvette for recording the induction curve. A weak modulated light [12 µmol(photon) m^{-2} s^{-1}] was given to get minimal fluorescence (F_0), followed by actinic light [53 µmol(photon) m^{-2} s^{-1}], and saturating pulse (SP) [6,000 µmol(photon) m^{-2} s^{-1}] to achieve maximum fluorescence (F_m). After the determination of F_0 and F_m, the induction curve was analyzed using *Dual PAM-100* software. The induction curve was recorded with SP for 5 min to achieve the steady state of the photosynthetic apparatus, and then the actinic light was turned off.

Proline content: Proline was extracted using 3% sulphosalicylic acid and estimated using L-proline as a standard as described in Bates *et al*. (1973). Briefly, harvested fresh algal biomass was homogenized in 3 mL of 3% sulphosalicylic acid and then centrifuged at 6,000 rpm for 10 min. The supernatant (1 mL) was heated with 1 mL of ninhydrin and 1 mL of glacial acetic acid at 100°C for 1 h. Proline was quantified spectrophotometrically at 440 nm by use of a standard curve of L-proline.

Total polyphenol content: Total phenolics were colorimetrically determined using Folin–Ciocalteu reagent as described by Cajnko *et al*. (2019) with slight modifications.

Statistical analysis: Data were analyzed by using *Graphpad Prism 5.01* software (La Jolla, CA, USA). Results were analyzed using a one-way analysis of variance (*ANOVA*) followed by the *Newman–Keuls* multiple comparison test. Significance was determined at $p<0.001$(* $p<0.05$, ** $p<0.01$, *** $p<0.001$), and results were expressed as mean values ± SD. All the experiments were done five times in replicates of three.

Results

Algal cell growth: The carrier solvent acetone had no adverse effects on cellular growth, physiological function, or photosynthetic efficiency of both algal species. Growth is a critical endpoint measure that indicates an overall vitality of a population under the examined conditions. When PYR was added to the culture medium, specific growth rates of both species were inhibited although the impact depended upon individual species and light conditions. In HL with PYR, the final biomass and growth of *C. vulgaris* were substantially lower than that of *S. acutus* (Fig. 1). In *C. vulgaris* cells during PYR in HL conditions, biomass was 0.125 mg mL^{-1}, which was the lowest of all treatments.

Biomolecules of algal cells: In the present study, the impact on cell protein, lipid, and carbohydrate content in response to LL and HL was measured in the presence of PYR (Fig. 2). In *S. acutus* cells, protein content was 248 µg mg^{-1}(DM) in control (C) and LL and 183 µg mg^{-1}(DM) in PYR and LL conditions while it was 432 µg mg^{-1}(DM) in C and HL and 434 µg mg^{-1}(DM) in PYR-exposed cells after 7 d of cultivation (Fig. 2*A*). In case of *C. vulgaris*, protein content was 294 µg mg^{-1}(DM) in C and LL and 246 µg mg^{-1}(DM) in PYR and LL treatment. It was seen that the maximum amount of protein was obtained from *C. vulgaris* from C and HL conditions, while it decreased significantly in PYR and HL (Fig. 2*B*).

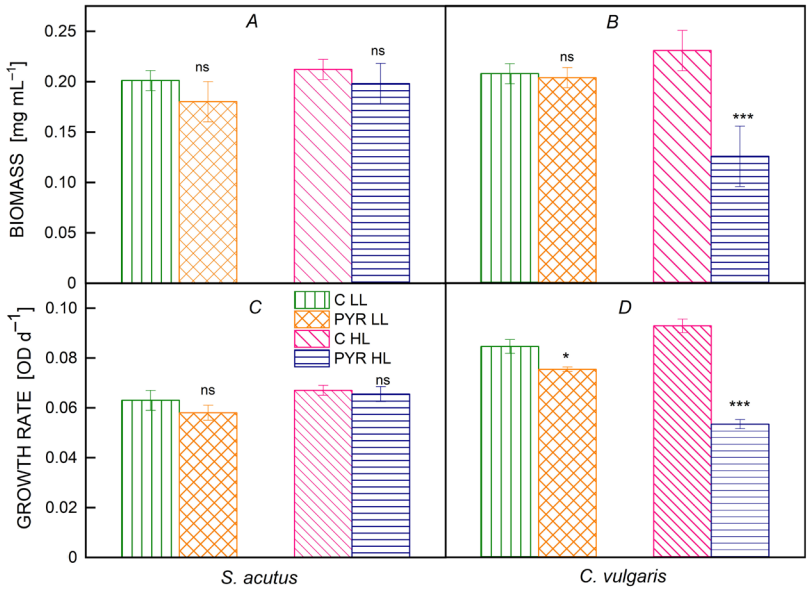

Fig. 1. Biomass (*A*,*B*) and growth rate (*C*,*D*) of *Scenedesmus acutus* and *Chlorella vulgaris* under LL and HL conditions with PYR exposure. Error bars represent standard deviation (n = 3). *** ($p<0.001$), ** ($p<0.01$), and * ($p<0.05$) represent significant differences between the control and respective treatment, ns = nonsignificant. C – control; HL – high light; LL – low light; PYR – pyrene.

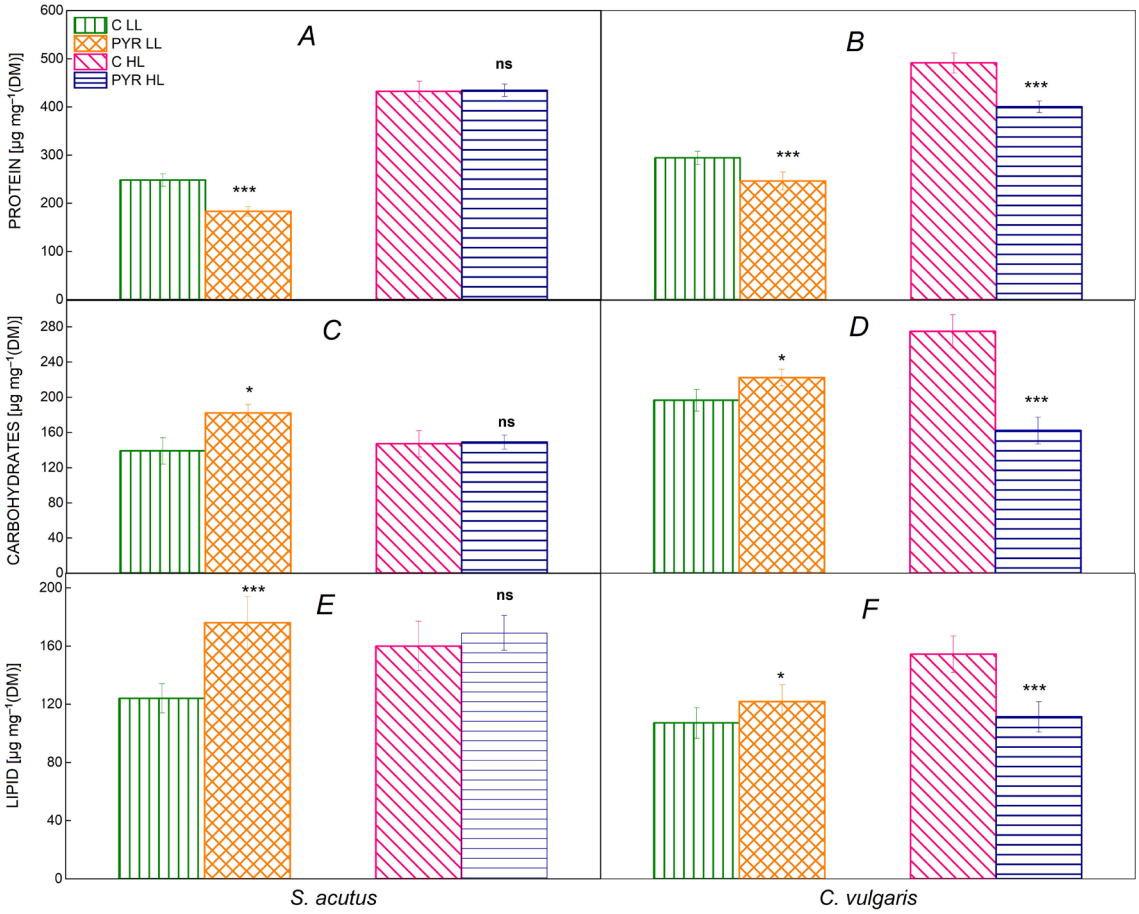

Fig. 2. Effects of different light intensities on the protein content (*A*,*B*), carbohydrate content (*C*,*D*), and lipid content (*E*,*F*) in *Scenedesmus acutus* and *Chlorella vulgaris* with PYR exposure. Error bars represent standard deviation (n = 3). *** (p<0.001), ** (p<0.01), and * (p<0.05) represent significant differences between the control and treatments, ns = nonsignificant. C – control; HL – high light; LL – low light; PYR – pyrene.

Carbohydrates and lipids also play important role in carbon partitioning, osmotic homeostasis, and metabolism of algal cells (Li *et al.* 2020). In the present study, carbohydrate content increased from 139 µg mg^{-1}(DM) (C and LL) to 182 µg mg^{-1}(DM) in PYR and LL while it was not affected by PYR and HL as compared to C and HL in *S. acutus* (Fig. 2*C*). Similarly, lipid content increased in PYR and LL conditions in both cells, while it was unaffected in PYR and HL in *S. acutus* (Fig. 2*E*,*F*). Interestingly, carbohydrate and lipid contents were extremely reduced in *C. vulgaris* in the PYR and HL conditions (Fig. 2*D*,*F*).

Proline and polyphenol content: The change in antioxidant molecules varied under different light conditions in both algal species. PYR LL and PYR HL treatments induced a considerable increase in proline concentration in *S. acutus* cells when compared with C LL and C HL as seen in Fig. 3. PYR LL treatment raised proline content by 75% as compared to C LL in *C. vulgaris* cells, but PYR HL treatment decreased proline content by 20% in comparison to C HL treatment. Additionally, in *S. acutus*,

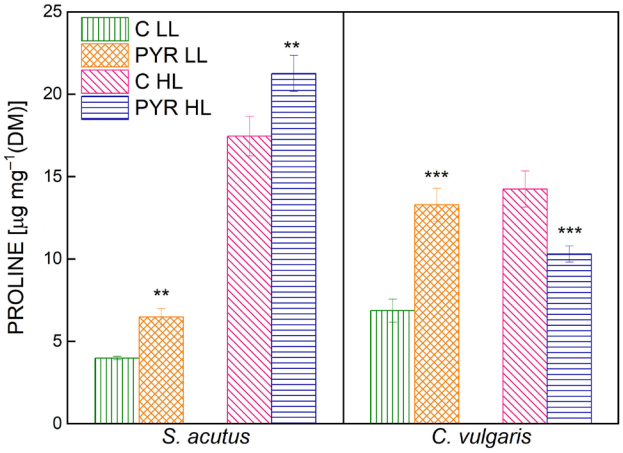

Fig. 3. Effects of different light intensities on the proline content of *Scenedesmus acutus* and *Chlorella vulgaris* with PYR exposure. Error bars represent standard deviation (n = 3). *** (p<0.001), ** (p<0.01), and * (p<0.05) represent significant differences between the control and treatments, ns = nonsignificant. C – control; HL – high light; LL – low light; PYR – pyrene.

PYR LL treatment resulted in a 9% drop in polyphenol content compared to C LL, but an increase was observed in PYR HL-treated cells compared to C HL (Fig. 4). On the other hand, in *C. vulgaris* cells, PYR LL caused a large rise in polyphenol content compared to C LL, whereas PYR HL caused a considerable drop (22%) in polyphenol content compared to C HL treatment (Fig. 4).

Photosynthetic pigments: To determine the effect of light intensity under PYR toxicity on photosynthesis, photosynthetic pigments (Chl *a*, Chl *b*, total Chl, and carotenoids) of *S. acutus* and *C. vulgaris* were measured (Table 1). Under LL and HL intensity along with PYR, the concentrations of Chl *a*, Chl *b*, and total Chl were reduced significantly in both species. However, this was lower in *S. acutus* in HL with PYR treatment (only 11% decrease as compared to C HL in *S. acutus*), compared to in *C. vulgaris* in PYR HL conditions (55% decrease as compared to C HL in *C. vulgaris*). Similarly, with PYR exposure, carotenoid concentration showed the same pattern in both species with both LL and HL (Table 1).

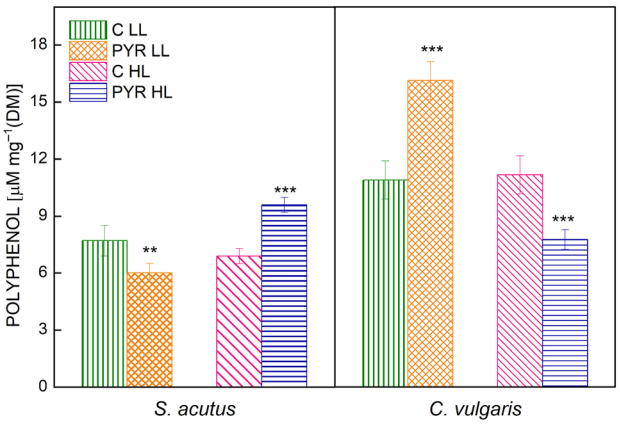

Fig. 4. Effects of different light intensities on the polyphenol content of *Scenedesmus acutus* and *Chlorella vulgaris* with PYR exposure. Error bars represent standard deviation (n = 3). *** (p<0.001), ** (p<0.01), and * (p<0.05) represent significant differences between the control and treatments, ns = nonsignificant. C – control; HL – high light; LL – low light; PYR – pyrene.

Efficiency of photosystem II in algal cells: The influence of light on PYR toxicity in both algal species was also evaluated using Chl *a* fluorescence kinetics. Table 2 shows the Chl *a* fluorescence parameters obtained with various treatments. In *S. acutus* in LL conditions, PYR exposure induced a significant increase in initial fluorescence (F_0), although it was unchanged in PYR HL treatment compared to C HL. It was also observed that F_0 was unaffected by PYR in *C. vulgaris* cells during the LL conditions but was reduced dramatically during the HL conditions (Table 2). Furthermore, maximal Chl *a* intensity (F_m) in *S. acutus* cells did not change in any of the conditions, whereas it declined severely in *C. vulgaris* cells during PYR HL and remained only 8% of C HL. In *S. acutus*, the quantum yield of PSII (F_v/F_m) decreased in the PYR LL treatment compared to the C LL treatment (Table 2). However, F_v/F_m was found to be the lowest with PYR HL treatment in *C. vulgaris*, as shown in Table 2. During both LL and HL, the value of F_v/F_0, which represents the efficiency of the oxygen-evolving complex, dropped considerably with PYR treatment in both algal species, however, the decline was deeper in *C. vulgaris*.

To evaluate the light energy-utilization efficiency of PSII, we compared the quantum yields of energy conversion within PSII in *S. acutus* and *C. vulgaris* cells in LL and HL with PYR exposure. The addition of PYR did not cause any significant change in $Y_{(II)}$ (quantum yield of PSII) in *S. acutus* during HL (Fig. 5) while, it was slightly reduced in PYR LL treatment (15% of C LL) (Fig. 5). In case of *C. vulgaris*, PYR caused a decrease in $Y_{(II)}$ and $Y_{(NO)}$ but not significantly in LL conditions, although the value of nonphotochemical quenching [$Y_{(NPQ)}$] increased (Fig. 6). However, a huge decrease was

Table 1. Effects on photosynthetic pigments content (Chl *a*, Chl *b*, total Chl, and carotenoids) in [µg mg^{-1}(DM)] in *Scenedesmus acutus* and *Chlorella vulgaris* under PYR exposure. Data are presented as the mean value of three replicates ± standard deviation. Significant differences were calculated according to *Newman–Keuls* multiple comparison test (ns = nonsignificant,*p<0.05, *p<0.01, and ***p<0.001). C – control; HL – high light; LL – low light; PYR – pyrene.

Treatment	Chl *a*	Chl *b*	Total Chl	Car (X+C)
Scenedesmus acutus				
C LL	19.4 ± 1.0	12.3 ± 1.6	31.7 ± 2.3	2.6 ± 0.4
PYR LL	14.2 ± 1.1*	9.1 ± 1.1*	23.3 ± 2.1*	2.2 ± 0.5^{ns}
C HL	19.5 ± 2.1	17.6 ± 1.41	37.1 ± 2.4	2.7 ± 0.1
PYR HL	17.2 ± 1.6*	10.4 ± 1.1***	27.6 ± 2.1***	2.7 ± 0.3^{ns}
Chlorella vulgaris				
C LL	25.5 ± 1.3	21.8 ± 2.1	47.4 ± 2.9	2.4 ± 0.2
PYR LL	17.1 ± 1.0*	14.3 ± 1.8**	31.4 ± 2.3***	1.5 ± 0.3***
C HL	28.3 ± 1.9	19.2 ± 1.5	47.5 ± 2.7	2.8 ± 0.1
PYR HL	12.6 ± 1.0***	10.9 ± 1.3***	23.5 ± 2.1***	0.6 ± 0.0***

Table 2. Effects of different light intensities on Chl *a* fluorescence parameters in *Scenedesmus acutus* and *Chlorella vulgaris* under PYR exposure. Data are presented as the mean value of three replicates ± standard deviation. Significant differences were calculated according to *Newman–Keuls* multiple comparison test (ns = nonsignificant, **p*<0.05, **p*<0.01, and ****p*<0.001). C – control; HL – high light; LL – low light; PYR – pyrene.

Treatment	F_0	F_m	F_v/F_m	F_v/F_0
Scenedesmus acutus				
C LL	0.06 ± 0.00	0.25 ± 0.01	0.76 ± 0.02	3.1 ± 0.5
PYR LL	0.07 ± 0.00**	0.25 ± 0.02^{ns}	0.71 ± 0.01*	2.4 ± 0.4**
C HL	0.07 ± 0.00	0.30 ± 0.01	0.76 ± 0.01	3.2 ± 0.4
PYR HL	0.08 ± 0.02^{ns}	0.31 ± 0.01^{ns}	0.73 ± 0.02*	2.7 ± 0.1***
Chlorella vulgaris				
C LL	0.10 ± 0.00	0.30 ± 0.01	0.66 ± 0.04	1.97 ± 0.32
PYR LL	0.10 ± 0.00^{ns}	0.27 ± 0.01*	0.64 ± 0.11*	1.72 ± 0.61*
C HL	0.09 ± 0.00	0.32 ± 0.02	0.73 ± 0.05	2.69 ± 0.41
PYR HL	0.01 ± 0.00***	0.03 ± 0.21***	0.56 ± 0.05***	1.27 ± 0.22***

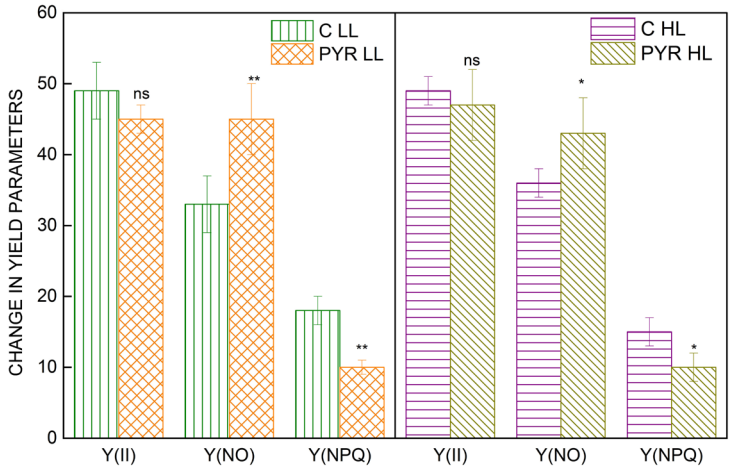

Fig. 5. Comparative analysis of quantum yields of *Scenedesmus acutus* with LL and HL under PYR exposure. Data are presented as the mean value of three replicates ± standard deviation. Significant differences were calculated according to *Newman–Keuls* multiple comparison test (ns = nonsignificant,**p*<0.05, **p*<0.01, and ****p*<0.001). C – control; HL – high light; LL – low light; PYR – pyrene.

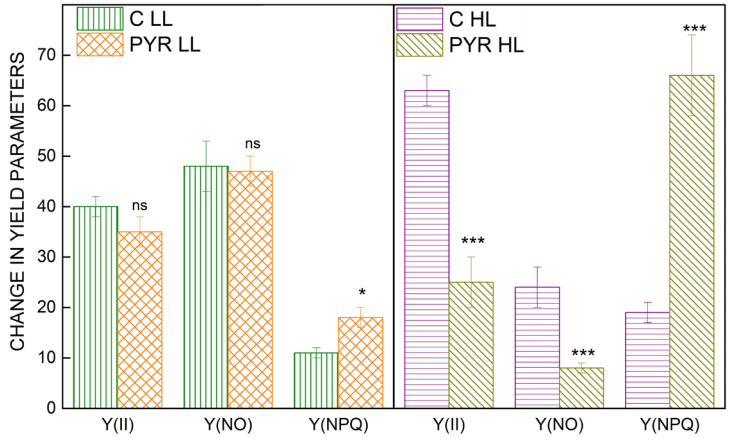

Fig. 6. Comparative analysis of quantum yields of *Chlorella vulgaris* with LL and HL under PYR exposure. Data are presented as the mean value of three replicates ± standard deviation. Significant differences were calculated according to *Newman–Keuls* multiple comparison test (ns = nonsignificant,**p*<0.05, **p*<0.01, and ****p*<0.001). C – control; HL – high light; LL – low light; PYR – pyrene.

observed in $Y_{(II)}$ and $Y_{(NO)}$ with PYR HL treatment which was related to a higher degree of PSII inhibition (Fig. 6). Compared with the C HL, PYR HL-exposed cells showed 3.5 times higher value of $Y_{(NPQ)}$ in *C. vulgaris* cells.

Discussion

This paper presents for the first time comparative data on the photosynthetic efficiency of two algal species grown

under PYR toxicity in response to low and high light intensities. Most of the toxicity data available are related to the effects of PYR on freshwater microalgae and are based on growth inhibition, while here we explained the light regulation of photosynthetic parameters as well. The level of growth and biomass suppression caused by PYR exposure in *C. vulgaris* was higher than that of *S. acutus* in both light intensities (LL and HL). Moreover, the extent of growth inhibition varied depending on the light intensity of individual tested species (Fig. 1). The growth rate and biomass were almost stable with LL but a drastic growth inhibition was observed when *C. vulgaris* cells were grown with HL. An accumulation of PAH in the lipid component of cells (Tomar and Jajoo 2021) and consequent alteration in membrane properties (Kottuparambil and Park 2019) may be responsible for the reduction in growth. The growth of *S. acutus* cells was better than that of *C. vulgaris* in PYR HL treatment. This result indicates that the *S. acutus* cells could tolerate PYR more in HL conditions.

PAHs have a significant influence on biomolecules of algae which change to adapt to different environmental conditions. Because PYR is hydrophobic, its harmful effects on green algae could be due to interference with cell biomolecules (Tomar and Jajoo 2021). The biochemical composition of algae is also influenced by variations in light intensity, for example, changes in the content of lipids, carbohydrates, and pigments, as well as the growth of microalgae (Tang *et al*. 2011, Paliwal *et al*. 2017). It is speculated that PYR could disrupt the functioning of algal cells as it rapidly interacts with proteins and lipids *in vivo* (Croxton *et al*. 2015). The obtained data revealed that PYR LL treatments altered biomolecules in *S. acutus*, but during HL, the number of biomolecules did not change significantly. In this investigation, exposure to PYR resulted in a much lower lipid and carbohydrate content in *C. vulgaris* in PYR HL conditions compared to PYR LL. Reduced carbohydrates and lipid content with PYR HL could be attributed to osmotic imbalance and cell division suppression (Cheng and He 2014). These findings suggest that PYR under HL has a greater impact on the synthesis of various biomolecules in *C. vulgaris* cells and it is unable to safeguard its metabolic functions. It is suggested that under different light intensities, microalgae may have various processes for carbon partitioning, which could change biomolecule levels (Nzayisenga *et al*. 2020).

To cope with oxidative stress, various antioxidant molecules contribute to survival and tolerance mechanisms in algae. Proline and polyphenol are two important osmolytes and antioxidants produced during stress in algae. Higher production of proline in *S. acutus* cells shows that it has a protective role through scavenging free radicals, stabilizing subcellular structure, and maintaining redox imbalance and homeostasis of the cell (Meena *et al.* 2019). Similar to proline, polyphenols are also regarded as strong antioxidants (Lee *et al*. 2015). Polyphenols suppress the generation of free radicals and act as direct radical scavengers of the lipid peroxidation chain reactions (chain breakers) (Tsao 2010). In *S. acutus* PYR LL and HL treatment caused the increase in proline content, which indicates its better ability to scavenge ROS. Similarly, an increase in proline and polyphenol content in *C. vulgaris* under PYR LL was observed, however, PYR HL showed lower proline and polyphenol content. It might be due to the activation of polyphenol oxidase enzyme by PYR during HL which oxidizes polyphenols by removing electrons and leaving them unstable.

In this study, Chl *a*, Chl *b*, and carotenoid contents were significantly affected by PYR under LL and HL conditions in both algal species (Table 2). It is well documented that exposure of green algae and plants to different toxicants leads to alteration in photosynthetic pigment contents and photosynthetic efficiency (Aksmann *et al.* 2011, Jajoo *et al*. 2014, Tomar and Jajoo 2014, 2021). Moreover, light is important for Chl and other pigment syntheses that absorb light of different wavelengths (Zarmi *et al*. 2020). It was found that light intensity significantly influenced the pigment content of *S. acutus* (Table 2). However, the lowest values of Chl and carotenoids were observed in *C. vulgaris* under PYR HL condition as compared to other treatments. In *C. vulgaris*, under PYR HL condition, PYR was probably more absorbed by the algal cells and inhibited the synthesis of photosynthetic pigments or resulted in pigment degradation.

Apart from changes in the growth of algal cells and photosynthetic pigment contents, the light intensity also affected the photosynthetic efficiency, specially PSII activity. Chl *a* fluorescence measurements were performed to examine the impact of light intensity with PYR toxicity on PSII in both algal species. F_0 is minimal fluorescence level when all antenna pigment complexes associated with the photosystem are assumed to be open (dark-adapted) and F_m is maximal fluorescence level when a high-intensity flash has been applied and all antenna sites are assumed to be closed. A drop in these fluorescence parameters (F_0 and F_m) in *C. vulgaris* during PYR HL treatment might be a result of many combined processes, such as enhancement in the capacity of light-harvesting complex II (LHCII) or an increase in the number of inactive RCs of PSII, which indicated a reduction in cells photosynthetic performance due to downregulation of photochemical efficiency (Elsheery and Cao 2008, Elsheery *et al.* 2008). In this study, F_m decreased drastically in response to PYR HL stress, probably due to disorganization of Chl structure (Hazrati *et al.* 2016). In contrast, *S. acutus* maintained F_0 and F_m values in PYR HL treatment, however, an increase was observed in F_0 during PYR LL treatment. The efficiency of the oxygen-evolving complex (F_v/F_0) decreased in both algal cells with all treatments, implying that PYR at low and high light intensity affected negatively the donor side of PSII. The F_v/F_m value is the ratio of variable fluorescence to maximal fluorescence and calculated as $F_m - F_0/F_m$. It measures the maximum efficiency of PSII when all PSII centers are open (Mathur *et al*. 2019, Jain and Jajoo 2020). This value can be used to estimate the potential efficiency of PSII by taking dark-adapted measurements. We found that *S. acutus* can maintain maximum efficiency of PSII (F_v/F_m) in both light treatments while it decreased drastically in *C. vulgaris* during the PYR HL condition. This indicates that PYR toxicity is exerted also by inhibition of cytochrome b_6/f complex as well as photooxidative damage to PSII during high light intensity. Our results suggest that

S. acutus has a more efficient protective mechanism for PSII which enables it to tolerate PYR even at HL intensity.

We further measured the photochemical quantum yield of PSII, which gives information about the response of PSII photochemistry, to PYR toxicity with low and high light intensities (Fig. 6). $Y_{(II)}$ represents the fraction of excitation energy used for photochemistry at PSII. The remaining fraction, $1 – Y_{(II)}$, tells about the dissipation of remaining energy. It is the sum of the yields of regulated dissipation [$Y_{(NPQ)}$], and unregulated dissipation, [$Y_{(NO)}$] (Mathur *et al*. 2019, Jain and Jajoo 2020). As evident from the results, PYR HL reduced the F_v/F_m ratio in *C. vulgaris* and the energy requirements for the reduction of quinone decreased largely. This accounts for the reduction in $Y_{(II)}$ and a corresponding increase in $Y_{(NPQ)}$ with PYR HL. Higher $Y_{(NPQ)}$ suggests inhibition of electron transport exerted by PYR HL. These PSII quantum yield parameters collectively indicated the reduced efficiency of photochemical energy regulation imposed by PYR exposure in *C. vulgaris* during HL. We noted that during low light intensity, PYR did not show major photosynthetic toxicity in *C. vulgaris* at the PSII level. The drop in $Y_{(NO)}$ and high rise in $Y_{(NPQ)}$ in PYR HL-exposed cells reflect damage to PSII but still with a protective mechanism through NPQ to protect itself against photochemical damage. A decrease in $Y_{(II)}$ also reflects a reduction in the number of active PSII centers and it may also be associated with a decrease in Chl *a* content in PYR HL-treated cells (Khpalwak *et al*. 2018, Tomar and Jajoo 2015, 2019). Moreover, a significant increase in Chl *a*/*b* ratio was reported, which indicated that antenna size was affected (Dinç *et al*. 2012). This change may be partially attributed to the augmentation of the NPQ values. During PYR exposure, an increase in $Y_{(NPQ)}$ is often reflected by a decrease of $Y_{(NO)}$ which can compensate for a decrease in PSII activity. Surprisingly, under PYR LL and HL, a higher $Y_{(NO)}$ and lower $Y_{(NPQ)}$ were observed in *S. acutus*, while the $Y_{(II)}$ was not significantly changed. Thus, a balance exists between $Y_{(NPQ)}$ and $Y_{(NO)}$ to maintain $Y_{(II)}$. Some other mechanisms, such as cyclic electron transport and the water–water cycle, might be involved in the protection and regulation of photosynthetic efficiency in *S. acutus* cells (Sun *et al*. 2020). However, the mechanisms underlying the interaction between $Y_{(NPQ)}$ and $Y_{(NO)}$ remain elusive.

It is concluded that light intensity of 50–60 and 100–110 μmol(photon) m^{-2} s^{-1}, are both suitable for the growth of *S. acutus* under PYR toxicity. Further, *S. acutus* was able to maintain its biomolecule composition when cultured under HL exposure. In this study, the biomolecule content of *C. vulgaris* was comparatively lower in PYR HL treatment than that of PYR LL culture condition. In *C. vulgaris* with PYR exposure, significantly low cell density, growth, and biomass values were observed in high light intensity, which is also supported by the negatively modulated photosynthetic process. It can be suggested that during HL, PYR may be absorbed rapidly and accumulated in cell membranes and organelles. PYR accumulation in membranes probably results in increased proton permeability, as well as an expansion of the membrane surface area, inhibiting primary ion pumps and causing the electrical potential and pH gradient to dissipate, inhibiting cellular growth (Petersen and Dahllöf 2007, Croxton *et al*. 2015). More important, because these are strongly connected processes, each being a result of the usage of energy from light and nutrients, a reduction in photosynthesis can lead to reduced growth. It was reported that some of the metabolites of PYR are quinones (Alegbeleye *et al*. 2017, Bukowska and Duchnowicz 2022) which can cause modifications in the photosynthetic machinery and can result in a significant decrease in energy output within chloroplasts. In this study, *S. acutus* exhibited higher pigment content and quantum yield of PSII as compared to *C. vulgaris* during PYR HL conditions. However, *S. acutus* shows better performance of all measured parameters in both high and low light intensities. Another interesting finding, which warrants further mechanistic and physiological attention, is the fine regulation of $Y_{(NO)}$ and $Y_{(NPQ)}$ in *S. acutus* for the protection of PSII. Therefore, *S. acutus* seems to be a more promising candidate for the removal of pyrene from the environment under varying light conditions.

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