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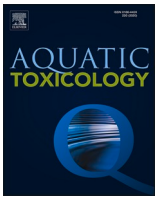

Light modulates the effect of antibiotic norfloxacin on photosynthetic processes of *Microcystis aeruginosa*

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ABSTRACT

Norfloxacin is one of the widely used antibiotics, often detected in aquatic ecosystems, and difficultly degraded in the environment. However, how norfloxacin affects the photosynthetic process of freshwater phytoplankton is still largely unknown, especially under varied light conditions. In this study, we investigated photosynthetic mechanisms of *Microcystis aeruginosa* in responses to antibiotic norfloxacin (0-50 μg/L) for 72 h under low (LL; 50 μmol photons m^{-2} s^{-1}) and high (HL; 250 μmol photons m^{-2} s^{-1}) growth light regimes. We found that environmentally related concentrations of norfloxacin inhibited the growth rate and operational quantum yield of photosynthesis system II (PSII) of *M. aeruginosa* more under HL than under LL, suggesting HL increased the toxicity of norfloxacin to *M. aeruginosa*. Further analyses showed that norfloxacin deactivated PSII reaction centers under both growth light regimes with increased minimal fluorescence yields only under HL, suggesting that norfloxacin not only damaged reaction centers of PSII, but also inhibited energy transfer among phycobilisomes in *M. aeruginosa* under HL. However, non-photosynthetic quenching decreased in the studied species by norfloxacin exposure under both growth light regimes, suggesting that excess energy might not be efficiently dissipated as heat. Also, we found that reactive oxygen species (ROS) content increased under norfloxacin treatments with a higher ROS content under HL compared to LL. In addition, HL increased the absorption of norfloxacin by *M. aeruginosa*, which could partly explain the high sensitivity to norfloxacin of *M. aeruginosa* under HL. This study firstly reports that light can strongly affect the toxicity of norfloxacin to *M. aeruginosa*, and has vitally important implications for assessing the toxicity of norfloxacin to aquatic microorganisms.

1. Introduction

Norfloxacin (NOR), as a member of the fluoroquinolone antibiotic family, is one of the most important class of second-generation synthetic antibiotics. Due to its broad-spectrum antibacterial effects on Gram-negative and Gram-positive bacteria, it is widely used in medicine, agriculture and aquaculture (Nie et al., 2009; Lian et al., 2016; Moreau et al., 2018; Yuan et al., 2019). Norfloxacin is unavoidably released into freshwater through urine and feces as it is extensively used and is partially metabolized in the body. Moreover, norfloxacin cannot be completely degraded by conventional biological wastewater treatment plants and is not known to be biodegradable (Ou et al., 2016). Therefore, norfloxacin concentrations found in the freshwater range from nanogram to microgram per liter, which could represent a major threat to the health of freshwater organisms (Kümmerer, 2008; Zhang et al., 2015; Li et al., 2018).

Phytoplankton as primary producers plays a key role for driving energy and material cycles in freshwater ecosystems (Välitalo et al., 2017). It was found that norfloxacin could inhibit the growth of *Microcystis aeruginosa* and decreased its cell size (Du et al., 2018). Moreover, high concentrations (>10 mg/L) of norfloxacin could induce irreversible damages to *M. aeruginosa* and finally lead to its death (Du

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et al., 2018). On the other hand, norfloxacin decreased the formation of colonies in *Scenedesmus quadricauda* (Pan et al., 2017a), and these varied colony sizes affected interspecies interactions between phytoplankton species. For example, alteration of *Scenedesmus obliquus* colony size by norfloxacin was found to further affect the stability and persistence of plankton systems (Pan et al., 2017b; Pan et al., 2020). Although several studies investigated the effect of antibiotics (such as chlortetracycline, enrofloxacin and florphenicol) on photosystem II (PSII) photosynthetic activity of various phytoplankton species (such as *Tetraselmis suecica, Microcystis flos-aquae* and *Pseudokichneriella subcapitata*) (Seoane et al., 2014; Wan et al., 2014,2015; Carusso et al., 2018; Du et al., 2018), the effect of norfloxacin on photosynthetic processes and underlying mechanisms is not clear. It is therefore urgent to investigate the effect of norfloxacin on photosynthetic processes in phytoplankton (Li et al., 2018).

The toxicity of pollutants, such as trace metals, herbicides and antibiotics, can be modulated by environmental factors, such as light and temperature (Ota et al., 2015; Xu and Juneau, 2016; Guo et al., 2016; Gomes and Juneau, 2017; Xu et al., 2019). Light is one of the most important environmental factors, which drives photosynthesis, the cornerstone of metabolism for phototrophs to convert carbon dioxide into organic matter (Pilon et al., 2011; Wahidin et al., 2013). In freshwater ecosystems, phytoplankton can adjust its photosynthetic states under varied light conditions such as season/weather change (MacIntyre et al., 2002). The physiological status induced by different light regimes in phytoplankton, may further affect its sensitivity to pollutants. It has been found that phytoplankton grown under strong light reduces their sensitivity to atrazine (Deblois et al., 2013). This observed effect is supposed to be a result of diluting atrazine toxicity by increasing the availability of binding sites (quinones) and increasing the ability of the light-regulating process. On the other hand, our previous studies found that high light increased mesotrione toxicity to *Chlamydomonas reinhardtii* compared to low light conditions (Xu et al., 2019), and higher light could increase zinc toxicity to *M. aeruginosa* by increasing zinc absorption (Xu and Juneau, 2016). Those studies suggest that light can decrease/increase the toxicity of pollutants to phytoplankton by changing their absorption or photosynthetic status. However, to the best of our knowledge, there is no information about how phytoplankton responds to antibiotics under varied light conditions.

In this research, we aimed to understand the effect of norfloxacin on photosynthetic processes, such as light absorption efficiency, electron transport, and PSII activity under varied growth light regimes in *M. aeruginosa*, a target aquatic phytoplankton as its cell membrane is similar as Gram-negative bacteria's. It was hypothesized that light could modify the toxicity of norfloxacin to *M. aeruginosa* by changing its absorption and/or photosynthetic status under different light conditions. We exposed *M. aeruginosa* to different concentrations of norfloxacin for 72 h under two growth light regimes to address the above questions. *M. aeruginosa* is one of the most widely distributed phytoplankton species in freshwater ecosystems, which is a common strain for toxicity test of pollutants (Deng et al., 2015; Liu et al., 2015; Guo et al., 2016; Wang et al., 2018). We found that norfloxacin could quickly and sharply decreased the PSII activity with stronger inhibitory effects under high growth light conditions in *M. aeruginosa*. This study advances our understanding of toxic effects of norfloxacin and possible mechanisms on photosynthetic processes, and has important implications for assessing ecotoxicological impacts of norfloxacin to phytoplankton in freshwater ecosystems.

2. Materials and methods

2.1. The material and culture conditions

Microcystis aeruginosa FACHB-905 was provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences, Wuhan, China. The antibiotic, norfloxacin was purchased from Sigma-Aldrich, Missouri, USA in the powder form with a purity of higher than 98% (Fig.1). The phytoplankton was grown in 250 mL Erlenmeyer flasks containing 150 mL of BG11 medium (Stanier et al., 1971) in growth chambers. The cultures were adapted to two different light intensities of 50 (LL) and 250 μmol photons m^{-2} s^{-1} (HL) with a 14 h/10 h light/dark cycle for at least four weeks (16 generations) before norfloxacin exposure experiments. The temperature in the growth chambers was 24 ± 1℃. The exposure norfloxacin concentrations were 5, 10, 20, 30 and 50 μg/L, which are related to environmental concentrations of norfloxacin in freshwater ecosystems. The setting concentrations of norfloxacin were all confirmed by HPLC in this study (see 2.6).

2.2. Phytoplankton growth

Phytoplankton cell number was examined every day after the addition of norfloxacin to the medium (sterile MQ water for control) during the three-day experiment. Cells were kept at their exponential growth phases and cell number was determined using a Multisizer™ 3 Coulter Counter® (Beckman Coulter Inc., Brea, USA). Their growth rates (μ) were calculated from the cell number over the 3-day periods:

$$\mu = \frac{\ln N_t - \ln N_0}{T} \times 100\%$$

where N_t is the cell number after a 3-day exposure, N_0 is the initial cell number at time 0 day, and T represents the exposure time (here 3 days).

2.3. Determination of fluorescence parameters

After 72-h exposure of different concentrations of norfloxacin, phytoplankton cells were taken and acclimated to actinic light condition (same intensity as growth light) for 5 min, the maximal fluorescence yield F'_m and minimal fluorescence yield F under actinic light were measured by a WATER-PAM Chlorophyll Fluorometer (Walz GmbH, Effeltrich, Germany). The operational PSII quantum yield, Φ'_M, was obtained by the equation of $(F'_m - F) / F'_m$ (Kitajima and Butler, 1975). Another sample was dark acclimated for 15 min, and then the light response curves were measured with 8 light steps (1 min for each step) with intensity from 49 to 808 μmol photons m^{-2} s^{-1}. At the end of the light response curve measurement, 10 μM diuron (DCUM) was added to obtain the maximal fluorescence yield F_{mDCMU} (Campbell et al., 1998). The maximal PSII quantum yield was calculated as follow: $\Phi_M = (F_{mDCMU} - F_0)/F_{mDCMU}$ (Genty et al., 1989). The relative electron transport rate (rETR) was calculated by the formula: $rETR = \Phi'_M \times PAR$ (Ralph et al., 2002). The maximal rate of rETR ($rETR_{max}$) and photosynthetic efficiency (α) were obtained by fitting this light response curve to an exponential function modified from (Jassby and Platt, 1976): $rETR = rETR_{max} \times [1 - \exp(-\alpha \times PAR/rETR_{max})]$. The light-saturation parameter (I_k) was calculated by $I_k = rETR_{max}/\alpha$. We also calculated

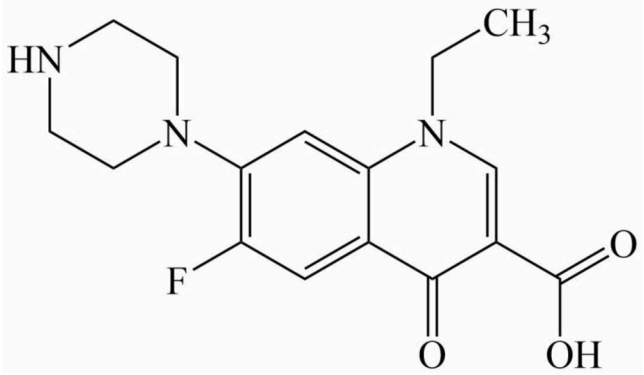

Fig. 1. The chemical structure of norfloxacin.

the non-photochemical quenching (NPQ), representing all quenching processes of the PSII chlorophyll fluorescence not directly related to photochemistry, with the equation of $(F_{mDCMU}-F'_m)/F'_m$ (Schreiber et al., 1986). The relative unquenched fluorescence (UQF_{rel}), representing the reduction state of PSII at steady-state electron transport was also calculated by the equation: $(F_S-F'_0)/(F_{mDCMU}-F'_0)$ (Juneau et al., 2005).

At the same time, a Photon System Instruments FL6000 fluorometer system (Brno, Czech Republic) was used to measure the rapid and polyphasic chlorophyll *a* fluorescence transient under 3600 μmol $m^{-2}s^{-1}$ of light with another dark sample acclimated for 15 min. The antenna size of an active RC of PSII (ABS/RC) was calculated as followed: $ABS/RC = (M_0/V_J)/[(F_m-F_{50\mu s})/F_m]$, where $M_0 = 4 \times (F_{300\mu s}-F_{50\mu s})/(F_m-F_{50\mu s})$ and $V_J = (F_{2ms}-F_{50\mu s})/(F_m-F_{50\mu s})$ (Krüger et al., 1997). Effective dissipation of an active RC (DI_0/RC) was calculated by the equation of $(ABS/RC)-(M_0/V_J)$ (Krüger et al., 1997). The kinetics was normalized to the relative variable fluorescence after subtracting $F_{50\mu s}$ for every data point $(F=(F_t-F_{50\mu s})/(F_m-F_{50\mu s})$, where F_t is the fluorescence at time t and F_m the maximal fluorescence of the curve.

The ratio (treatment / control) of active PSII centers was calculated using the following equation:

$$RC_a/(RC_a)_{ct} = [(M_0/V_J)_{ct}/(1-F_{50\mu s}/F_m)_{ct}] / [(M_0/V_J)/(1-F_{50\mu s}/F_m)]$$

Where RC_a represents the number of active PSII centers of the treated sample and $(RC_a)_{ct}$ of the control sample (Stirbet and Govindjee, 2011).

2.4. Measurement of intracellular reactive oxygen species

After 72-h exposure, reactive oxygen species (ROS) were measured according to the instructions supplied with the Reactive Oxygen Species Assay Kit. In this kit, the fluorescence probe 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) was used to measure the ROS level of algal cells. DCFH-DA can form 2',7'-dichlorodihydrofluorescein (DCFH) catalyzed by esterase when the probe is taken up by living cells. DCFH is non-fluorescent but highly sensitive to ROS, which oxidizes it to the highly fluorescent 2',7'-dichlorofluorescein (DCF).

After 72-h exposure, phytoplankton cells were collected by centrifugation (8000 r/min, 24 ℃, 10 min) and resuspended in the DCFH-DA. And they were incubated for 30 min in dark, and then washed three times with PBS to remove the DCFH-DA in the liquid. Finally, the phytoplankton cells were resuspended with PBS and the fluorescence emission at 525 nm was measured using an exciting light at 488 nm with a Microplate Reader (Thermo Fisher Scientific, Waltham, USA).

2.5. Pigment determination

The pigment profiles of *M. aeruginosa* grown at each norfloxacin concentration and both light regimes for 72-h were measured spectrophotometrically. The samples were harvested by centrifugation (8000 r/min, 24 ℃, 10 min), re-suspended in 95% ethanol, and the extracts subsequently stored overnight at -20℃. The supernatant was obtained by centrifugation, and the absorbance was then measured with a spectrophotometer (Shimadzu, Japan) at 664.1, 648.6 and 470.0 nm. The contents of chlorophyll *a* (Chl *a*) and carotenoids (Car) were calculated by the following equations (Lichtenthaler and Buschmann, 2001):

$$Chl\ a\ (\mu g/mL) = 13.36\ A_{664.1} - 5.19\ A_{648.6}$$

$$Car\ (\mu g/mL) = (1000\ A_{470} - 2.13\ Chl\ a)\ /\ 209$$

2.6. Measurement of norfloxacin concentrations

At the start and end of 72-h exposure (20, 30 and 50 μg/L) with and without phytoplankton cells, norfloxacin concentrations were detected on a high-performance liquid chromatography (HPLC; Agilent, USA), a LC-20AD chromatograph equipped with fluorescence detector and Xtimate C18 column (Shimadzu, Japan). The mobile phase was 0.025 mol/L phosphate buffer and acetonitrile with a volume ratio of 85/15, and the flow rate was set to 1.0 mL/min with a column temperature at 30℃. The excitation wavelength of 280 nm and the emission wavelength of 480 nm were chosen to measure the content of norfloxacin.

2.7. Toxicity test of degradation products of norfloxacin

We illuminated the medium containing 50 μg/L of norfloxacin and for 48-h under the light intensity of 250 μmol photons $m^{-2}\ s^{-1}$. We then found that the concentration of norfloxacin in the medium was reduced to 10 μg/L, which also contained degradation products confirmed by HPLC. At the same time, the medium with the same concentration of norfloxacin (10 μg/L) but without degradation products was set as a control. We cultured *M. aeruginosa* with the degraded medium (10 ug/L norfloxacin + degradation production) and the control medium (10 ug/L norfloxacin) for 72-h. After 72-h exposure, the growth rate, Φ'_M and Φ_M in the studied species of different treatments were determined.

2.8. Statistical analysis

Concentration-response curves for Φ'_M parameter and determination of Φ'_M-EC_{50} were obtained using non-linear logistic regressions generated by GraphPad Prism version 8.00 software for Windows. One-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test were employed to identify significant differences between the control and treatment groups using the IBM SPSS statistical package version 25. Normality of residuals was tested with a Shapiro-Wilk's test and homogeneity of variances was tested with a Levene's test. Statistical significance was set at $P < 0.05$. All measurements were performed at least three replicates and all figures were produced using Origin 2018 (OriginLab Corporation, Northampton, USA).

3. Results

3.1. Growth rate of M. aeruginosa under two light regimes

To examine the effect of norfloxacin on *M. aeruginosa* under two light regimes, we measured the growth rate of *M. aeruginosa* under five concentrations of norfloxacin. The results showed that norfloxacin significantly (Tukey's HSD, $P < 0.05$) reduced the specific growth rate of *M. aeruginosa* under two light intensities (50 and 250 μmol photons $m^{-2}\ s^{-1}$), which appeared to be concentration-dependent (Fig. 2). Under the high light intensity, the specific growth rate of *M. aeruginosa* decreased by 3% (5 μg/L), 10% (10 μg/L), 26% (20 μg/L), 44% (30 μg/L), and 63%

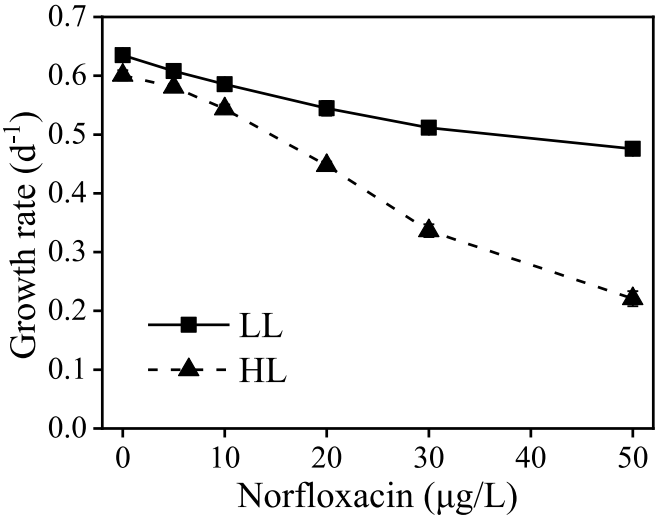

Fig. 2. Growth rate of *M. aeruginosa* grown under two light intensities after 72 h exposure to different norfloxacin concentrations. Data expressed as means ± SD (n=6). HL: high light; LL: low light.

(50 μg/L), whereas it was decreased by 4%, 8%, 14%, 19% and 25% as the concentration of norfloxacin increase under low light intensity compared to the corresponding control.

3.2. The effects of norfloxacin on photosynthetic activity

To understand if different concentrations of norfloxacin affected photosynthetic activity of *M. aeruginosa* grown under two light regimes, PSII photosynthetic parameters (Φ_M, Φ'_M, F_0 and F_m) under five concentrations of norfloxacin were measured. Under the low light intensity, norfloxacin significantly (Tukey's HSD, $P < 0.05$) decreased the Φ_M value by 6%, 12%, 35%, 45% and 63% at concentration of 5, 10, 20, 30 and 50 μg/L, respectively (Fig. 3a and b); under high light intensity, norfloxacin caused a decrease of Φ_M by 9%, 18%, 47%, 68% and 99%, indicating that high light exacerbated the impact of norfloxacin on Φ_M at concentrations of higher than 20 μg/L. The effect of norfloxacin on Φ'_M showed a similar trend (Fig. 3a and b). The half maximal effective concentration (EC_{50}) of operational quantum yield (Φ'_M) of *M. aeruginosa* under low light was found to be 35 μg/L, which was significantly (Tukey's HSD, $P < 0.05$) higher than it under high light with 23 μg/L.

We also found that the highest norfloxacin concentration significantly (Tukey's HSD, $P < 0.05$) increased the minimal fluorescence yields (F_0) and maximal fluorescence yields (F_m) by 177% and 45%, respectively, in the studied species grown under high light. On the other hand, Norfloxacin exposures did not significantly (Tukey's HSD, $P > 0.05$) affect the F_0 of *M. aeruginosa* grown under low light intensity (Fig. 3c and d). However, the highest norfloxacin concentration (50 μg/L) treatment significantly (Tukey's HSD, $P < 0.05$) decreased the F_m by 40% compared to the corresponding control, although low norfloxacin

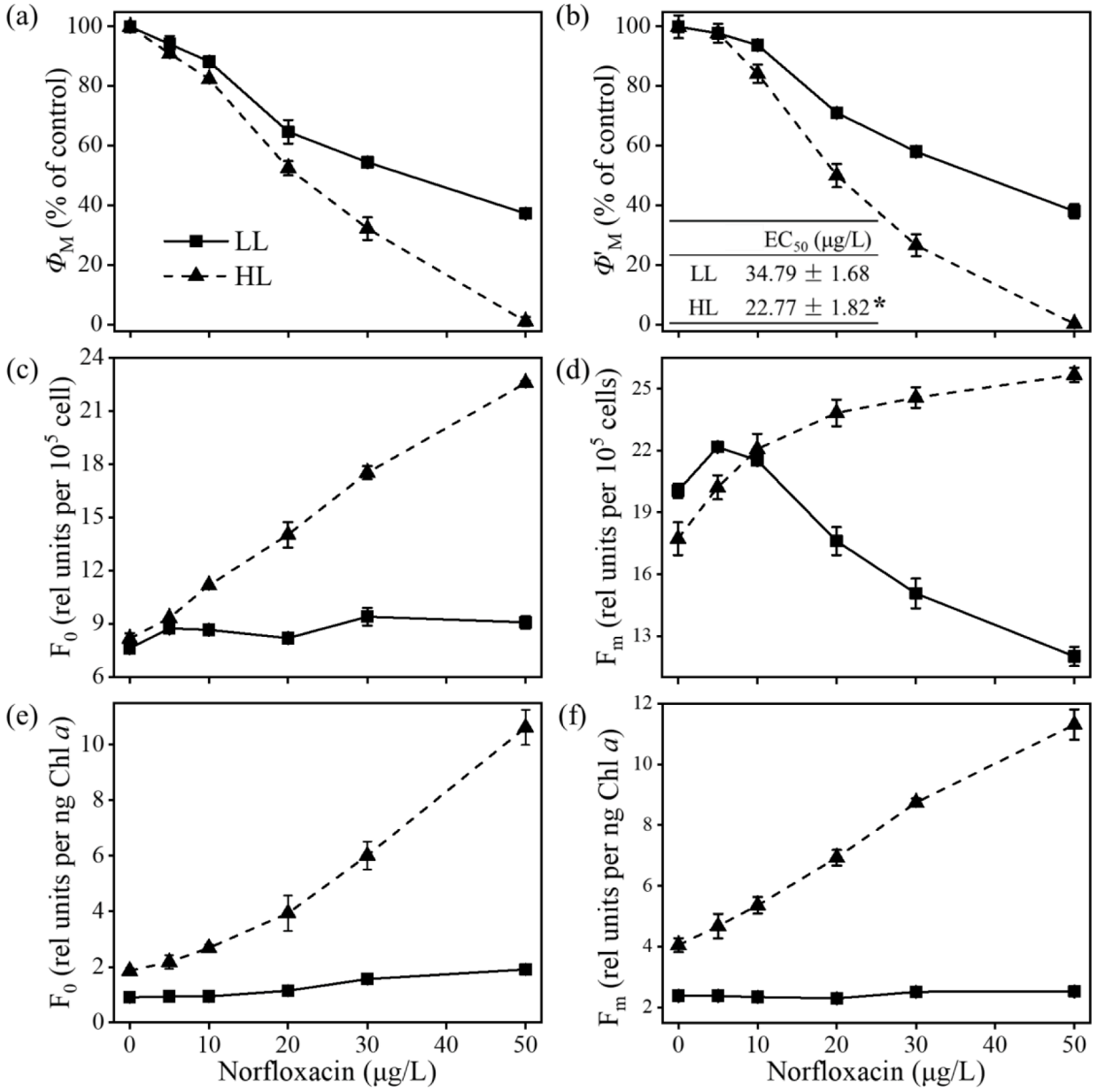

Fig. 3. Maximal PSII quantum yield (Φ_M), operational PSII quantum yield (Φ'_M), minimal fluorescence yield (F_0) and maximal fluorescence yield (F_m) of *M. aeruginosa* grown under two light intensities after 72 h exposure to different norfloxacin concentrations. The initial values of *M. aeruginosa* for Φ_M were 0.540 ± 0.001 (HL) and 0.620 ± 0.003 (LL); for Φ'_M, were 0.369 ± 0.003 (HL) and 0.515 ± 0.003 (LL). The insets show norfloxacin effective concentration (μg/L) required for 50% inhibition of PSII operational quantum yield (Φ'_M) in *M. aeruginosa* under two light intensities. The symbol of star on the error bar represents the significantly different of EC_{50} between the two light intensities ($p < 0.05$). Data expressed as means ± SD ($n = 6$).

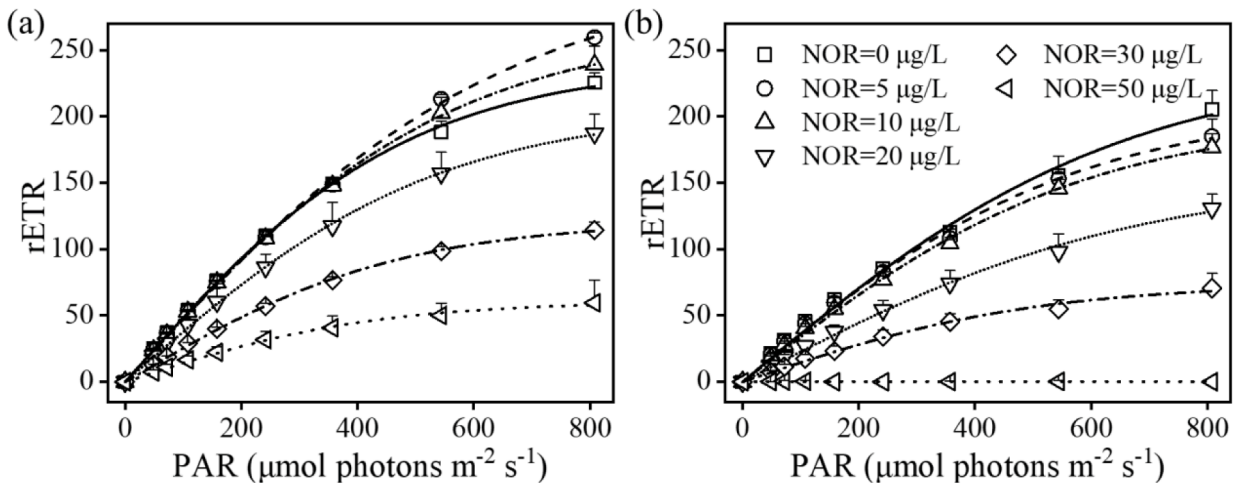

Fig. 4. The light response curves (LCs) of *M. aeruginosa* grown under two light intensities after 72 h exposure to different norfloxacin concentrations. Panel (a): grown under 50 μmol photons $m^{-2}s^{-1}$ illumination; Panel (b): grown under 250 μmol photons $m^{-2}s^{-1}$ illumination. Data expressed as means ± SD (n = 6).

treatment (5 and 10 μg/L) increased the F_m under low light condition compared to the corresponding control (Tukey's HSD, $P < 0.05$). After we normalize the value of F_0 and F_m to chlorophyll *a*, we could only find that norfloxacin increased these two parameters in *M. aeruginosa* under HL, but not under LL (Fig. 3e and f).

3.3. Effects of norfloxacin on the relative electron transport rate

To understand if different concentrations of norfloxacin affected photosynthetic electron transport rates of *M. aeruginosa* grown under two light regimes, photosynthetic transport rate and related parameters ($rETR_{max}$, *a* and I_k, respectively) were measured under five concentrations of norfloxacin. Norfloxacin significantly (Tukey's HSD, $P < 0.05$) decreased the relative electron transport rate (rETR) of *M. aeruginosa* with concentrations higher than 10 μg/L under the two light intensities (Fig. 4). However, high light combined with norfloxacin concentrations ranging from 20 to 50 μg/L obviously decreased rETR more compared to low light. It is worth noting that rETR was reduced to almost zero under high light intensity when *M. aeruginosa* was exposed to the highest norfloxacin concentration (50 μg/L). The three parameters fitted from the light response curves clearly showed the effect of norfloxacin on the electron transport rate in *M. aeruginosa* under the two studied light intensities (Fig. 4; Table 1).

Under the low light intensity, Norfloxacin (≥ 20 μg/L) significantly (Tukey's HSD, $P < 0.05$) decreased the light energy utilization efficiency (α) by 21% (20 μg/L), 46% (30 μg/L) and 69% (50 μg/L), the maximum electron transfer rate ($rETR_{max}$) by 49% (30 μg/L) and 74% (50 μg/L), and the light-saturation parameter (I_k) by 15% (50 μg/L) in the studied species (Table 1); however, under the high light intensity, norfloxacin decreased more of the α value by 11% (10 μg/L), 54% (20 μg/L), 62% (30 μg/L) and 98% (50 μg/L), the $rETR_{max}$ value by 56% (20 μg/L), 66% (30 μg/L) and 100% (50 μg/L) and the I_k value by 78% (50 μg/L) as compared to their corresponding controls (Table 1).

3.4. Rapid fluorescence kinetics

To examine how different concentrations of norfloxacin affected photosynthetic energy transfer of *M. aeruginosa* grown under two light regimes, rapid fluorescence kinetics curve (OJIP curve) and related parameters (ABS/RC, DI_0/RC and $RC_a/(RC_a)_{ct}$) under five concentrations of norfloxacin were measured. In the OJIP curve, each inflection point represents the different redox state of the plastoquinone transporter in the photosynthetic electron transport chain. Norfloxacin increased the J level of *M. aeruginosa* under both growth light intensities, and decreased the I-P level (Fig. 5). This trend of OJIP curves increased obviously as the concentration of norfloxacin increased in the studied species under both growth light intensities.

We further examined the PSII energy flux distributed among absorption, trapping, electron transport and dissipation by calculating parameters from this curve (Table 2). There was an obvious interaction between both factors for the absorption flux per reaction center (ABS/RC), which is an indication of the antenna size per reaction center of PSII. We noticed that norfloxacin (50 μg/L) had a significant (Tukey's HSD, $P < 0.05$) effect on *M. aeruginosa*, by increasing the PSII antenna size per reaction center to 198% and 256% under low and high light intensities, respectively. It was quite obvious that norfloxacin from 20 to

Table 1
Effects of norfloxacin on parameters of photosynthesis-irradiance curves for *M. aeruginosa* under two light intensities.

Light intensity	c (μg/L)	α	$rETR_{max}$	I_k (μmol photons $m^{-2}s^{-1}$)
LL	Control	0.501 ± 0.003^{a}	$225.4 \pm 22.3^{a,b}$	450.2 ± 44.2^{b}
	5	0.496 ± 0.005^{a}	259.6 ± 2.7^{a}	523.2 ± 10.2^{a}
	10	0.489 ± 0.004^{a}	$238.9 \pm 17.1^{a,b}$	$488.0 \pm 31.7^{a,b}$
	20	0.397 ± 0.075^{b}	207.9 ± 44.3^{b}	521.8 ± 19.4^{a}
	30	0.270 ± 0.006^{c}	114.5 ± 5.0^{c}	$424.1 \pm 20.3^{b,c}$
	50	0.154 ± 0.024^{d}	59.5 ± 14.0^{d}	383.5 ± 33.5^{c}
HL	Control	0.424 ± 0.013^{a}	205.2 ± 14.3^{a}	483.5 ± 20.9^{a}
	5	0.406 ± 0.001^{a}	186.4 ± 21.2^{a}	458.7 ± 51.0^{a}
	10	0.376 ± 0.011^{b}	176.7 ± 20.1^{a}	469.1 ± 39.3^{a}
	20	0.197 ± 0.002^{c}	91.0 ± 8.6^{b}	462.6 ± 40.3^{a}
	30	0.162 ± 0.029^{d}	70.6 ± 11.3^{b}	436.6 ± 12.0^{a}
	50	0.009 ± 0.004^{e}	1.0 ± 0.4^{c}	108.2 ± 0.3^{b}

a-e, those with different superscript letters for each growth light in the same column are significantly different (Tukey's HSD, $P < 0.05$). Data are means ± SD (n = 6). c, the norfloxacin concentration; α, photosynthetic efficiency; I_k, light-saturation parameter; $rETR_{max}$, maximal relative electron transport rate.

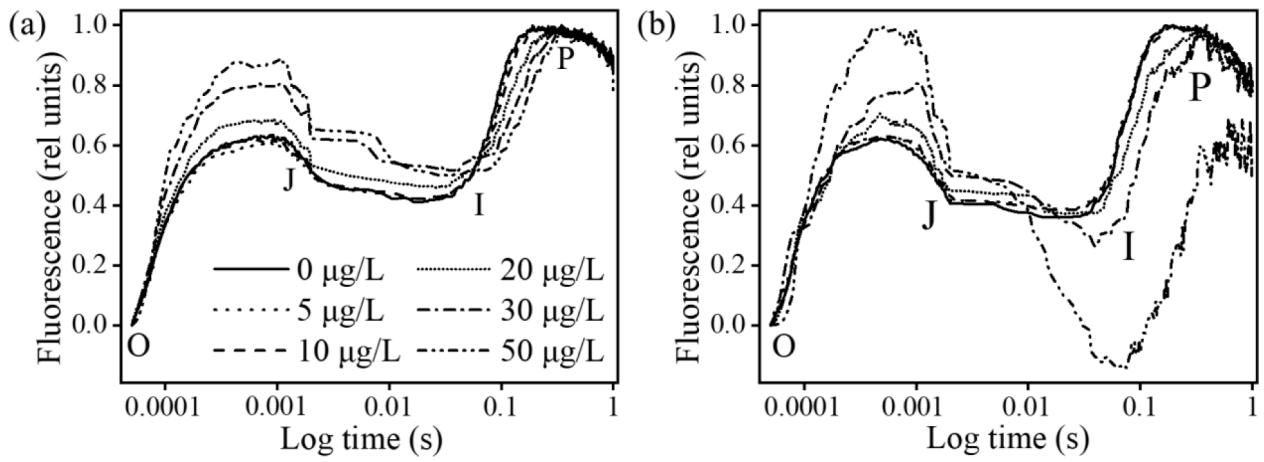

Fig. 5. Rapid fluorescence kinetics of *M. aeruginosa* grown under two light intensities after 72 h exposure to different norfloxacin concentrations. Panel (a): low light (50 μmol photons $m^{-2}s^{-1}$ illumination); Panel (b): high light (250 μmol photons $m^{-2}s^{-1}$ illumination).

Table 2
Effect of norfloxacin concentration on ABS/RC, DI_0/RC, UQF_{rel}, $RC_a/(RC_a)_{ct}$ and NPQ for *M. aeruginosa* under two light intensities.

Light intensity	c (μg/L)	ABS/RC	DI_0/RC	UQF_{rel}	$RC_a/(RC_a)_{ct}$	NPQ
LL	Control	12.59 ± 0.28^{d}	8.14 ± 0.22^{d}	0.10 ± 0.01^{c}	1.00 ± 0.02^{a}	$0.15 \pm 0.01^{a,b}$
	5	12.36 ± 0.14^{d}	8.01 ± 0.13^{d}	0.10 ± 0.00^{c}	1.02 ± 0.01^{a}	$0.17 \pm 0.01^{a,b}$
	10	$13.69 \pm 0.28^{c,d}$	9.17 ± 0.19^{d}	0.10 ± 0.00^{c}	0.92 ± 0.02^{b}	0.18 ± 0.01^{a}
	20	18.75 ± 0.96^{c}	14.19 ± 0.73^{c}	0.12 ± 0.03^{c}	0.67 ± 0.04^{c}	0.14 ± 0.03^{b}
	30	28.78 ± 1.33^{b}	24.10 ± 1.15^{b}	0.19 ± 0.02^{b}	0.44 ± 0.02^{d}	0.10 ± 0.01^{c}
	50	37.56 ± 5.46^{a}	32.68 ± 4.89^{a}	0.29 ± 0.05^{a}	0.34 ± 0.06^{e}	0.06 ± 0.01^{d}
HL	Control	13.89 ± 0.78^{d}	8.80 ± 0.55^{d}	0.14 ± 0.01^{b}	1.00 ± 0.06^{a}	0.29 ± 0.01^{a}
	5	14.05 ± 1.37^{d}	8.50 ± 0.93^{d}	0.13 ± 0.01^{b}	$0.93 \pm 0.04^{a,b}$	0.29 ± 0.01^{a}
	10	$16.01 \pm 0.44^{c,d}$	10.85 ± 0.14^{d}	0.13 ± 0.01^{b}	0.87 ± 0.02^{b}	0.29 ± 0.02^{a}
	20	22.96 ± 1.17^{c}	17.72 ± 0.96^{c}	0.14 ± 0.04^{b}	0.61 ± 0.03^{c}	0.21 ± 0.02^{b}
	30	32.57 ± 4.23^{b}	27.37 ± 3.74^{b}	0.14 ± 0.05^{b}	0.43 ± 0.06^{d}	0.14 ± 0.01^{c}
	50	49.42 ± 6.47^{a}	45.26 ± 6.32^{a}	0.54 ± 0.03^{a}	0.29 ± 0.04^{e}	0.03 ± 0.03^{d}

a-d, those with superscript different letters for each growth light in the same column are significantly different (Tukey's HSD, $P < 0.05$). Data are means ± SD ($n = 6$).

50 μg/L increased the dissipation of excess energy per reaction center (DI_0/RC) by 43%, 196% and 302% under low light, but by 211% (30 μg/L) and 414% (50 μg/L) under high light. Under low light intensity, norfloxacin significantly (Tukey's HSD, $P < 0.05$) increased the relative unquenched fluorescence (UQF_{rel}) by 95% (30 μg/L) and 197% (50 μg/L), the $RC_a/(RC_a)_{ct}$ ratio by 33% (20 μg/L), 56% (30 μg/L) and 66% (50 μg/L); under high light intensity, norfloxacin significantly (Tukey's HSD, $P < 0.05$) increased UQF_{rel} by 282% (50 μg/L), the $RC_a/(RC_a)_{ct}$ ratio by 40% (20 μg/L), 57% (30 μg/L) and 72% (50 μg/L) as compared to their corresponding controls.

Also, 30 and 50 μg/L norfloxacin decreased NPQ by 33% and 60% in the studied species under low light condition (Tukey's HSD, $P < 0.05$); under high light condition, the concentrations decreased NPQ by 50% and 91% in the studied species, respectively (Tukey's HSD, $P < 0.05$). However, the NPQ of *M. aeruginosa* was not significantly (Tukey's HSD, $P > 0.05$) affected by the low concentration of norfloxacin (from 5 to 10 μg/L) under both growth light conditions (Table 2).

3.5. The effect of norfloxacin on reactive oxygen species

In order to identify whether norfloxacin induces oxidative stress in *M. aeruginosa* under both light intensities, we measured the reactive oxygen species (ROS) content in the studied species exposed to five concentrations of norfloxacin. Norfloxacin induced an increase in the intracellular content of ROS in *M. aeruginosa* under both light intensities (Fig. 6). Under low light intensity, norfloxacin significantly (Tukey's HSD, $P < 0.05$) increased ROS by 36% (10 μg/L), 40% (20 μg/L), 46% (30 μg/L) and 52% (50 μg/L), but under high light condition, only high concentration of norfloxacin significantly (Tukey's HSD, $P < 0.05$) increased intracellular ROS content by 45% (20 μg/L), 112% (30 μg/L) and 241% (50 μg/L), and no effect was found for low concentrations of norfloxacin (5 and 10 μg/L).

3.6. Pigment content

In order to explore the effect of norfloxacin on *M. aeruginosa* grown under two light regimes, we then measured the pigment content in the studied species. As shown in Table 3, under low light intensity, low concentrations of norfloxacin (5 and 10 μg/L) significantly (Tukey's HSD, $P < 0.05$) increased Chl *a* content by 11% and 9% respectively, but did not influence Car content, thus decreasing the Car/Chl *a* ratio by 5% and 6%. However, norfloxacin at high concentrations of 20, 30 and 50 μg/L significantly (Tukey's HSD, $P < 0.05$) decreased Chl *a* by 14%, 29% and 44% respectively, and Car by 8%, 21% and 34% respectively, increasing Car/Chl *a* ratio by 8%, 11% and 18%. Under high light intensity, low concentrations of norfloxacin at 5 and 10 μg/L did not induce a significant effect on Chl *a* and Car/Chl *a* (Tukey's HSD, $P > 0.05$). However, at concentrations of 20, 30 and 50 μg/L, norfloxacin significantly (Tukey's HSD, $P < 0.05$) decreased Chl *a* by 18%, 39% and 49% respectively, and Car by 16%, 30% and 40% respectively, resulting in a significant increase of Car/Chl *a* by 14% (30 μg/L) and 19% (50 μg/L).

3.7. . Light-induced norfloxacin degradation/uptake

In order to explore the absorption and/or degradation of norfloxacin by *M. aeruginosa* under two growth light intensities, we measured the content of norfloxacin in the medium with or without cyanobacterial

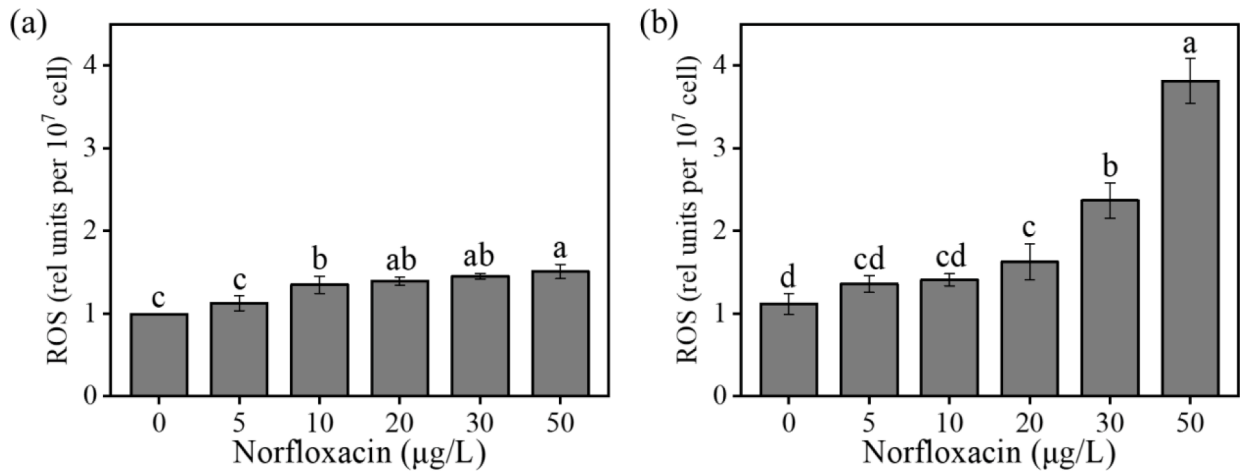

Fig. 6. Intracellular ROS content of *M. aeruginosa* after exposure to different norfloxacin concentrations for 72 h: (a) low light intensity; (b) high light intensity. Results are presented as mean values ± standard deviation of three independent assays. Different letters above adjacent bars indicate a significant difference (Tukey's HSD, $P < 0.05$) among different concentrations of norfloxacin.

Table 3
Pigment contents of *M. aeruginosa* grown under two different light intensities exposed to different norfloxacin concentrations for 72 h. Chlorophyll *a* (Chl *a*) and carotenoid (Car) contents are expressed as μg/10^8 cells.

Light intensity	c (μg/L)	Chl *a*	Car	Car/Chl *a*
LL	Control	8.42 ± 0.16^{b}	4.00 ± 0.07^{a}	0.47 ± 0.01^{c}
	5	9.31 ± 0.15^{a}	4.22 ± 0.09^{a}	$0.45 \pm 0.01^{c,d}$
	10	9.20 ± 0.29^{a}	4.10 ± 0.10^{a}	0.45 ± 0.01^{d}
	20	7.22 ± 0.66^{c}	3.69 ± 0.16^{b}	0.51 ± 0.03^{b}
	30	6.01 ± 0.08^{d}	3.16 ± 0.03^{c}	0.53 ± 0.00^{b}
	50	4.76 ± 0.20^{e}	2.66 ± 0.15^{d}	0.56 ± 0.01^{a}
HL	Control	4.24 ± 0.24^{a}	4.40 ± 0.06^{a}	1.04 ± 0.04^{b}
	5	4.02 ± 0.20^{a}	4.04 ± 0.13^{b}	1.01 ± 0.05^{b}
	10	3.98 ± 0.12^{a}	$4.23 \pm 0.12^{a,b}$	1.06 ± 0.03^{b}
	20	3.48 ± 0.31^{b}	3.71 ± 0.16^{b}	1.07 ± 0.08^{b}
	30	2.60 ± 0.10^{c}	3.09 ± 0.09^{c}	1.19 ± 0.01^{a}
	50	2.15 ± 0.29^{d}	2.64 ± 0.28^{d}	1.23 ± 0.06^{a}

a-d, those with different superscript letters for each growth light in the same column are significantly different (Tukey's HSD, $P < 0.05$). Data are means ± SD ($n = 6$).

cells after 72 h (Fig. 7). Under low light condition, norfloxacin at concentrations of 20, 30 and 50 μg/L was reduced by 24%, 36% and 36%, respectively without phytoplankton cells after 72 h (Tukey's HSD, $P < 0.05$). However, norfloxacin was decreased by 5%, 30% and 31%, respectively in the presence of *M. aeruginosa* cells (Tukey's HSD, $P < 0.05$). Under high light intensity, norfloxacin at concentrations of 20, 30 and 50 μg/L was degraded by 45%, 58% and 56%, respectively without cyanobacterial cells after 72 h (Tukey's HSD, $P < 0.05$). However, norfloxacin was decreased by 51%, 71% and 92%, respectively with *M. aeruginosa* (Tukey's HSD, $P < 0.05$). The results indicated an obvious impact of light intensity in the degradation and uptake of norfloxacin.

3.8. . The effect of norfloxacin degradation products

In order to explore whether the toxicity of norfloxacin depends on itself or its degradation products, we conducted a comparative study between norfloxacin plus degradation production and norfloxacin treated samples. As shown in Fig. S1, exposure to 10 μg/L of norfloxacin significantly reduced the growth rate and photosynthetic efficiency of *M. aeruginosa* (Tukey's HSD, $P < 0.05$). However, when *M. aeruginosa* was exposed to the degraded medium (10 μg/L + degraded production), its growth rate and photosynthetic efficiency are not significantly different from those of *M. aeruginosa* exposed to 10 μg/L norfloxacin (Tukey's HSD, $P > 0.05$).

4. Discussion

Understanding the toxic effect of environmentally relative concentrations of norfloxacin on cyanobacterium *M. aeruginosa* under varied light regimes is one of the central issues for environmental risk assessment of antibiotics in aquatic ecosystems. In this study, we found that

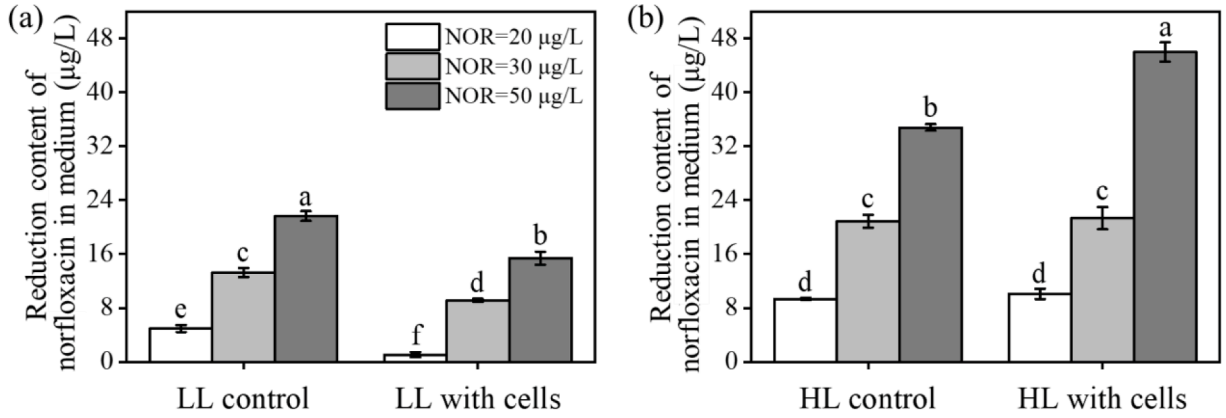

Fig. 7. The reduction of norfloxacin in the medium with or without *M. aeruginosa* under two growth light conditions after 72 h. Different letters above adjacent bars indicate a significant difference (Tukey's HSD, $P < 0.05$) among different concentrations of norfloxacin with or without *M. aeruginosa*.

norfloxacin inhibited growth and photosynthesis of *M. aeruginosa* by altering photosynthetic activity and energy transfer, and by increasing oxidative stress. We also noticed a stronger inhibition under HL compared to LL. We proposed that possible mechanisms for such observations might include the changes of photosynthetic processes, such as increased Car/Chl *a*, NPQ and ROS, and norfloxacin absorption for the studied species under HL. These results generally support our core hypothesis about combined effects of norfloxacin and light on *M. aeruginosa*.

4.1. Effects of light intensity on M. aeruginosa

In the natural environment, phytoplankton can adjust photosynthetic processes and growth to adapt/acclimate different light conditions as the season and cloudy conditions change (Huisman et al., 2004; Dubinsky and Stambler, 2009; Deblois and Juneau, 2012). In this study, the specific growth rate and operational PSII quantum yield of *M. aeruginosa* decreased under HL compared to LL, suggesting that the studied strain decreased light absorption efficiency under high light to keep similar growth rates under low light. This phenomenon was also found with another *M. aeruginosa* strain in one of our previous studies (Xu and Juneau, 2016).

Non-photochemical quenching is known to be an efficient photoprotective mechanism in cyanobacteria under excess light conditions (Karapetyan, 2007; Kirilovsky, 2007). In cyanobacteria, NPQ is mainly linked to orange carotenoid protein (OCP)-related quenching and state transitions (Kirilovsky, 2007). The higher NPQ of cells grown under HL than that under LL in this study, suggests that more energy was dissipated to protect PSII of the studied species under HL conditions (Kirilovsky, 2015; Du et al., 2019). Moreover, the increased Car/Chl *a* ratio under HL conditions due to a stronger decrease of Chl *a* compared to Car, suggests that carotenoids related quenching (such as OCP-related quenching) could be more induced compared to LL conditions (Deblois and Juneau, 2012; Sedoud et al., 2014). State transition, as another major component of NPQ, is controlled by the redox state of PQ in cyanobacteria (Kirilovsky, 2014). We demonstrated that the relative unquenched fluorescence (UQF_{rel}) of *M. aeruginosa* was lower, when no or low concentrations of norfloxacin were present, under LL conditions than that under HL conditions, suggesting that electron transport carriers beyond PSII are more efficient to drain electrons, and that the PQ pool is more oxidized when light intensity is lower. And the PQ pool of *M. aeruginosa* under high light was more reduced. This more reduced PQ could decrease the ability of state transition in *M. aeruginosa* under HL conditions. Indeed, we have indicated that *M. aeruginosa* had a decreased capacity to perform state transition under HL conditions compared to LL conditions in previous study (Xu et al., 2013). Therefore, the higher NPQ observed under HL conditions could be resulted from its higher Car/Chl *a* ratio, which could lead to higher carotenoid energy quenching (such as OCP-related NPQ).

4.2. Effects of norfloxacin exposure on photosynthetic processes of M. aeruginosa

We demonstrated here that norfloxacin inhibited photosynthesis of *M. aeruginosa*, in agreement with previous studies, showing that norfloxacin inhibited photosynthesis and growth in different cyanobacterial species at a concentration range from 2 μg/L to 1.44 mg/L (Isidori et al., 2005; Nie et al., 2009; González-Pleiter et al., 2013; Wan et al., 2015; Fu et al., 2017). Increased F_0 mainly implies that energy transfer among phycobilisomes (and/or from phycobilisomes to reaction centers) can be blocked (Chaloub et al., 2005), and this energy transfer inhibition was mainly related to biochemical alternations of PSII RCs, such as dissociation of phycobilisomes from RC (Sluchanko et al., 2017; Krasilnikov et al., 2020). On the other hand, decreased or increased F_m is generally related to RC damages (Stirbet and Govindjee, 2011). For example, a decrease of Chl *a* per cell under norfloxacin treatments could result in a decrease of F_0 and F_m; however, the value of F_0 and F_m per cell per Chl *a* still showed a similar trend of F_0 and F_m per cell under norfloxacin treatment, which strongly suggests an energy transfer inhibition as discussed above. Therefore, our results indicated that F_0 increased, and F_m increased or decreased in *M. aeruginosa* responses to norfloxacin, suggesting an inhibition of energy transfer among phycobilisomes in addition to damage to the electron transport chain by norfloxacin in *M. aeruginosa*.

Generally, the non-photochemical quenching is assumed to increase under stress conditions to protect PSII, but it was notable that NPQ decreased at high concentrations of norfloxacin for *M. aeruginosa*. This situation was also found in other cyanobacterial species exposed to high concentrations of metals (e.g., zinc) and herbicides (e.g., mesotrione) (Xu and Juneau, 2016; Newby et al., 2017; Xu et al., 2019). As discussed above, the higher value of UQF_{rel} was induced by norfloxacin, indicating more reduced PQ as discussed above. The results further suggest that state transition was inhibited under norfloxacin stress conditions. It is expected that the observed decrease of NPQ may partly result from a lower state transition ability under norfloxacin exposure in *M. aeruginosa*.

We also found the fluorescence intensity of J-I step in *M. aeruginosa* exposed to norfloxacin was increased in this study, which represents the electron transfer between Q_A and Q_B is partially inhibited (Force et al., 2003; Chalifour and Juneau, 2011; Stirbet and Govindjee, 2011; Stirbet et al., 2018). The significant reduction in $RC_a/(RC_a)_{ct}$ and initial slope (α) of the light response curve indicate the presence of norfloxacin has caused damages to the photoreactive system of cyanobacteria, resulting in the inactivation of reaction centers and a decrease in its efficiency in light usage (Xu et al., 2017). These results are in agreement with the changes of F_0 and F_m in the studied species under norfloxacin exposure as discussed above. These damages to the light system also led to a reduction in the ability of the cyanobacteria to withstand strong light (I_k). In addition, the deactivation of reaction centers also caused the effective dissipation per unit reaction center (DI_0/RC), and the absorption flux per reaction center (ABS/RC) increased (Xu et al., 2019).

It was found that norfloxacin can affect the metabolism of lipid and protein in *Scenedesmus obliquus* (Chu et al., 2020), which may also be induced in *M. aeruginosa* and therefore affect the stability of thylakoid membrane, and then inhibit energy (exciton) transfer from phycobilisomes to reaction centers. We clearly showed that energy (exciton) transfer from phycobilisomes to reaction centers and the inhibition of electron transfer from Q_A to Q_B, which may lead to excess energy/electron transfer to oxygen and finally produce ROS under norfloxacin exposure (He et al., 2012; Li et al., 2015). Indeed in this study, we found that norfloxacin induced an increase in ROS content although Carotenoid content (relative to Chl) increased (as possible antioxidative mechanisms). This indicates that *M. aeruginosa* was not able to efficiently scavenge produced ROS under norfloxacin exposure.

4.3. Effects of light intensities on norfloxacin toxicity

We were able to show here that high light intensity increased the toxic effect of norfloxacin in *M. aeruginosa*. This could be firstly related to a higher absorption of norfloxacin in *M. aeruginosa* under HL conditions compared to it under LL conditions. Indeed, it was previously shown that Cd toxicity was higher under high light conditions compared to low light conditions for species *Synechocystis* sp. since that higher light intensity could increase the uptake efficiency (Du et al., 2019). Although light may increase norfloxacin degradation, *M. aeruginosa* decreased the degradation of this antibiotic under LL conditions. This may be related to the reduction (absorption and shading effects) of light intensity by *M. aeruginosa* in the medium. However, the studied species did not decrease the degradation of norfloxacin under HL conditions, indicating that HL could increase norfloxacin absorption by *M. aeruginosa* and therefore induced a higher toxicity as the degraded products of norfloxacin were not toxic. The similar results were also

found in the higher toxicity of zinc to *M. aeruginosa* by higher absorption of zinc under high growth light compared to low growth light (Xu et al., 2016).

It is anticipated that norfloxacin with higher toxicity under HL conditions may be related to photosynthetic status changes compared to LL conditions. Under LL conditions, phytoplankton cells may not be limited by the availability of quinones and other electron transporters, while under HL conditions, they may become limited (Anderson et al., 1995; MacIntyre et al., 2002). Therefore, when cyanobacterial cells adapted to LL conditions, a fixed number of norfloxacin molecules would prevent a small portion of quinones required for photosynthesis with a small effect. This explains that LL is less sensitive to norfloxacin compared to HL-adapted cyanobacterial cells. However, when *M. aeruginosa* cells are exposed to HL, all available quinones are needed to maximize the use of light, and norfloxacin may be more toxic by increasing quinone limitation.

Our results indicate that phytoplankton can develop different protective strategies to deal with norfloxacin pollution under two different light intensities. First, we were able to show that non-photochemical quenching energy dissipation processes were enhanced in *M. aeruginosa* cells grown under HL conditions, contributing to their ability to dissipate excess energy and cope with light stress conditions. Second, the enhanced Car/Chl *a* ratio indicated that carotenoids (directly or indirectly) could participate in scavenging ROS produced under HL and norfloxacin stress conditions when *M. aeruginosa* cells were grown under HL conditions. However, those protective strategies developed by *M. aeruginosa* under the two light intensities were not sufficient to protect the photosynthetic apparatus when norfloxacin concentrations were high.

5. Conclusions

This study investigated the effect of norfloxacin on the physiological and photosynthetic mechanisms of *M. aeruginosa* grown under two light regimes. Our results indicated that norfloxacin at environmentally relevant concentrations could inhibit growth and photosynthesis activity in *M. aeruginosa* mainly by the inhibition of photosynthetic electron transport rate and energy/excitons transfer from phycobilisomes to reaction centers. Moreover, the sensitivity of *M. aeruginosa* to norfloxacin was increased under HL. Such a light-dependent toxicity of norfloxacin to *M. aeruginosa* was not only related to higher absorption of norfloxacin under HL, but also related to the different photosynthetic status under the two light regimes. Under HL, we found that the inhibition of photosynthetic electron transport rate induced more excess electrons/energy, which could react with oxygen and generate more ROS than LL conditions. Even the higher Car / Chl *a* can help *M. aeruginosa* to cope with excess energy/ROS stress, it was not successful to overcome this stress condition and finally induce irreversible inhibition of growth of the studied species. These results revealed the combined effect of light and norfloxacin on *M. aeruginosa* and its related photosynthetic protective mechanisms. This study suggests that the interaction of light intensity and norfloxacin concentrations should be considered in the evaluation of ecotoxicological effects of antibiotics on freshwater microorganisms.

CRediT authorship contribution statement

Libin Zhao: Conceptualization, Methodology, Writing - original draft, Visualization, Data curation. **Kui Xu:** Conceptualization, Methodology, Funding acquisition, Writing - review & editing. **Philippe Juneau:** Writing - review & editing. **Peihuan Huang:** Visualization, Investigation. **Yingli Lian:** Validation, Investigation. **Xiafei Zheng:** Validation. **Qiuping Zhong:** Data curation. **Wei Zhang:** Data curation. **Fanshu Xiao:** Data curation, Validation. **Bo Wu:** Writing - review & editing. **Qingyun Yan:** Writing - review & editing. **Zhili He:** Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

Declaration of Competing Interest

All authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2021.105826.

References

Anderson, J.M., Chow, W.S., Park, Y., 1995. The grand design of photosynthesis: acclimation of the photosynthetic apparatus to environmental cues. Photosynth. Res. 46 (1), 129–139. https://doi.org/10.1007/BF00020423.

Campbell, D., Hurry, V., Clarke, A. K., Gustafsson, P. and Öquist, G. (1998). Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. *Microbiol. Mol. Biol. Rev.*, 62 (3), 667-683. https://doi.org/10.1128/MMBR.62.3.667-683.1998.

Carusso, S., Juárez, A.B., Moretton, J., Magdaleno, A., 2018. Effects of three veterinary antibiotics and their binary mixtures on two green alga species. Chemosphere 194, 821–827. https://doi.org/10.1016/j.chemosphere.2017.12.047.

Chalifour, A., Juneau, P., 2011. Temperature-dependent sensitivity of growth and photosynthesis of *Scenedesmus obliquus, Navicula pelliculosa* and two strains of *Microcystis aeruginosa* to the herbicide atrazine. Aquatic. Toxicol. 103 (1), 9–17. https://doi.org/10.1016/j.aquatox.2011.01.016.

Chaloub, R.M., de Magalhães, C.C.P., Dos Santos, C.P., 2005. Early toxic effects of zinc on PSII of *synechocystis aquatilis* f. *aquatilis* (Cyanophyceae). J. Phycol. 41 (6), 1162–1168. https://doi.org/10.1111/j.1529-8817.2005.00143.x.

Chu, H., Ren, L., Yang, L., Chen, J., Zhou, X., Zhang, Y., 2020. Metabolomics reveals a lipid accumulation mechanism involving carbon allocation in Scenedesmus obliquus under norfloxacin stress. Renew. Energy 157, 585–592. https://doi.org/10.1016/j.renene.2020.05.051.

Deblois, C.P., Dufresne, K., Juneau, P., 2013. Response to variable light intensity in photoacclimated algae and cyanobacteria exposed to atrazine. Aquatic. Toxicol. 126, 77–84. https://doi.org/10.1016/j.aquatox.2012.09.005.

Deblois, C.P., Juneau, P., 2012. Comparison of resistance to light stress in toxic and non-toxic strains of *Microcystis aeruginosa* (Cyanophyta). J. Phycol. 48 (4), 1002–1011. https://doi.org/10.1111/j.1529-8817.2012.01191.x.

Deng, C., Pan, X., Zhang, D., 2015. Influence of ofloxacin on photosystems I and II activities of *Microcystis aeruginosa* and the potential role of cyclic electron flow. J. Biosci. Bioeng. 119 (2), 159–164. https://doi.org/10.1016/j.jbiosc.2014.07.014.

Du, J., Qiu, B., Pedrosa Gomes, M., Juneau, P., Dai, G., 2019. Influence of light intensity on cadmium uptake and toxicity in the cyanobacteria *Synechocystis* sp. PCC6803. Aquatic. Toxicol. 211, 163–172. https://doi.org/10.1016/j.aquatox.2019.03.016.

Du, Y., Wang, J., Zhu, F., Mai, D., Xiang, Z., Chen, J., Guo, R., 2018. Comprehensive assessment of three typical antibiotics on cyanobacteria (*Microcystis aeruginosa*): the impact and recovery capability. Ecotoxicol. Environ. Saf. 160, 84–93. https://doi.org/10.1016/j.ecoenv.2018.05.035.

Dubinsky, Z., Stambler, N., 2009. Photoacclimation processes in phytoplankton: mechanisms, consequences, and applications. Aquat. Microb. Ecol. 56 (2-3), 163–176. https://doi.org/10.3354/ame01345.

Force, L., Critchley, C., van Rensen, J.J., 2003. New fluorescence parameters for monitoring photosynthesis in plants. Photosynth. Res. 78 (1), 17. https://doi.org/10.1023/A:1026012116709.

Fu, L., Huang, T., Wang, S., Wang, X., Su, L., Li, C., Zhao, Y., 2017. Toxicity of 13 different antibiotics towards freshwater green algae *Pseudokirchneriella subcapitata* and their modes of action. Chemosphere 168, 217–222. https://doi.org/10.1016/j.chemosphere.2016.10.043.

Genty, B., Briantais, J., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. et Biophys. Acta (BBA) - Gen. Subjects 990 (1), 87–92. https://doi.org/10.1016/S0304-4165(89)80016-9.

Gomes, M.P., Juneau, P., 2017. Temperature and light modulation of herbicide toxicity on algal and cyanobacterial physiology. Front. Environ. Sci. 5, 50. https://doi.org/10.3389/fenvs.2017.00050.

González-Pleiter, M., Gonzalo, S., Rodea-Palomares, I., Leganés, F., Rosal, R., Boltes, K., Marco, E., Fernández-Piñas, F., 2013. Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: implications for environmental risk assessment. Water Res. 47 (6), 2050–2064. https://doi.org/10.1016/j.watres.2013.01.020.

Guo, J., Selby, K., Boxall, A.B.A, 2016. Effects of antibiotics on the growth and physiology of chlorophytes, cyanobacteria, and a diatom. Arch. Environ. Contam. Toxicol. 71 (4), 589–602. https://doi.org/10.1007/s00244-016-0305-5.

He, D., Dorantes-Aranda, J.J., Waite, T.D., 2012. Silver nanoparticle—algae interactions: oxidative dissolution, reactive oxygen species generation and synergistic toxic effects. Environ. Sci. Technol. 46 (16), 8731–8738. https://doi.org/10.1021/es300588a.

Huisman, J., Sharples, J., Stroom, J.M., Visser, P.M., Kardinaal, W.E.A., Verspagen, J.M. H., Sommeijer, B, 2004. Changes in turbulent mixing shift competition for light between phytoplankton species. Ecology 85 (11), 2960–2970. https://doi.org/10.1890/03-0763.

Isidori, M., Lavorgna, M., Nardelli, A., Pascarella, L., Parrella, A., 2005. Toxic and genotoxic evaluation of six antibiotics on non-target organisms. Sci. Total Environ. 346 (1), 87–98. https://doi.org/10.1016/j.scitotenv.2004.11.017.

Jassby, A.D., Platt, T., 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnol. Oceanogr. 21 (4), 540–547. https://doi.org/10.4319/lo.1976.21.4.0540.

Juneau, P., Green, B.R., Harrison, P.J., 2005. Simulation of pulse-amplitude-modulated (PAM) fluorescence: limitations of some PAM-parameters in studying environmental stress effects. Photosynthetica 43 (1), 75–83. https://doi.org/10.1007/s11099-005-5083-7.

Karapetyan, N.V., 2007. Non-photochemical quenching of fluorescence in cyanobacteria. Biochem. (Moscow) 72 (10), 1127. https://doi.org/10.1134/S0006297907100100.

Kirilovsky, D., 2007. Photoprotection in cyanobacteria: the orange carotenoid protein (OCP)-related non-photochemical-quenching mechanism. Photosynth. Res. 93 (1), 7. https://doi.org/10.1007/s11120-007-9168-y.

Kirilovsky, D., 2015. Modulating energy arriving at photochemical reaction centers: orange carotenoid protein-related photoprotection and state transitions. Photosynth. Res. 126 (1), 3–17. https://doi.org/10.1007/s11120-014-0031-7.

Kitajima, M., Butler, W.L., 1975. Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. Biochim. et Biophys. Acta (BBA) - Bioenergetics 376 (1), 105–115. https://doi.org/10.1016/0005-2728(75)90209-1.

Krasilnikov, P.M., Zlenko, D.V., Stadnichuk, I.N., 2020. Rates and pathways of energy migration from the phycobilisome to the photosystem II and to the orange carotenoid protein in cyanobacteria. FEBS Lett. 594 (7), 1145–1154. https://doi.org/10.1002/1873-3468.13709.

Krüger, G.H.J., Tsimilli-Michael, M., Strasser, R.J, 1997. Light stress provokes plastic and elastic modifications in structure and function of photosystem II in camellia leaves. Physiol. Plant. 101 (2), 265–277. https://doi.org/10.1111/j.1399-3054.1997.tb00996.x.

Kümmerer, K., 2008. Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks. Springer Science & Business Media.

Li, F., Liang, Z., Zheng, X., Zhao, W., Wu, M., Wang, Z., 2015. Toxicity of nano-TiO_2 on algae and the site of reactive oxygen species production. Aquatic. Toxicol. 158, 1–13. https://doi.org/10.1016/j.aquatox.2014.10.014.

Li, S., Shi, W., Liu, W., Li, H., Zhang, W., Hu, J., Ke, Y., Sun, W., Ni, J., 2018. A duodecennial national synthesis of antibiotics in China's major rivers and seas (2005–2016). Sci. Total Environ. 615, 906–917. https://doi.org/10.1016/j.scitotenv.2017.09.328.

Lian, Z., Pan, R., Wang, J., 2016. Microencapsulation of norfloxacin in chitosan/chitosan oligosaccharides and its application in shrimp culture. Int. J. Biol. Macromol. 92, 587–592. https://doi.org/10.1016/j.ijbiomac.2016.07.074.

Lichtenthaler, H.K., Buschmann, C. (2001). Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. Curr. *Protocols Food Anal. Chem.*, 1 (1), F3-F4. https://doi.org/10.1002/0471142913.faf0403s01.

Liu, Y., Wang, F., Chen, X., Zhang, J., Gao, B., 2015. Influence of coexisting spiramycin contaminant on the harm of Microcystis aeruginosa at different nitrogen levels. J. Hazard. Mater. 285, 517–524. https://doi.org/10.1016/j.jhazmat.2014.11.027.

MacIntyre, H.L., Kana, T.M., Anning, T., Geider, R.J., 2002. Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria1. J. Phycol. 38 (1), 17–38. https://doi.org/10.1046/j.1529-8817.2002.00094.x.

Moreau, R., Elkrief, L., Bureau, C., Perarnau, J., Thévenot, T., Saliba, F., Louvet, A., Nahon, P., Lannes, A., Anty, R., Hillaire, S., Pasquet, B., Ozenne, V., Rudler, M., Ollivier-Hourmand, I., Robic, M.A., D'Alteroche, L., Di Martino, V., Ripault, M., Pauwels, A., Grangé, J., Carbonell, N., Bronowicki, J., Payancé, A., Rautou, P., Valla, D., Gault, N., Lebrec, D., 2018. Effects of long-term norfloxacin therapy in patients with advanced cirrhosis. Gastroenterology 155 (6), 1816–1827. https://doi.org/10.1053/j.gastro.2018.08.026.

Newby, R., Lee, L.H., Perez, J.L., Tao, X., Chu, T., 2017. Characterization of zinc stress response in Cyanobacterium *Synechococcus* sp. IU 625. Aquatic. Toxicol. 186, 159–170. https://doi.org/10.1016/j.aquatox.2017.03.005.

Nie, X., Gu, J., Lu, J., Pan, W., Yang, Y., 2009. Effects of norfloxacin and butylated hydroxyanisole on the freshwater microalga *Scenedesmus obliquus*. Ecotoxicology 18 (6), 677–684. https://doi.org/10.1007/s10646-009-0334-1.

Ota, M., Takenaka, M., Sato, Y., Smith Jr, R.L., Inomata, H., 2015. Effects of light intensity and temperature on photoautotrophic growth of a green microalga, *Chlorococcum littorale*. Biotechnol. Reports 7, 24–29. https://doi.org/10.1016/j.btre.2015.05.001.

Ou, H., Ye, J., Ma, S., Wei, C., Gao, N., He, J., 2016. Degradation of ciprofloxacin by UV and UV/H2O2 via multiple-wavelength ultraviolet light-emitting diodes: effectiveness, intermediates and antibacterial activity. Chem. Eng. J. 289, 391–401. https://doi.org/10.1016/j.cej.2016.01.006.

Pan, Y., Dong, J., Wan, L., Sun, S., MacIsaac, H.J., Drouillard, K.G., Chang, X., 2020. Norfloxacin pollution alters species composition and stability of plankton communities. J. Hazard. Mater. 385, 121625 https://doi.org/10.1016/j.jhazmat.2019.121625.

Pan, Y., Liu, C., Li, F., Zhou, C., Yan, S., Dong, J., Li, T., Duan, C., 2017a. Norfloxacin disrupts Daphnia magna-induced colony formation in *Scenedesmus quadricauda* and facilitates grazing. Ecol. Eng. 102, 255–261. https://doi.org/10.1016/j.ecoleng.2017.02.037.

Pan, Y., Yan, S., Li, R., Hu, Y., Chang, X., 2017b. Lethal/sublethal responses of Daphnia magna to acute norfloxacin contamination and changes in phytoplankton-zooplankton interactions induced by this antibiotic. Sci. Rep. 7 (1), 40385. https://doi.org/10.1038/srep40385.

Pilon, L., Berberoğlu, H., Kandilian, R., 2011. Radiation transfer in photobiological carbon dioxide fixation and fuel production by microalgae. J. Quant. Spectrosc. Radiat. Transf. 112 (17), 2639–2660. https://doi.org/10.1016/j.jqsrt.2011.07.004.

Ralph, P., Gademann, R., Larkum, A., Kühl, M., 2002. Spatial heterogeneity in active chlorophyll fluorescence and PSII activity of coral tissues. Mar. Biol. 141 (4), 639–646. https://doi.org/10.1007/s00227-002-0866-x.

Schreiber, U., Schliwa, U., Bilger, W., 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth. Res. 10 (1), 51–62. https://doi.org/10.1007/BF00024185.

Sedoud, A., López-Igual, R., Ur Rehman, A., Wilson, A., Perreau, F., Boulay, C., Vass, I., Krieger-Liszkay, A., Kirilovsky, D., 2014. The cyanobacterial photoactive orange carotenoid protein is an excellent singlet oxygen quencher. Plant Cell 26 (4), 1781. https://doi.org/10.1105/tpc.114.123802.

Seoane, M., Rioboo, C., Herrero, C., Cid, Á., 2014. Toxicity induced by three antibiotics commonly used in aquaculture on the marine microalga *Tetraselmis suecica* (Kylin) Butch. Mar. Environ. Res. 101, 1–7. https://doi.org/10.1016/j.marenvres.2014.07.011.

Sluchanko, N.N., Slonimskiy, Y.B., Maksimov, E.G., 2017. Features of protein−protein interactions in the cyanobacterial photoprotection mechanism. Biochem. (Moscow) 82 (13), 1592–1614. https://doi.org/10.1134/S000629791713003X.

Stanier, R.Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G., 1971. Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriol. Rev. 35 (2), 171–205.

Stirbet, A., Govindjee, 2011. On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and photosystem ii: basics and applications of the OJIP fluorescence transient. J. Photochem. Photobiol. B 104 (1), 236–257. https://doi.org/10.1016/j.jphotobiol.2010.12.010.

Stirbet, A., Lazár, D., Kromdijk, J., 2018. Chlorophyll a fluorescence induction: can just a one-second measurement be used to quantify abiotic stress responses? Photosynthetica 56 (1), 86–104. https://doi.org/10.1007/s11099-018-0770-3.

Välitalo, P., Kruglova, A., Mikola, A., Vahala, R., 2017. Toxicological impacts of antibiotics on aquatic micro-organisms: a mini-review. Int. J. Hyg. Environ. Health 220 (3), 558–569. https://doi.org/10.1016/j.ijheh.2017.02.003.

Wahidin, S., Idris, A., Shaleh, S.R.M, 2013. The influence of light intensity and photoperiod on the growth and lipid content of microalgae *Nannochloropsis* sp. Bioresour. Technol. 129, 7–11. https://doi.org/10.1016/j.biortech.2012.11.032.

Wan, J., Guo, P., Peng, X., Wen, K., 2015. Effect of erythromycin exposure on the growth, antioxidant system and photosynthesis of *Microcystis flos-aquae*. J. Hazard. Mater. 283, 778–786. https://doi.org/10.1016/j.jhazmat.2014.10.026.

Wan, J., Guo, P., Zhang, S., 2014. Response of the cyanobacterium *Microcystis flos-aquae* to levofloxacin. Environ. Sci. Pollution Res. 21 (5), 3858–3865. https://doi.org/10.1007/s11356-013-2340-3.

Wang, Z., Chen, Q., Hu, L., Wang, M., 2018. Combined effects of binary antibiotic mixture on growth, microcystin production, and extracellular release of *Microcystis aeruginosa*: application of response surface methodology. Environ. Sci. Pollution Res. 25 (1), 736–748. https://doi.org/10.1007/s11356-017-0475-3.

Xu, K., Li, Z.K., Liu, S.W., Qiu, B.S., 2017. Effects of iron deficiency on the growth and photosynthesis of three bloom-forming cyanobacterial species isolated from Lake Taihu. Phycol. Res. 65, 151–159. https://doi.org/10.1111/pre.12166.

Xu, K., Li, Z.K., Qiu, B.S., Juneau, P., 2013. Different responses to high light stress of toxic and non-toxic microcystis aeruginosa acclimated under two light intensities and zinc concentrations. Toxicol. Environ. Chem. 95 (7), 1145–1156. https://doi.org/10.1080/02772248.2013.849347.

Xu, K., Juneau, P., 2016. Different physiological and photosynthetic responses of three cyanobacterial strains to light and zinc. Aquatic. Toxicol. 170, 251–258. https://doi.org/10.1016/j.aquatox.2015.11.015.

Xu, K., Racine, F., He, Z., Juneau, P., 2019. Impacts of hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor (mesotrione) on photosynthetic processes in *Chlamydomonas reinhardtii*. Environ. Pollut. 244, 295–303. https://doi.org/10.1016/j.envpol.2018.09.121.

Yuan, J., Ni, M., Liu, M., Zheng, Y., Gu, Z., 2019. Occurrence of antibiotics and antibiotic resistance genes in a typical estuary aquaculture region of Hangzhou Bay, China. Mar. Pollut. Bull. 138, 376–384. https://doi.org/10.1016/j.marpolbul.2018.11.037.

Zhang, Q., Ying, G., Pan, C., Liu, Y., Zhao, J., 2015. Comprehensive evaluation of antibiotics emission and fate in the river basins of China: source analysis, multimedia modeling, and linkage to bacterial resistance. Environ. Sci. Technol. 49 (11), 6772–6782. https://doi.org/10.1021/acs.est.5b00729.