Supporting Information for the manuscript entitled "The Effects of Hydrogen Peroxide on Cyanobacterium Microcystis aeruginosa in the Presence of Nanoplastics"

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Material and methods: Calculation of Nanoplastic Particle Number Concentration

The concentration levels of nanoplastics tested in present study ranged from 5 mg L^{-1} to 100 mg L^{-1}. Mass concentration is commonly used due to the sampling and characterization challenges of nanoplastics, so we conform to this norm. To calculate the particle number concentration of polystyrene nanoparticles in the solution,

$$\text{Particle Number Concentration} = \frac{\text{Mass Concentration}}{\text{Mass Per Nanoparticle}} = 3 * \frac{\text{Mass Concentration}}{4\pi \times r^3 \times \rho}$$

where, ρ = density of polystyrene = 1.04 g cm^{-3}, r = average radius of nanoparticles = 50 nm = 5×10^{-8} cm. Thus, the particle number concentration of polystyrene nanoparticles used in this study were: 9.18×10^{15}, 1.84×10^{16}, 5.51×10^{16}, 9.18×10^{16}, 1.84×10^{17} particles L^{-1}, equivalent to the mass concentration of 5, 10, 30, 50, 100 mg L^{-1}, respectively.

Materials and methods: Calculation of Relative H_2O_2 Decrements

In addition to the change of H_2O_2 concentration in 24 h, we also calculated the relative H_2O_2 decrements value of each treatment. We set the values of the change of H_2O_2 concentration in 0 mg L^{-1} nanoplastics treatment group as the reference group for both initial H_2O_2 concentration, and compared the values of 5, 10 mg L^{-1} nanoplastics treatment groups to the reference groups at their corresponding initial H_2O_2 concentration. As Figure illustrated in S9, the relative H_2O_2 decrement of 5 and 10 mg L^{-1} nanoplastics groups were all smaller than 1 at both initial H_2O_2 concentration, implying that the change of H_2O_2 concentration was less than compared with nanoplastic-free groups, in other words, nanoplastics inhibit the decomposition of H_2O_2 in BG-11 media.

Discussion: Calculation of Relative Irradiance Loss

We measured the optical density (absorbance) at 680 nm of solutions with different nanoplastics concentrations, calculated the relative irradiance light loss, and interpolated a curve between nanoplastics concentration and relative irradiance loss.

Optical density is defined as:

$$\text{Optical Density} = log_{10}(\frac{I_0}{I_1})$$

where I_0 is the intensity of visible light incident upon a small area of the film, and I_1 is the intensity of light transmitted by that region of the film. We can calculate $\frac{I_1}{I_0}$, which is the transmittance of solutions with different nanoplastics concentrations based on their corresponding optical density value. Then, the irradiance loss, which is defined as $1-\frac{I_1}{I_0}$, can be determined. Lastly, we subtracted the irradiance loss of solutions with nanoplastics added with that of solutions without nanoplastics added, and name this value the "Relative Irradiance Loss".

Discussion: Testing Effect of Irradiance Loss Induced by Nanoplastics on Cell Abundance

We tested how well the effects of light transmitted calculated based on the relative light loss explain the effects of nanoplastics on cell abundance, and found the AICc values of the new model, which replaced light transmitted with the nanoplastics and light intensity terms in the original model, was much more positive (18112) compared with the original model 1 (14158.9), implying that the effects of nanoplastics on *M. aeruginosa* abundance can only be partially described by their ability to affect transmitted light.

Table S1. Summary statistics for the linear regression model of the full-factorial experiment for the effects of four factors (H_2O_2, Nanoplastics, Light, and Temperature), and their full-factorial interactions on *M. aeruginosa* abundance. Exclude treatments with the highest nanoplastics (100 mg L^{-1}) and H_2O_2 (20 mg L^{-1}) level.

Term	Estimated Coefficient	F ratio	Prob > F
H_2O_2	-2.254	380.32	< .0001
Nanoplastics	-1.2000	82.00	< .0001
Temperature	-0.298	6.45	0.0111
Light	-0.049	0.14	0.7045
Temperature × H_2O_2	-0.881	38.71	< .0001
H_2O_2 × Nanoplastics	0.975	37.14	< .0001
Temperature × Nanoplastics	-0.717	19.47	< .0001
H_2O_2 × Light	-0.125	0.64	0.4233
Nanoplastics × Light	0.067	0.14	0.7092
Temperature × Light	0.027	0.03	0.8659
Temperature × H_2O_2 × Nanoplastics	-0.647	10.91	0.0010
Temperature × Nanoplastics × Light	-0.376	2.96	0.0853
H_2O_2 × Nanoplastics × Light	0.272	1.60	0.2064
Temperature × H_2O_2 × Light	-0.235	1.52	0.2174
Temperature × H_2O_2 × Nanoplastics × Light	-0.420	2.54	0.1114

Table S2. Summary statistics for the linear regression model of the full-factorial experiment for the effects of four factors (H_2O_2, Nanoplastics, Light, and Temperature), and their full-factorial interactions on total MC-LR and MC-dmLR production

Term	Estimated Coefficient	F ratio	prob > F
H_2O_2	-22.592	70.04	<.0001
Nanoplastics	-17.198	40.58	<.0001
Temperature	3.742	1.92	0.1759
Light	-2.839	1.11	0.3013
H_2O_2 × Nanoplastics	16.720	38.36	<.0001

Temperature × Nanoplastics	-4.199	2.42	0.1303
Temperature × Light	-2.273	0.71	0.4065
Temperature × H_2O_2	-1.699	0.40	0.5338
Nanoplastics × Light	-0.603	0.05	0.8249
H_2O_2 × Light	-0.293	0.01	0.9144
Temperature × H_2O_2 × Nanoplastics	4.255	2.48	0.1255
H_2O_2 × Nanoplastics × Light	1.619	0.36	0.5533
Temperature × Nanoplastics × Light	1.470	0.30	0.5901
Temperature × H_2O_2 × Light	1.218	0.20	0.6551
Temperature × H_2O_2 × Nanoplastics × Light	-0.407	0.02	0.8812

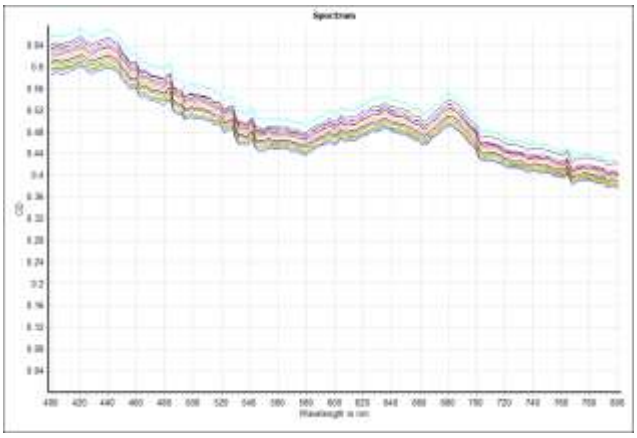


Figure S1. Absorbance spectra of *Microcystis aeruginosa*

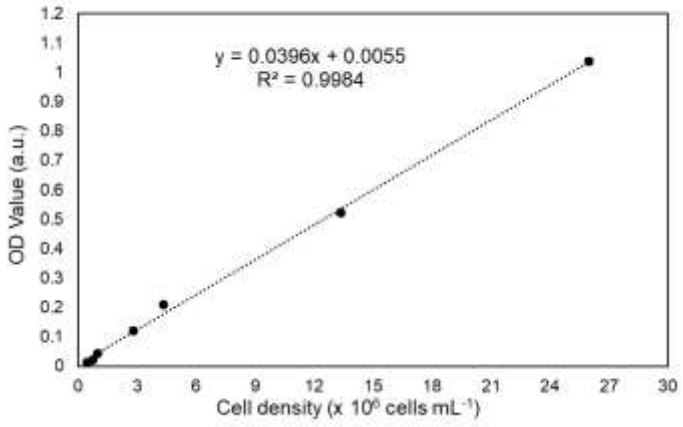


Figure S2. Linearly proportional correlation between *Microcystis aeruginosa* suspension of various cell densities and OD value at 680 nm. Data are presented as means (n = 4) ± SE

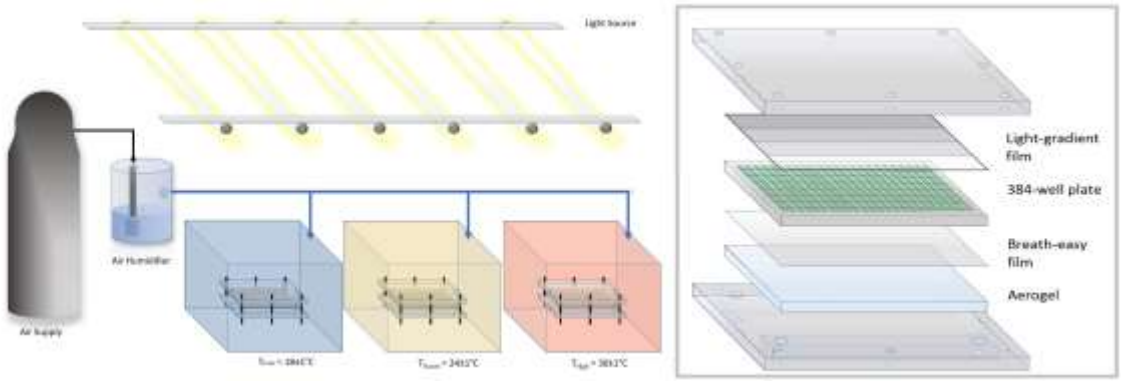


Figure S3. Systematic illustration of platform setup consisting of variables control and full-factorial screening devices. Inset: expanded schematic of the main components of one individual device.

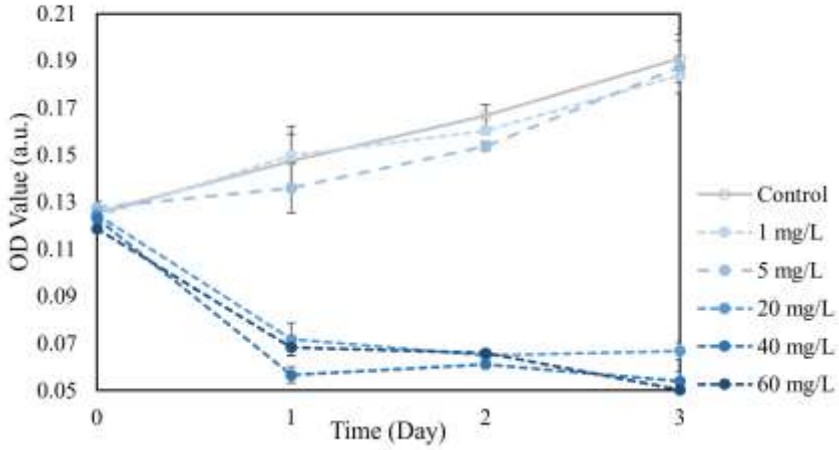
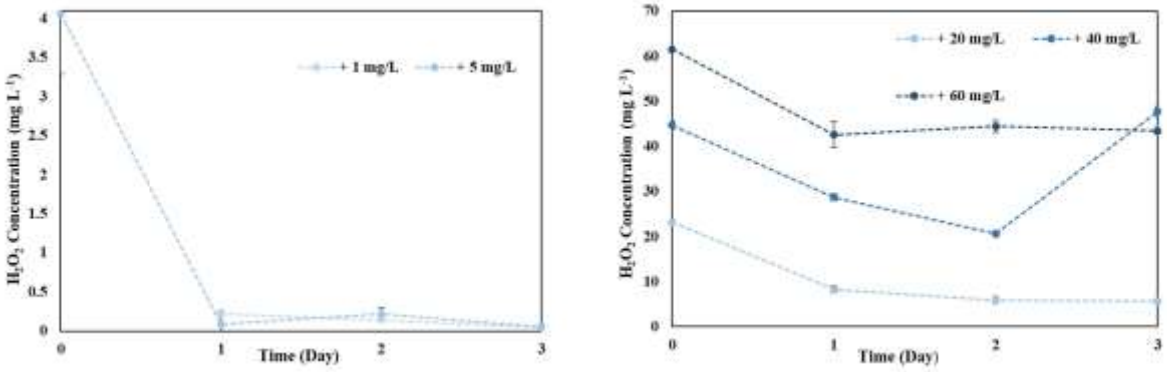


Figure S4. Reduction of *M. aeruginosa* abundance under different H_2O_2 concentrations (0, 1, 5, 20, 40, 60 mg L^{-1}). Cell density is expressed as the optical density value. Data are presented as means (n = 16) ± SE



(a) (b)

Figure S5. The remaining H_2O_2 concentration in *M. aeruginosa* cultures with an initial H_2O_2 dosage of (a) 1 and 5, (b) 20, 40 and 60 mg L^{-1} after 24, 48 and 72 h exposure. Data are presented as means (n = 2) ± SE

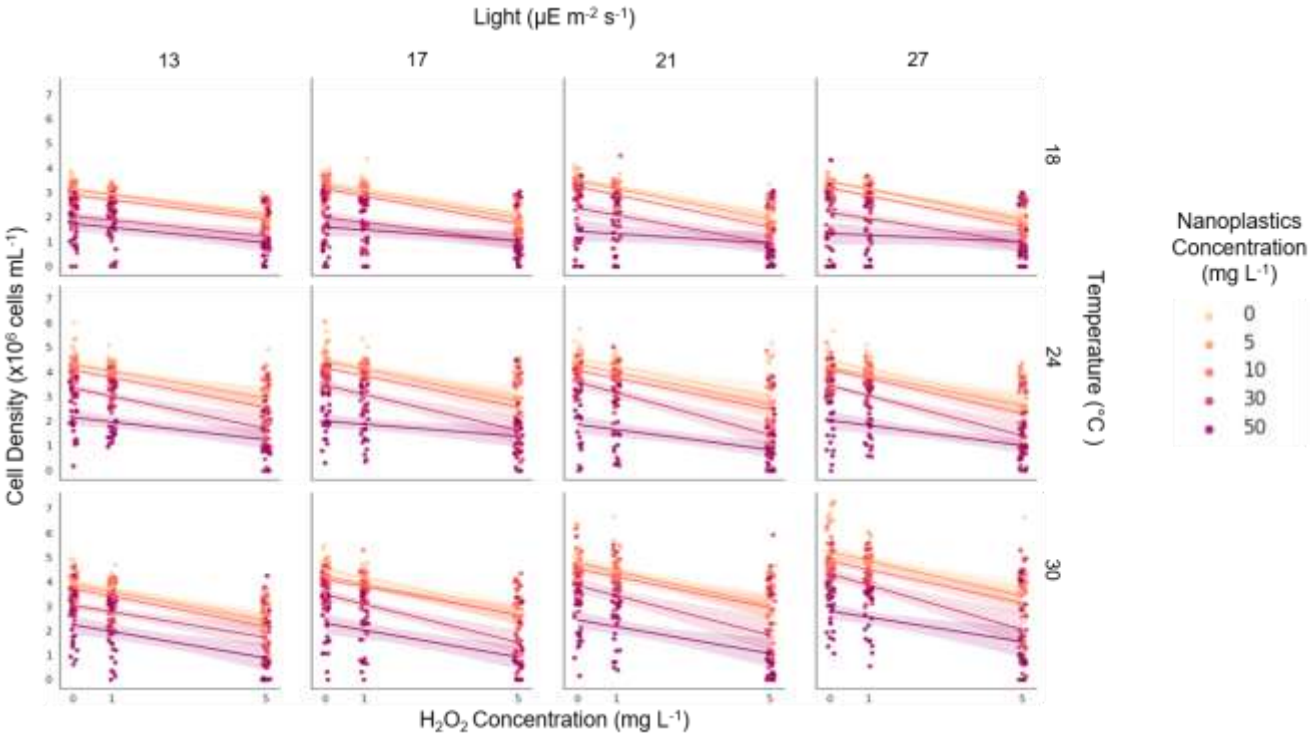


Figure S6. Cell density of *Microcystis aeruginosa* after 72 h exposure showing the interactive effects of H_2O_2 (0, 1 and 5 mg L^{-1}, x-axis), light (13, 17, 21, and 27 µmol $m^{-2}s^{-1}$, across columns), temperature (18, 24, and 30 °C, across rows), and nanoplastics (0-yellow, 5, 10, 30, 50-red mg L^{-1}; intermediate levels in intermediate colors), with the highest exposure level omitted for NPs and H_2O_2. Fitted model expectations for cell density are shown for each nanoplastic level with lines (mean) and shaded areas (95% CI).

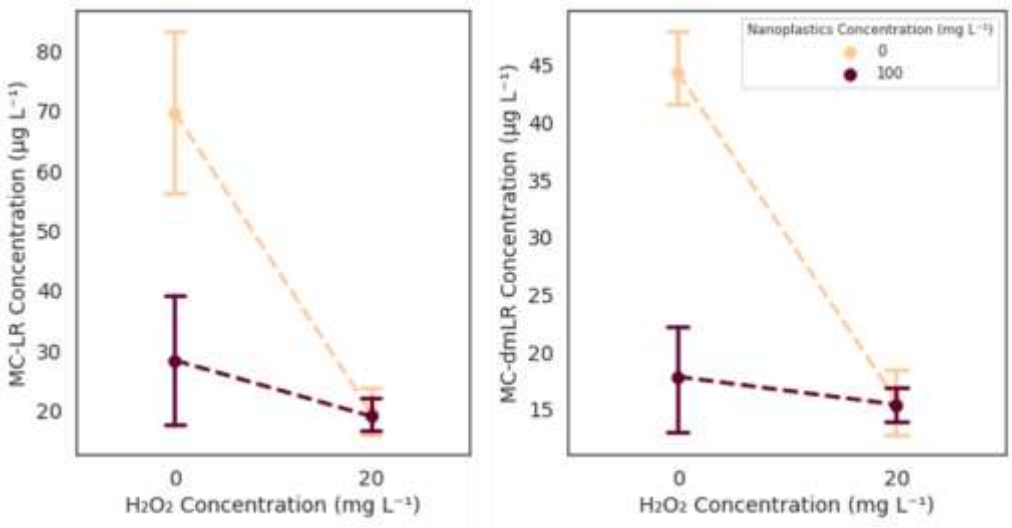


Figure S7. MC-LR and MC-dmLR concentration of *M. aeruginosa* after 72 h exposure showing the combined effects of nanoplastics (0, 100 mg L^{-1}) and H_2O_2 (0, 20 mg L^{-1}), across all light (13, 27 µmol $m^{-2}s^{-1}$), and temperature (18, 30 °C) conditions. a: MC-LR result; b: MC-dmLR result. Bars represent 95% CI.

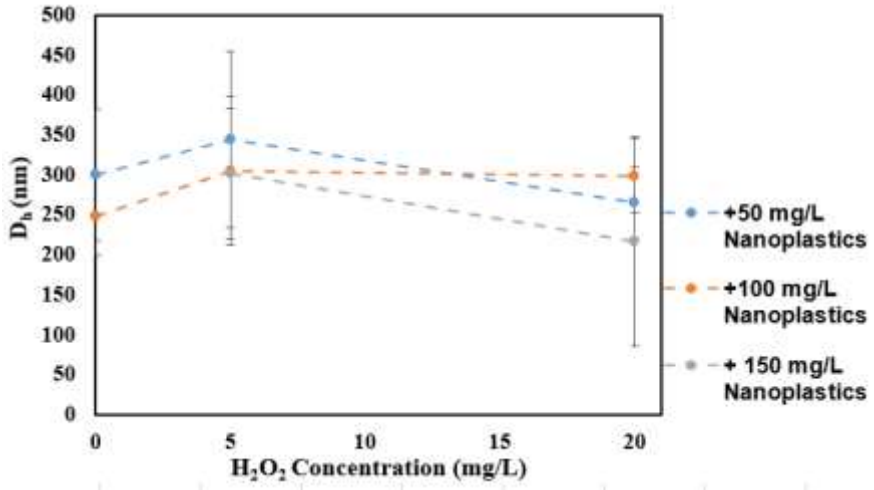


Figure S8. D_h of PS-NH_2 at 50, 100, 150 mg L^{-1} in the presence of different H_2O_2 concentration (0, 5, 20 mg L^{-1}) in BG-11 medium, as means ± SE.

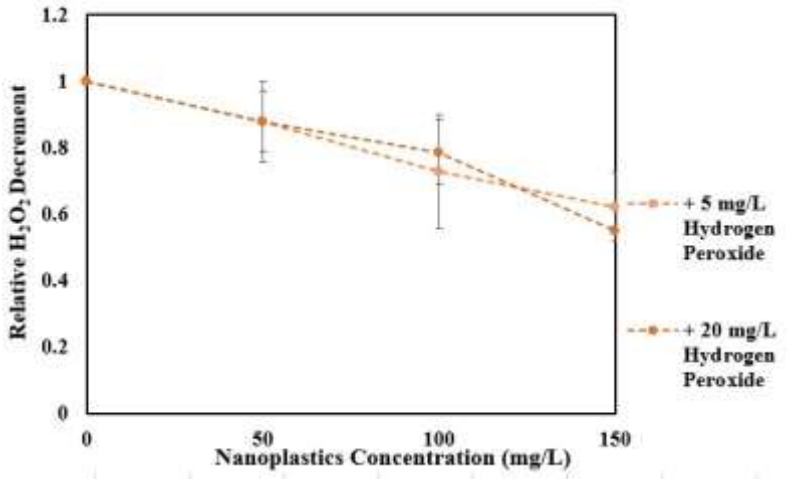
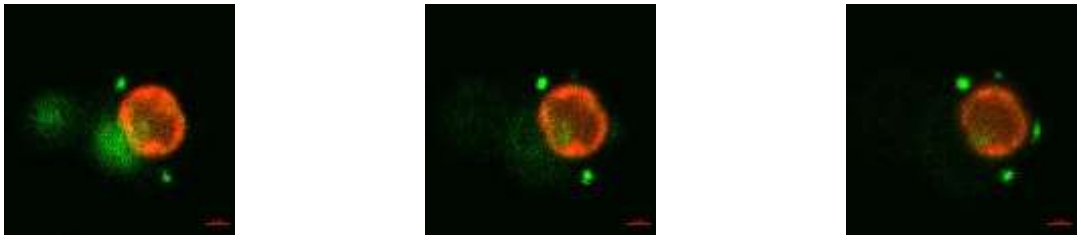


Figure S9. Relative H_2O_2 decrements in 24 h under different nanoplastics (0, 50, 100 and 150 mg L^{-1}) and initial H_2O_2 (5 and 20 mg L^{-1}) levels. Each response was the change of H_2O_2 concentration relative to that at zero nanoplastics conditions (for both initial H_2O_2 concentrations). Data are presented as means (n = 5) ± SE



(a) (b) (c)

Figure S10. Additional Z slices of *M. aeruginosa* cells from confocal microscopy showing the uptake of PS-NH_2 after 72 h of incubation at 10 mg L^{-1}. Uptake is indicated by stronger fluorescence in the slice from mid-cell (b), as opposed to slices from either side (a and c). Green fluorescence corresponds to PS-NH_2 beads, red to auto-fluorescent *M. aeruginosa*

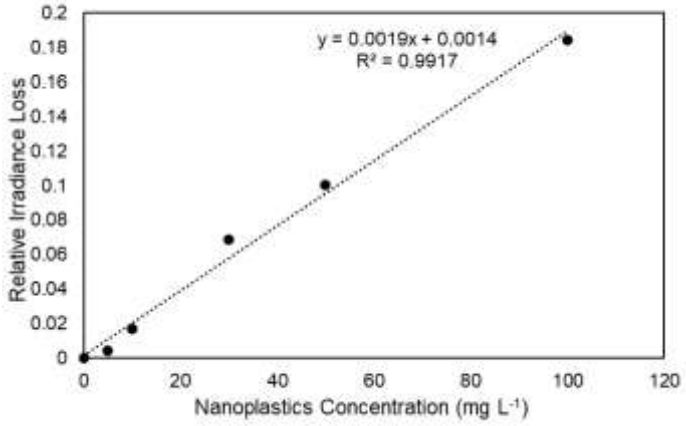


Figure S11. Linearly proportional correlation between nanoplastics concentration and relative irradiance loss.