Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Information

UV-B radiation enhances the toxicity of TiO_2 nanoparticles to the marine microalga *Chlorella pyrenoidosa* by disrupting the protection function of extracellular polymeric substances

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Supporting Text

Text 1. Acute toxicity tests with single and combined stressor

To perform exposure experiments, algal cells at the exponential phase of growth were inoculated in fresh f/2 medium at a cell density of 1.0×10^5 cells·mL^{-1}. An appropriate volume of the 10 g·L^{-1} TiO_2 NPs stock dispersion was added to the algae containing medium to achieve the final working concentrations of TiO_2 NPs dispersion for each specific experiment. The exposure experiments were started simultaneously with a 12:12 light-dark cycle and the UV-B irradiation was applied in addition at the beginning of each photoperiod. Algal cultures were provisionally transferred to petri dishes when they were exposed to UVBR and this would bring the algal cultures into contact with UVBR. After irradiation, the algal cells were subsequently cultured in 250 mL flasks containing 125 mL of culture media. The cultures in flasks were shaken three times per day.

The exposure concentrations of TiO_2 NPs in the single stress test (no UVBR) were set at 0, 20, 40, 80, 120 and 160 mg·L^{-1}. The UV-B exposure durations in the single stress test (no TiO_2 NPs) were 30, 60, 240, 480 and 720 min per day, which equivalent to a UV-B dose of 0.18, 0.36, 1.44, 2.88 and 4.32 kJ·m^{-2}·d^{-1}), respectively. After a 96 h exposure to the above treatments, the algal cell densities in each sample were counted using a light microscope (BX-51, Olympus, Japan) and the inhibition rate (*IR*) was calculated as follows: $IR(\%) = (N_c\text{-}N_t)/\mathrm{Nc}\times100\%$, where N_c and N_t are the average cell densities (cells·mL^{-1}) in the controls and treatments, respectively. Cell counting was used to quantify algae growth to overcome shading issues associated with other techniques.[1] The effective concentration of TiO_2 NPs or dose of UVBR leading to a 50% algal growth reduction compared to the control in the single stress tests (s-EC_{50}/s-ED_{50}), was calculated from the concentration/dose-response curves. The results of the single stress tests were used to inform the exposure conditions in the combined stress tests, where s-EC_{50} or s-ED_{50} was set as one Toxic Unit according to Brow[1] and Sprague[2] and where the TiO_2 NPs concentration and the UVBR dose were set as an equal toxicity ratio. The concentration of TiO_2 NPs was 0.150 s-EC_{50}, 0.225 s-EC_{50}, 0.300 s-EC_{50}, 0.375 s-EC_{50} and 0.450 s-EC_{50}, and the corresponding UV-B doses were 0.150 s-ED_{50}, 0.225 s-ED_{50}, 0.300 s-ED_{50}, 0.375 s-ED_{50} and 0.450 s-ED_{50}. Then the effective concentration/dose values leading to a 50% algal growth reduction in the combined stress tests (c-EC_{50}/c-ED_{50}) were calculated.

References:

1 J. Farkas and A. M. Booth, Are fluorescence-based chlorophyll quantification methods suitable for algae toxicity assessment of carbon nanomaterials? *Nanotoxicology*, 2017, **11**, 569–577.

2 V. M. Brown, The calculation of the acute toxicity of mixtures of poisons to rainbow trout, *Water Res.*, 1968, **2**, 723–733.

3 J. B. Sprague, Measurement of pollutant toxicity to fish. ii. Utilizing and applying bioassay results, *Water Res.*, 1970, **4**, 3–32.

Table S1. Physicochemical characteristics of TiO_2 nanoparticles.

Physicochemical characteristic	Source	Result
Primary particles size	Transmission electron microscopy (TEM; JEM-1200EX, JEOL, Japan)	21.4±2.9 nm
Purity	Manufacturer	≥99.5%
BET surface area	Micromeritics ASAP 2460 analyzer (USA)	51.10 m^2/g
Crystalline structure	X-ray diffractometer (XRD; SmartLab Rigaku, Japan)	Anatase: 89.45% Rutile: 10.55%

Table S2. Characteristics of sterile filtered seawater.

Characteristic	Source	Result
Ionic strength	Multi-parameter water quality analyzer (ProPlus, YSI, USA); Convert from conductivity	7.10±0.01 $mol \cdot L^{-1}$
Salinity	Multi-parameter water quality analyzer (ProPlus, YSI, USA)	31.63±0.10 ‰
pH	pH meter (PB-10, Sartorius, Germany)	8.26±0.02
Natural organic matter	TOC analyzer (TOC-V CPN analyzer, Shimadzu, Japan)	1.45±0.01 $mg \cdot L^{-1}$

Table S3. Hydrodynamic diameters of TiO_2 nanoparticles in seawater under different treatments over a 24 h period.

Treatment	LT	LUT	HT	HUT
0 h	1420.38±31.92^{A}	1411.01±53.24^{A}	1808.86±41.29^{a}	1803.26±34.00^{a}
0.5 h	1535.84±66.04^{A}	1395.98±70.04^{A}	1791.23±92.31^{a}	1762.24±21.67^{a}
1 h	1532.70±35.16^{A}	1390.86±28.67^{B}	1863.88±64.12^{a}	1721.43±44.97^{b}
1.5 h	1682.24±14.94^{A}	1403.04±54.01^{B}	1758.85±37.13^{a}	1648.63±55.22^{b}
2 h	1730.39±38.40^{A}	1410.53±51.69^{B}	1743.55±54.14^{a}	1624.60±31.35^{b}
4 h	1753.77±65.00^{A}	1550.05±38.04^{B}	1736.82±49.09^{a}	1398.37±43.52^{b}
6 h	1610.68±22.88^{A}	1496.94±77.63^{A}	1407.24±67.95^{a}	1497.23±16.16^{a}
8 h	1283.03±39.41^{A}	1301.84±11.56^{A}	1300.96±42.73^{a}	1268.96±43.82^{a}
12 h	1056.51±23.18^{A}	1050.07±31.24^{A}	1227.31±32.37^{a}	1153.94±57.79^{a}
24 h	1034.34±50.00^{A}	1039.34±38.90^{A}	1022.60±25.35^{a}	1055.53±20.71^{a}

Table S4. Two-way ANOVA summary on interactive effects of UVBR and TiO_2 nanoparticles on Ti contents, EPS contents, intracellular ROS levels, MDA contents and ABS/CS_0 values of *Chlorella pyrenoidosa*.

Index	**Source**	**df**	**UV**	**TiO_2**	**UV*TiO_2**
			1	**1**	**1**
Ti	H	MS	32.898	0.303	0.290
		F	3181.506	29.308	28.091
		p	<0.001	0.001	**0.001**
EPS	H	MS	8.111	35.498	18.732
		F	5.993	26.228	13.840
		p	0.031	<0.001	**0.003**
Intracellular ROS	L	MS	5.279	121.241	4.607
		F	40.835	937.877	35.635
		p	<0.001	<0.001	**<0.001**
	H	MS	37.613	1463.971	37.570
		F	96.201	3744.391	96.093
		p	<0.001	<0.001	**<0.001**
MDA	L	MS	9.915	3.972	4.941
		F	53.246	21.330	26.533
		p	<0.001	0.002	**0.001**
	H	MS	63.964	23.205	17.139
		F	280.729	101.842	75.219
		p	<0.001	<0.001	**<0.001**
ABS/CS_0	H	MS	5.02210^6	2.217×10^7	4.538×10^5
		F	91.292	403.054	8.250
		p	<0.001	<0.001	**0.021**

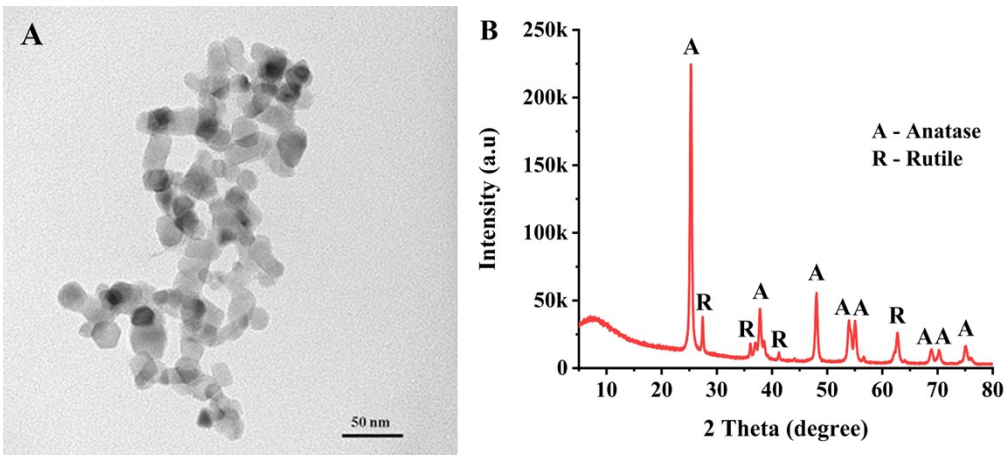

Fig. S1. TEM images (A) and XRD spectrum (B) of TiO_2 NPs.

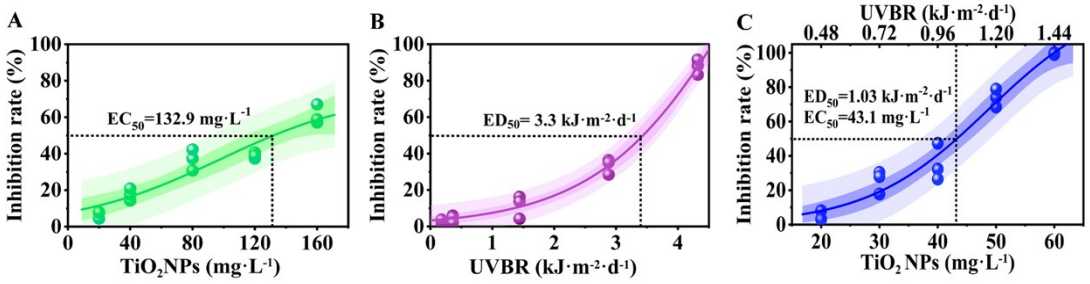

Fig.S2. Acute toxic effects of single TiO_2 nanoparticles (A) or UVBR (B) and their combination (C) on *C. pyrenoidosa* after 96-h exposure.

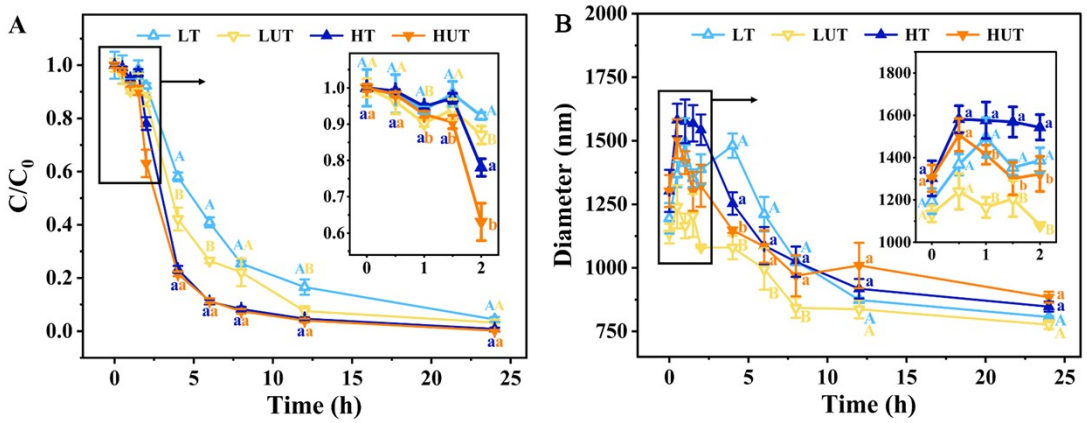

Fig.S3. Sedimentation (A) and z-average diameter kinetics (B) of TiO_2 NPs in f/2 medium under different treatments over a 24 h period (n=3). Different uppercase letters (A–B) indicate significant differences between the 15 mg·L^{-1} TiO_2 NPs treatments before and after exposure to UVBR ($p < 0.05$). Different lower case letters (a–b) denote significant differences between the 35 mg·L^{-1} TiO_2 NPs treatments before and after exposure to UVBR ($p < 0.05$).

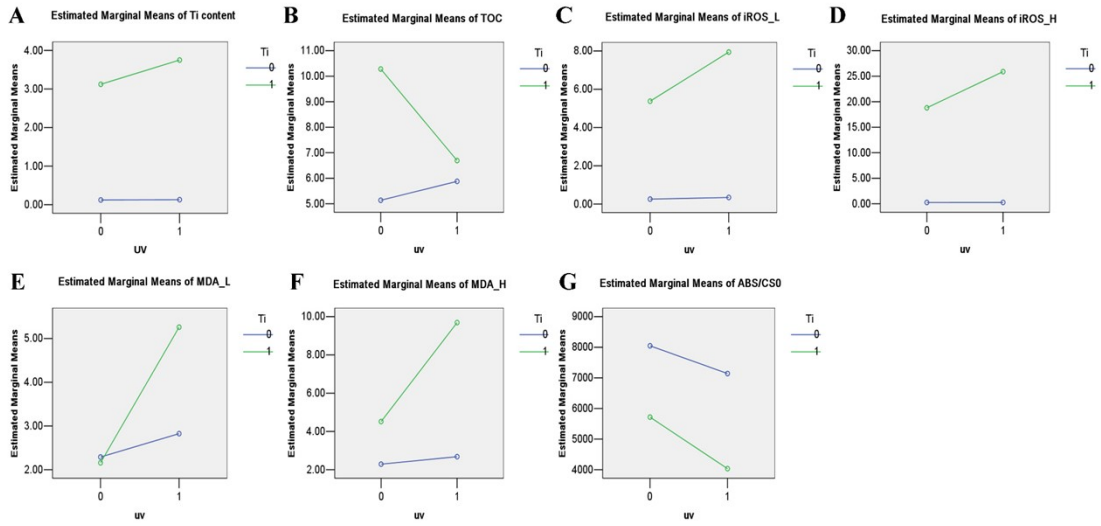

Fig. S4. Interaction plots show interaction between UVBR and TiO_2 NPs on cellular Ti contents (A), EPS contents (B), intracellular ROS levels (D), MDA contents (F) and ABS/CS_0 values (G) of *C. pyrenoidosa* cells from high combined treatments, and intracellular ROS levels (C) and MDA contents (E) of algal cells from low combined treatments.

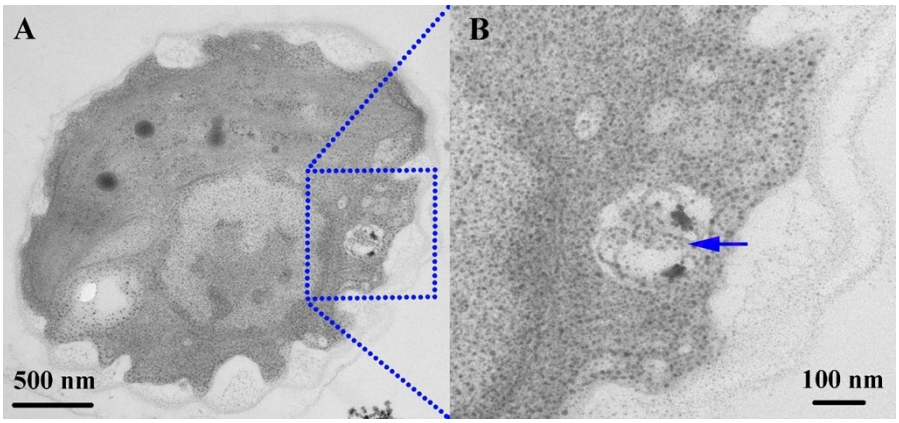

Fig. S5. TEM images of *C. pyrenoidosa* cells treated with 35 mg·L^{-1} TiO_2 NPs (HT; A). The magnification from (A) marked with blue square frame is shown in (B), where the blue arrow indicates an endocytic vesicle.