Investigating the impact of cerium oxide nanoparticles upon the ecologically significant marine cyanobacterium *Prochlorococcus*

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Keywords: *Prochlorococcus*, Ecotoxicology, Nanomaterials, Cerium oxide, Marine pollution, Phytoplankton

Supplementary Information

SI.1 Pro99 Media

To culture *Prochlorococcus sp.* MED4, cyanobacteria were grown in Pro99 media.[1] Briefly, media was prepared as follows; to 500 mL autoclaved and filtered natural oligotrophic seawater, 0.8 mL NH_4Cl (0.5 M), 1 mL $NaH_2PO_4 \bullet H_20$ (0.025 M) and 50 μL trace metal stock was added and mixed by inversion three times. Each component was filter sterilized using a 0.02 μm filter prior to addition to seawater. Ionic strength of the NSW used during experiments are within the range of natural seawater in relevant regions (~40-60 mS).[2]

SI.2. Characterisation of $nCeO_2$: Additional Information

SI.2.1 TEM primary particle size distribution

As described in section 2.2 (main text), $nCeO_2$ were imaged using transmission electron microscopy (TEM). To determine primary particle size, the diameter of 100 nanoparticles was measured using Image J v.3.2 software. The size distribution of primary $nCeO_2$ particles is presented in Fig SI.1.

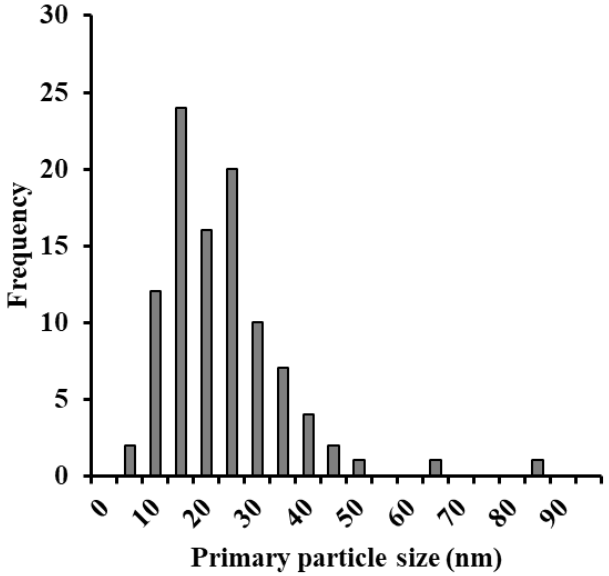

Figure SI.1. Size distribution histogram (diameter) of primary $nCeO_2$ particles measured using Image J v.3.2 software (n=100).

SI.2.2 UV-vis spectrophotometry

UV-visible spectra (Fig SI.2) were collected using an Agilent Cary 60 UV-vis spectrophotometer of a sample of 100 mg L^{-1} $nCeO_2$ in MilliQ ultrapure water.

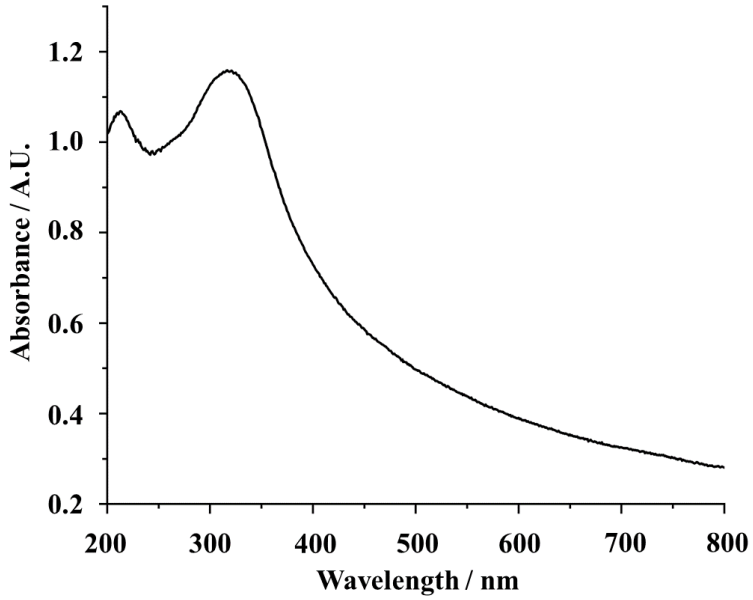

Figure SI.2. UV-vis spectra of $nCeO_2$ utilised in experimental work.

SI.2.3 DLS Additional results

a) Count rate data

Dynamic light scattering (DLS) was carried out on $nCeO_2$ (1 and 100 mg L^{-1}) added to NSW as described in section 2.2 (main text). The mean count data obtained at each timepoint during the 240-h experiment is presented in Table SI.1.

Table SI.1. Mean count rate recorded during DLS analysis of $nCeO_2$ in NSW

	Mean Count Rate (kcps)	
Time (h)	**1 mg L^{-1}**	**100 mg L^{-1}**
0	311 ± 35	225 ± 90
1	255 ± 51	207 ± 150
2	208 ± 108	226 ± 85
4	256 ± 65	117 ± 12
24	229 ± 26	214 ± 136
48	189 ± 112	172 ± 55
72	299 ± 114	246 ± 176
168	178 ± 118	223 ± 70
240	249 ± 100	108 ± 54

b) Sedimentation images

During DLS analysis, $nCeO_2$ was observed to precipitate out of the water column at both test concentrations. Deposited material was visible by eye (Fig SI.3) after 24 h and continued to be evident throughout the 240-h experiment.

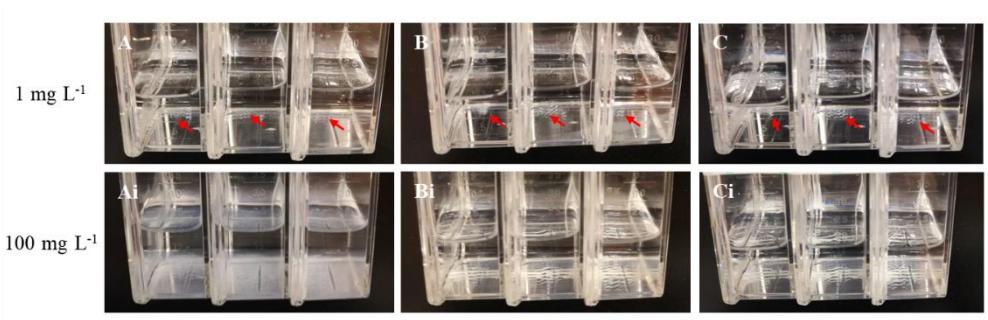

Figure SI.3. Photographic observation of $nCeO_2$ (1 and 100 mg L^{-1}) sedimentation during DLS analysis; A – 24 h, B – 168 h, C – 240 h. Red arrows indicate where deposited material is not immediately obvious in 1 mg L^{-1} samples.

SI.3 Toxicity testing: Initial cell density

Short- (72 h) and extended (240 h) exposures were utilized to examine the effect of $nCeO_2$ exposure upon the marine cyanobacterium *Prochlorococcus sp.* MED4 (sections 2.3 and 2.4, main text). A summary of experimental conditions used in this work is provided in Table SI.2.

Table SI.2. Experimental conditions summary for toxicity testing with *Prochlorococcus sp.* MED4

Experiment	**Exposure length (h)**	**Media**	**$nCeO_2$ concentration**	**Initial cell density (cells mL^{-1})**
Short-term (section 2.3)	72	NSW	0, 1, 10 and 100 µg L^{-1}	2.8×10^4
Short-term (section 2.3)	72	Pro99	0, 1, 10 and 100 µg L^{-1}	8.9×10^5
Extended exposure (section 2.4)	240	NSW	0, 1, 10 and 100 µg L^{-1}; 1, 10 and 100 mg L^{-1}	1.7×10^5
Extended exposure (section 2.4)	240	Pro99	0, 1, 10 and 100 µg L^{-1}; 1, 10 and 100 mg L^{-1}	6.6×10^5

SI.4 Fluorescent microscopy: additional images

Fluorescent microscopy was utilized to examine the occurrence of hetero-aggregation between $nCeO_2$ and cyanobacteria (section 2.5, main text). The entrapment of cyanobacteria within large aggregates of $nCeO_2$ could be seen in both NSW and Pro99 media. Additional images to supplement those presented in Fig 5 (main text) are presented in Fig SI.4.

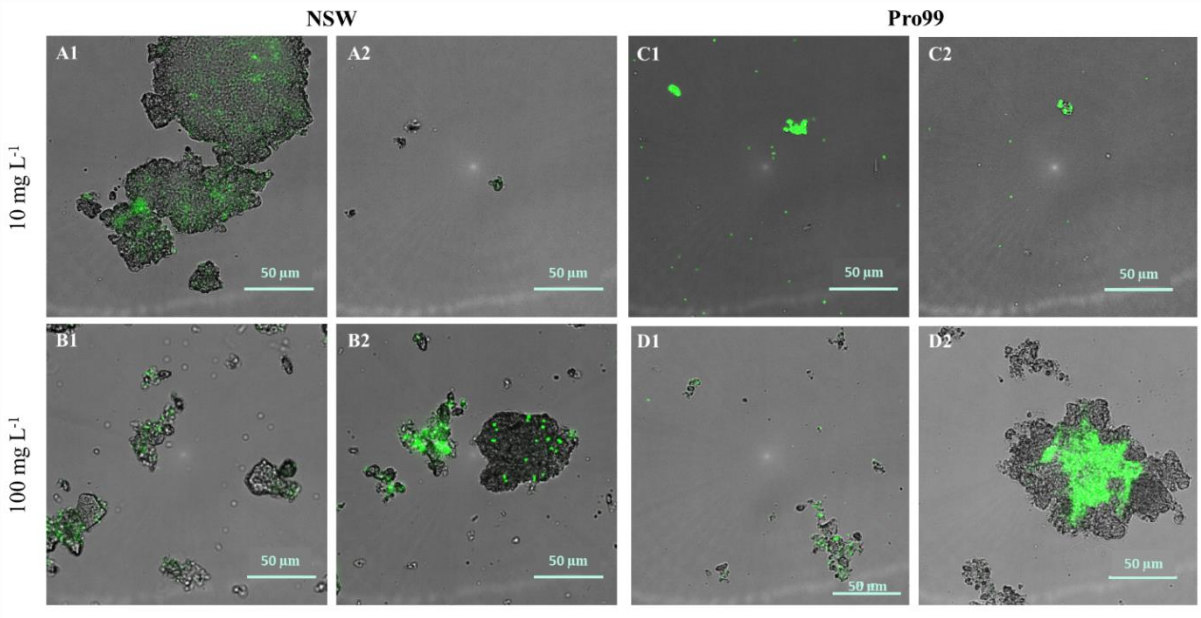

Figure SI.4. Hetero-aggregation of *Prochlorococcus sp.* MED4 (green) and $nCeO_2$ (10 and 100 mg L^{-1}) formed in natural oligotrophic seawater (A; 10 mg L^{-1} and B; 100 mg L^{-1}) and Pro99 media (C; 10 mg L^{-1} and D; 100 mg L^{-1}). Images were captured using fluorescent microscopy under brightfield and GFP fluorescent and subsequently were merged.

SI.5 References

1. Moore, L. R.; Post, A. F.; Rocap, G.; Chisholm, S. W., Utilization of different nitrogen sources by the marine cyanobacteria Prochlorococcus and Synechococcus. *Limnology and Oceanography* **2002,** *47* (4), 989-996.

2. Tyler, R. H.; Boyer, T. P.; Minami, T.; Zweng, M. M.; Reagan, J. R., Electrical conductivity of the global ocean. *Earth Planets Space* **2017,** *69*, 156.