

Supporting Information

MANUSCRIPT TITLE: Microplastic Impacts on Microalgae Growth: Effects of Size and Humic Acid

AUTHORS: Ge Liu, Ruifen Jiang, Jing You, Derek C. G. Muir, Eddy Y. Zeng

ADDRESS: Guangdong Key Laboratory of Environmental Pollution and Health, School of Environment, Jinan University, Guangzhou 511443, China and National Water Research Institute, Environment Canada, 867 Lakeshore Road, Burlington, Ontario L7R 4A6, Canada

NO. OF TEXTS: 2

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NO. OF FIGURES: 6

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Text S1. Scanning Electron Microscopy

The morphology of PS and algae after exposure in different treatments was obtained by scanning electron microscopy (SEM; Hitachi SU8010, Japan). The microalgae cells were exposed in suspensions with different treatments for 24 h. The concentrations of PS beads and HA were 50 mg L⁻¹ and 10 mg L⁻¹, respectively. Then the cells were collected by centrifugation (15000 rpm; 5 min), fixed with 2.5% glutaraldehyde for 5 h, and then washed by phosphate buffer saline buffer with pH=7.4. The fixed algae sample was dehydrated by ethanol solutions with a concentration gradient of 30%, 50%, 70%, 90%, 95%, and 100%. One milliliter of each ethanol solution was added into the sample, and removed by centrifugation after 15 min incubation. After dehydration, the samples were freeze-dried at -80 °C for 12 h before SEM imaging.

Text S2. Reactive Oxygen Species Assay

Intracellular reactive oxygen species (ROS) levels produced by *S. obliquus* exposed to five PS suspensions with concentration of 50 mg L⁻¹ were measured after 24 h using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) oxidant-sensitive probe following the instructions of the ROS kit. First, *S. obliquus* cells were separated from the exposure suspension by centrifuging at 12000 rpm for 5 min. Second, the cells were suspended in 1 mL 10 µM DCFH-DA solution, incubated at 37 °C for 20 min, and thoroughly mixed every 3–5 min. Third, the microalgae cells were washed three times with a phosphate saline buffer solution, and residual DCFH-DA outside the cells was removed. Flow cytometry with Kaluza analysis software (Beckmann Kurt, Brea, USA) was used to measure the fluorescence intensity of each sample with excitation and emission wavelengths at 488 and 525 nm, respectively. The concentration of algae used in this experiment was 2 × 10⁶ cells mL⁻¹.

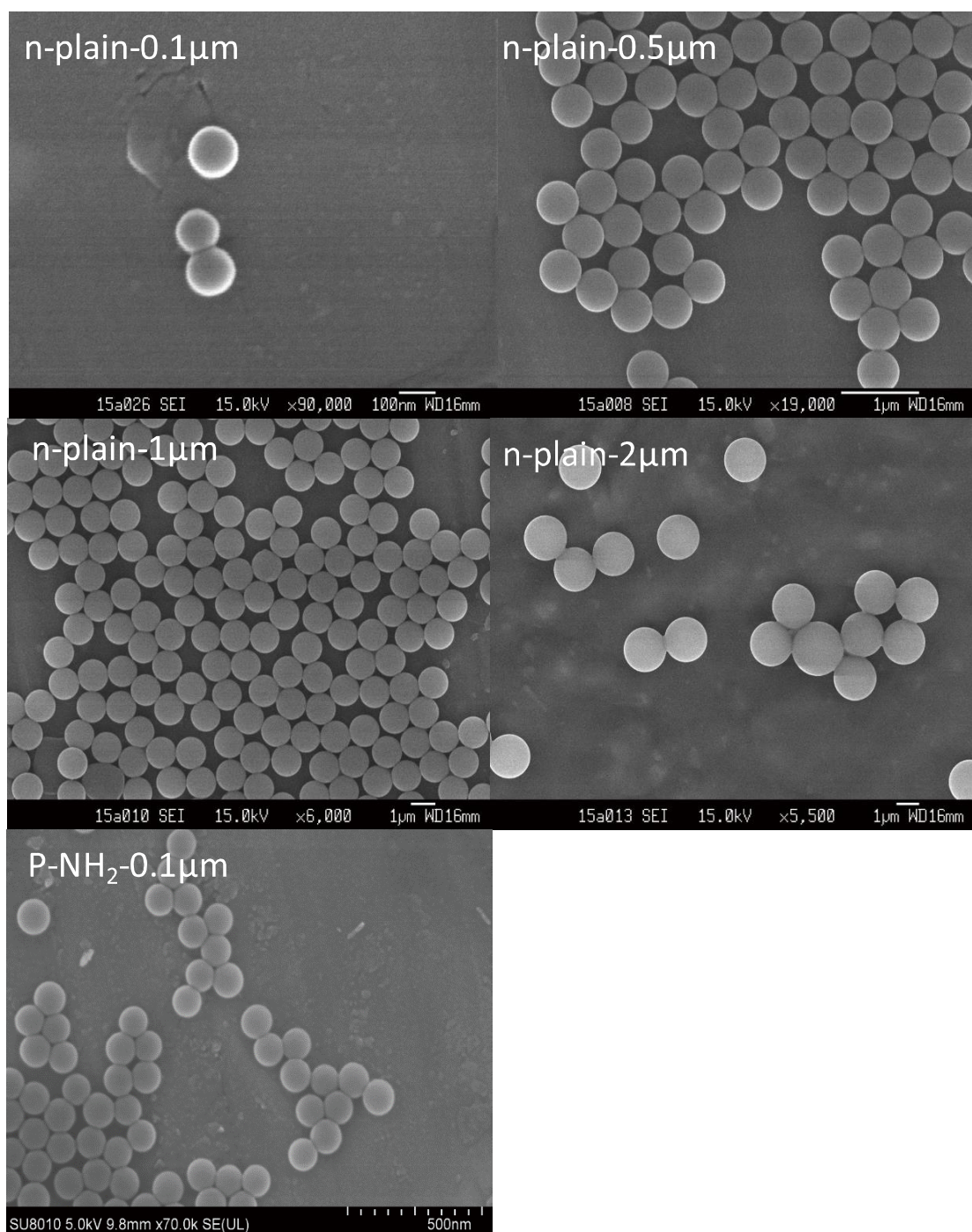
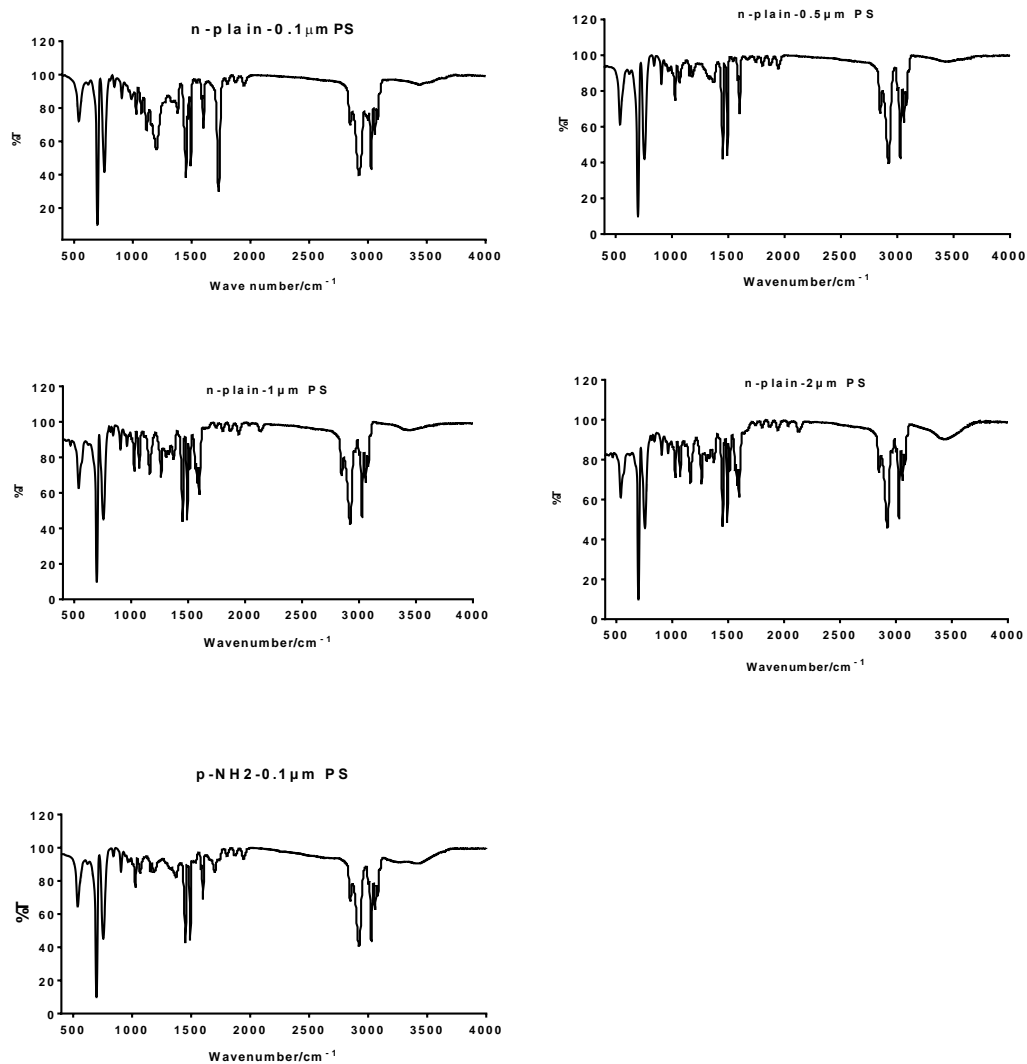


Figure S1. Morphology of 5 types of MPs



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Figure S2. Infrared spectra of 5 types of MPs.

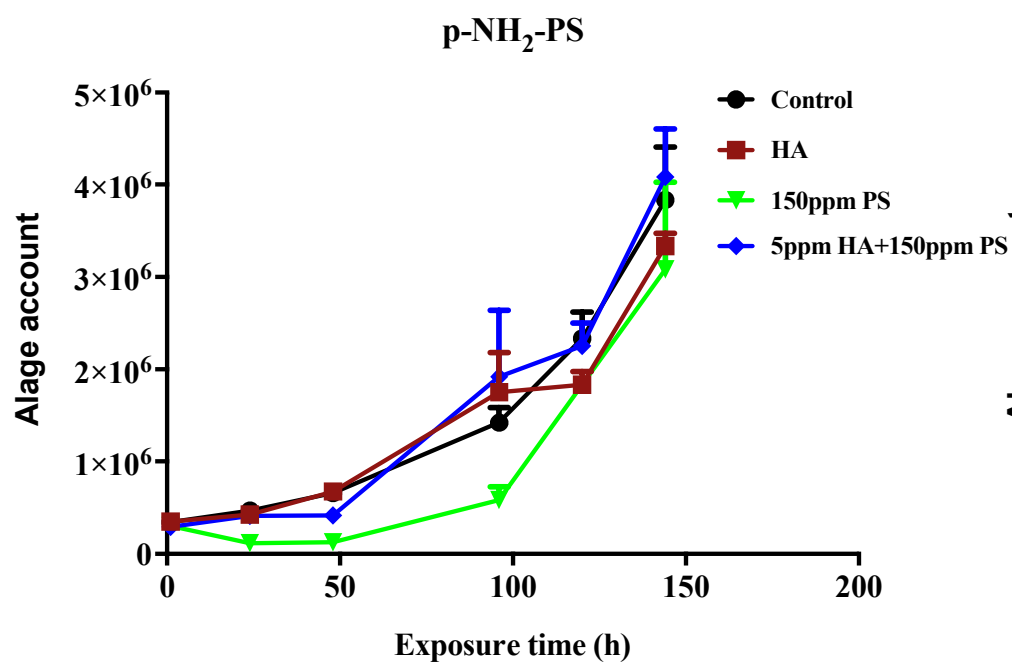


Figure S3. Growth curves of *S. obliquus* in suspensions with and without p-NH₂-PS.

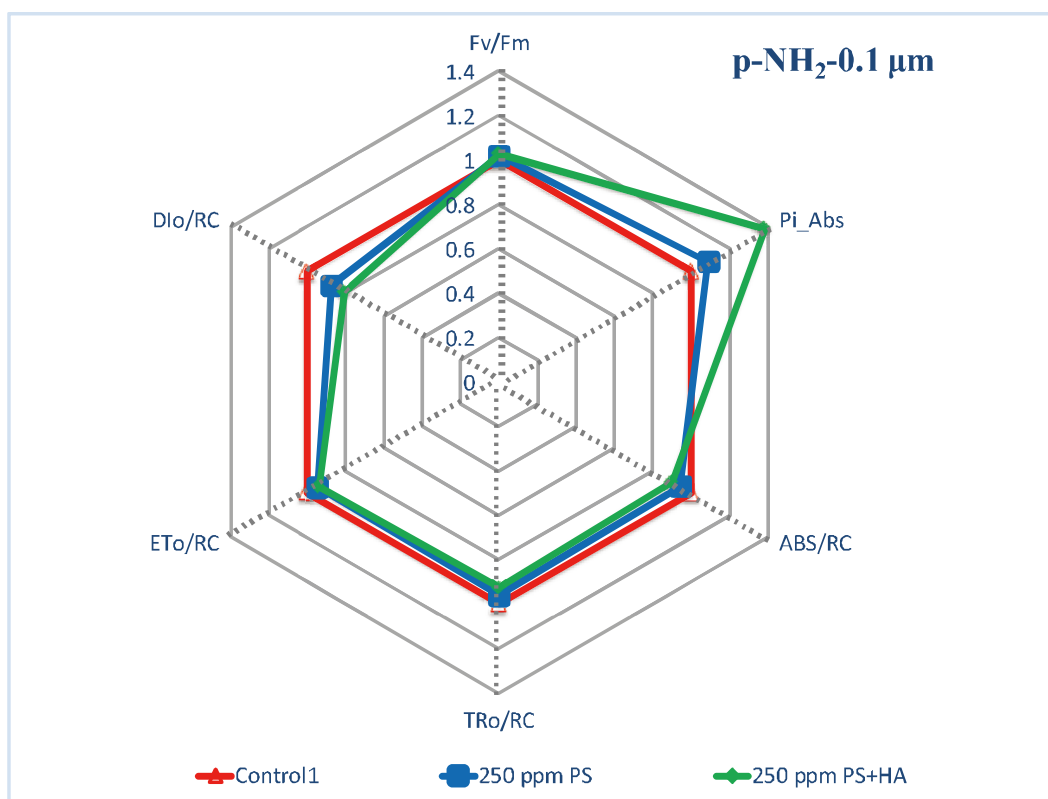


Figure S4. OJIP parameters of microalgae exposure in p-NH₂-0.1 μm suspension after 48 h. Each value was defined as the ratio of control. The concentration of HA, PS and *S. obliquus* was 15 mg L⁻¹, 250 mg L⁻¹ and 2×10^6 cells mL⁻¹, respectively.

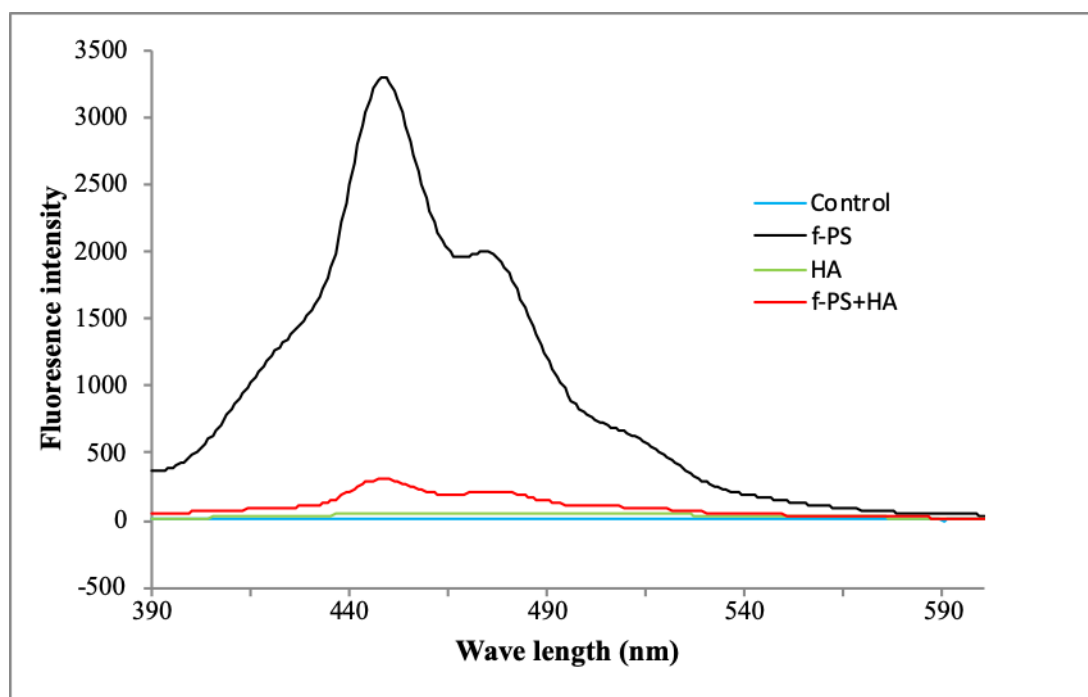


Figure S5. Fluorescence intensity of f-PS-0.1 μm in the presence or absence of HA, wherein the concentrations of f-PS and HA were 100 mg L^{-1} and 50 mg L^{-1} , respectively.

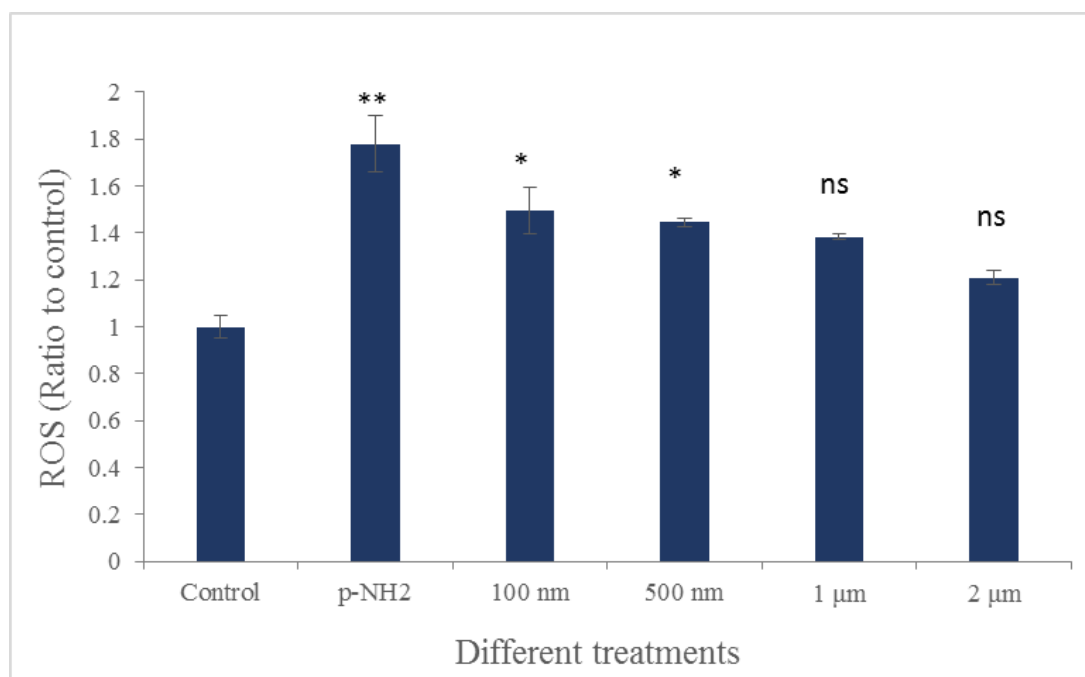


Figure S6. ROS produced in different treatments compared to the control group. The exposure concentration of MPs was 50 mg L⁻¹, and the algae concentration was 2×10⁶ cells mL⁻¹. (**, p<0.01; *, p<0.05)

61 **Table S1 Diameters and Characteristic of the PS beads in this study.**

Plastic Type	Diameter (nm)	HD (nm)	ζ -p (mV)	IR50 (mg L ⁻¹)
n-plain-PS-0.1 μ m	100	111	-28	61(28-133)
n-plain-PS-0.5 μ m	500	592	-23	7.5(5.8-9.7)
n-plain-PS-1 μ m	1000	2160	-12	33(14-80)
n-plain-PS-2 μ m	2000	4296	-24	22(6.8-70)
p-NH ₂ -PS-0.1 μ m	100	183	42	24(10-53)

62 HD: Hydrodynamic diameter; IR50: 50% growth inhibition rate; ζ -p: ζ -potential

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