Supporting Information

Factors determining the toxicity of engineered nanomaterials to *Tetrahymena thermophila* in freshwater: the critical role of organic matter

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The accumulation of MWCNTs in *T. thermophila*

The accumulation of MWCNTs in *T. thermophila* was measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)[1, 2]. Cell pellets were homogenized with 0.2mL solution containing 1% SDS, 1 mM $MgCl_2$ and 1 mM $CaCl_2$ by using an ultrasonic cell processor. Then, the homogenate was incubated at 37℃ for 2 h. Next, the homogenate was treated with 20 μg deoxyribonuclease I at 37 °C for 2 h to further degrade the DNA and reduce the viscosity. Next, the homogenate was centrifuged at 16,000rpm for 20min, and the precipitate was resuspended with 0.2mL 20% glycerin. The samples were added to the wells of gel, and a 100 V electric current was constantly operated for 1 h. Finally, the optical images of the gels were taken.

TEM imaging of *T. thermophila*[3]

After 6 h exposure, the agglomerated nanomaterials in the exposure medium were removed by centrifugation at 1000rpm for 1min. And the cells exposed to nanomaterials alone were collected by centrifugation at 2500rpm for 5min and

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Electronic supplementary information (ESI) available

resuspended with 1mL PBS containing 2.5% v/v glutaraldehyde and fixated overnight. Then the cells were stained using osmium acid (1%) for 2 h and then carefully washed with 0.1 mol/L PBS (pH 7.0) for 3 times. The cell pellets were dehydrated with gradually increasing ethanol gradient and acetone. Next, the cell pellets were embedded at 70 ℃ for 12 h, and the embedded block was cut into ultrathin sections of 70-90 nm and placed on the copper grid and stained with lead citrate and 4% uranium dioxide acetate for 10 min respectively. Finally, the distribution of nanoparticles in cells was observed by TEM (Hitachi H7650, Japan) at 120 kV.

The determination of ROS content using 2',7'-dichlorodihydrofluorescein diacetate

T. thermophila in ultrapure water was incubated with a 25 μmol/L solution of H_2DCF-DA for 15 min at 25 ℃ before being exposed to nanomaterials. After incubation, the exposed solution containing the fluorescence probe was removed by centrifugation at 2500 rpm for 5 min, and the collected cells were subsequently washed with ultrapure water two times. Then, the cells were resuspended to 1 x 10^6 cells/mL in freshwater and mixed with the exposed medium. After 6 h exposure, the agglomerated nanomaterials in the exposure medium were removed by centrifugation at 1000 rpm for 1 min. The exposure medium was removed by centrifugation at 2500 rpm for 5 min and the cell pellets were resuspended with 1 mL freshwater. The fluorescence spectrum of dichlorofluorescein (DCF) was determined on a fluorescent spectrometer (F-7000, HITACHI, Japan) at Ex/Em = 485/523 nm. The results are presented as the value of the exposure group divided by the control group in freshwater.

The determination of MDA content by thiobarbituric acid (TBA) method[4]

After 6 h exposure, the exposed medium was removed by centrifugation as before and the cells were broken with 1 mL PBS in an ice bath for 1 min using an ultrasonic cell processor. The homogenate was centrifuged at 16,000 rpm for 20 min, and the supernatant was used for subsequent experiments. Next, the MDA contents and protein concentrations of the samples were measured by using MDA and total protein quantitative assay kits (Nanjing Jiancheng Bioengineering Institute, China)

following the manufacturer's instructions.

The determination of cell membrane damage using ethidium bromide

After 6 h exposure, the exposed medium was removed by centrifugation as before. Then, the cell pellets were incubated with 1 mL freshwater containing 1 mg/L EB for 20 min at 25 ℃ at 80 rpm. After incubation, the exposure medium was removed by centrifugation at 2500 rpm for 5 min and the cells were carefully washed with 1 mL PBS twice. Next, the cell pellets were resuspended in 1 mL freshwater, and the fluorescence spectrum was determined on a fluorescent spectrometer at Ex/Em = 510/600 nm. The results are presented as the value of the exposure group divided by the control group in freshwater.

The determination of lysosomal membrane damage using neutral red

After 6 h exposure, the exposed medium was removed by centrifugation as before. Next, the collected cells were incubated with 0.5 mL of a 500 mg/L NR solution for 5 min at 25 ℃. After incubation, the exposure medium was removed by centrifugation at 2500 rpm for 5 min and the cells were carefully washed with 1 mL PBS twice. The cell pellets were fixed with 0.5 mL of a fixative containing 1% v/v formaldehyde and 10 g/L $CaCl_2$. NR was extracted from the lysosomes by adding 0.5 mL of a lysate solution containing 1% v/v acetic acid and 50% v/v ethanol. The solution was placed overnight and then centrifugated at 16,000G for 5 min, and the absorbance of the supernatant was determined at 570 nm. The results are presented as the value of the control group divided by the exposure group in freshwater.

Table S1 the components of Artificial, moderately hard freshwater

	$NaHCO_3$	$CaSO_4$	$MgSO_4$	KCl
Concentration (mg/L)	72	35.6	45	3

Table S2 Physicochemical properties of PS NPs and fPS NPs in the absence or presence of organic matter. *represents $p < 0.05$ compared to the control group in fresh water without organic matter

Samples	Particle size (nm)	Surface coating	Organic matter	Hydrodynamic diameters (nm)	Zeta Potential (mV)
PS NPs	50	None	None	48.27 ± 0.99	- 21.33 ± 1.50
			HA	51.12 ± 0.89*	- 18.33 ± 0.86*
			BSA	49.23 ± 0.71	- 18.63 ± 1.20*
			DB	50.34 ± 0.76*	- 18.70 ± 0.36*
fPS NPs	51	None	None	51.88 ± 0.23	- 22.93 ± 1.70
			HA	51.91 ± 0.59	- 23.73 ± 1.60
			BSA	52.84 ± 0.15*	- 23.90 ± 0.66
			DB	52.17 ± 0.33	- 24.27 ± 0.71

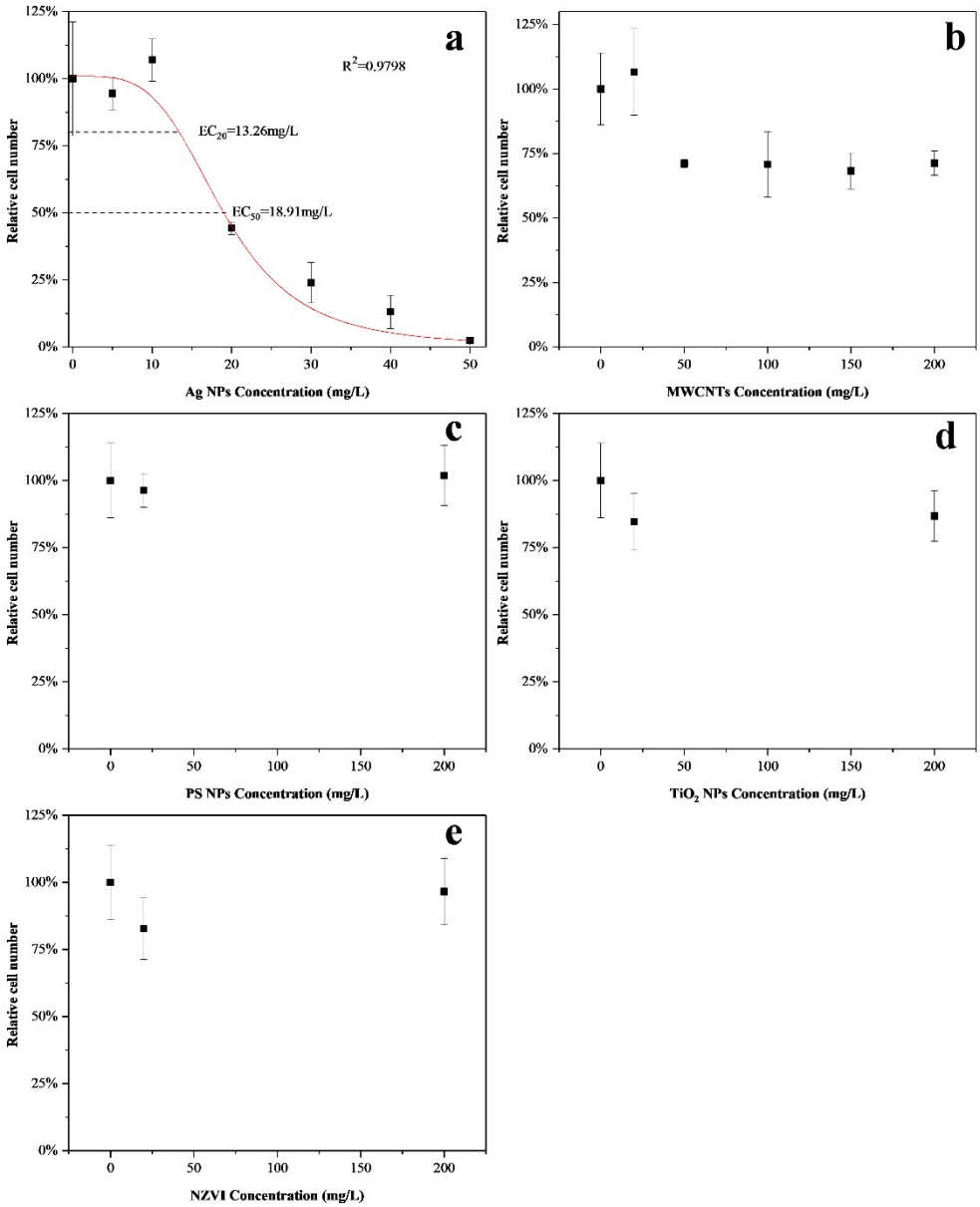

Figure S1 Mortality of *T. thermophila* in the presence of different: a) Ag NPs, b) MWCNTs, c) PS NPs, d) TiO_2 NPs, e) NZVI concentrations. Values are means ± S (n = 3). EC20 and EC50 of Ag NPs for *T. thermophila* were determined using a logistic model by Origin 10.

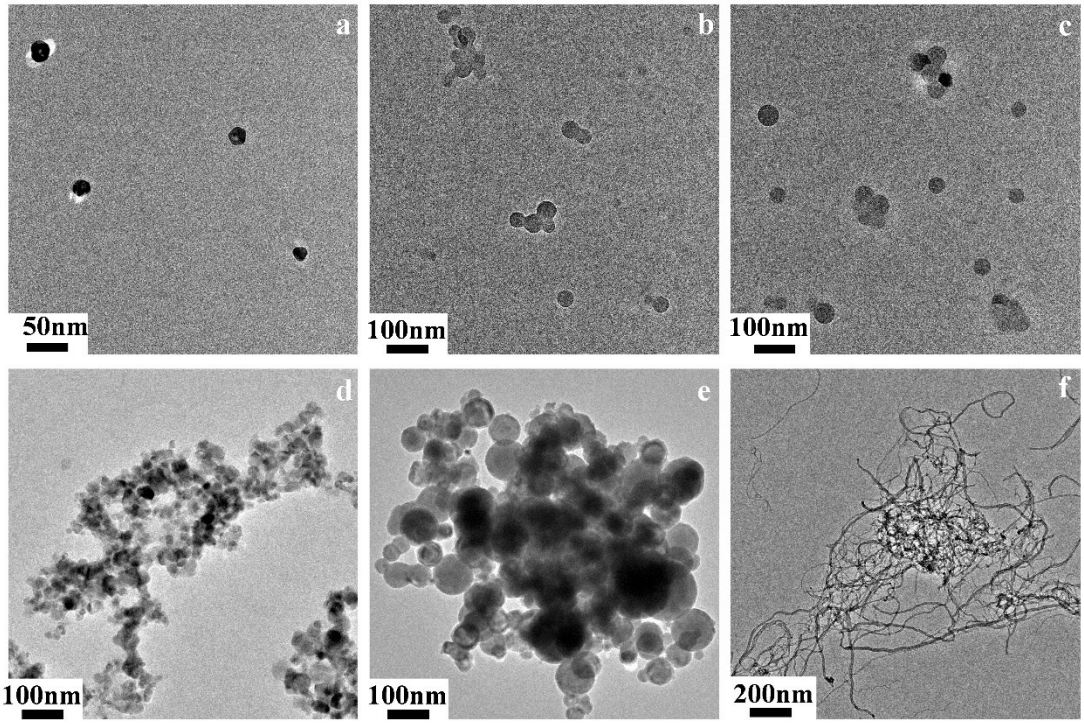

Figure S2 TEM images of nanomaterials in freshwater: a) 5 mg/L Ag NPs, b) 200 mg/L PS NPs, c) 200 mg/L fPS NPs, d) 200 mg/L TiO_2 NPs, e) 200 mg/L NVZI and f) 20 mg/L MWCNTs

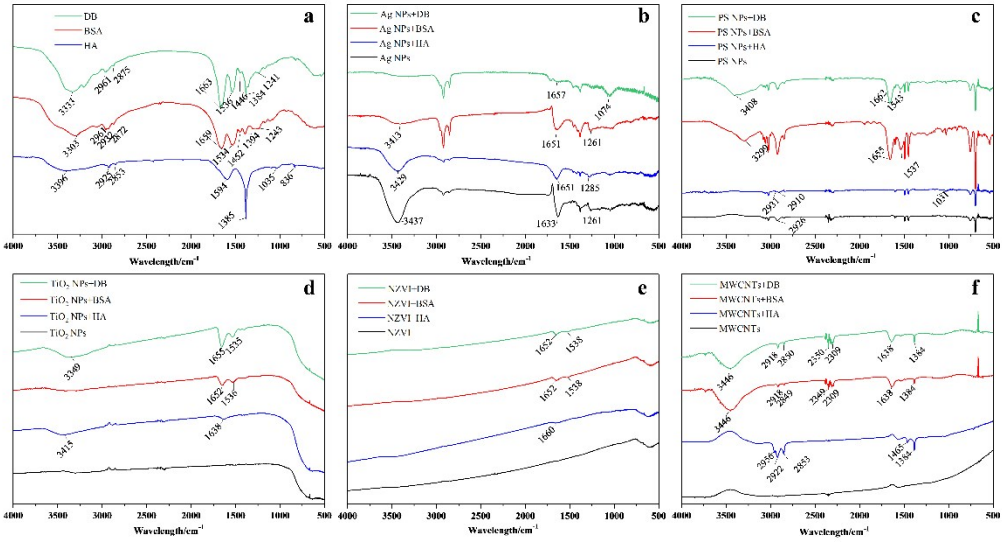

Figure S3 Infrared spectra of: a) organic matter, b) organic matter coated Ag NPs, c) organic matter coated PS NPs, d) organic matter coated TiO_2 NPs, e) organic matter coated NZVI, f) organic matter coated MWCNTs

Table S3 Ion dissolution proportion of nanomaterials after 12 h incubation.

Samples	Elements Determination	None	+HA	+BSA	+DB
Ag NPs	Ag	1.97±0.13%	2.22±0.27%	1.69±0.08%	1.89±0.24%
NZVI	Fe	-	-	-	-
TiO_2 NPs	Ti	-	-	-	-
PS NPs	Ni, Mn, Cu, Cr, Cd	-	-	-	-
fPS NPs	Ni, Mn, Cu, Cr, Cd	-	-	-	-
MWCNTs	Ni, Mn, Cu, Cr, Cd	-	-	-	-

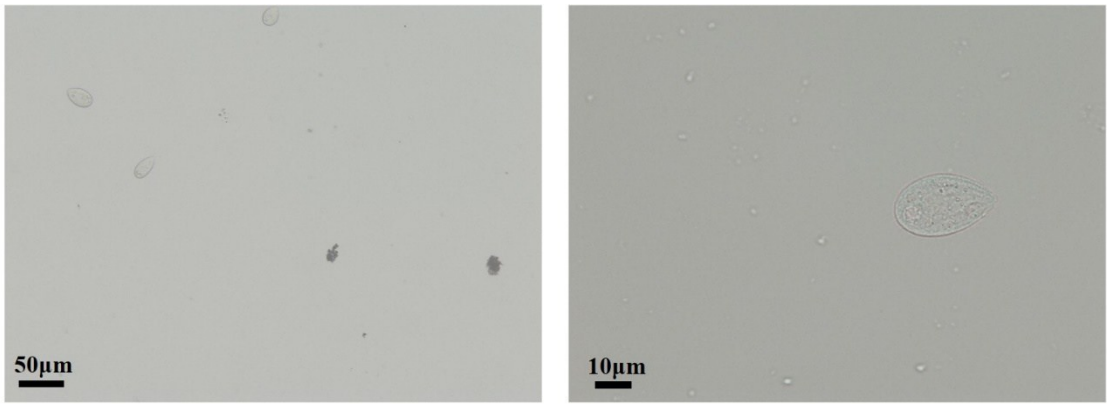

Figure S4 Bright field images of *T. thermophila* exposed to MWCNTs for 6 h.

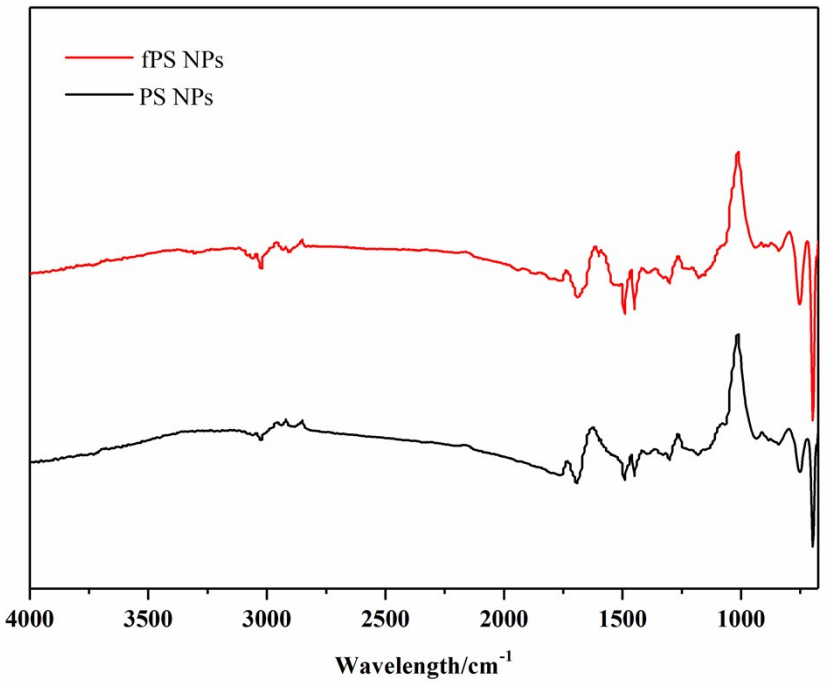

Figure S5 Infrared spectra of of PS NPs and fPS NPs

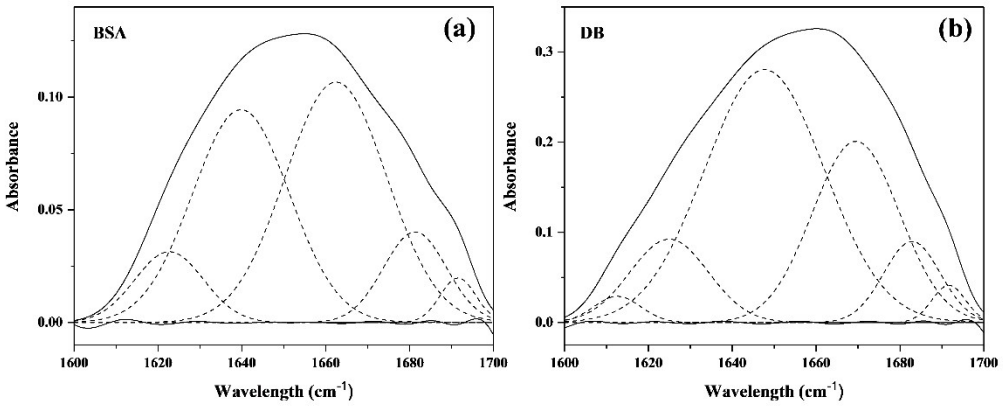

Figure S6 Infrared spectra of: a) BSA, b) DB in the amide I together with the best fitted individual component bands. full and dotted lines indicate the experimental data and fitting Gaussian components, respectively.

Table S4 Infrared spectra peak analysis of BSA and DB in the amide I

Samples	Center wavelength (cm^{-1})	Peak Area Proportion (%)	Samples	Center wavelength (cm^{-1})	Peak Area Proportion (%)
BSA	1623	8.50	DB	1613	2.04
	1640	36.09		1625	10.93
	1662	42.74		1648	50.78
	1681	9.94		1669	26.47
	1692	2.73		1683	7.70
	-	-		1692	2.07

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