Supporting Information

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Importance of sample pretreatment on the bacterial bioassay for toxicity of ZnO nanoparticles

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Table S1: Summary of the various methods applied in previous studies including ZnO NPs characterization and toxicity tests (LB: Luria-Bertani, TS: Tryptic soy, DI: Distilled water, AW: Artificial wastewater)

Study	Type of NPs	Sample preparation		Exposure concentration of ZnO NPs	Test medium properties	Microorganism
		For characterization	For toxicity			
Jiang et al., 2009	Al_2O_3, SiO_2, TiO_2, ZnO	NPs were sterilized in an oven at 160 ^{0}C for 3 h, then dispersed in filter-sterilized DI water to make stock suspensions of 2000 mg/L. The suspensions were placed in an ultra-sound water bath for 30 min to break aggregates before diluting them to the exposure concentrations. NPs stock suspension was diluted to 200 mg/L in 1 g/L NaCl, divided into 10 aliquots of 10 mL, and adjusted to various pH values by 0.01 M HCl or NaOH solution. The samples were stored for 2 h to achieve equilibrium, and put into an ultra-sound bath to disperse aggregates before the zeta potential measurements	For the NPs treatment, 10 mL bacterial suspension was added to a test tube. Then 100 mL NP stock suspension was added to achieve an exposure concentration of 20 mg/L. Bacteria and NPs were fully mixed by vortexing, then the tubes were incubated for 2 h at 30 ^{o}C in a dark shaker. The toxicity was evaluated by comparing the number of colony forming units on TS agar plates with the control after 24 h incubation.	20 mg/L	TS agar	*B. subtilis*, *E. coli*, *P. fluorescens*
Padmavathy and Vijayaraghavan, 2011	ZnO	ZnO NPs were dispersed in 2-propanol.	LB containing ZnO NPs were inoculated with overnight culture of bacteria	10-150 mg	LB broth	*E. coli*, *S. aureus*, *B. subtilis*, *Klebsiella oxytoca*, *Proteus mirabilis*
Baek and An, 2011	CuO, NiO, Sb_2O_3, ZnO	Used as purchased	Used as purchased	0-350 mg/L	LB agar prepared with and without NPs addition	*E. coli*, *B. subtilis*, *S. aureus*
Bellanger et al., 2015	ZnO	Pellets of ZnO were dispersed in water and vortexed prior to use	Suspensions dispersed in water were sonicated for 1 min in culture medium	$7x10^{-6}$ - $2x10^{-3}$ M Zn eq.	LB broth	*E. coli*, *C. metallidurans*
Baek et al., 2017	ZnO	Not stated	Sequential dilution in LB broth followed by a minute of sonication	20-1280 mg/L	LB broth and agar	*E. coli*
Mallevra et al., 2014	Ag, TiO_2, ZnO,	NPs were received conditioned under argon and preserved in the dark until use. Stock suspensions were freshly prepared in matrices of exposure (LB and AW) prior to each experiment, sonicated, then serially diluted to give final concentrations per well ranging from 0 to 200 mg/L.	NPs was tested in 96-well flat bottomed black microtitre plates by real time monitoring of the emitted luminescence evolution (i.e. reduction). Assays were realised in 100% LB or 100% AW with final element and cell concentrations of 0-200 mg/L and 108 cfu/mL, respectively, in a final volume of 100 mL per well. Plates were incubated at 28 ^{o}C in the reader and the luminescence monitoring undertaken in a kinetic mode with measurements taken every 15 min for 120 min.	0-200 mg/L	Bacteria were pre-cultured either in LB laboratory rich medium or in AW consisting of AB mineral medium supplemented with 0.5% (w/v) of glucose as carbon source). AB mineral medium with 0.5% glucose has been proposed as AW due to being similar in composition to AW	*P. putida*

Baysal et al., 2019	TiO_2, ZnO	NPs were treated with sea water and 1:10 diluted sea water with ultra-pure watre for 24 h, non-treated NPs were used as control.	Growth inhibition of bacteria including NPs were treated with Nurient agar prepared with, ultra-pure water, sea water and sea water diluted with1:10 ultra-pure water colony counting method. 24 h incubation	5-100 mg/L	Nutrient agar prepared with (i) ultra-pure water, (ii) sea water (iii) sea water diluted with1:10 ultra-pure water	*E. coli,* *P. aeruginosa,* *B. subtilis,* *S. aureus*
Baysal et al., 2018	TiO_2, ZnO	NPs were treated with 2.5 µg/L and 25µg/L of PM2.5 airborne particles for 24 h, non-treated NPs were used as control.	Growth inhibition of bacteria including NPs were treated with Nurient agar prepared with, ultra-pure water, 2.5 µg/L and 25µg/L of PM2.5 airborne particles colony counting method. 24 h incubation	5-100 mg/L	Nutrient agar prepared with (i) ultra-pure water, (ii) 2.5 µg/L PM2.5 airborne particulate in aqueous solutions (iii) 25 µg/L PM2.5 airborne particulate in aqueous solutions	*E. coli,* *P. aeruginosa,* *B. subtilis,* *S. aureus*
Bondarenko et al., 2016	Ag Au CuO TiO_2 MWCNTs SiO_2 ZnO	The stock suspensions of NPs (except MWCNTs, Ag and Au NMs) were prepared at nominal concentrations of 5000 mg metal/L in sterile DI, homogenized using ultrasonic probe immediately after the preparation of the suspensions at continuous mode without temperature adjustment and left for 2 days to equlibrate. Stock suspensions were stored in the dark at room temperature for up to 2 weeks. 100 mg/L NPs were used for the characterization.	The initial optical density (OD_{600}) of bacterial suspension was measured, and the 96-well plates were incubated statically at 30 °C during assay and were shaken once before each measurement. 4 h incubation	0.1- 100 mg/L	LB medium without NaCl. Before the test, bacterial culture was diluted in NaCl-free LB medium.	*E. coli* *S.aureus* *B. subtilis* *P.putida* *P.aeruginosa* *V. fischeri*
Aruoja et al., 2015	Al_2O_3, Co_3O_4, CuO, Fe_3O_4, MgO, Mn_3O_4, Sb_2O_3, SiO_2, TiO_2, ZnO, WO_3 Pd	Suspensions were vortexed and sonicated and diluted in three chosen media for stability, and zeta potential	Suspensions were vortexed and sonicated and diluted in three chosen media and the 96-well plates were incubated at 25 °C during assay without shaking in the dark. 24 h incubation	0.01-100 mg/L	Nutrient agar prepared with (i) distilled water, (ii) algal test medium, (iii) 2% NaCl	*V. fischeri,* *E.coli,* *S. aureus,* *Tetrahymena thermophila,* *Pseudokirchneriella subcapitata*

Table S2: Chemical parameters analyzed in seawater samples, and related information about the procedure

Parameter	Method	Instrument	Reference
SO_4^{2-}	Turbidimetric as barium sulfate (375.4): Sulfate ion is converted to a barium sulfate suspension under controlled conditions. The resulting turbidity is determined spectrophotometrically at 420 nm.	UV-VIS spectrometry (Biochrom Libra S70 spectrophotometer)	Water and Environmental Analysis 2010; Environmental Monitoring Systems Laboratory (EMSL) 1983
NO_3^-	Sulfanilamide/ethylenediamine with Cd reduction (353.3): The nitrite (that originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is measured spectrophoto-metrically at 540 nm	UV-VIS spectrometry (Biochrom Libra S70 spectrophotometer)	Water and Environmental Analysis. Perkin Elmer. 2010; Environmental Monitoring Systems Laboratory (EMSL) 1983; American Public Health Association 1992.
NH_4^+	Nesselerization (APHA 4500): The sample is buffered at a pH of 9.5 with a borate in order to decrease hydrolysis of cyanates and organic nitrogen compounds and is then distilled into a solution of boric acid. The ammonia in the distillate is determined colorimetrically by Nesslerization at 425.0 nm by spectrometrically.	UV-VIS spectrometry (Biochrom Libra S70 spectrophotometer)	Water and Environmental Analysis. Perkin Elmer. 2010; Environmental Monitoring Systems Laboratory (EMSL) 1983; American Public Health Association 1992
Cl^-	Chromatographic separations were performed at 30 °C with a Dionex IonPac AS20 analytical column (2 × 250 mm). In addition, guard column and cartridge using ultra-pure (UP) water obtained from Dionex. The gradient programme: 10 mM of KOH for 6 min; linear increase of the KOH concentration from 10 mM to 25 mM for 15 min; 25 mM of KOH for 4 min; linear increase of the KOH concentration from 25 mM to 40 mM for 5 min; 40 mM of KOH for 5 min; linear decrease of the KOH concentration from 40 mM to 10 mM for 2 min. A 75 μL-aliquot of the sample/standard solution was loaded into the eluent stream. Flow rate of 2.5 mL/min.	Ion chromatograpy (Dionex ICS-3000)	Baysal et al. 2017 doi:10.1007/s10661-017-5982-7
Na, K	Direct analysis of seawater samples according to the EPA 200.5	ICP-OES (Spectro, SpectroBlue)	EPA Method 200.5, Determination of Trace Elements in Drinking Water by Axially Viewed Inductively Coupled Plasma-Atomic Emission Spectrometry
PO_4^-	Ammonium molybdate solution acidified with H_2SO_4 was added onto the extracted samples along with excess ascorbic acid. The formation of the green/blue color was observed after heating them in the water bath. Colorimetric measurements were taken both at 822 nm and 650 nm for the purpose of comparison.	UV-VIS spectrometry (Biochrom Libra S70 spectrophotometer)	EPA Method 365.3: Phosphorous, All Forms (Colorimetric, Ascorbic Acid, Two Reagent)

Table S3. Chemical properties of applied sea water as a treatment solution (N:3, SD<10)

Chemical Property	**Results**
pH	8.28±0.82
Na (mg/L)	6693±535
K (mg/L)	230.6±9.2
NO_3 (mg/L)	0.255±0.015
NO_2 (mg/L)	0.0052±0.0005
NH_3-N (mg/L)	0.916±0.064
SO_4 (mg/L)	2282.8±190.4
PO_4 (mg/L)	ND
Cl^- (g/L)	15.98±0.61

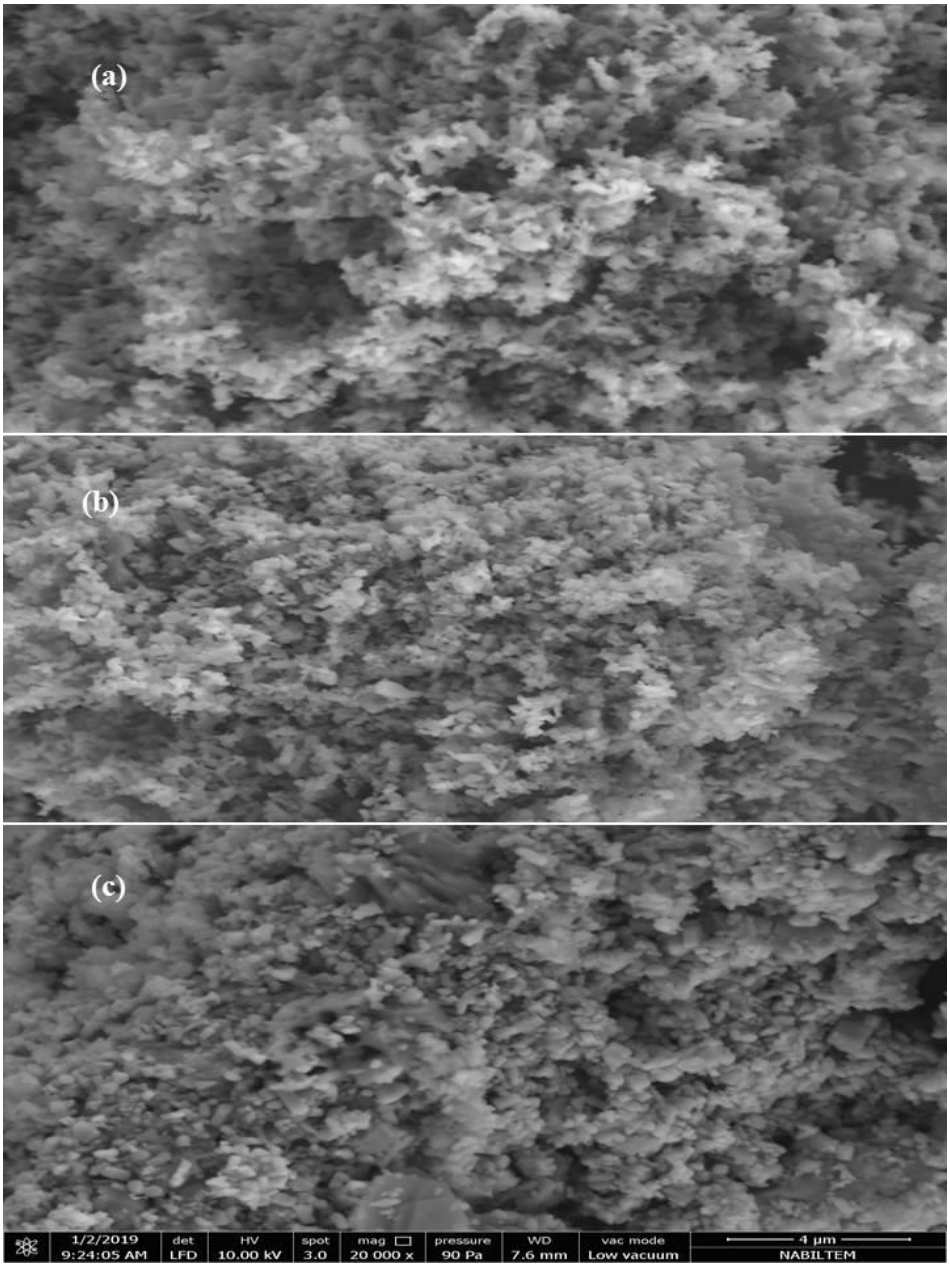

Figure S1: SEM images of ZnO NPs with respect to sample pretreatment

(**a**) Method I, (**b**) Method II, (**c**) Method III.