Salinity-dependent toxicities of zinc oxide nanoparticles to the marine diatom *Thalassiosira pseudonana*

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Introduction & Objectives

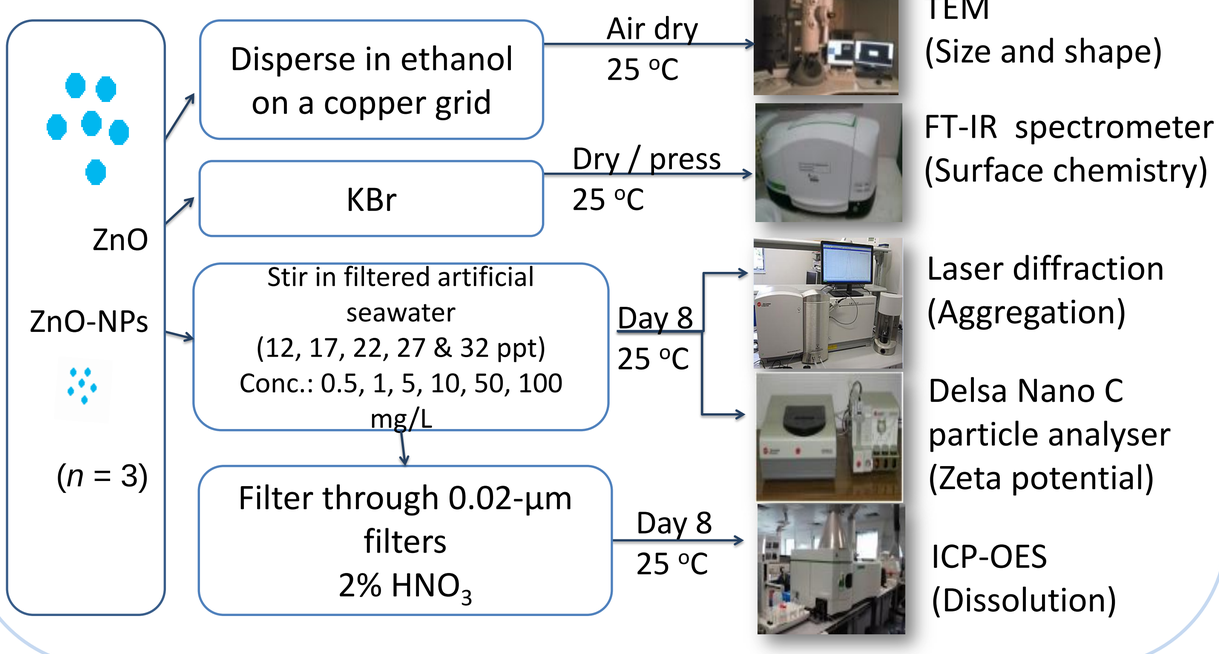
- Engineered zinc oxide nanoparticles (ZnO-NPs), which effectively block the ultraviolet light, have been used extensively in sunscreens.
- Annually, at least 4000 tonnes of sunscreens are directly washed off into the marine environment during swimming [1] and sunscreens can contain up to 25% ZnO-NPs [2].
- The toxicities of ZnO-NPs is suggested to be attributed to the dissolved zinc ions and the nanoparticles [3,4].
- Aggregation and dissolution of ZnO-NPs, hence their toxicities, are related to the salinity of water medium [5,6]. However, little is known about the salinity effect on physicochemical properties of ZnO-NPs and their consequent toxicities to marine organisms.
- The study aims to:

(1) investigate the influences of salinity, exposure concentration and time on the aggregate sizes, surface charge and dissolution of ZnO-NPs in seawater; and

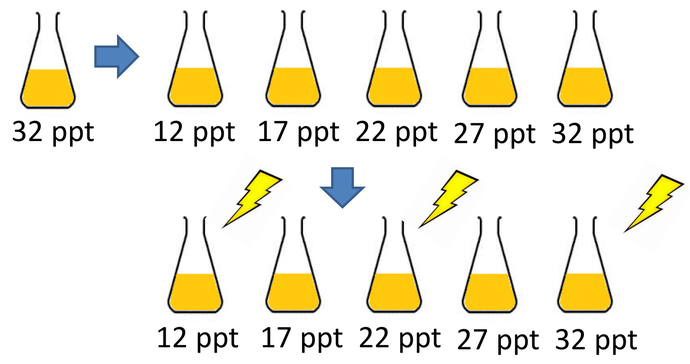
(2) examine the interacting effect of salinity and waterborne exposure of ZnO-NPs to the marine diatom *Thalassiosira pseudonana*.

Materials & Methods

- Physicochemical characterization



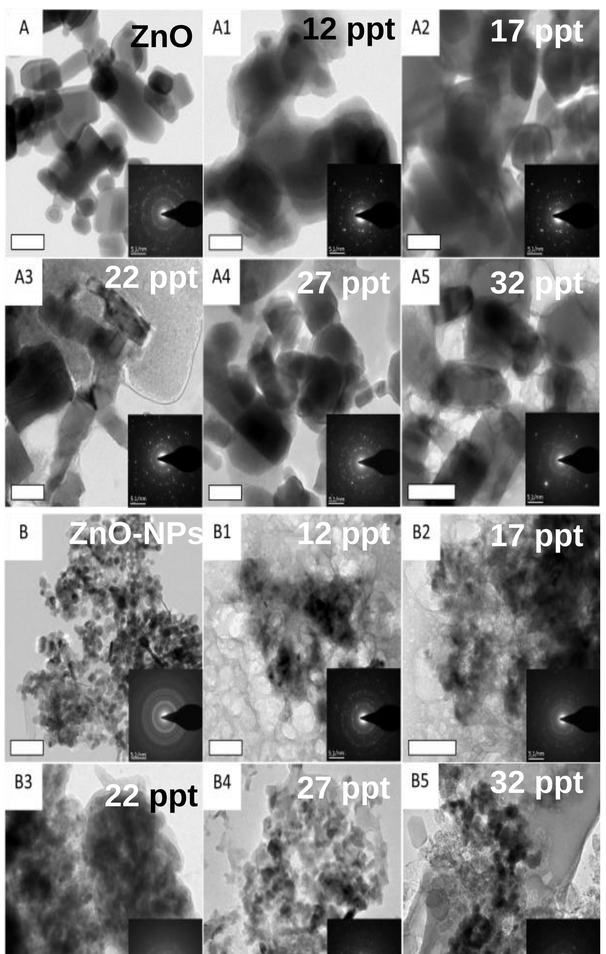
- 96-h algal growth inhibition test



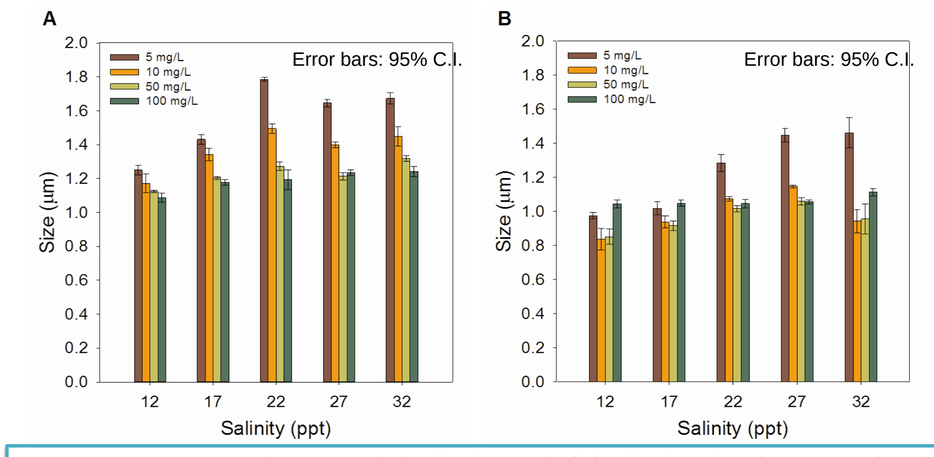
T. pseudonana was acclimated to test salinities for > 5 generations (25 ± 1 °C).

Diatoms were exposed to ZnO, ZnO-NPs and ZnO-NPs ultrafiltrate at concentration 0, 0.5, 1, 5, 10, 50 mg/L. Test concentrations of $ZnCl_2$ matched the $[Zn^{2+}]$ from ZnO-NPs at test concentration and salinity after 8 days (n = 4).

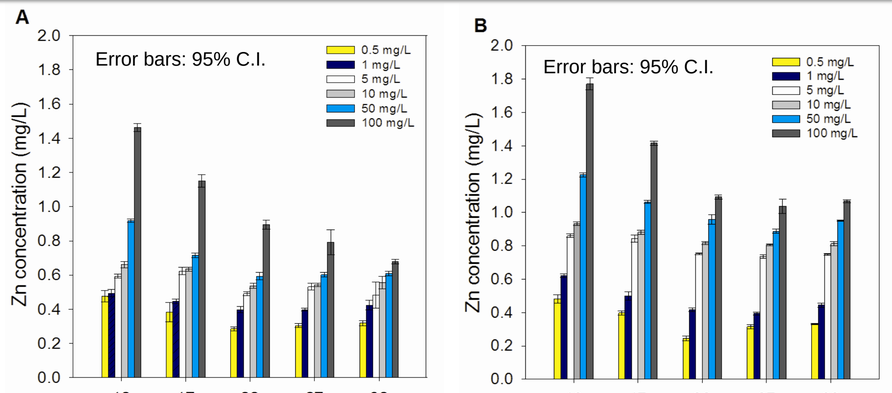
Results & Discussion



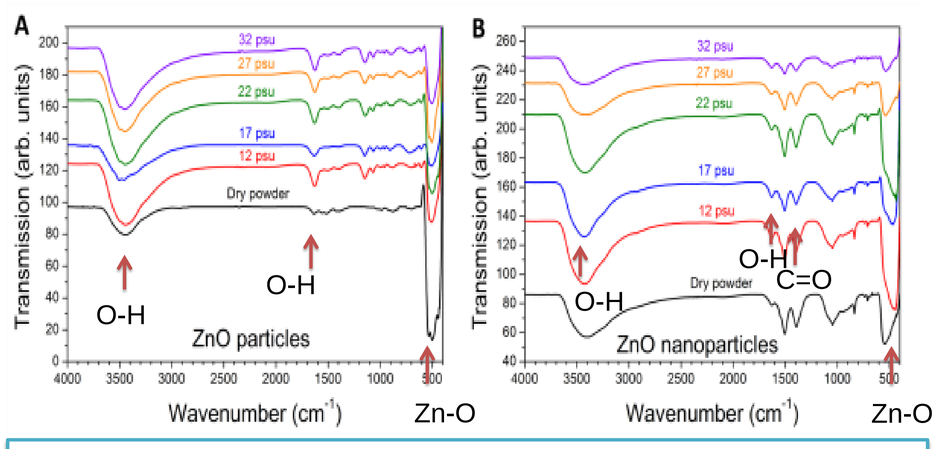
Primary particle size of (A) ZnO was significantly larger than (B) ZnO-NPs (t-test; p < 0.001). Both ZnO and ZnO-NPs formed aggregates in filtered artificial seawater at all test salinities (A1-5; B1-5). Inset: electron diffraction pattern of the particles.
Scale bar = 100 nm.



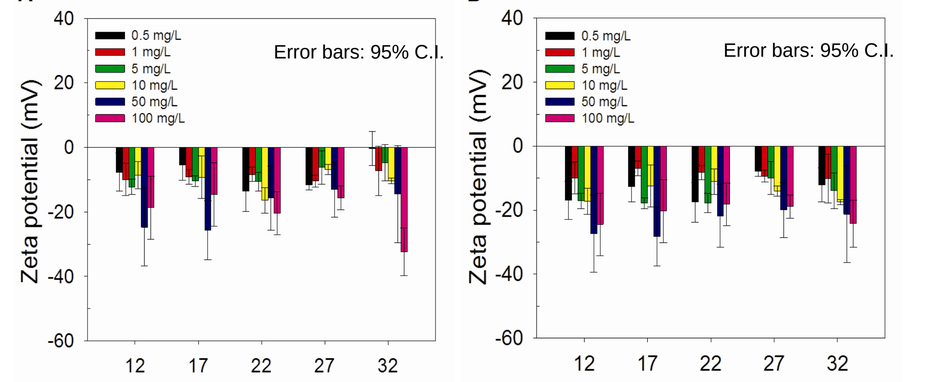
Mean aggregate sizes of (A) ZnO and (B) ZnO-NPs increased with increasing salinities from 12 to 22 ppt (Three-way ANOVA: particle size x concentration x salinity; p < 0.001) due to ionic strength effect, but there was no significant increase in aggregate size from 22 to 32 ppt (Tukey's post-hoc test; p > 0.05), probably because of the particle interfacial electrostatic repulsion [7].



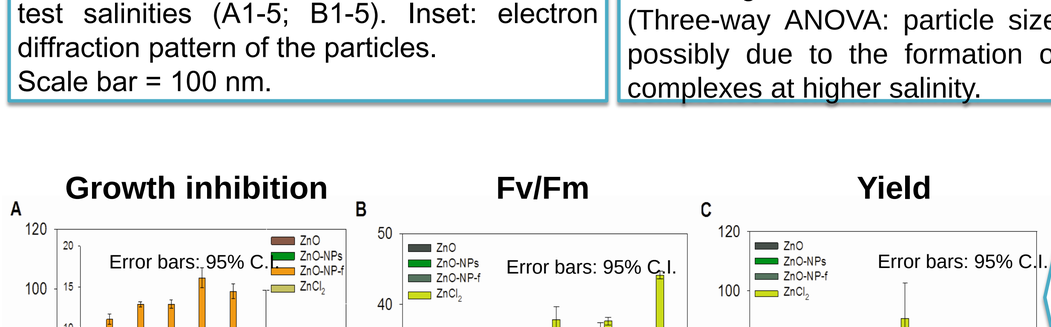
Zn^{2+} ions dissolved from (A) ZnO and (B) ZnO-NPs decreased with increasing salinities from 12 to 22 ppt but levelled off from 22 to 32 ppt (Three-way ANOVA: particle size x concentration x salinity; p < 0.001), possibly due to the formation of larger aggregates and insoluble zinc complexes at higher salinity.



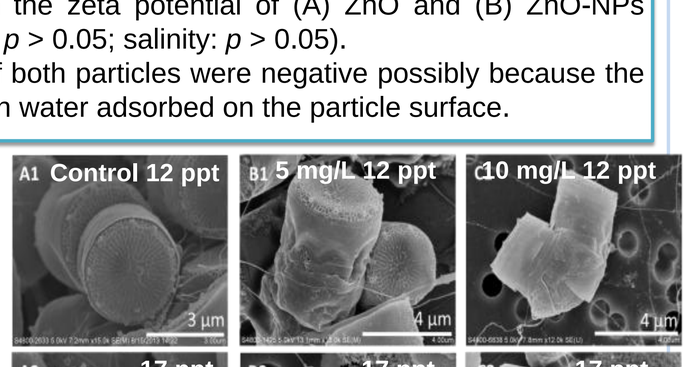
The peaks at 1350 – 1600 cm^{-1}, which corresponded to the vibrations of the C-H, C-O and C=O, in the FT-IR spectra of (B) ZnO-NPs suggested the presence of residue of zinc acetate, a precursor material for ZnO-NPs synthesis [8].



Salinity, concentration and particle size did not have significant interaction effect on the zeta potential of (A) ZnO and (B) ZnO-NPs (Three-way ANOVA; p > 0.05; salinity: p > 0.05).
The zeta potential of both particles were negative possibly because the SO_4^{2-} and OH^- ions in water adsorbed on the particle surface.



- Toxicities of the 4 test zinc compounds on *T. pseudonana* generally decreased with increasing salinities from 12 to 27 ppt (Two-way ANOVA: salinity x chemical;
  growth: p < 0.001; Fv/Fm: p < 0.001; yield: p < 0.001).
- Toxicities: ZnO-NPs > ZnO > ZnO-NPs ultrafiltrate > $ZnCl_2$ (Tukey's test, p < 0.05).
- Toxicity of ZnO-NPs could be partially explained by their size and Zn^{2+} release.



- *T. pseudonana* in control group at 5 test salinities (A1-5) had intact cell surface and girdle band. The diatoms exposed to ZnO-NPs at 5 mg/L (B1-5) and 10 mg/L (C1-5) showed girdle band deformation and irregular cell outlines.
- ZnO-NPs aggregates adsorbed onto the diatom frustule surface. Interaction between nanoparticles and diatom may also contribute to the toxicity of ZnO-NPs.

Conclusions

- Change in salinity altered the physicochemical properties of ZnO-NPs in seawater, hence their toxicities to marine organisms.
- Increase in salinity led to the formation of larger aggregates and reduction of free Zn^{2+} concentration in seawater.
- The toxicities of 4 test zinc compounds on the *T. pseudonana* generally decreased with increasing salinity.
- The bioavailable zinc ion dissolved from ZnO-NPs could partially illustrate the toxic mechanisms of ZnO-NPs.
- Adsorption of ZnO-NPs onto the diatom frustule surface suggested the interaction between the nanoparticles and the cell surface may lead to the growth inhibition and influence normal cell activities as illustrated above.

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Cited references: [1] Danovaro et al. (2008) *Environ Health Perspect* 116:441-447. [2] SCCS (2012) *COLIPA S 76*. [3] Miao et al. (2010) *Environ Toxicol Chem* 29:2814-2822. [4] Manzo et al. (2013) *Sci Total Environ* 445-446:371-376. [5] Nugegoda & Rainbow (1989) *Mar Ecol Prog Ser* 55:149-157. [6] Auffan et al. (2014) *Nanotoxicol* 8(S1):167-176. [7] Brant et al. (2007) *Environ Nanotechnol*; McGraw-Hill, 231-294. [8] Hsieh (2007) *J Chin Chem Soc* 54:31-34.