

The role of titanium dioxide nanoparticles in the elevated uptake and retention of cadmium and zinc in *Daphnia magna*

Cheng Tan^{1,2}, Wen-Hong Fan¹ and Wen-Xiong Wang^{2,*}

¹*Department of Environmental Science and Engineering, Beihang University, Beijing 100083, China*

²*Division of Life Science, The Hong Kong University of Science and Technology (HKUST), Clear Water Bay, Kowloon, Hong Kong*

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Number of Tables: 1 (Table S1)

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Detailed Method:

Quantify nano-TiO₂ concentration in daphnids

After exposure, for each replicate, 20 daphnids were removed and rinsed in M7 medium for about 2 min. The daphnids were subsequently dried in an oven at 80 °C to a constant weight, and then digested in 4 mL concentrated HNO₃ with the addition of 1 mL of hydrogen peroxide to aid tissue digestion. The digests were evaporated to dryness at 110 °C. The TiO₂ concentrations in the digested samples were then determined using the method described by Zhang et al (24). In brief, TiO₂ released by digestion were decomposed into titanium (IV) ion by heating with 1 ml of the sulphuric acid-ammonium sulphate solution. After cooling down, the above solution was transferred quantitatively to a 25 ml volumetric flask. TiO₂ concentration in digested samples was determined by ICP-AES (IRIS Intrepid- II). The recovery for this TiO₂ quantification technique was ranged from 88% to 107%.

(24) Zhang, X.; Sun, H.; Zhang, Z.; Niu, Q.; Chen, Y.; Crittenden, J.C. Enhanced bioaccumulation of cadmium in carp in the presence of titanium dioxide nanoparticles. *Chemosphere*, , **2007**, 67, 160 – 166.

Table S1. Experimental purposes, time conditions and main results

Experiment	Purpose	Exp. Time	Dep. Time	Main results	Compare with dissolved phase
Uptake	Quantify the influx rate of Cd and Zn adsorbed on nano-TiO ₂ in <i>D. magna</i> .	3 h	none	(k_u : L/g/h) Cd: 5.07±0.79 Zn: 8.37±0.97	Cd: 80.6 times higher Zn: 185 times higher (26)
AE	Quantify the influences of nano-TiO ₂ concentrations on Cd and Zn assimilation efficiency in <i>D. magna</i> .	15 min	48 h	(AE: %) Cd: 44.5±2.4-24.6±3.7 Zn: 51.8±5.0-30.4±3.4	
Efflux	Quantify the efflux rate of Cd and Zn adsorbed on nano-TiO ₂ in <i>D. magna</i> .	3 d	7 d	(k_e : /d) Cd: 0.0377±0.008 0.0383±0.018 Zn: 0.087±0.019 0.091±0.022	Cd: 70% lower Zn: 62% lower

a.*Exp.=Exposure and Dep.=Depuration.

b. Values in table S1 are 95% confidence intervals.

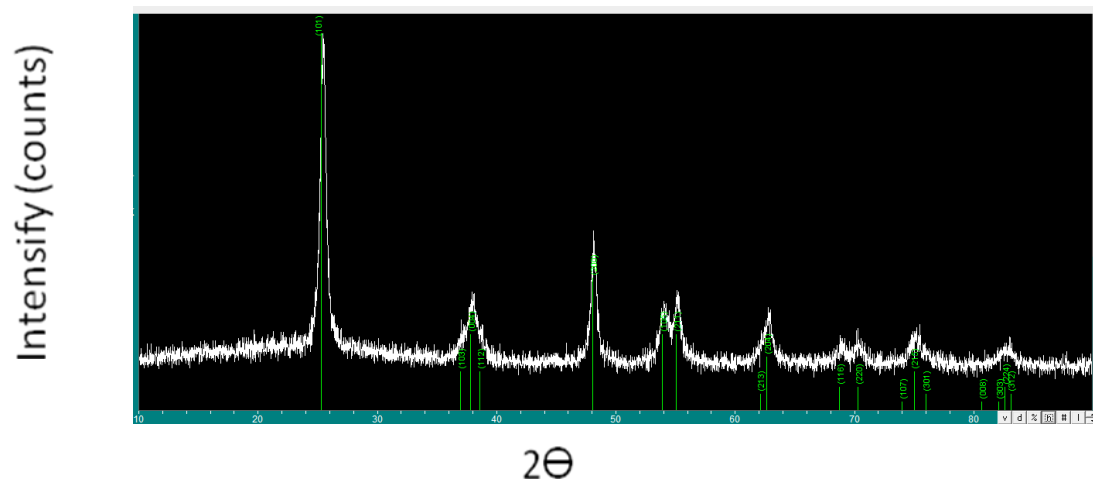


Figure S1. X-ray diffraction (XRD) pattern of purchased commercial titanium dioxide nanoparticles (white curve) compared with standard reference data for titanium dioxide of anatase (green lines, number: 21-1272, space group: $2/m/2$).

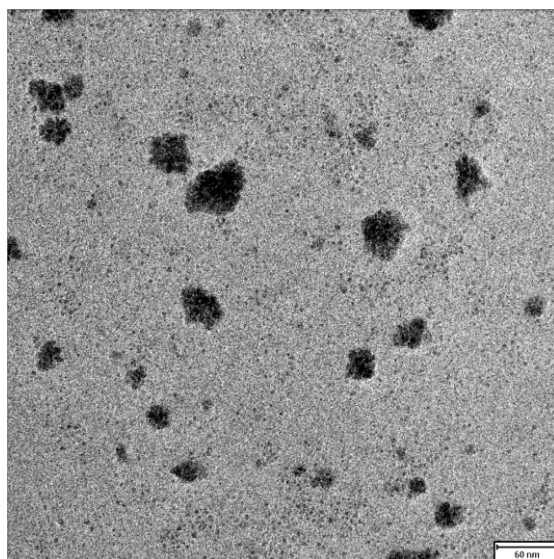


Figure S2. Transmission electron microscopy (TEM) images of titanium dioxide nanoparticles in the SM7 medium.

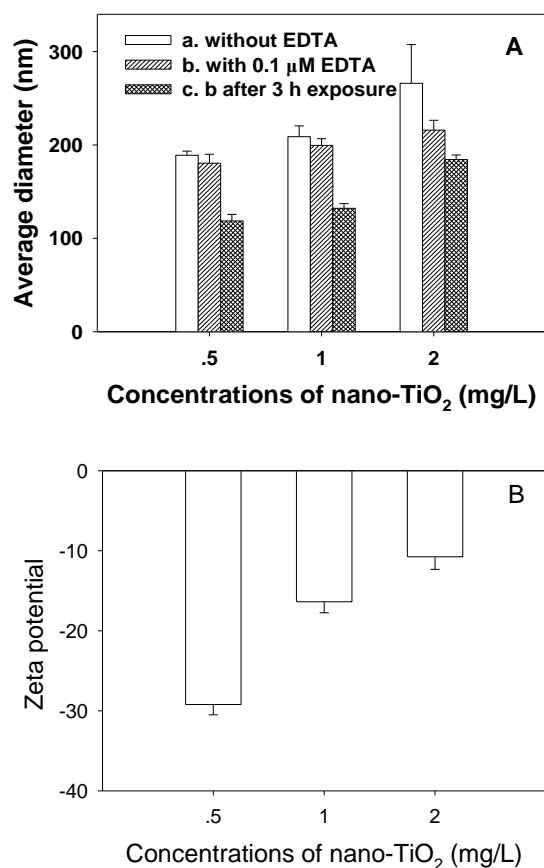


Figure S3. (A) Average diameters of nano-TiO₂ (0.5 to 2 mg/L) in simplified Elendt M7 medium without EDTA, with 0.1 μ M EDTA, and with 0.1 μ M EDTA after 3 h exposure according to the DLS data. (B) Zeta potential of nano-TiO₂ (0.5 to 2 mg/L) in simplified Elendt M7 medium.

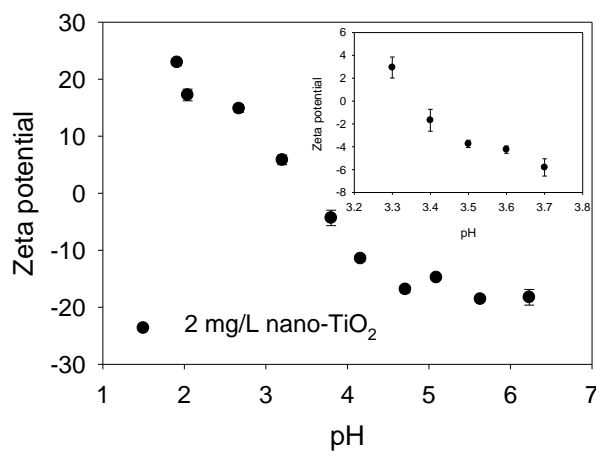


Figure S4. The relationship between zeta potential and pH of 2 mg/L nano-TiO₂ in simplified Elendt M7 medium.

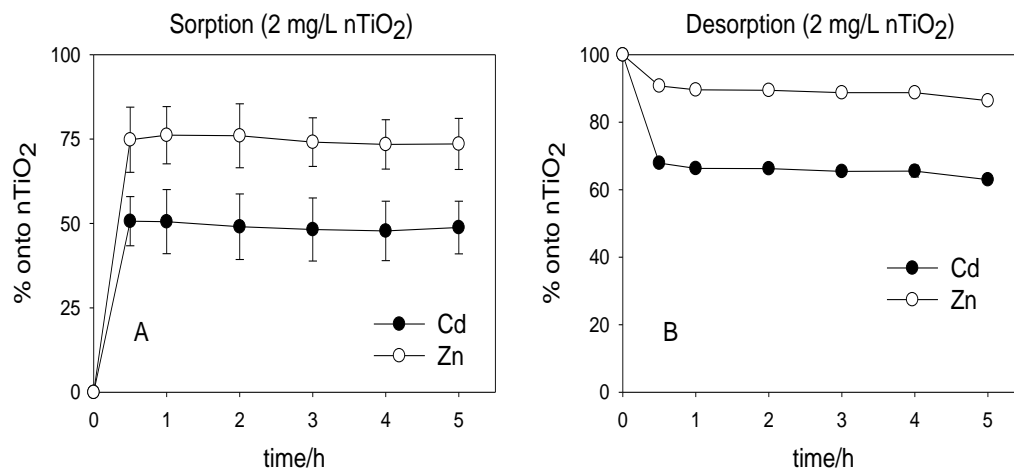


Figure S5. (A) Sorption kinetics of ¹⁰⁹Cd and ⁶⁵Zn on nano-TiO₂. (B) Desorption kinetics of ¹⁰⁹Cd and ⁶⁵Zn from nano-TiO₂. Values are mean ± SD (n=3).

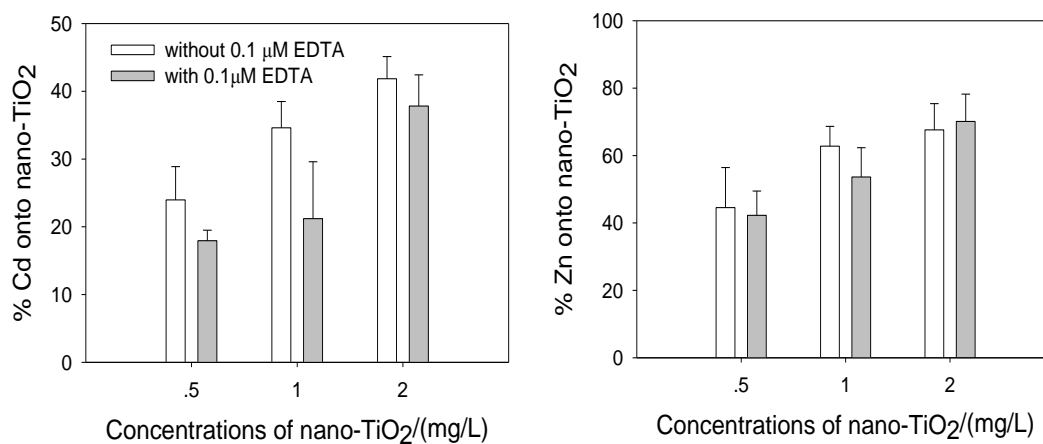


Figure S6. The percentages of Cd (A) and Zn (B) adsorbed onto nano-TiO₂ in simplified Elendt M7 medium with and without 0.1 μM EDTA. Values are mean ± SD (n=3).

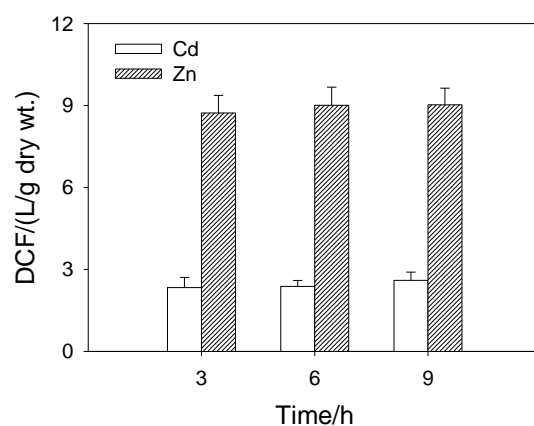


Figure S7. The uptake of ^{109}Cd and ^{65}Zn adsorbed on 1 mg/L nano- TiO_2 in *D.magna* during the 9-h exposure, and the exposure medium was renewed at 3 and 6 h. Values are mean \pm SD (n=3).

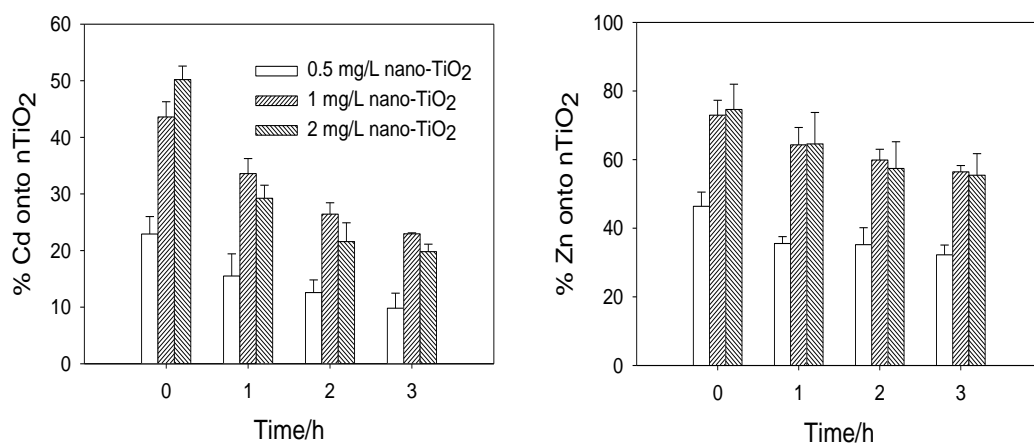


Figure S8. (A) and (B) Percentages of Cd and Zn adsorbed on nano- TiO_2 (0.5, 1, and 2 mg/L) during the 3-h exposure in simplified Elendt M7 medium containing 0.1 μM EDTA. Values are mean \pm SD (n=3).

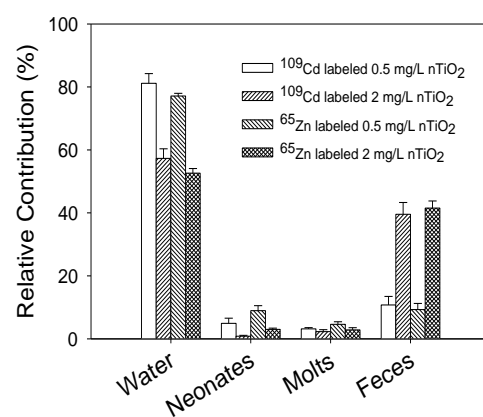


Figure S9. The relative contributions of metal loss from *D. magna* during the 7-day depuration period. Values are mean \pm SD (n=3).