Supporting Information

Accumulation, distribution and toxicity of arsenate associated with titanium dioxide nanoparticles in *Daphnia magna*

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Number of Pages: 6

Number of Tables: 1 (Table S1)

Number of Figures: 3 (Figure S1 to Figure S3)

Supplementary Information for Accumulation Analysis of nano-TiO_2 and *As*.

For particle content (body burden of arsenic and titanium in daphnia) analyses, sampled daphnia were treated following a modified method of Nathalie Adam et al. (Adam et al. 2014). They were washed for a few seconds in pure water to wash off the surrounding exposure medium, then washed in 5 mM Na_2EDTA for 1 min to remove externally bound nanoparticles and aggregates. Then we used 0.1 M potassium phosphate buffer (pH 7.0) to remove arsenic. After quickly washing in ultrapure water to remove the EDTA and potassium phosphate buffer, we put the daphnids samples into bullet vials. All vials were placed in a dry oven at 60℃ for at least 48 h until a constant dry weight. To each vial, containing dried daphnids, 50 μL HNO_3 (69%) and (after 12 h) 50 μL HF(40%) was added. The daphnids were dissolved 4 h later by microwave digestion (4 min 100 W, 3min 180 W, 2min 180 W, 2 min 300 W, 2 min 300 W, 2 min 450 W), after which the samples were diluted to 1-2 % HNO_3. The *As* and Ti concentration of the daphnids was measured by ICP-MS (Thermo Scientific Element 2 XR). For water samples, they were directly evaporated to dryness and then measured following the protocol described above. The Agilent 7500a ICP-MS was used at the conditions described in Table 1. Internal standards were rhodium and indium (1000 μg mL^{-1}, SCP Science) and the monitored masses were 75*As*, 115In, 103Rh and 82Se for equation correction of interferences. The deviation of the instrument was below 2% during the analysis and the limit of detection of the instrument for arsenic was calculated as 0.2 μg L^{-1}. For quality control, two standard solutions of 5 and 20 μg L^{-1} arsenic were analyzed every 10 samples as calibration check solutions.

Adam N, Schmitt C, Galceran J, Companys E, Vakurov A, Wallace R, Knapen D, Blust R (2014): The chronic toxicity of ZnO nanoparticles and ZnCl2 to Daphnia magna and the use of different methods to assess nanoparticle aggregation and dissolution. Nanotoxicology 8, 709-717

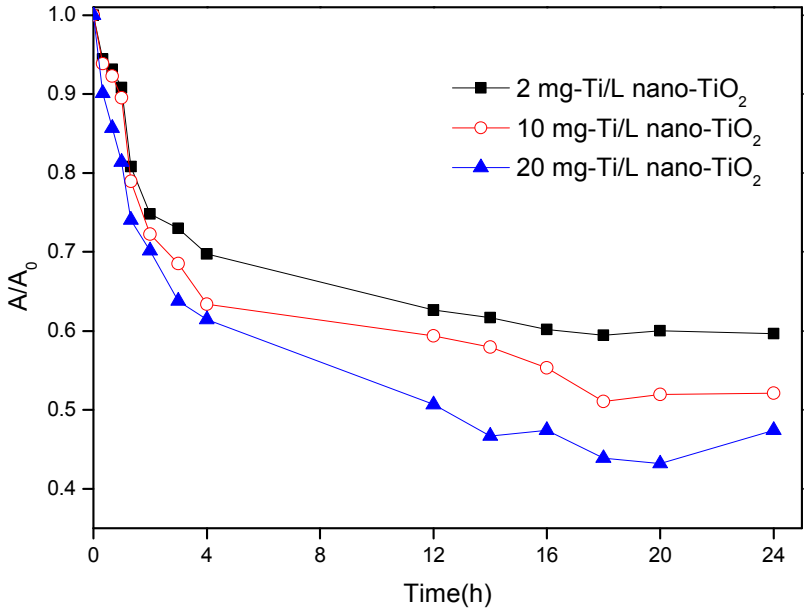

Fig.S1. Sedimentation of nano-TiO2 under different concentration in SM7.

Table S1. Typical operation conditions for LA-ICP-MS analysis

ICP-MS conditions	
RF power	1350 W
Plasma gas	14 L min^{-1} Ar
Auxiliary gas	0.9 L min^{-1} Ar
Make-up gas	0.8 L min^{-1} Ar
Sampling depth	5.4 mm
Detector	Dual (pulse and analog counting)
Dwell time/mass	6 ms
Laser parameters	
Wavelength	193 nm
Energy density	14 J cm^{-2}
Carrier gas	He (optimized to get the highest sensitivity)
Ablation style	Single spot
Ablation spot size	32 μm
Repetition rate	8 Hz
Laser pulse	480

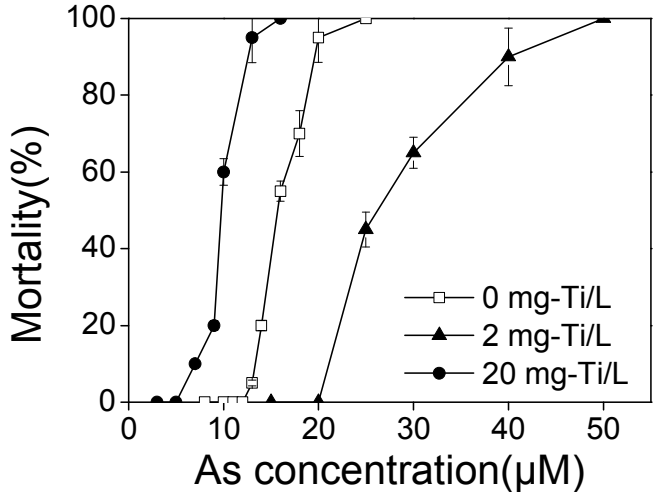

Fig. S2. Toxic effect of As(V) in the presence of nano-TiO_2.

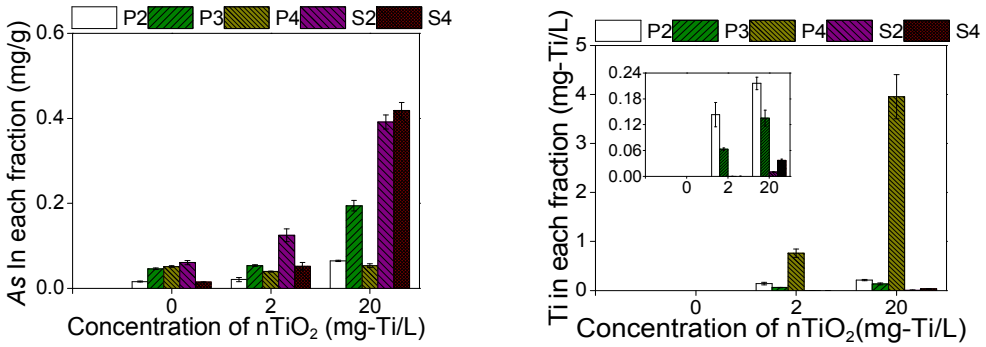

Fig. S3. Subcellular distribution of total arsenic and Ti in *Daphnia magna* after exposure to different nano-TiO_2 concentrations for 3 h. Mean ± standard deviation (n=3). P2- Metal-Rich Granules. P3- Organelles. P4- Heat-Sensitive Protein. S2- Cellular Debris. S4- Heat-Denatured Protein.