Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting Information for:

Bioconcentration of TiO_2 nanoparticles by the freshwater nematode *Plectus aquatilis* disobeys equilibrium partitioning

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This Supplemental Information contains two tables, four figures, and two sections of text:

Tables

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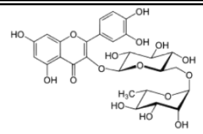
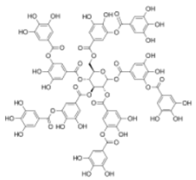
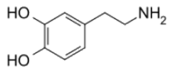
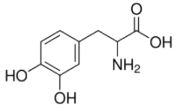
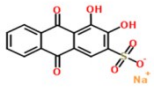
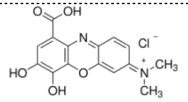
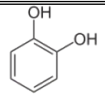
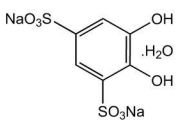
Table SI 1) Chemicals used for surface functionalization of TiO_2 NP and parameters describing particle behavior in Chriesbach water.

Chemical	Surface property	Relevance	Structure	Size in Chriesbach water (nm)	Sedimentation rate (hr^{-1})	ζ-potential (mV)
(+) - Rutin Trihydrate		Environmental		68.3 ± 1.1	0.00 ±0.00	-16.2 ±0.7
Tannic Acid				5400 ±480	0.06 ± 0.02	-17.4 ±0.5
Dopamine	Cation	Biological		2500 ± 350	0.21±0.01	-16.0 ± 0.5
3,4-Dihydroxy-DL-phenylalanine	Zwitterion			810 ±25	0.18 ± 0.03	-15.1 ± 0.7
Alizarin Red	Anion	Dye molecule		890 ± 20	0.02 ± 0.00	-17.5 ±0.4
Gallocyanine	Cation			1300 ± 110	0.11 ± 0.01	-16.1 ± 0.7
Catechol	Non polar			3200 ± 640	0.09 ± 0.01	-16.1 ± 0.9

1,3-Benzenedisulfonic acid, 4,5-dihydroxy-, sodium salt	(strong) Anion			2000 ± 240	0.27 ± 0.02	-16.4 ± 0.1
P-25	NA	Commercial	NA	1400 ± 110	0.11 ± 0.00	-18.7 ± 0.5
Nb Doped TiO_2	NA		NA	1400 ± 91	0.08 ± 0.01	-17.6 ± 0.5

Chemicals which were used for surface functionalization of TiO_2 NP, with summarized classification based on the relevance and the chemical properties. Chemical structure were adapted from the producers information.

Table SI 2) Composition of Chriesbach water.

DOC [mg C/l]	Cl^- [mM]	NO_3^- [mM]	SO_4^{2-} [mM]	K^+ [mM]	Na^+ [mM]	Ca^{2+} [mM]	Mg^{2+} [mM]	Ionic Strength [mM]
3.72	0.97	0.36	0.25	0.11	0.84	2.59	0.62	8.05

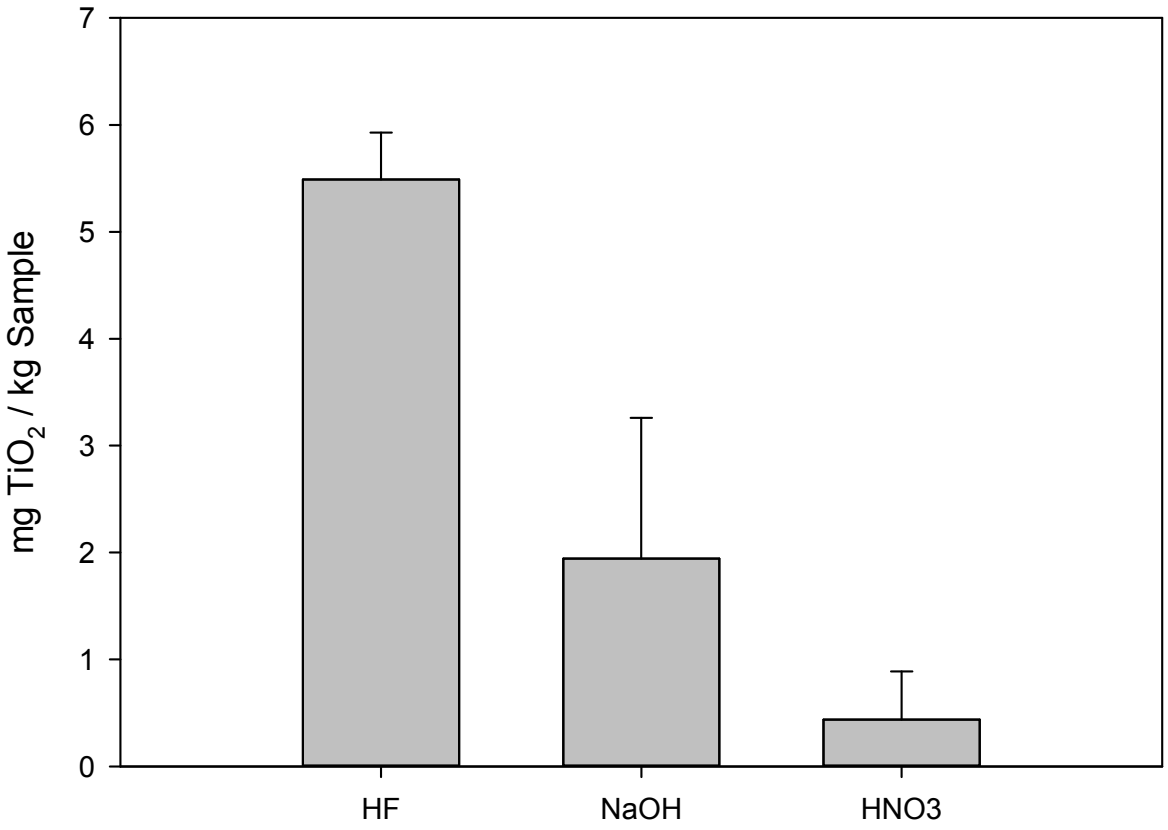


Figure SI 1) Comparison of different digestion methods for recovering TiO_2 from nematodes: HF – (combination of hydrofluoric acid and nitric acid), NaOH: sodium hydroxide and HNO_3: nitric acid digestion.

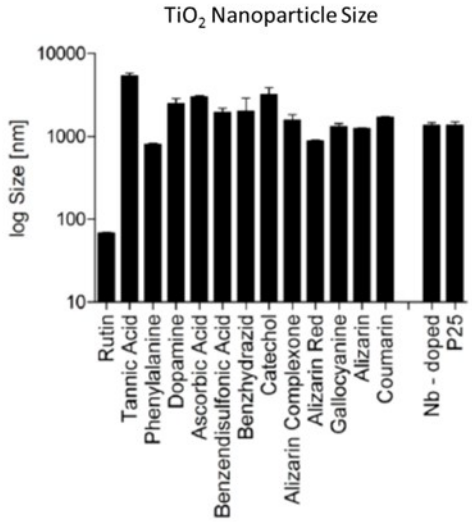


Figure SI 2) Coated TiO_2 nanoparticle sizes in Chriesbach River water.[1]

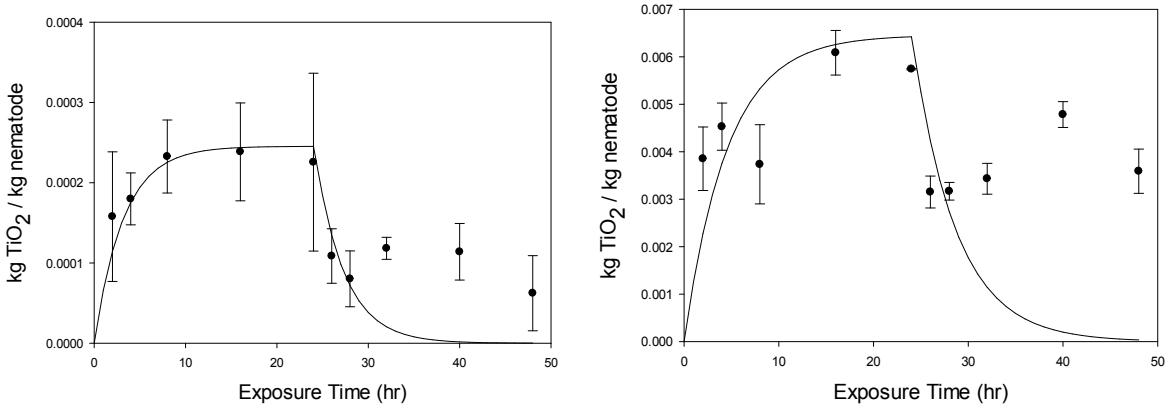


Figure SI 3) Uptake and elimination of TiO_2 NP (data as in Figure 1 B and C) modeled with single elimination phase.

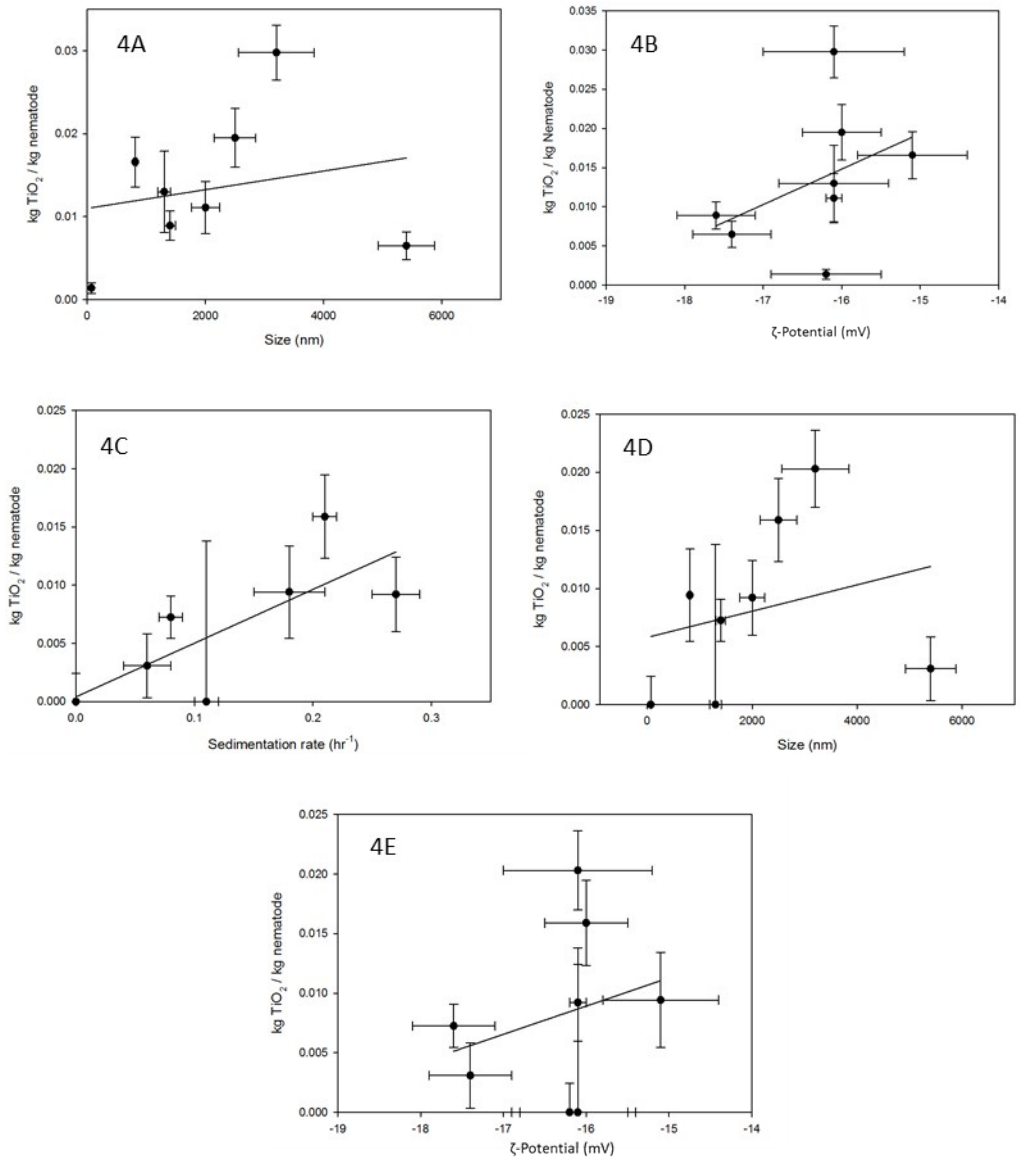


Figure SI 2) Uptake of surface functionalized TiO_2 NPs by the nematode *Plectus aquatilis* (n=3 ± standard deviation): A) Poor of correlation between TiO_2 associated with the nematode and TiO_2 aggregate size (r=0.21), B) Poor of correlation between TiO_2 NP ζ-potential and TiO_2 associated with the nematode (r=0.42), and C) Good correlation between TiO_2 internalized by the nematode and TiO_2 NP sedimentation rate (r=0.75), D) Poor of correlation between TiO_2 internalized by the nematode and TiO_2 aggregate size (r=0.26) , and E) Poor of correlation between TiO_2 internalized by the nematode and TiO_2 NP ζ-potential (r= 0.26) (for C-E, average internalization of rutin and gallocyanine coated TiO_2 NP less than the attached TiO_2 content internalized, TiO_2 content set to zero).

Text SI 1) DNA barcodes for the nematode *Plectus aquatilis.*

Plectus_aquatilus_LSU

TGAAACACGGACCAAGGAGTCTAACATGTGCGCGAGTCATTGGGTGTCAAACCTAAAGGCGGAATGAAAGTGAA
GATCGGCTCGACCGGTTGATATGGGATCCGTTCGGTCACGGCCGAGCGGCGCACCATAGCCCCGTCTCGACTGCT
TGCAGTGGGGCGGAGGCAGAGCGTACACGTTGGGACCCGAAAGATGGTGAACTATGCCTGAGCAGGACGAAGC
CAGAGGAAACTCTGGTGGAGGTCCGAAGCGGTTCTGACGTGCAAATCGATCGTCAGACTTGGGTATAGGGGCGA
AAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCTGAAGTTTCCCCCAGGATAGCTGGAGCTCTTTAGAGCAGTT
GTATCCGGTAAAGCGAATGATTAGAGGCCTTGGGGACGAAACGACCTCAACCTATTCTCAAACTTTCAATGGGTAC
GAAGTCCCGGTTGCTTGACTCGAACCGACGGACAGTGAATGTGAGCTCCAAGTGGGCCATTTTTGGTAAGCA

Plectus_aquatilus_SSU

CGAATATGGTGAAGCCGCGAATGGCTCATTACAACAGCCACTGTTTACTTGATCTTGATTATCCTACTTGGATAACT
GTGGTAATTCTAGAGCTAATACACGCAATAAAGCTCCGACCTTACGGGACGAGCGCATTTATTAGACCAAAACCAA
TCGGGCTTCGGCCTGAACGGTGGTGACTCTGAATAACTGAGCTGATCGCATGGTCTTTGTACCGGCGACGCATCTT
TCAAGTGTCTGCCTTATCAACTTTCGATGGTAGTTTATGTGCCTACCATGGTTGTTACGGGTAACGGAGAATAAGG
GTTCGACTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAC
TCTCGGCACGAGGAGGTAGTGACGAAAAATAACGAGGCGGTTCTCTAAGAGGCCCGCTATCGGAATGGGTACAA
TTTAAACCCTTTAACGAGGACCTATGAGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCTCAAGGT
GTATATCGCCATTGCTGCGGTTAAAAAGCTCGTAGTTGGATCTGCGCCTTCGGACTCGGTCCGCCCAACGGGTGTG
AACTGAGATCCAAGGCTTATACTGCTGGTTTTCCCTTGATGCTCTTCACTGGGTGTCTTGGGTGGCTAGCGAGTTTA
CTTTGAAAAAATTAGAGTGCTTAACACAGGCTATCGCCTGAATACTTGTGCATGGAATAATAGAATAAGACCACGG
CTCTATTTTATTGGTTTTCGGAACTGTGATAATGGTTAAGAGGGACAGACGGGGGCATTCGTATCGCT

Text SI 2) Separation of nematodes from growth media on sucrose gradients.

Nematodes were pelleted from the growth media by centrifugation. The nematodes were then suspended in 60 % ice cold sucrose and then centrifuged at 800 g for 20 minutes. After separation the nematodes were washed 3 times with culture media to remove excess sucrose solution. The nematodes were then allowed to recover overnight, before nanoparticle exposures were conducted.

1. H. Schug, C. W. Isaacson, L. Sigg, A. Amman and K. Schirmer, *Environ. Sci. Technol.*, 2014, **48**, 11620-11628.