

Quantity and quality of natural organic matter influence the ecotoxicity of
titanium dioxide nanoparticles

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Appendix A. **Supplementary data**

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Table S1: Mean particle size (\pm SD; n=3) and the respective polydispersity indices (=PI) of the nTiO₂ product A-100 (at 1 and 4 mg nTiO₂/L) in presence of increasing levels of seven NOMs (Sigma Aldrich Humic Acid (SA HA), Seaweed Extract (SW), Suwannee River (SR) Natural Organic Matter (SR NOM), SR Humic Acid (SR HA), SR Fulvic Acid (SR FA), Leonardite (LEO) and Pahokee Peat (PP)) after 0 (t_{0h}) and 96 (t_{96h}) h of the acute toxicity experiment with *Daphnia*. NA indicates invalid DLS measurements due to insufficient scattered light intensities.

NOM type	TOC (mg/L)	nTiO ₂ (mg/L)	Size (nm) t _{0h}	Size (nm) t _{96h}	PI
SA HA	0.00	1	346 (\pm 81)	NA	0.216 - 0.282
	0.04		325 (\pm 34)	NA	0.254 - 1.843
	0.40		210 (\pm 57)	NA	0.155 - 0.270
	4.00		508 (\pm 25)	315 (\pm 47)	0.260 - 0.276
	0.00	4	528 (\pm 99)	NA	0.257 - 0.290
	0.04		530 (\pm 85)	NA	0.266 - 0.287
	0.40		498 (\pm 96)	NA	0.249 - 0.267
	4.00		212 (\pm 47)	124 (\pm 3)	0.156 - 0.257
SW	0.00	1	NA	NA	0.545 - 0.634
	0.04		NA	NA	0.518 - 0.736
	0.40		NA	NA	0.589 - 0.732
	4.00		554 (\pm 42)	471 (\pm 41)	0.219 - 0.281
	0.00	4	NA	NA	NA
	0.04		NA	NA	NA
	0.40		NA	NA	0.624 - 0.741
	4.00		316 (\pm 38)	490 (\pm 0)	0.190 - 0.302
SR NOM	0.00	1	NA	NA	0.615 - 0.703
	0.04		NA	NA	0.429 - 0.705
	0.40		NA	NA	0.417 - 0.716
	4.00		298 (\pm 4)	306 (\pm 12)	0.226 - 0.292
	0.00	4	NA	NA	0.582 - 0.769
	0.04		NA	NA	0.433 - 0.704
	0.40		NA	NA	0.489 - 0.765
	4.00		227 (\pm 4)	1042 (\pm 59)	0.209 - 0.391
SR HA	0.00	1	1194 (\pm 50)	NA	0.400 - 0.690
	0.04		1191 (\pm 40)	NA	0.371 - 0.778
	0.40		256 (\pm 4)	NA	0.246 - 0.600
	4.00		266 (\pm 4)	255 (\pm 6)	0.237 - 0.445
	0.00	4	NA	NA	0.395 - 0.765
	0.04		NA	NA	0.436 - 0.601
	0.40		NA	NA	0.467 - 0.654
	4.00		213 (\pm 4)	289 (\pm 2)	0.216 - 0.275

NOM type	TOC (mg/L)	nTiO ₂ (mg/L)	Size (nm) t _{0h}	Size (nm) t _{96h}	PI
SR FA	0.00	1	913 (±13)	NA	0.316 - 0.688
	0.04		1030 (±32)	NA	0.343 - 0.670
	0.40		807 (±4)	1223 (±126)	0.273 - 0.771
	4.00		236 (±2)	369 (±22)	0.189 - 0.281
	0.00	4	NA	NA	0.404 - 0.752
	0.04		NA	NA	0.399 - 0.743
	0.40		NA	NA	0.424 - 0.680
	4.00		255 (±3)	860 (±63)	0.209 - 0.375
LEO	0.00	1	346 (±81)	NA	0.216 - 0.282
	0.04		308 (±39)	NA	0.266 - 0.276
	0.40		153 (±2)	633 (±76)	0.221 - 0.319
	4.00		171 (±9)	211 (±18)	0.200 - 0.248
	0.00	4	528 (±99)	NA	0.257 - 0.290
	0.04		567 (±147)	NA	0.257 - 0.306
	0.40		477 (±82)	NA	0.235 - 0.272
	4.00		129 (±45)	111 (±2)	0.105 - 0.272
PP	0.00	1	346 (±81)	NA	0.216 - 0.282
	0.04		298 (±30)	NA	0.279 - 0.306
	0.40		150 (±34)	625 (±172)	0.107 - 0.341
	4.00		157 (±96)	324 (±29)	0.130 - 0.291
	0.00	4	528 (±99)	NA	0.257 - 0.290
	0.04		554 (±96)	NA	0.290 - 0.294
	0.40		336 (±96)	NA	0.258 - 0.274
	4.00		104 (±1)	121 (±12)	0.192 - 0.262

Table S2: Mean particle size (\pm SD; n=3) and the respective polydispersity indices (=PI) of the nTiO₂ product P25 (at 1 and 4 mg nTiO₂/L) in presence of increasing levels of seven NOMs (Sigma Aldrich Humic Acid (SA HA), Seaweed Extract (SW), Suwannee River (SR) Natural Organic Matter (SR NOM), SR Humic Acid (SR HA), SR Fulvic Acid (SR FA), Leonardite (LEO) and Pahokee Peat (PP)) after 0 (t_{0h}) and 96 (t_{96h}) h of the acute toxicity experiment with *Daphnia*. NA indicates invalid DLS measurements due to low scattered light intensities.

NOM type	TOC (mg/L)	nTiO ₂ (mg/L)	Size (nm) t _{0h}	Size (nm) t _{96h}	PI
SA HA	0.00	1	251 (\pm 30)	NA	0.207 - 0.270
	0.04		243 (\pm 43)	NA	0.206 - 0.222
	0.40		112 (\pm 2)	NA	0.108 - 0.266
	4.00		215 (\pm 51)	131 (\pm 3)	0.240 - 0.418
	0.00	4	418 (\pm 77)	NA	0.234 - 0.257
	0.04		450 (\pm 86)	NA	0.214 - 0.253
	0.40		103 (\pm 2)	NA	0.134 - 0.173
	4.00		298 (\pm 67)	109 (\pm 10)	0.237 - 0.280
SW	0.00	1	502 (\pm 12)	NA	0.235 - 0.766
	0.04		643 (\pm 32)	NA	0.259 - 0.726
	0.40		197 (\pm 7)	NA	0.243 - 0.734
	4.00		191 (\pm 17)	227 (\pm 49)	0.117 - 0.244
	0.00	4	1370 (\pm 56)	NA	0.407 - 0.428
	0.04		NA	NA	0.451 - 0.486
	0.40		1449 (\pm 11)	NA	0.430 - 0.435
	4.00		173 (\pm 5)	905 (\pm 83)	0.200 - 0.379
SR NOM	0.00	1	1127 (\pm 64)	NA	0.427 - 0.778
	0.04		1272 (\pm 96)	NA	0.433 - 0.779
	0.40		221 (\pm 18)	NA	0.127 - 0.721
	4.00		267 (\pm 18)	291 (\pm 11)	0.152 - 0.254
	0.00	4	NA	NA	0.480 - 0.527
	0.04		NA	NA	0.468 - 0.652
	0.40		1534 (\pm 4)	NA	0.464 - 0.728
	4.00		163 (\pm 2)	549 (\pm 15)	0.169 - 0.247

NOM type	TOC (mg/L)	nTiO ₂ (mg/L)	Size (nm) t _{0h}	Size (nm) t _{96h}	PI
SR HA	0.00	1	697 (±2)	NA	0.290 - 0.725
	0.04		327 (±13)	NA	0.164 - 0.777
	0.40		193 (±1)	996 (±21)	0.210 - 0.457
	4.00		139 (±3)	144 (±2)	0.138 - 0.253
	0.00	4	NA	NA	0.543 - 0.674
	0.04		NA	NA	0.602 - 0.663
	0.40		198 (±2)	NA	0.198 - 0.657
	4.00		139 (±1)	144 (±3)	0.123 - 0.191
SR FA	0.00	1	1202 (±30)	NA	0.410 - 0.790
	0.04		1102 (±51)	NA	0.381 - 0.579
	0.40		637 (±17)	NA	0.270 - 0.658
	4.00		143 (±1)	617 (±56)	0.162 - 0.320
	0.00	4	1202 (±30)	NA	0.410 - 0.721
	0.04		1575 (±105)	NA	0.462 - 0.554
	0.40		1285 (±30)	NA	0.368 - 0.588
	4.00		155 (±1)	1061 (±81)	0.192 - 0.471
LEO	0.00	1	251 (±30)	NA	0.207 - 0.270
	0.04		228 (±77)	NA	0.201 - 0.245
	0.40		161 (±55)	657 (±22)	0.204 - 0.391
	4.00		176 (±65)	127 (±9)	0.222 - 0.309
	0.00	4	418 (±77)	NA	0.234 - 0.257
	0.04		402 (±77)	NA	0.239 - 0.267
	0.40		199 (±28)	NA	0.197 - 0.239
	4.00		153 (±27)	102 (±5)	0.236-0.256
PP	0.00	1	251 (±30)	NA	0.207 - 0.270
	0.04		215 (±22)	NA	0.208 - 0.257
	0.40		124 (±4)	NA	0.170 - 0.702
	4.00		129 (±15)	136 (±6)	0.270 - 0.302
	0.00	4	418 (±77)	NA	0.234 - 0.257
	0.04		445 (±101)	NA	0.218 - 0.252
	0.40		128 (±7)	NA	0.153 - 0.192
	4.00		99 (±2)	108 (±2)	0.195 - 0.264

Table S3: Mean zeta potential (\pm SD; n=3) of A-100 and P25 (both assessed at 4.00 mg nTiO₂/L) in ASTM medium in presence of increasing NOM quantities with deviating quality (i.e., Sigma Aldrich Humic Acid (SA HA), Seaweed Extract (SW), Suwannee River (SR) Natural Organic Matter (SR NOM), SR Humic Acid (SR HA), SR Fulvic Acid (SR FA), Leonardite (LEO) and Pahokee Peat (PP)).

NOM type	TOC (mg/L)	A-100	P25
		Zeta Potential (mV)	Zeta Potential (mV)
ASTM - Blank	0.00	-4.92 (\pm 0.10)	-10.79 (\pm 0.46)
SA HA	0.04	-13.03 (\pm 0.10)	-13.60 (\pm 2.21)
	0.40	-17.91 (\pm 2.03)	-22.51 (\pm 1.69)
	4.00	-19.89 (\pm 0.27)	-22.21 (\pm 1.06)
SW	0.04	-4.09 (\pm 0.16)	-9.77 (\pm 0.24)
	0.40	-9.4 (\pm 0.13)	-21.48 (\pm 0.80)
	4.00	-20.79 (\pm 0.33)	-21.22 (\pm 0.26)
SR NOM	0.04	-6.65 (\pm 1.01)	-12.52 (\pm 0.43)
	0.40	-12.48 (\pm 0.80)	-20.23 (\pm 0.67)
	4.00	-22.14 (\pm 0.23)	-19.97 (\pm 1.08)
SR HA	0.04	-6.93 (\pm 0.25)	-14.94 (\pm 0.38)
	0.40	-16.16 (\pm 0.70)	-23.10 (\pm 0.62)
	4.00	-24.39 (\pm 0.23)	-23.61 (\pm 0.58)
SR FA	0.04	-3.59 (\pm 0.45)	-13.47 (\pm 0.26)
	0.40	-14.34 (\pm 0.44)	-19.93 (\pm 0.15)
	4.00	-21.68 (\pm 0.53)	-22.81 (\pm 0.79)
LEO	0.04	-12.09 (\pm 0.10)	-12.88 (\pm 1.15)
	0.40	-21.02 (\pm 2.60)	-28.62 (\pm 3.67)
	4.00	-12.09 (\pm 0.12)	-24.97 (\pm 3.66)
PP	0.04	-13.91 (\pm 0.77)	-18.20 (\pm 2.10)
	0.40	-22.30 (\pm 1.00)	-24.33 (\pm 2.89)
	4.00	-22.57 (\pm 1.00)	-22.11 (\pm 3.02)

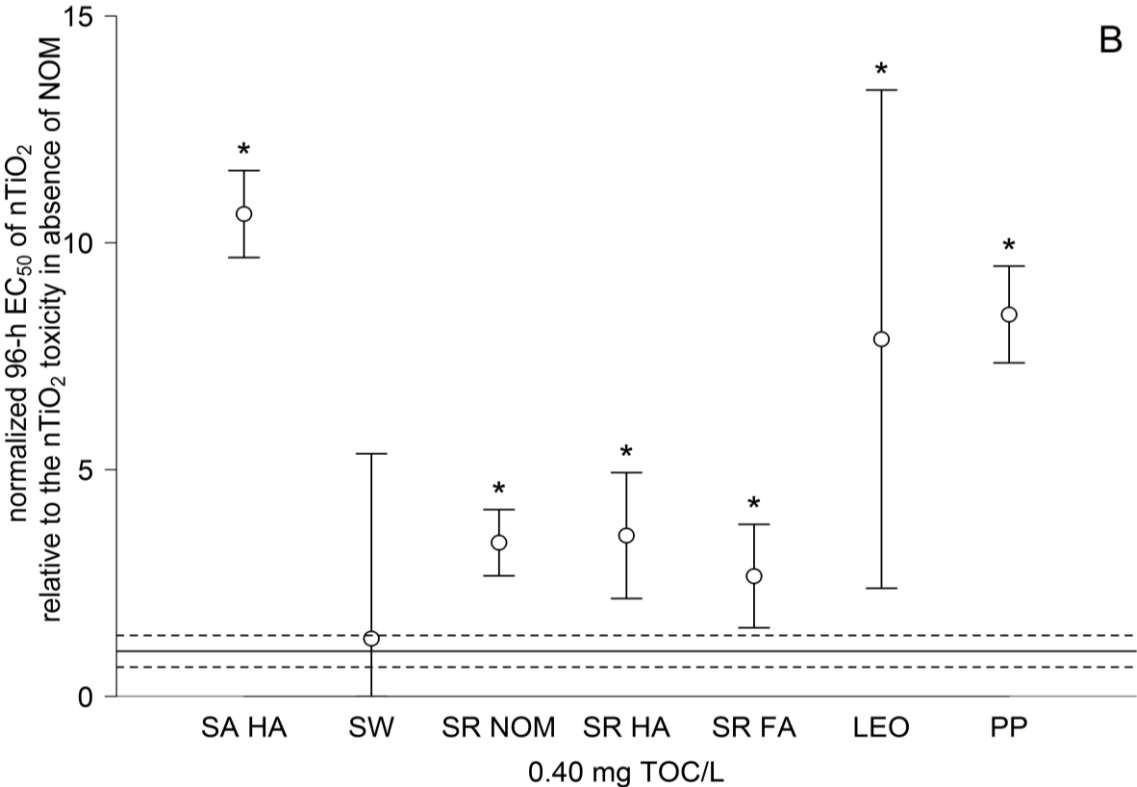
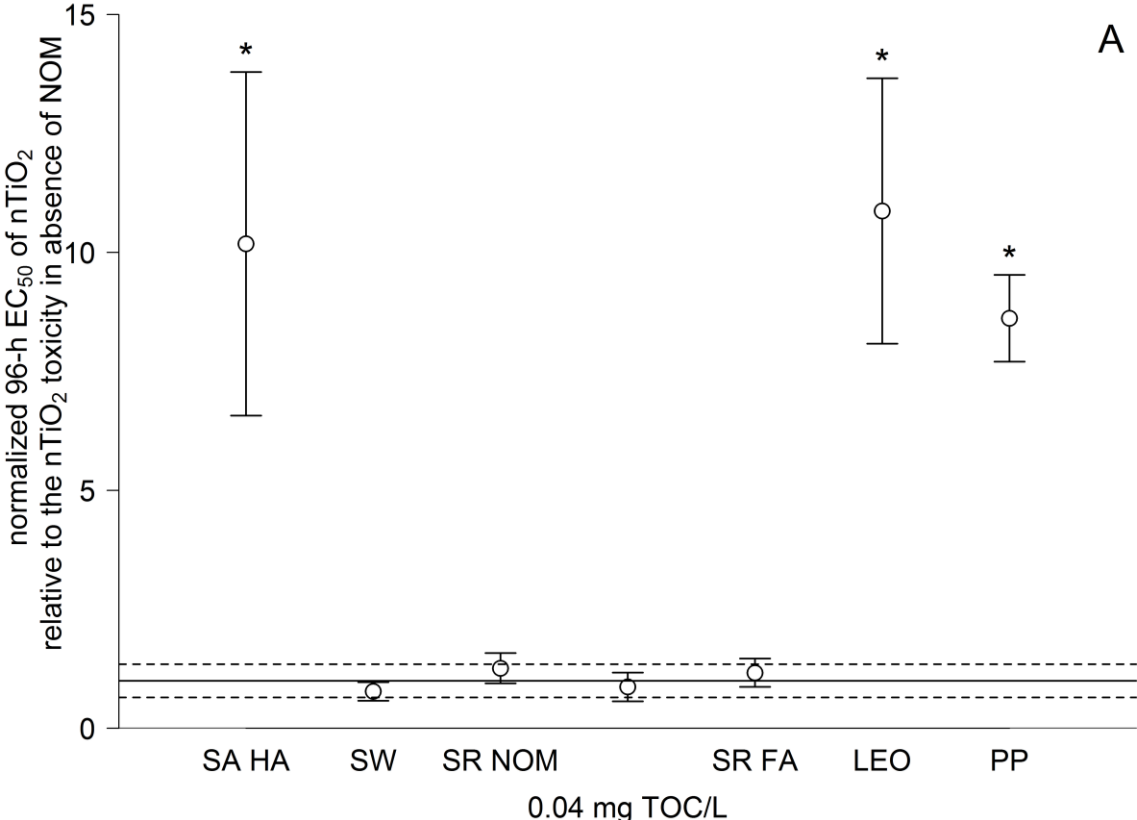
Table S4: Properties of the used nTiO₂ products P25 and A-100 according to the manufacturer and own measurements

Properties	Method	A-100	P25
Producer		Crenox GmbH (Germany)	Evonik (Germany)
Crystalline structure composition		99% anatase	~70% anatase/ ~30% rutile
Primary particle size (nm)	advertised ^a	6.00	21.00
Surface area	BET/advertise d ^a	~230.00	50.00 (±15.00)
Ave. diameter in dispersion (nm)	DLS ^b	87.30 (±2.40)	95.40 (±1.10)
PI in dispersion	DLS ^b	0.132-0.189	0.139–0.443
Zeta potential in dispersion (mV)	DLS ^b	-4.92 (±0.10)	-10.79 (±0.46)
Concentration of stock dispersion	ICP-MS ^b	2.00	2.00

^aProducer information

^bDispersed in H₂O dest., stabilized by a low pH

Figure S1



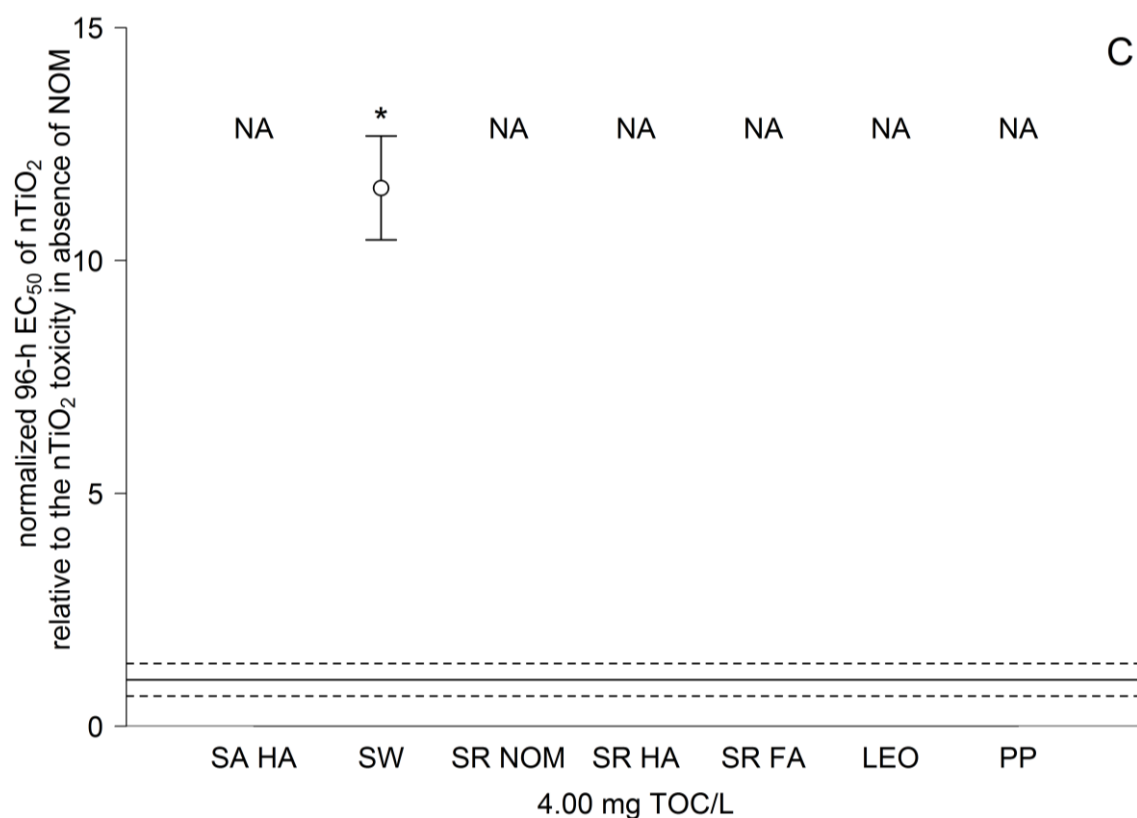
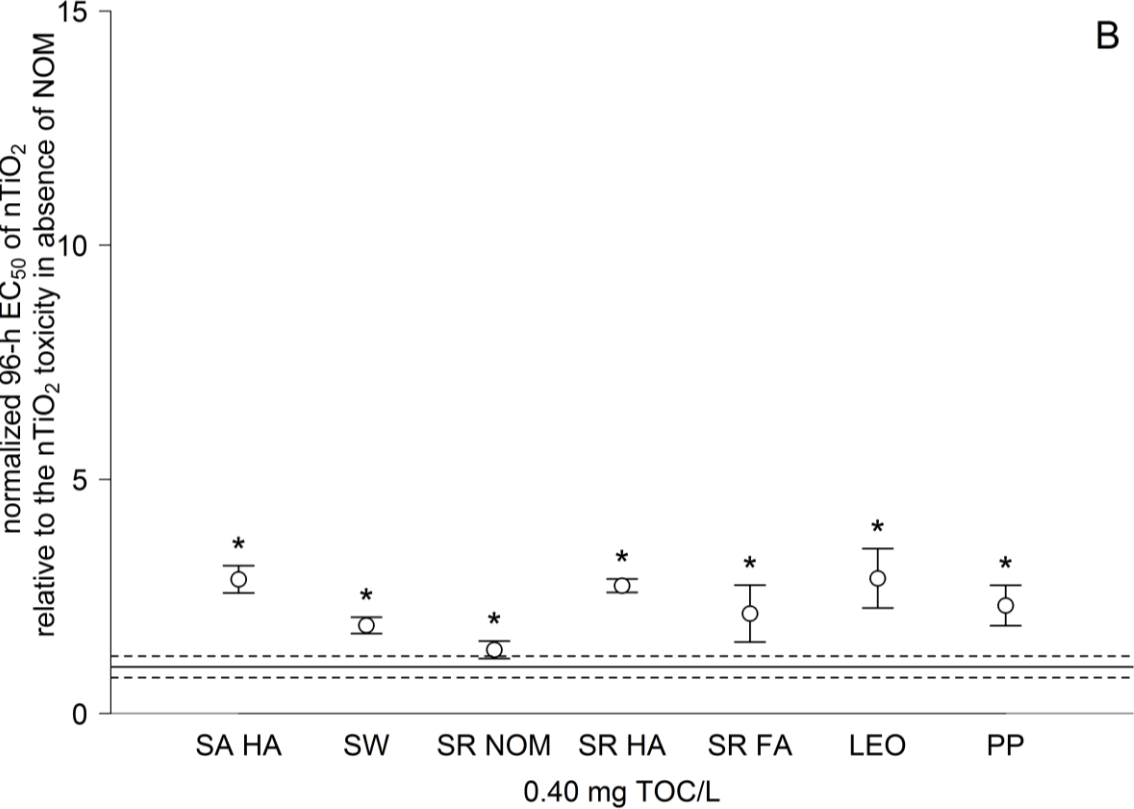
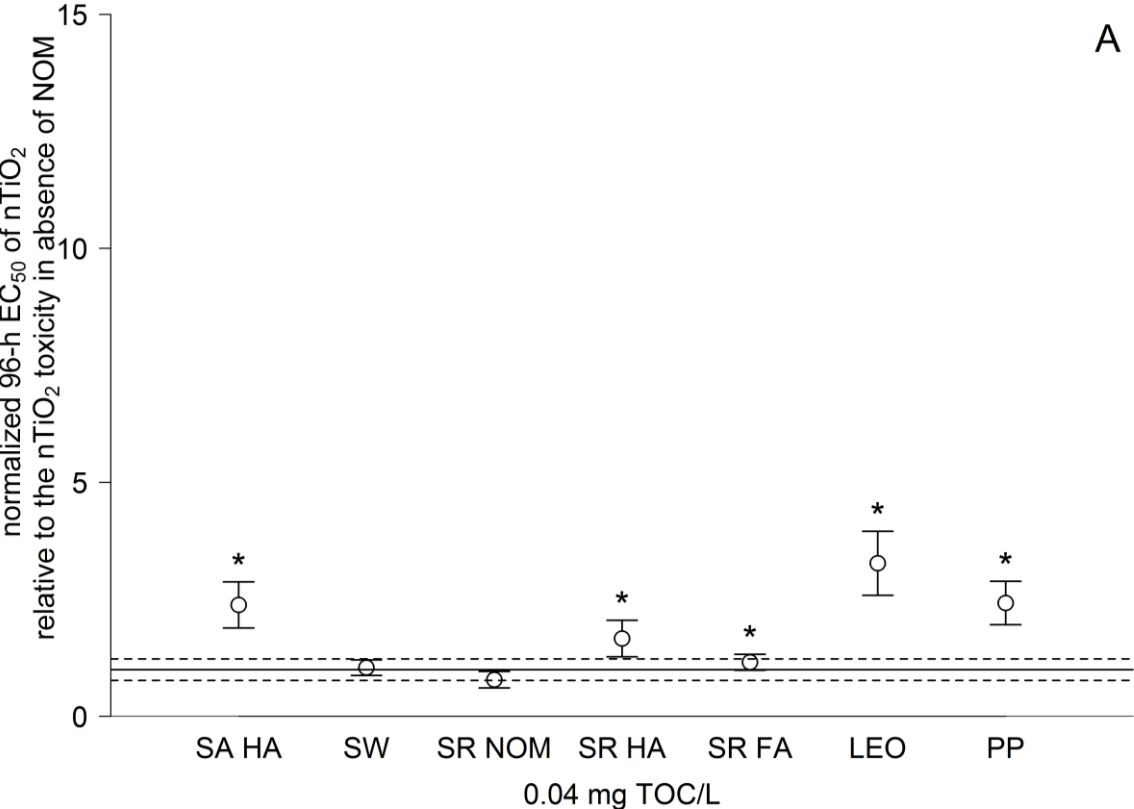


Figure S1 A-C: Normalized 96-h EC₅₀ values (with 95% CIs) for the immobilization data of *D. magna*: Gained 96-h EC₅₀ values of A-100 at concentrations of (A) 0.04, (B) 0.40 and (C) 4.00 mg TOC/L NOM (Seaweed Extract (SW), Suwannee River (SR) Natural Organic Matter (SR NOM), SR Humic Acid (SR HA), SR Fulvic Acid (SR FA), Leonardite (LEO) and Pahokee Peat (PP)) were expressed relative to the 96-h EC₅₀ determined for the same nTiO₂ product at 0.00 mg TOC/L NOM (black line).

Figure S2



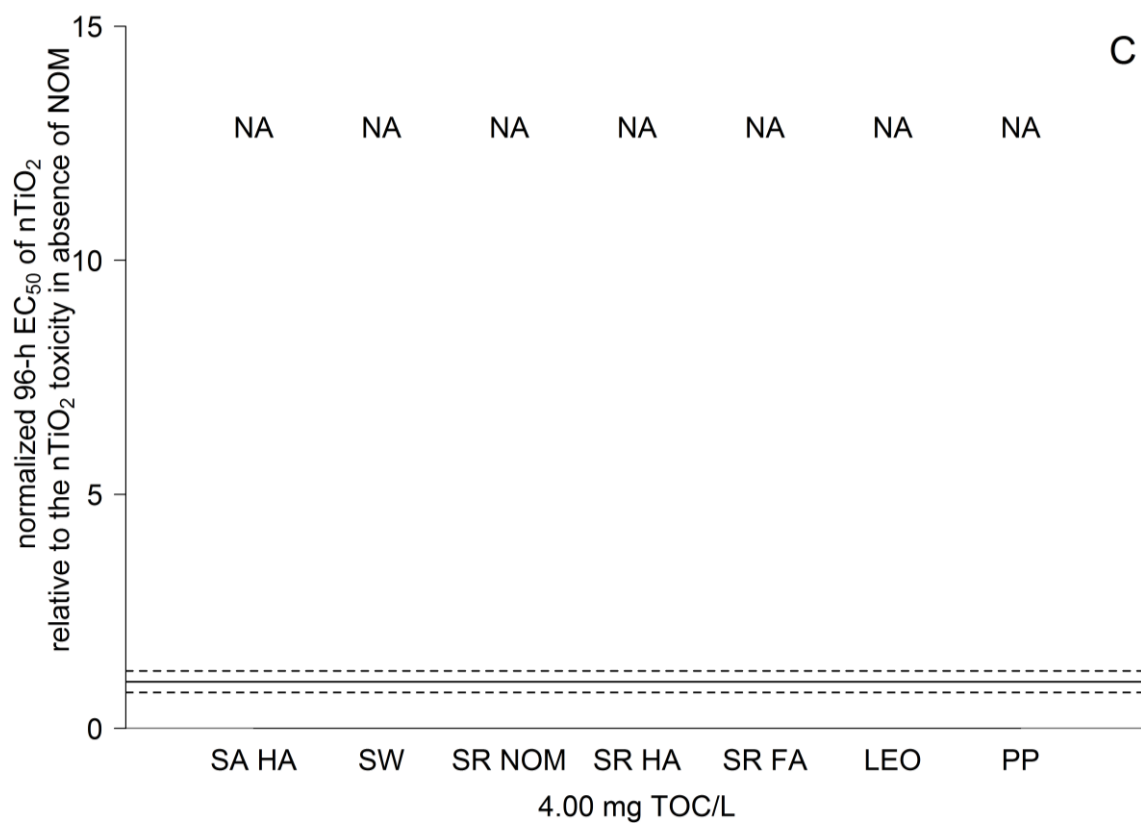


Figure S2 A-C: Normalized 96-h EC₅₀ values (with 95% CIs) for the immobilization data of *D. magna*: Gained 96-h EC₅₀ values of P25 at concentrations of (A) 0.04, (B) 0.40 and (C) 4.00 mg TOC/L NOM (Seaweed Extract (SW), Suwannee River (SR) Natural Organic Matter (SR NOM), SR Humic Acid (SR HA), SR Fulvic Acid (SR FA), Leonardite (LEO) and Pahokee Peat (PP)) were expressed relative to the 96-h EC₅₀ determined for the same nTiO₂ product at 0.00 mg TOC/L NOM (black line).

Figure S3

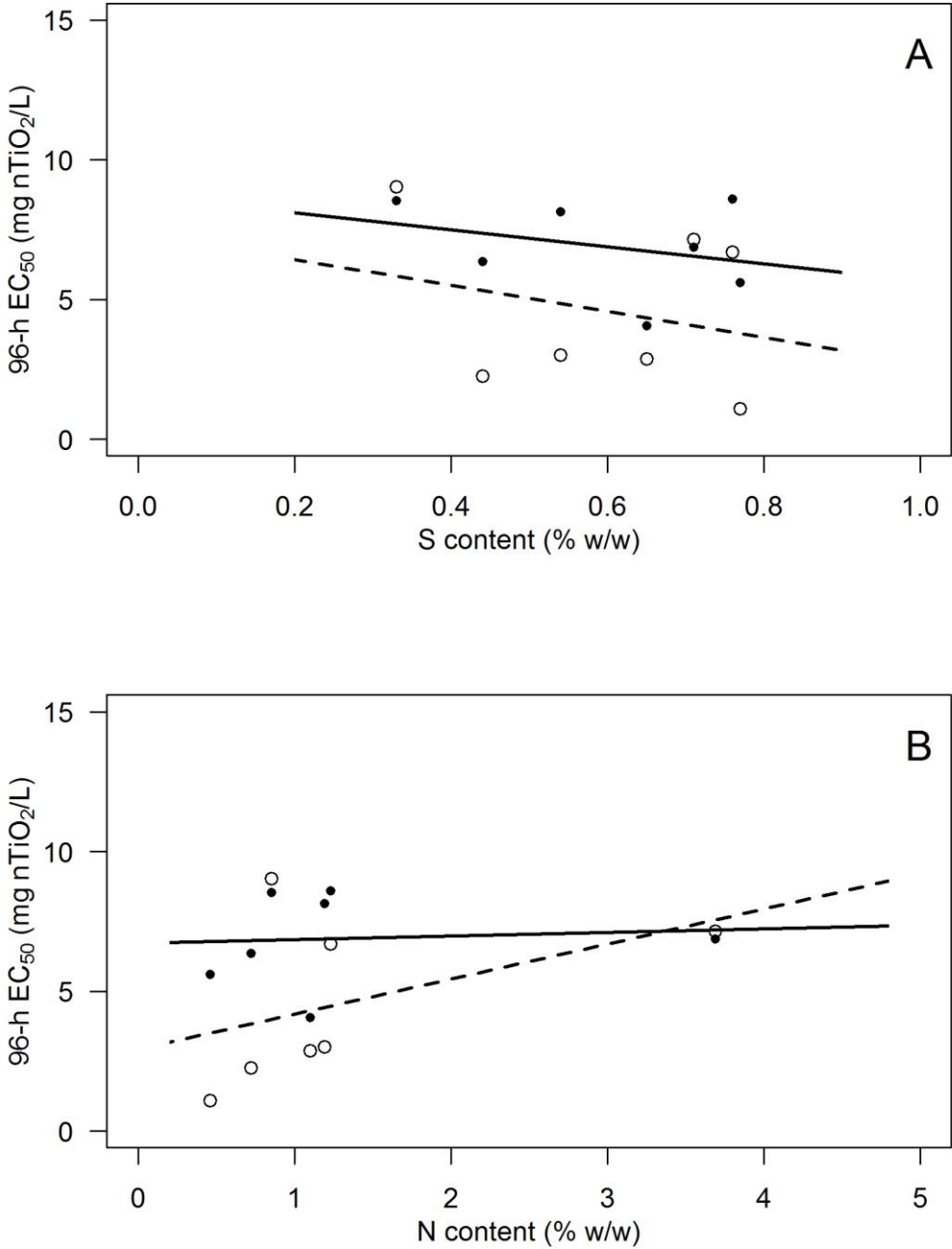


Figure S3 A-B: Linear correlation models of the NOM-sulfur (A) or nitrogen (B) content with 96-h EC₅₀ values of the nTiO₂ products A-100 (white circles; dashed line) and P25 (black circles; continuous line).