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

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Appendix A. **Supplementary data**

3 Figures

4 Tables
Table S1: Mean particle size (±SD; n=3) and the respective polydispersity indices (=PI) of the nTiO$_2$ product A-100 (at 1 and 4 mg nTiO$_2$/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 (±81)          | NA                 | 0.216 - 0.282 |
|           | 0.04       | 325 (±34)       | NA                 | 0.254 - 1.843      |
|           | 0.40       | 210 (±57)       | NA                 | 0.155 - 0.270      |
|           | 4.00       | 508 (±25)       | 315 (±47)          | 0.260 - 0.276      |
|           | 0.00       | 528 (±99)       | NA                 | 0.257 - 0.290      |
|           | 0.04       | 530 (±85)       | NA                 | 0.266 - 0.287      |
|           | 0.40       | 498 (±96)       | NA                 | 0.249 - 0.267      |
|           | 4.00       | 212 (±47)       | 124 (±3)           | 0.156 - 0.257      |
| SW        | 0.00       | NA              | NA                 | NA                 |
|           | 0.04       | NA              | NA                 | 0.518 - 0.736      |
|           | 0.40       | NA              | NA                 | 0.589 - 0.732      |
|           | 4.00       | 554 (±42)       | 471 (±41)          | 0.219 - 0.281      |
| SR NOM    | 0.00       | NA              | NA                 | NA                 |
|           | 0.04       | NA              | NA                 | 0.624 - 0.741      |
|           | 0.40       | NA              | NA                 | 0.489 - 0.765      |
|           | 4.00       | 316 (±38)       | 490 (±0)           | 0.190 - 0.302      |
| SR HA     | 0.00       | NA              | NA                 | NA                 |
|           | 0.04       | NA              | NA                 | 0.615 - 0.703      |
|           | 0.40       | NA              | NA                 | 0.429 - 0.705      |
|           | 4.00       | 298 (±4)        | 306 (±12)          | 0.226 - 0.292      |
|           | 0.00       | 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 (±4)        | 1042 (±59)         | 0.209 - 0.391      |
|           | 0.00       | 1194 (±50)      | NA                 | 0.400 - 0.690      |
|           | 0.04       | 1191 (±40)      | NA                 | 0.371 - 0.778      |
|           | 0.40       | 256 (±4)        | NA                 | 0.246 - 0.600      |
|           | 4.00       | 266 (±4)        | 255 (±6)           | 0.237 - 0.445      |
|           | 0.00       | 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 (±4)        | 289 (±2)           | 0.216 - 0.275      |
| NOM type | TOC (mg/L) | nTiO₂ (mg/L) | Size (nm) t₀h | Size (nm) t₉₆h | PI   |
|----------|------------|---------------|---------------|---------------|------|
|          | 0.00       | 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 |
| SR FA    | 0.00       | 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 |
|          | 0.00       | 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 |
| LEO      | 0.00       | 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 |
|          | 0.00       | 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 |
| PP       | 0.00       | 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 (±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 (t₀h) and 96 (t₉₆h) h of the acute toxicity experiment with *Daphnia*. NA indicates invalid DLS measurements due to low scattered light intensities.

| NOM type       | TOC (mg/L) | nTiO₂ (mg/L) | Size (nm)  | Size (nm)  | PI     |
|----------------|------------|--------------|------------|------------|--------|
|                |            |              | ₉₀₄₆h      | ₉₀₄₆h      |        |
| SA HA          | 0.00       | 1            | 251 (±30)  | NA         | 0.207 - 0.270 |
|                | 0.04       | 0.4          | 243 (±43)  | NA         | 0.206 - 0.222 |
|                | 0.40       | 4            | 112 (±2)   | NA         | 0.108 - 0.266 |
|                | 4.00       |              | 215 (±51)  | 131 (±3)   | 0.240 - 0.418 |
| SW             | 0.00       | 1            | 418 (±77)  | NA         | 0.234 - 0.257 |
|                | 0.04       | 4            | 450 (±86)  | NA         | 0.214 - 0.253 |
|                | 0.40       | 0.4          | 103 (±2)   | NA         | 0.134 - 0.173 |
|                | 4.00       |              | 298 (±67)  | 109 (±10)  | 0.237 - 0.280 |
| SR NOM         | 0.00       | 1            | 502 (±12)  | NA         | 0.235 - 0.766 |
|                | 0.04       | 4            | 643 (±32)  | NA         | 0.259 - 0.726 |
|                | 0.40       |              | 197 (±7)   | NA         | 0.243 - 0.734 |
|                | 4.00       |              | 191 (±17)  | 227 (±49)  | 0.117 - 0.244 |
|                | 0.00       |              | 1370 (±56) | NA         | 0.407 - 0.428 |
|                | 0.04       |              | NA         | NA         | 0.451 - 0.486 |
|                | 0.40       |              | 1449 (±11) | NA         | 0.430 - 0.435 |
|                | 4.00       |              | 173 (±5)   | 905 (±83)  | 0.200 - 0.379 |
|                | 0.00       | 1            | 1127 (±64) | NA         | 0.427 - 0.778 |
|                | 0.04       | 4            | 1272 (±96) | NA         | 0.433 - 0.779 |
|                | 0.40       |              | 221 (±18)  | NA         | 0.127 - 0.721 |
|                | 4.00       |              | 267 (±18)  | 291 (±11)  | 0.152 - 0.254 |
|                | 0.00       |              | NA         | NA         | 0.480 - 0.527 |
|                | 0.04       |              | NA         | NA         | 0.468 - 0.652 |
|                | 0.40       |              | 1534 (±4)  | NA         | 0.464 - 0.728 |
|                | 4.00       |              | 163 (±2)   | 549 (±15)  | 0.169 - 0.247 |
| NOM type | TOC (mg/L) | nTiO₂ (mg/L) | Size (nm) t₀h | Size (nm) t₉₆ₖ | PI     |
|----------|------------|--------------|---------------|---------------|--------|
|          |            |              |               |               |        |
|          | 0.00       | 697 (±2)     | NA            |               | 0.290 - 0.725 |
| SR HA    | 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 |
|          | 4.00       | NA           | NA            |               | 0.543 - 0.674 |
| SR FA    | 0.00       | 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 |
|          | 4.00       | 1202 (±30)   | NA            |               | 0.410 - 0.721 |
|          | 0.04       | 1157 (±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       | 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       | 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       | 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       | 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 (±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 Zeta Potential (mV) | P25 Zeta Potential (mV) |
|--------------|------------|----------------------------|-------------------------|
| ASTM - Blank | 0.00       | -4.92 (±0.10)              | -10.79 (±0.46)          |
|              | 0.04       | -13.03 (±0.10)             | -13.60 (±2.21)          |
|              | 0.40       | -17.91 (±2.03)             | -22.51 (±1.69)          |
|              | 4.00       | -19.89 (±0.27)             | -22.21 (±1.06)          |
| SA HA        | 0.04       | -4.09 (±0.16)              | -9.77 (±0.24)           |
|              | 0.40       | -9.4 (±0.13)               | -21.48 (±0.80)          |
|              | 4.00       | -20.79 (±0.33)             | -21.22 (±0.26)          |
| SW           | 0.04       | -6.65 (±1.01)              | -12.52 (±0.43)          |
|              | 0.40       | -12.48 (±0.80)             | -20.23 (±0.67)          |
|              | 4.00       | -22.14 (±0.23)             | -19.97 (±1.08)          |
| SR NOM       | 0.04       | -6.93 (±0.25)              | -14.94 (±0.38)          |
|              | 0.40       | -16.16 (±0.70)             | -23.10 (±0.62)          |
|              | 4.00       | -24.39 (±0.23)             | -23.61 (±0.58)          |
| SR HA        | 0.04       | -3.59 (±0.45)              | -13.47 (±0.26)          |
|              | 0.40       | -14.34 (±0.44)             | -19.93 (±0.15)          |
|              | 4.00       | -21.68 (±0.53)             | -22.81 (±0.79)          |
| SR FA        | 0.04       | -12.09 (±0.10)             | -12.88 (±1.15)          |
|              | 0.40       | -21.02 (±2.60)             | -28.62 (±3.67)          |
|              | 4.00       | -12.09 (±0.12)             | -24.97 (±3.66)          |
| LEO          | 0.04       | -13.91 (±0.77)             | -18.20 (±2.10)          |
|              | 0.40       | -22.30 (±1.00)             | -24.33 (±2.89)          |
|              | 4.00       | -22.57 (±1.00)             | -22.11 (±3.02)          |
| PP           | 0.04       | -13.91 (±0.77)             | -18.20 (±2.10)          |
|              | 0.40       | -22.30 (±1.00)             | -24.33 (±2.89)          |
|              | 4.00       | -22.57 (±1.00)             | -22.11 (±3.02)          |
Table S4: Properties of the used nTiO$_2$ 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 $^d$ | ~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         |

$^a$Producer information

$^b$Dispersed in H$_2$O dest., stabilized by a low pH
Figure S1 A-C: Normalized 96-h EC<sub>50</sub> values (with 95% CIs) for the immobilization data of <i>D. magna</i>: Gained 96-h EC<sub>50</sub> 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 EC<sub>50</sub> determined for the same nTiO<sub>2</sub> product at 0.00 mg TOC/L NOM (black line).
Figure S2 A-C: Normalized 96-h EC$_{50}$ values (with 95% CIs) for the immobilization data of *D. magna*: Gained 96-h EC$_{50}$ 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 EC$_{50}$ determined for the same nTiO$_2$ product at 0.00 mg TOC/L NOM (black line).
Figure S3 A-B: Linear correlation models of the NOM-sulfur (A) or nitrogen (B) content with 96-h EC$_{50}$ values of the nTiO$_2$ products A-100 (white circles; dashed line) and P25 (black circles; continuous line).