Supporting material

Attachment efficiency of nanomaterials to algae as an important criterion for ecotoxicity and grouping

Kerstin Hund-Rinke^{1*}, Tim Sinram¹, Karsten Schlich¹, Carmen Nickel², Hanna Paula Dickehut³, Matthias Schmidt³, Dana Kühnel³

- ¹ Fraunhofer Institute for Molecular Biology and Applied Ecology; Auf dem Aberg 1, 57392 Schmallenberg, Germany
- ² Institute for Energy and Environmental Technology. V.(IUTA), Bliersheimer Straße 58-60, 47229 Duisburg, Germany
- ³Helmholtz Centre for Environmental Research (UFZ), Permoserstr. 15, 04318 Leipzig, Germany
- * Correspondence: kerstin.hund-rinke@ime.fraunhofer.de

1 Attachment of CeO₂ NM-212 to the green algae *Raphidocelis subcapitata*



Figure S 1: Attachment of CeO_2 NM-212 to algae. This phase-contrast image (left = 400×, right = 1000×) was captured during a 72-h growth inhibition test with 2.5 mg/L CeO₂ NM-212.



Figure S2: Attachment of CeO_2 NM-212 to algae. This phase-contrast image (left = 400×, right = 1000×) was captured during a 72-h growth inhibition test with 10 mg/L CeO₂ NM-212.



Figure S3: Attachment of CeO_2 NM-212 to algae. This phase-contrast image (left = 400×, right = 1000×) was captured during a 72-h growth inhibition test with 40 mg/L CeO₂ NM-212.

2 Attachment of CeO₂ NM-211 to the green algae *Raphidocelis subcapitata*



Figure S4: Attachment of CeO₂ NM-211 to algae. This phase-contrast image (1000× magnification) was captured after 3 h incubation with 100 mg/L CeO₂ NM-211.

3 Attachment of CeO₂ NM-213 to the green algae *Raphidocelis subcapitata*



Figure S5: Attachment of CeO_2 NM-213 to algae. This phase-contrast image (left = 400×, right = 1000×) was captured during a 72-h growth inhibition test with 10 mg/L CeO₂ NM-213.



Figure S6: Attachment of CeO_2 NM-213 to algae. This phase-contrast image (left = 400×, right = 1000×) was captured during a 72-h growth inhibition test with 24 mg/L CeO₂ NM-213.



Figure S7: Attachment of CeO2 NM-213 to algae. This phase-contrast image (left = $400 \times$, right = $1000 \times$) was captured during a 72-h growth inhibition test with 80 mg/L CeO₂ NM-213.



Figure S8: Attachment of CeO₂ NM-213 to algae, showing transparent, algae-shaped structures (red arrow). This phase-contrast image (1000× magnification) was captured during a 72 h growth inhibition test with 80 mg/L CeO₂ NM-213.

4 Control algae *Raphidocelis subcapitata*



Figure S9: <u>Control algae Raphidocelis subcapitata. Upper panel: scanning electron microscopy</u> (SEM) images. Lower panel: helium ion microscopy (HIM) images. Middle insert: SEM image of CeO₂ NM212 exposed algae cells.

5 Attachment of TiO₂ non-doped (91 % anatase; 9 % rutile) to the green algae *Raphidocelis subcapitata* – short-term test



Figure S10: Attachment of non-doped TiO₂ (91% anatase and 9% rutile) to algae, showing large agglomerates with embedded algal cells. This phase-contrast image (upper 400×, lower 1000×) was captured after 3 h incubation with 100 mg/L non-doped TiO₂. 6 Attachment of Eu-doped TiO₂ to the green algae *Raphidocelis subcapitata* – short-term test

Figure S11: Attachment of Eu-doped TiO_2 to algae revealing attachment to transparent, sheath-like structure around algal cells (red arrow). This phase-contrast image (left = 1000×, right = 100×) was captured after 3 h incubation with 100 mg/L.

Figure S12: Attachment of Eu-doped TiO₂ to algae, revealing particles spread over a small area with incorporated algae cells (red arrow). This phase-contrast image (1000× magnification) was captured after 3 h incubation with 100 mg/L Eu-doped TiO₂.

7 Attachment of Fe-doped TiO₂ to the green algae *Raphidocelis subcapitata* – short-term test

Figure S13: Attachment of Fe-doped TiO₂ to algae (red arrow). This phase-contrast image (1000× magnification) was captured after 3 h incubation with 100 mg/L Fe-doped TiO₂.

8 Attachment of TiO₂ NM-105 to the green algae *Raphidocelis subcapitata* – short-term test

Figure S14: Attachment of TiO₂ NM-105 to algae, showing nanoparticle attachment to algal cells and transparent, algae-shaped structures (red arrow). This phase-contrast image (1000× magnification) was captured after 3 h incubation with 100 mg/L TiO₂ NM-105.

9 Attachment of TiO₂ NM-104 to the green algae *Raphidocelis subcapitata* – short-term test

Figure S15: Attachment of TiO₂ NM-104 to algae, showing loose formation of smaller agglomerates. This phase-contrast image (1000× magnification) was captured after 3 h incubation with 100 mg/L TiO₂ NM-104. 1 0 Attachment of Eu-doped TiO₂ to the green algae *Raphidocelis subcapitata* – growth inhibition test

Figure S16: Attachment of Eu-doped TiO₂ to algae. This phase-contrast image (1000× magnification) was captured during a 72-h growth inhibition test with 2 mg/L Eu-doped TiO₂.

Figure S17: Attachment of Eu-doped TiO_2 to algae. This phase-contrast image (1000× magnification) was captured during a 72-h growth inhibition test with 18 mg/L Eu-doped TiO_2 .

1 1 Attachment of TiO₂ NM-104 to the green algae *Raphidocelis subcapitata* – growth inhibition test

Figure S18: Attachment of TiO_2 NM-104 to algae. This phase-contrast image (left = 400×, right = 1000×) was captured during a 72-h growth inhibition test with 7.5 mg/L TiO_2 NM-104.

Figure S19: Attachment of TiO_2 NM-104 to algae. This phase-contrast image (left = 400×, right = 1000×) was captured during a 72-h growth inhibition test with 30 mg/L TiO₂ NM-104.

Figure S20: Attachment of TiO_2 NM-104 to algae. This phase-contrast image (left = 400x, right = 1000x) was captured during a 72-h growth inhibition test with 120.0 mg/L TiO_2 NM-104.