

Influence of alumina coating on characteristics and effects of SiO₂ nanoparticles in algal growth inhibition assays at various pH and organic matter contents

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1 **Abstract** – Silica nanoparticles (NPs) belong to the industrially most important NP types.
2 In a previous study it was shown that amorphous SiO₂ NPs of 12.5 and 27.0 nm are stable
3 in algal growth inhibition assays and that their ecotoxic effects are related to NP surface
4 area. Here, it was hypothesized and demonstrated that an alumina coating completely alters
5 the particle-particle, particle-test medium and particle-algae interactions of SiO₂ NPs.
6 Therefore, stability and surface characteristics, dissolution, nutrient adsorption and effects
7 on algal growth rate of both alumina coated SiO₂ NPs and bare SiO₂ NPs in OECD algal
8 test medium in function of pH (6.0-8.6) and natural organic matter (NOM) contents (0-12
9 mg C/l) were investigated. Alumina coated SiO₂ NPs aggregated in all media and adsorbed
10 phosphate depending on pH and NOM concentration. On the other hand, no aggregation or
11 nutrient adsorption was observed for the bare SiO₂ NPs. Due to their positive surface
12 charge, alumina coated SiO₂ NPs agglomerated with *Pseudokirchneriella subcapitata*.
13 Consequently, algal cell density measurements based on cell counts were unreliable and
14 hence fluorescent detection of extracted chlorophyll was the preferred method. Alumina
15 coated SiO₂ NPs showed lower toxicity than bare SiO₂ NPs at concentrations \geq 46 mg/l,
16 except at pH 6.0. At low concentrations, no clear pH effect was observed for alumina
17 coated SiO₂ NPs, while at higher concentrations phosphate deficiency could have
18 contributed to the higher toxicity of those particles at pH 6.0-6.8 compared to higher pH
19 values. Bare SiO₂ NPs were not toxic at pH 6.0 up to 220 mg/l. Addition of NOM
20 decreased toxicity of both particles. For SiO₂ NPs the 48 h 20 % effect concentration of
21 21.8 mg/l increased 2.6-21 fold and a linear relationship was observed between NOM
22 concentration and effective concentrations. No effect was observed for alumina coated SiO₂
23 NPs in presence of NOM up to 1000 mg/l. All experiments point out that the alumina
24 coating completely altered NP interactions. Due to the difference in surface composition the
25 SiO₂ NPs, which had the smallest surface area, were more toxic to the alga than the alumina

26 coated SiO₂ NPs. Hence, surface modification can dominate the effect of surface area on
27 toxicity.

28

29 **Keywords** – SiO₂, coating, nanoparticles, algae, NOM

30 1. INTRODUCTION

31 Scientific research involving nanotechnology has grown exponentially (Braun et al., 1997).
32 This led to the development of engineered nanoparticles (NPs) and nanoparticle containing
33 consumer products with improved performances compared to non-nanotechnology based
34 products. The special feature of nanoparticles is their small size, between 1 and 100 nm in
35 two or three dimensions (ASTM, 2006), and related high amount of specific surface area,
36 which enhances their reactivity (Oberdörster et al., 2005). Due to production, use and
37 disposal of NPs or NP containing products, they are expected to be released into the
38 environment. Therefore, concerns about the potential environmental risks posed by NPs
39 have been raised (Colvin et al, 2003). In addition to the establishment of effect
40 concentrations for NPs, other research priorities were to assess the importance of general
41 NP characteristics, like size, surface area, chemical composition, solubility and aggregation
42 behaviour for their (eco)toxicity (Tran et al., 2005). An often overlooked parameter is the
43 presence of a surface coating. To our knowledge, no ecotox studies have systematically
44 investigated the influence of a surface coating on the physical and toxicological properties
45 of NPs.

46 In this study, we describe the differential characteristics and effects of a bare SiO₂ and an
47 alumina coated SiO₂ NP. Silicon dioxide (SiO₂) nanoparticles are among the most
48 important industrially engineered NPs (Rittner, 2003). They are used in paints and coatings
49 for an improved rheology, attachment and scratch-resistance and in printer toners they serve
50 as anti-binder (Mizutani et al., 2006; Zappa et al., 2009). Furthermore, these NPs are used
51 in chemical or mechanical polishing processes, among which dental polishing to prevent
52 tooth caries (Gaikwad et al., 2008). Other medical applications are the use of SiO₂ NPs as
53 carrier for therapeutic agents or for diagnostic purposes (Wang et al, 2006; Zhang et al.,
54 2008). Silica nanoparticles can act as a binding site for negatively charged ions when an

55 alumina coating is applied to their surface (Li and Stöver, 2008). Because of their numerous
56 applications and high production volumes, both silica and alumina were included in the list
57 of representative nanomaterials adopted by OECD's working party on manufactured
58 nanomaterials (OECD, 2010).

59 In a previous study, we demonstrated that bare SiO₂ NPs were stable in algal test medium
60 and that their ecotoxicity was related to their surface area. Furthermore, the NPs were found
61 attached to the algal cell wall and toxicity was not due to particle dissolution (Van Hoecke
62 et al., 2008). However, in the present study it was hypothesized that an alumina coating
63 might alter the particle-particle, particle-alga and particle-test medium interactions of SiO₂
64 NPs. Therefore, NP suspension stability was investigated in test media with varying pH and
65 natural organic matter (NOM) concentration. In algal growth inhibition assays with
66 *Pseudokirchneriella subcapitata* the influence of both parameters on SiO₂ and alumina
67 coated SiO₂ NP toxicity was assessed. In order to prevent erroneous conclusions due to
68 particle-alga interactions, algal growth was measured based on cell density (measured
69 directly with a cell counter) or on chlorophyll contents relative to a standard series of non
70 exposed cells with known cell densities. Finally, we investigated here the appearance of a
71 shading effect, which is the inhibition of algal growth due to light limitation caused by
72 absorption or scattering by the NPs, and particle-test medium interactions, i.e. the
73 dissolution of NPs and nutrient adsorption by the NPs. To exclude the contribution of any
74 impurities to the toxic effects, NP suspensions had been dialyzed with deionized water prior
75 to the experiments.

76

77 **2. MATERIALS AND METHODS**

78 **2.1. Chemicals and nanoparticle specifications**

79 All chemicals were of pro analytical grade supplied by VWR International (Leuven,
80 Belgium). An Al^{3+} standard was purchased at Sigma Aldrich (Bornem, Belgium) (No.
81 39435). Natural organic matter (NOM) was sampled from 'Le puisseau de St. Martain', a
82 creek in Bihain, Belgium, using a portable reverse-osmosis based device (PROS/2) in order
83 to concentrate NOM as described by Serkiz and Perdue (1990) and Sun et al. (1995). The
84 sampling procedure was described in more detail in De Schamphelaere et al. (2003).

85 Commercially available LUDOX[®] aqueous colloidal silica suspensions were obtained
86 from Sigma-Aldrich, i.e. the negatively charged LUDOX CL-X SiO_2 nanoparticles (NPs)
87 (No. 420891) and the positively charged alumina coated SiO_2 NPs LUDOX CL (No.
88 420883). The core material of the latter NP consists of amorphous SiO_2 to which an
89 approximately 1 nm layer coating of alumina was deposited (Vo et al., 2007). The alumina
90 coating arose through adsorption of Al^{3+} ions to the negatively charged SiO_2 NP surface.
91 With increasing pH, more Al ions adsorp to the surface, which precipitate as aluminium
92 hydroxide ($\text{Al}(\text{OH})_3$), forming a solid coating onto the SiO_2 NPs (Kuan et al., 2000). Van
93 der Meeren et al. (2004) observed a decreasing surface charge from pH 5 on and a switch in
94 zetapotential from positive to negative at pH 2.5. The latter observation suggests that at low
95 pH values, the solid coating dissolves and Al^{3+} ions adsorbed to the SiO_2 NP surface can be
96 substituted by protons (H^+) (James and Healy, 1972). Hence, the first iso-electric point
97 (IEP) at pH 2.5 is the point of zero charge of the bare SiO_2 NP core.

98 Except for the first experiment in which the toxicity of dialyzed and non dialyzed
99 suspensions was compared, dialyzed aqueous suspensions of both silica products were used
100 for further experiments. The dialysis of the NP suspensions was performed using dialysis
101 membranes (12.4 kDa) from Sigma Aldrich (No. D9652). The sample was placed inside the
102 dialysis tubes and submerged in 5 l of Milli-Q water. The sample was left for a minimum of
103 4 h each time and the water was renewed four times, since it was observed that the

104 conductivity of dialysis water did not further decrease after 4 dialysis cycles (supporting
105 information, Figure S1). Once the dialysis was complete, the concentration of the stock
106 suspension was determined gravimetrically after freeze drying 1 ml of suspension in a
107 freeze dryer overnight at -60 °C and 0.1 bar pressure. As a consequence, any impurities
108 possibly present in the industrial product could be removed. Nitrogen gas adsorption
109 experiments were used to measure the specific surface area of both particle types using a
110 Tristar 3000 BET instrument (Micrometrics, Norcross, GA, USA). Specific surface area of
111 the LUDOX CL-X SiO₂ NPs was 102 m²/g, while the LUDOX CL alumina coated SiO₂
112 NPs represented a specific surface area of 203 m²/g. Primary particle sizes calculated from
113 these measurements were 22 and 11 nm, respectively.

114 Nanoparticle suspensions in OECD ecotoxicity test medium were prepared from either the
115 original or dialyzed stock by spiking the silica NPs dropwise into the test media under
116 continuous stirring. Where appropriate, NOM and/or buffer solutions (final concentration
117 of 3.6 mM buffer) were added to the medium and pH was adjusted to the desired value
118 using 1 M NaOH or HCl solutions before spiking the NPs. To maintain a pH value of 6.0 2-
119 (N-Morpholino)ethanesulfonic acid (MES) buffer was used. Media pHs 6.6 and 7.6 were
120 stabilized with 3-(N-Morpholino)propanesulfonic acid (MOPS) buffer and pH 8.6 required
121 2-(Cyclohexylamino)ethanesulfonic acid (CHES) buffer.

122

123 **2.2. Suspension characterization**

124 Particle size distributions of both NP types in all test media were analyzed using dynamic
125 light scattering (DLS) four days after suspension preparation as described in Van Hoecke et
126 al. (2008). A concentration of 2 g/l silica NPs and 460 mg/l alumina coated silica NPs was
127 used for the DLS analysis with a PCS 4700 SM (Malvern Instruments, Worcestershire, UK)
128 equipped with a 5 mW HeNe laser. Scattered light was detected under an angle of 150 ° and

129 data was processed by a 7032 CN correlator (Malvern Instruments). Non negative least
130 squares analysis was used to determine the particle size distribution, whereas the harmonic
131 intensity weighed average hydrodynamic diameter, also referred to as the Z-average
132 diameter (Zave), was obtained by cumulant analysis option of the automeasure software
133 (Malvern Instruments). The zetapotential in OECD medium at various pH values was
134 determined using a Zetasizer 3000 HSA (Malvern Instruments, Worcestershire, UK).
135 During an algal growth inhibition test, pH was monitored each day using a pH electrode
136 (P407, Consort, Turnhout, Belgium) and adjusted from 0.2 pH units deviation on. The
137 media at pH 8.6 were checked twice a day. Natural organic matter concentrations were
138 monitored in a buffer free replicate in OECD algal test medium incubated under
139 experimental conditions and measured with A TOC-500 (Shimadzu, Duisburg, Germany)
140 carbon analyzer.

141

142 **2.3. Algal culturing and growth inhibition tests**

143 The alga *Pseudokirchneriella subcapitata* (Korshikov) Hindak was obtained from the
144 Culture Collection of Algae and Protozoa (CCAP 278/4, Oban, Scotland) and subcultured
145 in the laboratory. The culture medium consisted of ES-medium (Provasoli, 1966) at 1/2
146 strength which was added to carbon filtered aerated tap water, supplemented with 1.4 mg/l
147 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 15 mg/l $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 150 mg/l NaNO_3 and 2.35 mg/l $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$. Each
148 week, cultures were visually inspected for contamination using a light microscope. Four
149 days prior to the start of a growth inhibition experiment, a new algal culture was prepared
150 and allowed to grow on a shaking table at 20 ± 1 °C in continuous light ($70 \mu\text{E}/(\text{m}^2 \cdot \text{s})$).

151 The 72 h algal growth inhibition experiments were conducted in accordance with OECD
152 guideline No. 201 (OECD, 2006). Prior to the start of a test, all test concentrations ranging
153 between 4.6 and 1000 mg/l were equilibrated at 25 °C overnight. For each test

154 concentration three replicates and one background correction (no algae added) were
155 included. The replicates were inoculated with 10^4 algal cells/ml. During the 72 h test, all
156 flasks were incubated at a temperature of 25 °C under continuous illumination
157 ($70 \mu\text{E}/(\text{m}^2 \cdot \text{s})$) and were shaken manually three times a day. Every day, the algal cell
158 density was measured in all replicates based on both an electronic cell count and
159 chlorophyll contents. In the former method, a sample was introduced into a cell counter
160 (Beckman Coulter Counter, Gent, Belgium) and the number of cells/ml was determined.
161 The latter method consisted of adding 3 ml extraction mixture (dimethylsulfoxide
162 (DMSO):acetone 1:1) to a 0.75 ml sample, vortexing and allowing to stand in the dark for
163 20 minutes to extract the chlorophyll. Chlorophyll fluorescence was recorded with a
164 spectrophotometer (LS50B, Perkin Elmer, Zaventem, Belgium) in a 1 cm quartz cuvette at
165 a wavelength of 671 nm, using an excitation wavelength of 431 nm (Mayer et al., 1997).
166 Preliminary research indicated that the presence of both NP types in the concentrations used
167 in the experiments did not affect the fluorescent signal of chlorophyll (results not shown).
168 The algal cell density was determined against a concentration series of algal cells in OECD
169 medium that had been inoculated with the same culture and extracted under identical
170 conditions. Each day, a new algal concentration series was prepared and analyzed. The
171 average specific growth rate μ (1/d) was calculated as the slope of a linear regression of the
172 natural logarithm of the measured cell density (corrected for background or blank) versus
173 time. Data analyzed after 48 h were reported here, because experiments performed at pH
174 6.0 and 8.6 did not always meet the validity criteria prescribed by the OECD when
175 analyzed at 72 h. Where possible, data analyzed after 72 h is given in the supplementary
176 information section. A log-logistic or modified log-logistic concentration-response curve
177 was fitted to the toxicity data with Statistica 6.0 (Statsoft, Tulsa, OK).

178 Additionally, the occurrence of a shading effect, i.e. decrease in algal growth due to
179 limitation in the light availability caused by the nanoparticles, was assessed separately
180 using 1000 mg/l NP suspensions in OECD medium at pH 7.4. The experimental approach
181 consisted of two 96 well plates, i.e. an opaque plate fixed on top of a white plate. Basically,
182 the chlorophyll contents upon spatially separating particles and algal cells was compared to
183 the chlorophyll contents when algal cells and NPs were added to the same well. The same
184 set-up was formerly described in Hund-Rinke et al. (2006) and in Van Hoecke et al. (2009).

185 **2.4. Assessment of interactions between nanoparticles and test medium**

186 In NOM free medium, two types of possible interactions between particles and test
187 media were investigated, i.e. partial dissolution and adsorption of the nutrients ammonium
188 (NH_4^+) and phosphate (PO_4^{3-}) to the NP surface. Nanoparticle suspensions were prepared
189 and incubated in such a way that experimental conditions were identical to those during the
190 72 h algal growth inhibition tests, but without addition of algal cells. After 48 h, NPs were
191 removed from the suspensions. To this end, the alumina coated SiO_2 NP suspensions were
192 first centrifuged at 2000xg for 15 minutes in a swinging-bucket centrifuge (IEC centra-8
193 centrifuge, International Equipment Co, Needham, MA) to sediment large aggregates.
194 Then, 20 ml samples were forced through 10 kDa ultrafilters with polyethersulfon
195 membrane (Vivaspin 20, Sartorius, Goettingen, Germany) by a 10 minute centrifugation at
196 2000xg. The filtrate was used for further analysis. The ultrafilter performance, i.e. binding
197 of analytes to the filter material or leaching of analytes from either the filter material or
198 from particles retained by the filter, was checked by including additional controls after
199 every 80 ml run through the filter. More specifically, to check for possible binding of
200 analytes to the filter material, standard OECD medium controls were filtered and its filtrate
201 included in the colorimetric analysis. Leachage of analytes from the filter material or from
202 the retained particles on the filter into subsequent samples was checked by including

203 deionized water controls. Furthermore, the retention of Al by the filters was assessed at
204 various pH values with 0.5 mg Al/l standards in OECD medium without Fe and EDTA.
205 The concentration of reactive silica, aluminum, NH_4^+ and PO_4^{3-} was colorimetrically
206 assessed with an Aquamate spectrophotometer (Thermo Electron Corporation, Waltham,
207 MA, US). Reactive silica was complexed with ammonium molybdate and subsequently
208 reduced with sodium sulfite, based on ASTM procedure D859-00 (ASTM, 2000). The
209 absorbance of the blue complex was recorded at 700 nm in a 1 cm cuvette. Aluminum
210 concentration was measured in a 2 cm cuvette using a commercial analysis kit No
211 1.14825.0001 from Merck KgaA (Darmstadt, Germany). In addition, chemical speciation
212 of aluminum in OECD medium at various pH values was estimated with Visual MINTEQ
213 ver. 2.51 program (CEAM, EPA, US). Colorimetric analysis of NH_4^+ and PO_4^{3-} was
214 performed in a 1 cm cuvette using commercial analysis kits (no. 1.14848.0001 and no.
215 1.14752.0001).

216 In NOM containing buffer free medium at pH 7.4, the adsorption of both NOM and
217 phosphate to the surface of alumina coated SiO_2 NPs was investigated. Therefore, after 48 h
218 incubation under test conditions, 0, 4.6, 46 and 460 mg/l suspensions were centrifuged for
219 15 minutes at 2000xg to sediment the particle aggregates. The supernatant was used for
220 NOM and phosphate analysis using methods described above. Concentrations were
221 expressed relative to the identically treated control.

222

223 **3. RESULTS**

224 **3.1. Nanoparticle characterization**

225 The average diameters of SiO_2 and alumina coated SiO_2 NPs of original stock
226 dilutions in deionized water at the same pH of the original stock and of dialyzed stock
227 dilutions in OECD medium at various pH values and natural organic matter (NOM)

228 concentrations are given in **Table 1**. The SiO₂ NPs were stable in deionized water at pH
229 9.6, since the diameter obtained in the DLS measurements corresponded well with the BET
230 primary particle size of 22 nm. The average DLS diameters around 31 nm obtained in
231 OECD medium suggested that some aggregation occurred under test conditions. The
232 alumina coated SiO₂ NPs formed small aggregates in deionized water at pH 4, where a
233 mean diameter of 39 nm was obtained. In all other media, severe aggregation gave rise to
234 micrometer size particles, of which the actual size was too large to be accurately
235 determined by dynamic light scattering. The addition of NOM did not affect the stability of
236 the silica NP suspensions. The zeta potential values varied with pH, as reported in **Table 2**.
237 For both particles, the surface charge became more negative at higher pH values. However,
238 the SiO₂ NPs having a zeta potential between -23 and -47 mV were negatively charged over
239 the entire pH interval used in this study, while the zeta potential of the alumina coated SiO₂
240 NPs switched from positive to negative between pH 7.5 and 9.0. Those observations are
241 fully in line with the reported IEP values of SiO₂ and Al₂O₃, respectively.

242

243 **3.2. Algal growth inhibition assessments**

244 First, it was investigated if dialyzing the NP suspensions affected their toxicity. The
245 concentration-response curves of dialyzed and non dialyzed SiO₂ NPs collapsed, regardless
246 of the algal cell density measurement method. **Figure 1A** presents those concentration-
247 response curves. However, in case algal cell density was analyzed by fluorescence of
248 extracted chlorophyll, the effect on growth rate was much more severe compared to the cell
249 counting method. For example, the highest test concentration of 460 mg/l resulted in 56 %
250 or 92 % decrease in growth rate when assessed using cell counting or extracted chlorophyll
251 fluorescence, respectively. A different outcome was obtained when comparing
252 concentration-response curves of dialyzed and non dialyzed alumina coated SiO₂ NPs. Cell

253 counting resulted in non-monotonous concentration-response curves, with apparently high
254 toxicity at low NP concentrations. However, clusters of algal cells growing in flocs could
255 be visually observed, as illustrated in **Figure 2**. Hence, the measured cell count was much
256 lower compared to the real density of individual cells of which the clusters were composed.
257 Chlorophyll fluorescence analysis resulted in monotonous concentration-response curves,
258 with the non-dialyzed suspension causing a more severe effect on algal growth rate
259 compared to the dialyzed NP suspension. At the highest test concentration 55 % decrease
260 was observed for non-dialyzed suspensions against only 25 % for dialyzed suspensions.
261 Possibly, impurities present in LUDOX CL can partly explain the observed effects of non
262 dilayzed suspension. From Figure S1 in supporting information it can be observed that
263 more impurities were removed from the LUDOX CL nanoparticle suspensions during the
264 dialysis compared to the LUDOX CL-X nanoparticle suspensions. The four concentration-
265 response curves are given in **Figure 1B**. An overview of 48 h 10, 20 and 50 % effect
266 concentrations on growth rate, as well as No Observed Effect Concentrations (NOECs) and
267 Lowest Observed Effect Concentrations (LOECs) are given in **Table 3**. The 72 h values are
268 reported in **Table S1** in the supplementary information. For all subsequent experiments the
269 dialyzed suspensions were used.

270 The concentration-response curves with concentration both expressed as mass and
271 as surface area are given in **Figure S2**.

272 Secondly, the toxicity of both NPs with varying pH was assessed. All curves
273 obtained using fluorescence of extracted chlorophyll are given in **Figures 3A** and **3B**. The
274 concentration-response curves obtained using the cell counter are shown in the
275 supplementary information in **Figure S3A**. At the highest test concentration of 220 mg/l no
276 significant reduction in algal growth rate was observed for SiO₂ NPs at pH 6.0. At pH 6.8
277 and 8.6, concentration-response curves were similar, for which 10 % effect concentrations

278 (E_rC_{10}) of 12.3 and 13.2 mg/l were established, respectively. The highest toxicity was
279 observed at pH 7.6, with 48 h- E_rC_{10} of 6.1 mg/l. The alumina coated SiO₂ NPs showed a
280 different pH influence on toxicity (**Figure 3B**). At low test concentrations, no clear pH
281 effect was observed. At the higher test concentrations, pH 8.6 resulted in the lowest toxicity
282 with a NOEC of 100 mg/l. At pH values 6.0, 6.8 and 7.6, the NOECs were 4.6 mg/l. The
283 52 % decrease in algal growth rate in the highest test concentration at pH 6.0 was the only
284 exposure condition causing an effect > 50 %. The 48 h effect concentrations, NOECs and
285 LOECs can be found in **Table 4**. Concentration-response curves obtained using cell
286 counting and corresponding effect parameters are given in the supplementary information
287 in **Figure S3B** and **Table S2**.

288 In addition, the relation between toxicity and specific surface area of both particles
289 can be evaluated. Based on the specific surface area measurements (203 m²/g for the
290 alumina coated SiO₂ NPs and 102 m²/g for the SiO₂ NPs) only, the alumina coated SiO₂
291 NPs were expected to be most toxic. However, from **Tables 3 and 4** it is clear that the
292 difference in toxicity cannot be explained by surface area. For example, at pH values 6.8-
293 8.6 48 h- E_rC_{20} s of SiO₂ NPs ranged between 9.9 and 20.4 mg/l, while those of alumina
294 coated SiO₂ NPs were higher and ranged between 25.2 and 342.1 mg/l. This demonstrates
295 that here, in case of different surface chemistry, toxicity was not governed by specific
296 surface area only, and that coating characteristics can dominate toxic response.

297 Finally, the influence of natural organic matter (NOM) on the toxicity of both NP
298 types was assessed at pH 7.4. Addition of NOM to the test medium before spiking the NPs
299 decreased their toxicity. For SiO₂ NPs, 48 h- E_rC_{10} values were 25.6, 120.6 and 263.5 mg/l
300 in presence of 1.2, 4.7 and 9.0 mg C/l NOM, respectively, which represents a 2-19 fold
301 increase. All ecotoxicological effect parameters are summarized in **Table 5**. No toxicity
302 was observed at the highest test concentration of 1000 mg/l alumina coated SiO₂ NPs in

303 presence of any concentration of NOM. **Figure 4A** and **4B** show all concentration-response
304 curves assessed using chlorophyll detection. In **Table S1**, **Table S2** and **Figure S4** of the
305 supplementary information concentration-response curves based on cell counting and their
306 corresponding 48 h effect parameters, as well as 72 h effect parameters are included.

307 The 96 well plate experiments to assess the shading effect indicated that toxicity
308 was not due to light limitation. When algal cells and NP suspensions were spatially
309 separated, no decrease in chlorophyll contents relative to the control was observed.
310 However, when algal cells were directly exposed to the NP suspensions, chlorophyll
311 contents decreased with >80 % relative to the control. The results are graphically shown in
312 **Figure 5**.

313 **3.3. Interactions between nanoparticles and test medium**

314 First, the Vivaspin 20 filter performance was checked for retaining or leaching of
315 analytes. In four filtering processes with OECD medium no phosphate or ammonium was
316 retained by the filters. Mean (std. dev.) recoveries of 100.8 (2.4) and 100.2 (0.7) % were
317 obtained, respectively. On the other hand, leachage of these nutrients into filtered deionized
318 water was negligible because absorbance values were not significantly higher than those of
319 deionized water blanks that were not forced through the filters. The concentration of
320 dissolved silica in filtered OECD medium and deionized water run through a particle
321 containing filter was beneath the detection level of the colorimetric method (< 0.050 mg/l).
322 Finally, after filtering a 0.5 mg/l Al standard in OECD medium at pH 2, a recovery of 90.4
323 (9.8) % was obtained.

324 The SiO₂ NPs partially dissolved in OECD medium and their solubility was pH
325 dependent, as presented in **Table 6**. At pH 8.6, 68.6 mg SiO₂/l reactive silica was present in
326 the highest SiO₂ NP concentration of 460 mg/l, which is a reactive silica concentration 11
327 times higher compared to the same suspension at pH 6.0. On the other hand, the reactive

328 silica concentration in the alumina coated SiO₂ NP suspensions was low. At the highest NP
329 concentration of 460 mg/l, the reactive silica concentration was not higher than 1.1 mg/l as
330 SiO₂. Only in the filtrate of the 460 mg/l suspension at pH 8.6 a significant amount of
331 aluminum was detected (0.068 mg/l or 2.5x10⁻³ mM). However, the chemical speciation
332 program Visual MINTEQ indicated that only 3.12x10⁻⁵ mM ionic Al can be present in
333 OECD medium at pH 8.6. Any excess of Al was predicted to precipitate as the mineral
334 diaspore (α -AlO(OH)). On the other hand, Parent et al. (1996) reported 30 % reduction in
335 algal growth rate of the green alga *Chlorella pyrenoidosa* exposed to 6.2 μ M mononuclear
336 inorganic Al for 96 h. As a consequence, it is not likely that the decrease in algal growth
337 rate during exposure to alumina coated SiO₂ NPs was caused by the presence of inorganic
338 mononuclear dissolved Al. However, very small (< 10 kDa) secondary diaspore colloids in
339 460 mg/l suspensions at pH 8.6 could have passed the ultrafilter. Also, aluminum is known
340 to form polynuclear compounds (Parent et al., 1994). Such colloids and/or polynuclear
341 compounds could have been formed after partial dissolution of the Al(OH)₃ coating and
342 desorption of Al³⁺ and/or its dissolved hydroxides. Hence, it cannot be excluded that these
343 small secondary colloids did not contribute to the observed toxic effects.

344 Colorimetric Al analysis of 0.5 mg/l standards in OECD medium (without Fe and EDTA)
345 at various pH values before and after ultrafiltration experimentally confirmed the predicted
346 precipitation of the Visual MINTEQ program. Indeed, at pH 4.5, 7.6 and 11.0, recoveries
347 (std. dev. on the mean of five replicates) of 3.3 (3.6), 15.0 (14.4) and -0.1 (3.7) % were
348 obtained, which allowed to conclude that Al precipitated and did not pass the ultrafilter. On
349 the other hand, 48 h-NOECs \geq 100 μ g/l (3.7 μ M) were established for the (precipitated)
350 alumina standard tested in the pH range of 6.0-8.6. The lowest NOEC of 100 μ g/l was
351 observed at pH 6.8, while the highest NOEC of 460 μ g/l was observed at pH 7.6 and 8.6. At
352 the highest test concentration of 2200 μ g/l total Al, toxicity was highest at pH 6.0 and 6.8.

353 The latter toxicity parameters correspond well to the 96 h- E_rC_{50} of 576 $\mu\text{g/l}$ total Al
354 established by Call et al. (1984) for *Pseudokirchneriella subcapitata* at pH 7.25-7.89. This
355 indicates that Al toxicity was strongly influenced by its pH dependent speciation, as
356 described previously by Klöppel et al. (1997). Still, since it is currently unclear to what
357 extent the Al desorbed from the alumina coated SiO_2 NPs and subsequently precipitated, it
358 cannot be excluded from these experiments that secondary colloids contributed to the
359 decrease in algal growth rate. In the supplementary information section **Table S3** reports
360 the Al speciation of a 0.5 mg/l Al solution in OECD medium at various pH values. **Figure**
361 **S5** shows the concentration-response curves obtained for an Al standard in OECD medium
362 at pH 6.0-8.6.

363 Ammonium did not adsorb to the surface of both NP types. Phosphate, on the other
364 hand, did not adsorb to the SiO_2 NPs, but showed a strong pH dependent affinity towards
365 the alumina coated SiO_2 NP surface, with increased sorption at low pH. At pH 6.0 and 6.8,
366 a NP concentration of 460 mg/l decreased the available phosphate concentration to a value
367 below the detection level of the colorimetric analysis (0.03 mg $\text{PO}_4^{3-}/\text{l}$), which meant that
368 more than 97.3 % of the phosphate was adsorbed to the NP surface. The concentration of
369 available phosphate in function of pH and alumina coated SiO_2 NP concentration is
370 presented in **Figure 6**.

371 The concentration of NOM in centrifuged 460 mg/l alumina coated SiO_2 NP
372 suspensions was significantly lower compared to the NP free control for all three NOM
373 concentrations. At lower NP concentrations, no significant decrease was detected. At NOM
374 concentrations of 2.7, 7.4 and 12.5 mg C/l, the decrease in NOM concentration (std. dev., n
375 = 2) was 1.3 (0.1), 3.2 (0.2) and 4.3 (0.1) mg C/l, respectively. This corresponds to an
376 adsorption of NOM to the NP surface (std. dev., n = 2) of 2.7×10^{-3} (0.3×10^{-3}), 7.0×10^{-3}

377 (0.5×10^{-3}) and 9.3×10^{-3} (0.3×10^{-3}) mg C/mg NP. **Figure S6** in the supplementary
378 information summarizes the measured concentrations in all suspensions.

379 In the 7.4 and 12.5 mg C/l NOM suspensions, the phosphate adsorption was lower
380 compared to the NOM free medium. For example, in absence of NOM, 460 mg/l alumina
381 coated SiO₂ NPs adsorbed (mean (std.dev.) of 2 replicates) 93.1 (4.5) % of total phosphate.
382 However, in presence of 7.4 and 12.5 mg C/l NOM those suspensions adsorbed only 76.8
383 (1.5) and 66.9 (1.4) %, respectively. **Table S4** in the supplementary information gives an
384 overview of all phosphate measurements in function of NOM concentration.

385 **4. DISCUSSION**

386 Because of the identical composition of the core material, both particles are sold as
387 a colloidal silica suspension. However, from the characterization data, it is clear that the
388 alumina coating completely changed the particle surface characteristics and stability
389 behaviour. Consequently, our results emphasize the importance of detailed product
390 specification in nanoparticle containing products and in nanoparticle studies. The difference
391 in stability in the OECD medium can be explained using the zetapotential measurements.
392 Upon lowering the pH to 6.0, the surface charge on the bare silicon dioxide particles
393 became less negative, though was still large enough to prevent aggregation. Only at pH
394 values < 2.5 the SiO₂ NPs were expected to bare no charge (Van der Meeren et al., 2004).
395 Due to the low surface charge on the alumina coated SiO₂ NPs, the suspensions in OECD
396 medium were unstable and particles aggregated severely. The zetapotential measurements
397 suggested a point of zero charge between pH 7.5 and 9.0, which was in agreement with the
398 studies performed by Van der Meeren et al. (2004) and Jiang et al. (2009) in which iso-
399 electric points of 8-8.5 were obtained.

400 The bare SiO₂ NPs were more toxic to the alga compared to the alumina coated
401 SiO₂ NPs, despite the fact that the alumina coated SiO₂ NPs presented a larger amount of

402 specific surface area. Apparently, the alumina coating interacted differently with the alga.
403 As a consequence, the relation between toxicity and surface area, that was demonstrated for
404 12.5 and 27.0 nm SiO₂ NPs (Van Hoecke et al., 2008), was no longer valid when NPs bore
405 a chemically different coating on their surface. Again, it is clear that the surface dominated
406 NP characteristics. Hence, from a risk assessment point of view, NP coatings should be
407 taken into account, whereby physical and chemical as well as toxicological characteristics
408 of coated NPs cannot be directly extrapolated from knowledge on non-coated NPs.
409 However, since only one organism was used in the present study, care must be taken not to
410 generalize the higher toxicity of alumina coated silica NPs. More experimental data on
411 other organisms is to be obtained. Nevertheless, an in vitro study with bare and alumina
412 coated LUDOX[®] silica NPs also showed lower cytotoxicity, reactive oxygen species and
413 DNA double strand breaks when a human neuronal cell line was exposed to the alumina
414 coated NPs compared to the bare silica particles (Kim et al., 2010).

415 Chlorophyll analysis of algae exposed to the SiO₂ NPs indicated a much more
416 severe effect on algal growth rate compared to the cell count based data. In fact, at the
417 highest test concentration, the algal cells almost completely lacked chlorophyll. The
418 fluorescent signal in the 460 mg/l sample was 96 % lower compared to the control and at
419 46 mg/l 82 % decrease was found. These results therefore suggest that breakdown of
420 chlorophyll and/or chlorophyll synthesis inhibition was an important aspect of the
421 mechanistic toxic response induced by the SiO₂ NPs. A similar conclusion was also drawn
422 by Wei et al. (2010) who observed between 75 and 95 % decrease in chlorophyll upon
423 exposure of the alga *Scenedesmus obliquus* to silica NPs at 50 to 200 mg/l. Due to the
424 clustering between algal cells and alumina coated SiO₂ NPs and the resulting erroneously
425 low cell density measurement, it was not possible to draw conclusions on a similar direct
426 effect of these NPs on chlorophyll.

427 The clustering of algal cells and alumina coated SiO₂ NPs was caused by the
428 electrostatic attraction between the positively charged particles and the negatively charged
429 algal cells. The alumina coated SiO₂ NP aggregates acted as a binding agent between algal
430 cells. An identical observation was made by Jiang et al. (2009) and Simon-Deckers et al.
431 (2009) upon exposure of bacteria to Al₂O₃ NPs. Both articles mention the flocculation of
432 cell suspensions in presence of Al₂O₃ NPs. In view of the fact that *Pseudokirchneriella*
433 *subcapitata* naturally occur as singular cells (Nygaard et al., 1986), their ability to survive
434 and grow in a clustered structure is remarkable. However, from an ecological point of view
435 the cluster formation can indirectly affect the algal community and species at higher levels
436 of the aquatic ecosystem. At one hand, increased gravity of clusters of algal cells and
437 particle aggregates can enhance their sedimentation to deeper levels of the surface water
438 body, where light intensity is lower. This way, the cluster formation can indirectly affect
439 algal growth (Navarro et al., 2008). Due to the decreased availability of algal cells in the
440 pelagic part of the aquatic environment, species higher up the aquatic food chain may suffer
441 from food limitation. In a previous study, we experimentally confirmed this mode of action
442 principle for CeO₂ NP aggregates in chronic *Daphnia magna* survival and reproduction
443 tests (Van Hoecke et al., 2009). Due to the large negative surface charge of the SiO₂ NPs, a
444 similar clustering with algal cells was not expected and also not observed. Nevertheless, the
445 SiO₂ NPs were able to interact directly with the algal cells through adsorption to the cell
446 wall, as investigated in a previous study with similar LUDOX SiO₂ NP suspensions (Van
447 Hoecke et al., 2008).

448 It turned out that NP toxicity can be strongly pH dependent. In general, except at pH
449 6.0, the bare SiO₂ NPs were more toxic compared to the alumina coated ones. However, it
450 is currently not clear why no toxic effect was observed for the SiO₂ NPs at pH 6.0.
451 Possibly, the decreased surface charge diminished NP reactivity. Again, the large impact of

452 the alumina coating on SiO₂ NP characteristics was apparent from the algal growth
453 inhibition tests assessed at various pH values. Alumina coated SiO₂ NPs were shown to be
454 most toxic at pH 6.0 and 6.8 and least toxic at pH 8.6. Possibly, the pH dependent
455 phosphate adsorption contributed to the differential toxicity (**Figure 6**). Indeed, since no
456 phosphate was available at high test concentrations at pH 6.0 and 6.8, the algae could have
457 suffered from a lack of nutrients. Previously, it was shown that decrease in algal growth
458 rate occurred up to 50 % if no phosphate was available (Van Hoecke et al., 2009). The
459 chemical analysis data furthermore indicated that dissolution of the bare SiO₂ NPs was
460 substantial. This is not surprising, since the solubility of amorphous silica in water at 25 ° C
461 is 120 mg/l (Alexander et al., 1954). Hence, during the algal growth inhibition tests, no
462 equilibrium was reached between solid and dissolved silica. On the other hand, dissolution
463 of the silica core of alumina coated NPs was inhibited due to the presence of the coating.
464 The chemical analysis data furthermore indicated that dissolution of the silica core is
465 inhibited due to the Al(OH)₃ coating. In addition to the knowledge that dissolved Al species
466 precipitate from 0.03x10⁻³ to 0.8x10⁻³ mg/l on, it is not likely that toxicity of alumina
467 coated SiO₂ NPs was caused by the presence of dissolved aluminum species either.
468 Similarly, Jiang et al. (2009) concluded that bacterial toxicity of Al₂O₃ particles at 20 mg/l
469 was not due to dissolution, since concentrations were below the ICP-OES detection level.

470 The addition of NOM to the test media strongly decreased toxicity. Since the NOM
471 was able to adsorb to the alumina surface, the decrease in toxicity could be due to the
472 shielding of the NPs by NOM, preventing a direct interaction with algal cells, which caused
473 a decrease in bioavailability. Yang et al. (2009) experimentally demonstrated the adsorption
474 of humic acid, a component of NOM, to the Al₂O₃ NP surface and attributed this behaviour
475 to the NPs' large surface area, low hydrophilicity, few negative charges and the strong
476 physical interactions between humic acid and the NP surface, like electrostatic attraction

477 and ligand exchange. When analyzed using a cell counter, concentration-response curves of
478 alumina coated SiO₂ NPs were, again, different from those assessed using fluorescent
479 detection of extracted chlorophyll, as shown in **Figure S3**. For SiO₂ NPs the effect
480 concentrations in presence of NOM, listed in **Table 5**, were found to increase linearly with
481 increasing NOM concentration, which is illustrated in **Figure 7**. In **Figure S7**, the 72 h
482 toxicity data of these tests are also presented. **Table S5** lists the a, b and determination
483 coefficient (R²) parameters of the linear regressions, calculated using least squares analysis.
484 However, to date it is not clear through which mechanism the NOM decreased SiO₂ NP
485 toxicity. Unlike for the alumina coated SiO₂ NPs, it was difficult to separate the bare SiO₂
486 NPs from the NOM due to the colloidal stability of the suspension. Consequently, the NOM
487 adsorption to SiO₂ NPs could not be quantified. Humic acid, one of the components of
488 NOM, was not found to adsorb to SiO₂ NPs, which was explained through the high
489 hydrophilic nature of the SiO₂ surface (Yang et al., 2009). However, other compounds of
490 NOM can still adsorb to the SiO₂ NPs. For example, acid-base interactions between organic
491 acid groups in NOM and hydroxyl groups on the silica surface can establish the adsorption
492 (Considine et al., 2005). In addition, NOM is also able to bind to algal cells, which can in
493 turn shield the algal cell surface from direct interaction with NPs. Campbell et al. (1997)
494 demonstrated the binding of dissolved organic matter to the cell surface of phytoplankton.
495 The authors suggested that either a hydrogen-bonding sorption mechanism between
496 negatively charged functional groups in the DOM and on the cell surface or the formation
497 of hydrophobic bonds between both are involved.

498 In conclusion, the assessment of particle-particle, particle-test medium and particle-
499 alga interactions confirmed that the application of a thin layer of alumina onto the surface
500 of SiO₂ NPs completely altered their characteristics. First, due to the low surface charge,
501 alumina coated SiO₂ NPs aggregated in test medium, while bare SiO₂ NPs were stable.

502 Second, bare SiO₂ NPs were more toxic in standard OECD test medium and showed a
503 different pH dependent toxicity compared to the alumina coated ones. Third, dissolution
504 and nutrient adsorption characteristics were different. As a consequence, coating
505 formulations should be taken into account when performing risk assessments of engineered
506 NPs.

507

508 **Acknowledgement** – This research was funded by the European Union Sixth Framework
509 Programme NanoInteract (NMP4-CT-2006-033231) and the Agency for Innovation
510 through Science and Technology (IWT, Belgium). Furthermore, the authors would like to
511 thank Liesbet Baele, Krisztina Hracs and Gisèle Bockstael.

512

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628 **TABLES**

629 **Table 1** – Average particle diameters (Zave) and polydispersity indices (PI) obtained with
 630 dynamic light scattering in various media

631

Product	Medium	pH	Zave (nm) (std.dev.)	PI (std. dev.)
SiO ₂ NPs (LUDOX CL-X)	Deionized water	9.6	20.9 (0.1)	0.26 (0.01)
	OECD	6.0	33.3 (0.5)	0.09 (0.02)
	OECD	6.8	32.3 (1.0)	0.13 (0.10)
	OECD	7.6	31.4 (2.3)	0.19 (0.12)
	OECD	8.6	31.9 (2.2)	0.14 (0.13)
	OECD + 2 mg C/l NOM	7.4	32.7 (0.6)	0.10 (0.03)
	OECD + 6 mg C/l NOM	7.4	32.6 (1.6)	0.14 (0.08)
	OECD + 10 mg C/l NOM	7.4	32.9 (1.1)	0.12 (0.10)
Al(OH) ₃ coated SiO ₂ NPs (LUDOX CL)	Deionized water	3.7	39.4 (1.2)	0.24 (0.01)

632 **Table 2** – Zetapotential values of SiO₂ and alumina coated SiO₂ NPs in various media

Product	Medium	pH	Zetapotential (mV) (std.dev.)
SiO ₂ NPs (LUDOX CL-X)	OECD	6.0	-23.1 (0.4)
	OECD	7.5	-32.8 (1.1)
	OECD	9.0	-37.7 (5.5)
	Deionized water	9.6	-47.3 (5.2)
Al(OH) ₃ coated SiO ₂ NPs (LUDOX CL)	Deionized water	3.7	39.6 (3.2)
	OECD	6.0	20.6 (0.4)
	OECD	7.5	4.9 (0.5)
	OECD	9.0	-6.7 (0.2)

633

634 **Table 3** - NOECs, LOECs and 48 h- E_rC_x (95 % confidence interval) values of dialyzed and
 635 non dialyzed NP suspensions at pH 7.4 in standard OECD algal test medium. Both cell
 636 counting (CC) and fluorescence of extracted chlorophyll (Chl) were used to determine algal
 637 cell density.
 638

Particle	CC/ Chl	NOEC	LOEC	E_rC_{10}	E_rC_{20}	E_rC_{50}
		mg/l	mg/l	mg/l	mg/l	mg/l
SiO ₂ non dialyzed	CC	22	46	26.9 21.7-33.4	36.7 32.1-42.1	n.d.
	Chl	22	46	18.2 14.4-23.0	26.0 21.9-30.9	48.0 43.4-53.1
alumina coated SiO ₂ non dialyzed	CC	< 10	< 10	< 10	< 10	< 10
	Chl	10	22	> 460	14.2 9.3-21.6	35.1 26.6-46.1
SiO ₂ dialyzed	CC	22	46	28.3 22.0-36.5	36.7 31.5-42.9	n.d.
	Chl	22	46	14.0 11.1-17.8	21.8 18.3-26.1	46.4 41.9-51.5
alumina coated SiO ₂ dialyzed	CC	< 10	10	< 10	< 10	< 10
	Chl	22	46	46.2 33.7-63.3	120.9 88.6-165.0	> 460

639 **Table 4** - Ecotoxicological effect parameters (95 % confidence interval) of algal growth
 640 inhibition assays with SiO₂ and alumina coated SiO₂ NPs in standard OECD algal test
 641 medium at various pH values, analyzed after 48 h. Algal cell density was analyzed using
 642 fluorescent detection of extracted chlorophyll.

Particle	pH	NOE C mg/l	LOEC mg/l	E_rC₁₀ mg/l	E_rC₂₀ mg/l	E_rC₅₀ mg/l
SiO ₂ dialyzed	6.0	n.d.	> 220	> 220	> 220	> 220
	6.8	10	22	12.3 8.5-17.7	20.4 16.0-25.9	58.6 49.1-69.9
	7.6	4.6	10	6.1 4.6-8.1	9.9 8.1-12.1	25.7 22.7-29.1
	8.6	10	22	13.2 10.5-16.6	19.2 16.4-22.4	42.2 37.2-47.7
alumina coated SiO ₂ dialyzed	6.0	4.6	10	47.5 26.8-84.0	118.8 83.5-169.1	n.d.
	6.8	4.6	10	9.5 5.3-16.8	25.2 16.5-38.4	n.d.
	7.6	4.6	10	12.9 4.2-40.0	155.7 70.3-344.7	n.d.
	8.6	100	220	179.2 129.1- 248.6	342.1 290.3- 403.1	n.d.

643 **Table 5** - NOECs, LOECs and E_rC_x values (95 % confidence interval) of both NPs types in
 644 OECD algal test medium at pH 7.4, in presence of various NOM concentrations. Algal cell
 645 density was analyzed using fluorescence of extracted chlorophyll.

Particle	NOM	NOEC	LOEC	E_rC_{10}	E_rC_{20}	E_rC_{50}
	mg C/l	mg/l	mg/l	mg/l	mg/l	mg/l
Dilayzed SiO ₂ NPs	1.2	22	46	25.6 18.4-35.6	55.8 43.6-71.4	211.9 185.0-242.6
	4.7	100	220	120.6 100.3-145.0	218.9 192.4-249.0	606.6 567.2-648.7
	9.0	100	220	263.5 232.7-298.3	462.0 423.1-504.5	1206.9 1151.7-1264.7
Dialyzed alumina coated SiO ₂ NPs	1.3	> 1000	> 1000	> 1000	> 1000	> 1000
	4.9	> 1000	> 1000	> 1000	> 1000	> 1000
	9.1	> 1000	> 1000	> 1000	> 1000	> 1000

646 **Table 6** – Concentration of reactive (dissolved) silica in SiO₂ NP suspensions in OECD
 647 medium at various pH values, assessed after 48 h incubation under test conditions. Standard
 648 deviation on two replicated measurements are given in between parentheses. DL =
 649 detection limit = 0.050 mg SiO₂/l.

Conc. SiO ₂ NPs (mg/l)	Concentration of dissolved silica (mg SiO ₂ /l)			
	pH 6.0	pH 6.8	pH 7.6	pH 8.6
4.6	< DL	< DL	< DL	< DL
46	< DL	1.8 (0.3)	2.9 (0.0)	18.1 (0.3)
460	6.0 (0.4)	12.6 (0.3)	26.5 (0.2)	68.6 (0.3)

Figure legends

Figure 1 – Concentration-response curves of SiO₂ and Al(OH)₃ coated SiO₂ NPs in standard OECD test medium at pH 7.4. Algal density was measured using cell counting (CC) and using fluorescence of extracted chlorophyll (Chl). Algal growth rate was expressed relative to the control (% rtc) and error bars represent standard deviation on the mean growth rate (n = 3).

Figure 2 - Algae clusters in 10 mg/l alumina coated SiO₂ NPs on 3rd day of algal growth inhibition test.

Figure 3 – Concentration-response curves of SiO₂ and alumina coated SiO₂ NPs in OECD medium at various pH values. Algal cell density was analyzed using chlorophyll fluorescence. Error bars represent standard deviation on mean growth rate calculated after 48h.

Figure 4 – Concentration-response curves of SiO₂ and alumina coated SiO₂ NPs in OECD medium at pH 7.4 in presence of various natural organic matter concentrations. Algal cell density was analyzed using chlorophyll fluorescence. Error bars represent standard deviation on mean growth rate.

Figure 5 – 96 well plate experiment to assess the importance of light limitation. Algal cells were always spiked into the lower white plate. In the control, both the white and opaque plate contained standard OECD test medium. When algae and NPs were spatially separated, the white plate contained OECD medium while NPs in OECD medium were spiked into the opaque plate. Treatments where algal cells and NPs were in direct contact, the white plate contained both algal cells and NPs in OECD medium, while the opaque plate contained OECD medium only.

Figure 6 – Concentration of phosphate in ultrafiltered OECD medium in function of alumina coated SiO₂ NP concentration for various pH values, expressed as % relative to the standard OECD medium (% rtc).

Figure 7 – Illustration of linear relations between the NOM content of OECD medium and the established effect concentrations of SiO₂ NPs on algal growth rate, assessed after 48 h using chlorophyll analysis.

Figure 1

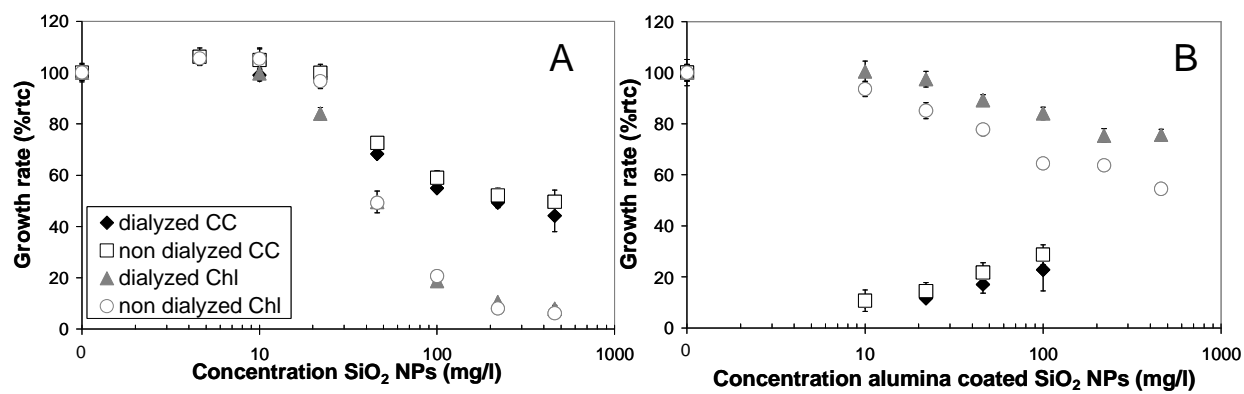


Figure 2

Color for publishing on the web :



Black and white for printing :



Figure 3

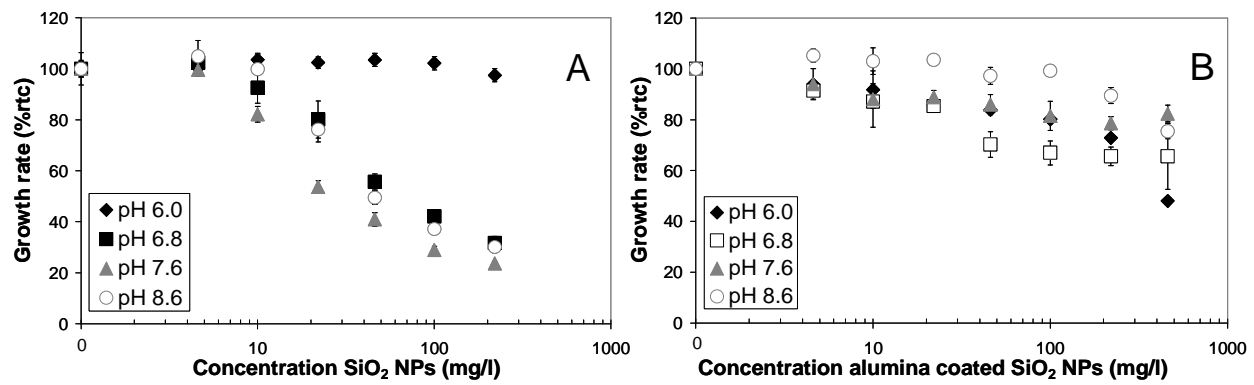


Figure 4

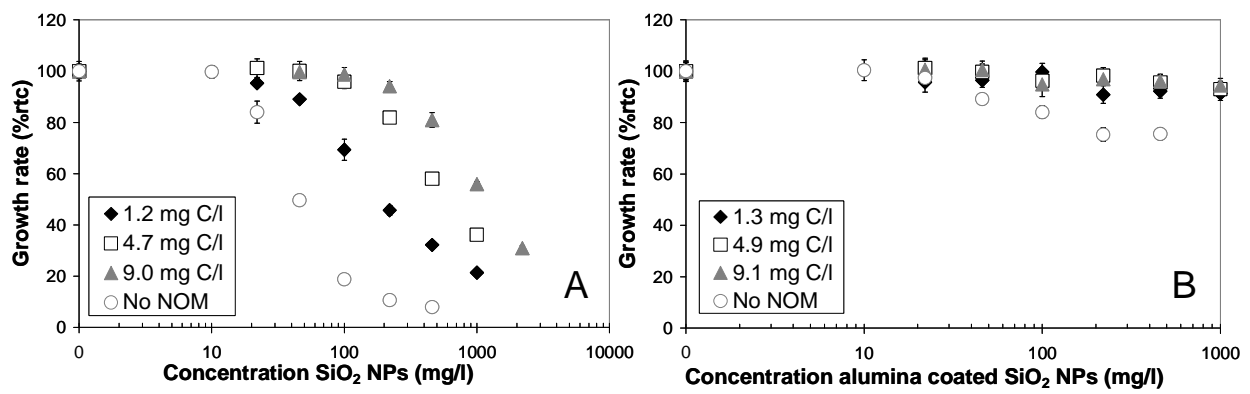


Figure 5

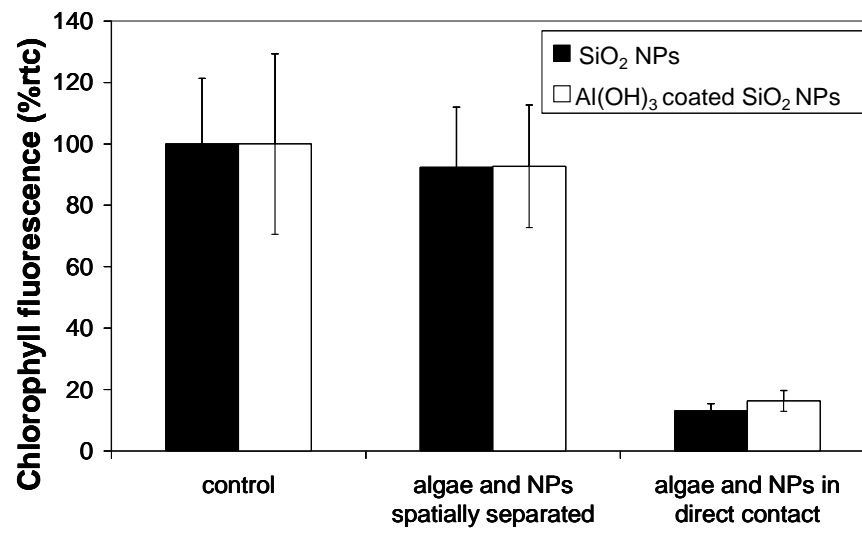


Figure 6

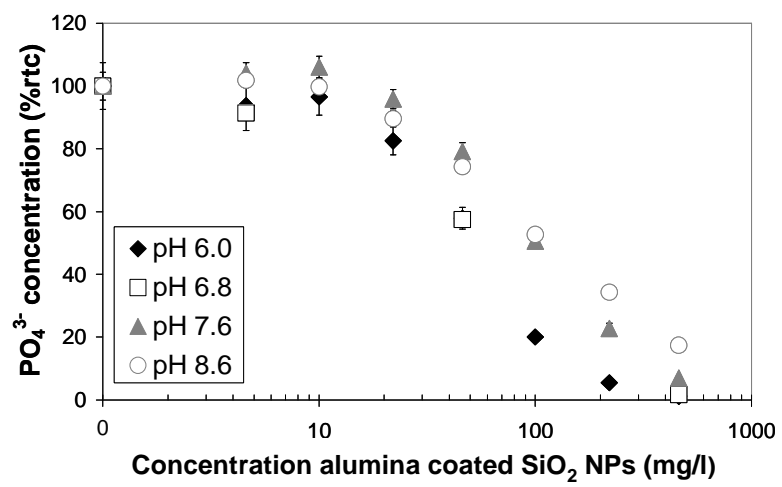


Figure 7

