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Effect of non-ageing and ageing ceria nanoparticles suspensions on fresh water micro-algae

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When assessing the hazards properties of nanomaterials in the environment, the main research challenges are numerous. Firstly, determining if nanomaterials are more or less toxic than the bulk forms of the same materials and the extent to which toxicity is governed or influenced by the physico-chemicals properties of the nanoparticles. Secondly, it appears necessary to study the effect of nanomaterials and nanoparticles throughout their life cycle including both initial forms and physico-chemically modified form (*i.e.* aggregated or agglomerated forms) resulting from an ageing process.

Our work focused on the effect of commercial ceria nanoparticle ($nCeO_2$) suspensions, towards freshwater micro-algae assessing the effect $n CeO_2$ suspensions with different agglomeration/aggregation state obtained by using an artificial ageing process. Both ageing and non-ageing $n CeO_2$ suspensions were fully characterized using dynamic light scattering (ZetaSizer, Malvern Instruments) or laser diffraction (MasterSizer, Malvern Instruments) and transmission electron microscopy (TEM). In addition, the interaction between NPs and algae were investigated using flow-cytometry and environmental scanning electron microscope technique (E-SEM).

The results obtained showed that the algae growth inhibition was similar after exposure to non-ageing or ageing $n CeO_2$ suspensions. The results obtained from flow-cytometry and E-SEM proved that the ceria NPs are able to tightly entrap the algae cells, which could in part contribute to the effect recorded. Those results also support the fact that aggregation or agglomeration has a few influences when focusing on the standardized algae ecotoxicity test. Moreover by comparison to our previous studies performed with other ceria suspensions, it was shown that the primary particle size and consequently the particle surface area is a relevant parameter in assessing the ecotoxicity of nanoparticles.

Key words: Ceria nanoparticles, *Pseudokirchneriella subcapitata*, agglomeration state, ageing suspensions

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1. Introduction

When assessing the hazards properties of nanomaterials to the environment, the effect of nanomaterials and nanoparticles including both initial form and altered form should be addressed. Thus, the present work aimed at investigating the effects of a commercial ceria nanoparticle composite suspension (*n*CeO_2) toward freshwater algae. The ecotoxic effects were investigated using both non-aging (freshly prepared) or aging (artificially altered) *n*CeO_2 suspensions. The standardized green algae *Pseudokirchneriella subcapitata* was used for the algae growth inhibition experiments. All the suspensions were fully characterized using dynamic light scattering (ZetaSizer, Malvern Instruments) or laser diffraction (MasterSizer, Malvern Instruments) and transmission electron microscopy (TEM). In addition, the interaction between NPs and algae were investigated using flow-cytometry and environmental scanning electron microscope technique (E-SEM).

2. Nano-ceria suspensions

Commercial *n*CeO_2 were obtained as 248 g/L stable suspension in water. A primary particle size of 10 nm was reported by the supplier. For the ecotoxicity tests, an initial *n*CeO_2 suspension (25 mg/L) was prepared by dilution of the commercial suspension into the algae growth media (OECD 201 growth media). This suspension was artificially aged under light and slow magnetic stirring for 3 days. Another freshly (non-ageing) *n*CeO_2 suspension was prepared 15 minutes prior to the experiment using the same dilution protocol but without ageing process. For the ecotoxicity tests, *n*CeO_2 suspensions with nominal concentrations from 0.195 to 25 mg/l (8 concentrations) were then prepared by dilution of the ageing and non-ageing initial *n*CeO_2 suspensions in the algae growth media. The average specific growth rate of *P. subcapitata* in each concentration was calculated each day up to 72h for both ageing and non-ageing *n*CeO_2 suspensions.

3. Characterization of non-ageing and ageing suspensions

Investigation of the initial *n*CeO_2 suspensions in algae growth medium showed that ageing and non-ageing suspensions mainly differ in term of agglomeration/aggregation state. As illustrated by figure 1, the non-ageing suspension were mainly composed of small *n*CeO_2 agglomerates or aggregates around 30 nm with some of them up to 500 nm. By comparison, the ageing suspension showed large and loose agglomerated particles up 10 µm (Figure 1).

4. Algae growth inhibition test

The results obtained clearly show that *n*CeO_2 are ecotoxic towards micro algae. Moreover, the effects recorded were similar after both ageing and non-ageing nano-ceria suspension. We have calculated EC_{50} values of 1.4 mg/L and 1.8 mg/L for the non-ageing and the ageing ceria suspensions, respectively. This observation suggested that whatever the agglomeration state, the algae growth inhibition is similar when focusing on the algae growth inhibition test. Moreover, compared with our previous works with other nano-ceria suspensions [1], our data support the view that the primary particle size and consequently the surface area might be an important parameter to take into account, as previously suggested by Van Hoecke *et al*. [2].

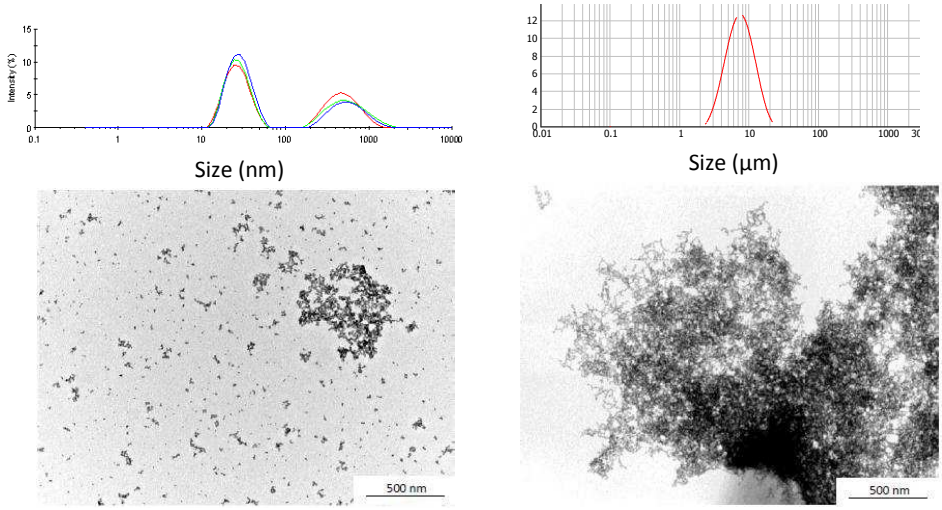


Figure 1: Particle size distribution and TEM micrographs of the non ageing (a) and ageing (b) nano-ceria suspensions in the OECD algae growth medium (25 mg/L).

5. Nanoparticles–Algae interaction

The interaction between ceria NPs and algae were investigated by flow-cytometry and using E-SEM) technique. The cytogram distributions showed an increase in cell complexity with increasing *n*CeO_2 concentration which suggests a potential adsorption onto the cell wall or a potential internalization into the cell. Without demonstrating the NPs internalization in cells, the results obtained from E-SEM confirmed that the ceria NPs are able to tightly entrap and wrap the algae cells (figure 2). These observations suggest that the algae ecotoxicity could, in part, be due to this close interaction by limiting the transport of metabolites and nutrients across the cell wall, or by inducing cell membrane disruptions and oxidative stress [3].

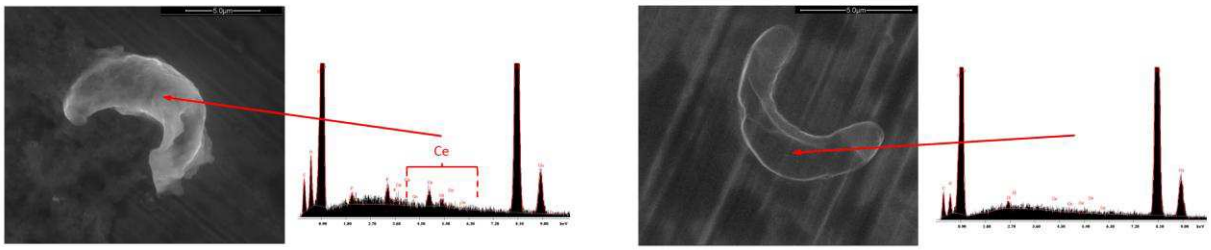


Figure 2: E-SEM micrographs of Pseudokirchneriella subcapitata exposed to $nCeO_2$ suspension (a) and in the control media without $nCeO_2$ (b)

6. References

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