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1. Introduction

The use of engineered nanomaterials(NMs) raises concern regarding their fate in environmental compartments (accumulation) and their potential effects on aquatic organisms. One of the singularities of these substances is their duality of action, both physical (ex. adhesion, adsorption, physical alteration of tissues) and chemical. In the chain of events leading to the occurrence of a deleterious effect, the interaction between a NM and a biological organism is one essential event. This may be an important parameter for the NMs fate in trophic transfer. Therefore, understanding the links between the properties of NMs and the physical interaction phenomenon (adhesion, internalization) with aquatic organisms, is essential in a context of *a priori* hazard assessment and for the categorization of these substances within a regulatory purpose.

The surface contact can be the first and most important step at the NMs-algae interaction [1]. In this context, it has been demonstrated that nanoparticles can adhere to the cell walls [2] and the toxic effects are described as: severe membrane damage, thickening of the extracellular polymeric substances layer [3], and a decrease of light access due to a shading effect causing nutrient intake limitations [4]. Also, it has been demonstrated that NMs can be internalized, transformed and stored in the cell causing ultra-structural damages and oxidative stress [3]. But the question regarding which of the NM characteristics (i.e. chemical composition, size, surface area, charge or others) is the primary cause of these effects, is not yet answered. In addition, the role of algae cell wall composition in the potential NMs-algae interaction is still unclear, however it may have high relevance in the NMs accumulation and trophic transfer into aquatic food-webs.

In this context, our work aims at determining the interaction of various NMs with freshwater microalgae, in order to gain a better understanding of its implication in bioaccumulation and biological effect. Finally, our motivation is to discuss the relevance of these parameters in the context of NMs grouping.

2. Materials and methods

Series of experiments were performed to identify the potential interaction between NMs and microalgae. Briefly, green algae with different cell wall composition (*Pseudokirchneriella subcapitata*, *Chlamydomonas reinhardtii* strain CC125 and muted cell wall strain CC400) were separately exposed for 15 minutes to five different NMs suspensions (Table 1). Each algal suspension was prepared at $0{,}5x10^6$ cell ml^{-1}.

All samples were analyzed using a flow cytometer, where the fluorescence (FL1 intensity) and the granularity or cell complexity (side scattering logarithm) were measured. The displacement of the cytogram outline of the tests samples was compared to the control profile. Additional microscopy analysis was performed to confirm the results.

Nanoparticle	CeO_2-25nm	CeO_2-10nm	TiO_2	TiO_2	TiO_2
OECD code	NM-213	-	NM-105	NM-101	NM-104
Crystalline structure	Cubic	Cubic	72.6% anatase, 18.4% rutile, 9% amorphic	Anatase	Rutile
Coating	-	Tri ammonium citrate	-	-	Al_2O_3
Size (nm)	<25	10	21	<10	20
Charge (mV) in algal medium	-30.47	-27.13	-20.77	-21.13	-5.35

Table 1: Nanoparticles characteristics

3. Results and discussion

An important influence of the cell wall composition has been observed with a different type of interaction for the three different algae. The results obtained for *C. reinhardtii* strain CC125 that is a glycoproteinic cell

walled algae, showed no significant modification in algal complexity (and all below 30%) for any of the NMs tested. On the other hand, for *P. subcapitata* the NM-algae interaction varied from 3% to 100% of granularity modification (both algae results showed on Figure 1). Finally, the mutated strain of *C. reinhardtii* CC400 with no cell wall had intermediate response.

In addition, the interaction seemed to be dependent on the physicochemical characteristics of NMs. Indeed, each NMs have interacted differently with *P. subcapitata,* as evident by the significant difference observed in the percentage of more complex cells depending on the NMs. Two of the TiO_2 NMs showed a greater interaction with the algae, modifying the granularity of almost all the cells. Oppositely, the CeO_2-10nm did not seem to affect the algal complexity even at the maximal concentration. Aditional microscopic analysis corelated the more complex algal profiles with images of cells being attached by more NMs forming NM-algae heteroagglomerates and, likewise, those that didn't showed a change in granularity with no altered cells. These results may be explained by the Zeta potential of NMs and microalgae cell wall. Given that the *P. subcapitata* cell surface is globally negatively charged, the negative charged NMs might not likely attach due to an electrostatic repulsion [3], but the NMs with a less negative charge showed a higher attachment to the cells causing heteroagglomerates, which was also shown to be correlated with greater toxicity [1].

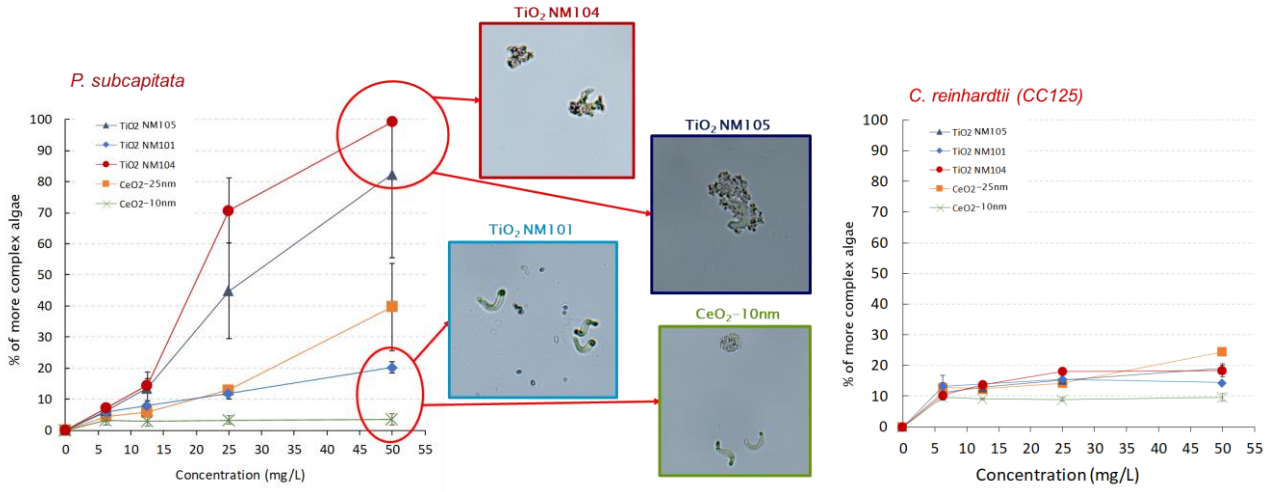

Figure 1: Increase of complex algae percentage for the tested NMs for P. subcapitata with microscope images (right) and for C. reinhardtii strain CC125 (left).

4. Conclusions

A dose-dependent response is observed for every type of NMs and an interaction specificity is showed. Flow cytometry and microscopic observation showed evidence that this interaction was dependent upon algae complexity. So far, two parameters were identifyed as having a major role in the NM-algae interaction: *i)* the cell wall composition, as each algae had a different response to the set of tested NMs; and *ii)* the NMs physico-chemical properties; because for a given algae cell wall type, a different degree of increased algal complexity was observed according to each NM. Finally, it can be concluded that the NM-algae interaction has a major role in bioaccumulation and subsequently trophic transfer since it will determine the quantity and localization of NM in the cells. Based on these parameters, the next step will be to study a larger diversity of NMs and algae for a NM grouping proposal.

5. References

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