Assessment of nanoceria toxicity in aquatic photosynthetic organisms

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The commercial applications of engineered nanoparticles (ENPs) have widely expanded over the last years with subsequent increased release into the environment. The particular physicochemical properties of nanoparticles with regards to the same non-nano compound have raised serious concerns about their potential environmental risks. Algae and cyanobacteria are ecologically relevant organisms which are at the base of aquatic food webs and have essential roles in nutrient cycling; both are ideal models to study potential effects of released ENPs.

Cerium oxide nanoparticles, which have widespread applications, are interesting nanomaterials due to their unique redox properties which are based in the mixed valence state of CeO_2 (Ce^{3+} and Ce^{4+}). Our group have reported that nanoceria exhibited strong toxicity to the green alga *Pseudokirchneriella subcapitata* and the cyanobacterium *Anabaena CPB4337*; in both organisms nanoceria exposure resulted in highly damaged cells with extensive membrane disruption [1]. We found no evidence of nanoparticle uptake by cells, but our observations suggested that their toxic mode of action required direct contact between nanoparticles and cells; in the case of the cyanobacterium, cells completely coated by layers of ceria nanoparticles were observed [1]. Free cerium was highly toxic for both organisms but the negligible amount found dissolved in the nanoparticle suspensions could not explain the observed toxic effect of nanoceria on the aquatic organisms [1].

In order to to gain insights into the mechanisms of the observed toxicity by nanoceria, the main bioenergetic process of these organisms, photosynthesis, was studied both by measuring oxygen evolution and chlorophyll a fluorescence emission parameters. Nanoceria significantly inhibited photosynthesis in the cyanobacterium in the whole range of concentrations tested (0.01 to 100mg/L); a dual effect of nanoceria was found in the green alga with slight stimulation at low concentrations and strong inhibition at the highest concentrations tested; chlorophyll a fluorescence experiments indicated that nanoceria had a significant impact on the primary photochemical processes of photosystem II (Fig. 1). The primary cause of the observed photosynthetic inhibition by nanoceria could be an excessive level of ROS formation; the results indicated a strong generation of reactive oxygen species (ROS) as indicated by an increase in DCF fluorescence, a general oxidative stress indicator (Fig. 2) which caused oxidative damage in both photosynthetic organisms. It is proposed that nanoceria increase the production of hydrogen peroxide (a normal ROS by-product of light-driven photosynthesis) in both the green alga and cyanobacterium; this ROS, through a Fenton-like reaction, may increase lipid peroxidation, compromising membrane integrity and also seriously impairing photosynthetic performance, eventually leading to cell death.

References

[1] Rodea-Palomares I, Boltes K, Fernández-Piñas F, Leganés F, García-Calvo E, Santiago J and Rosal R. Toxicological Sciences **119** (2011): 135-145.

Acknowledgements- This work was funded by Comunidad de Madrid grants S-0505/AMB/0321 and S-2009/AMB/1511 (Microambiente-CM) and by the Spanish Ministry of Science and Innovation [grant CGL2010-15675, sub-programme BOS].

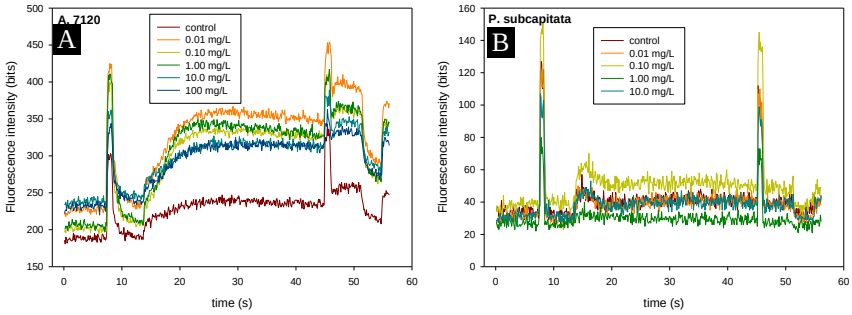

Fig. 1.Chorophyll fluorescence traces of the cyanobacterium (A) and the green alga (B) exposed to nanoceria.

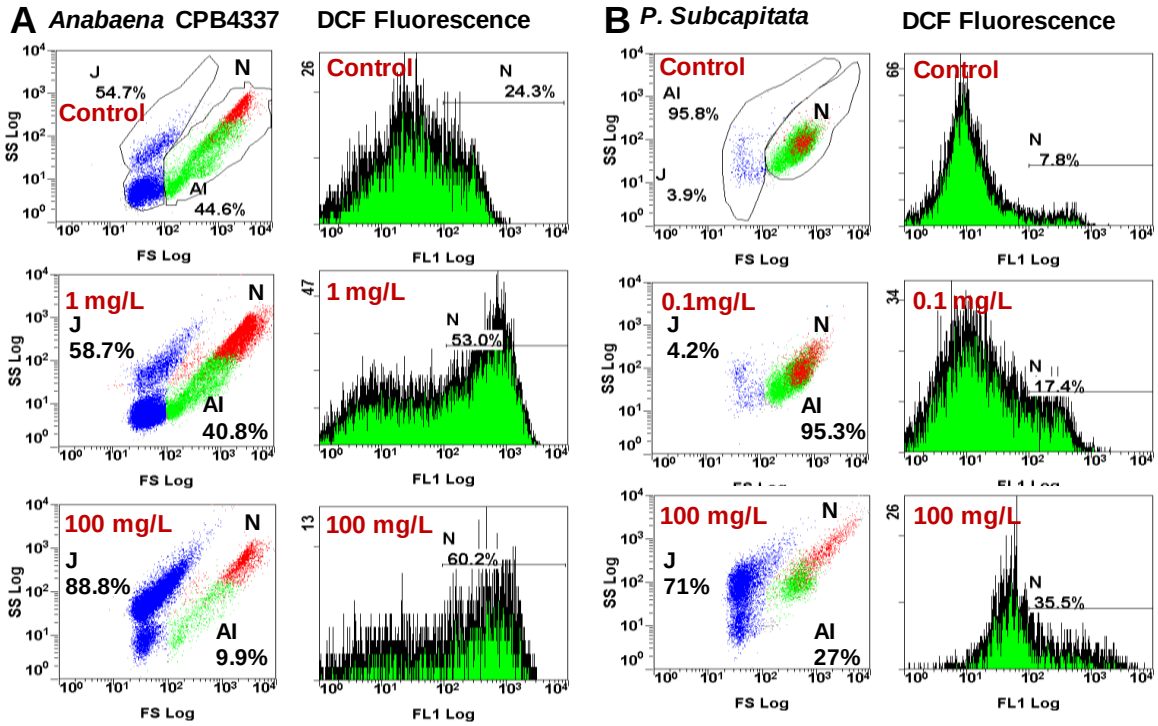

Fig. 2. Flow cytograms of cell/filament complexity (SS) as a function of cell/filament size (FS) and corresponding DCF fluorescence histograms of *Anabaena* CPB4337 (A) and *P. subcapitata* (B) exposed for 72 h to CeO_2 nanoparticles. Cell/filament subpopulations were identified based on size and complexity: AI = main subpopulation (green dots), N = subpopulation which included cells/filaments with the highest size, complexity and DCF fluorescence signal (red dots) and J = Cells/filaments with the smallest size/complexity and/or cell debris (blue dots).