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Uptake and biomagnification of multifunctional magnetic and NIR-sensitive nanoparticles by aquatic plants: electron spin resonance, two-photon and confocal microscopy studies

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Introduction

The steady growth of nanotechnology-enhanced products raises concerns about the interaction of engineered nanoparticles (ENPs) with biological systems. The aquatic environment is particularly at risk of exposure to ENPs, as it acts as a sink for most environmental contaminants. Herein, we implemented superparamagnetic iron oxide nanoparticles (SPIONs) and NIR-sensitive lanthanide-doped up-conversion nanophosphors (UCNPs) as local probes to study the uptake and internalization of ENPs by aquatic plants.

Materials and methods

Two model aquatic plants, *Egeria densa* and *Vallisneria spiralis,* were exposed to custom-synthesized ENPs: (i) γ-Fe_2O_3 SPIONs (functionalized with citric acid, Zeta-potential of -40 mV) and (ii) to three types of non-functionalized multipurpose luminescent and paramagnetic UCNPs, *i.e.* to nanoparticles of $NaYF_4$:Yb,Er,Gd and $Y_3Al_5O_{12}$:Yb,Er,Gd as well as Gd_2O_3:Yb,Er,Zn. To detect ENPs in plants, we used electron spin resonance (ESR) and two optical microscopy methods: confocal and two-photon.

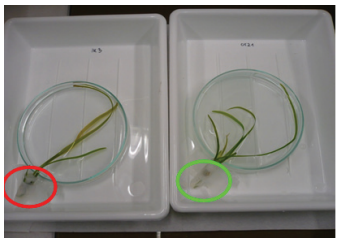

Figure 1. The experimental setup for exposing *Vallisneria spiralis* to waterborne ENPs *via* roots (left panel) and for the control experiment (right panel). Fluorescent paramagnetic upconversion nanoparticles (UCNPs) based on $NaYF_4$:Yb,Er,Gd at the concentration of 1 mg/mL were used in this experiment.

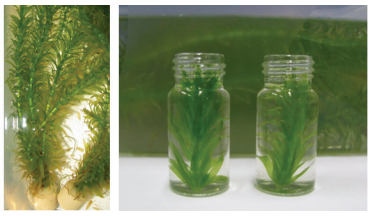

Figure 2. Exposure of *Egeria densa* to waterborne SPIONs: (**a**) the photograph of *Egeria densa*; (**b**) the top parts of *Egeria densa* positioned in 20-mL glass beakers and immersed in aqueous suspensions of strongly diluted SPIONs (6.0×10^{13} of SPIONs particles per 1 mL).

Confocal and two-photon microscopy studies

Confocal and two-photon microscopy performed under excitation with NIR light (λ_{ex}=980 nm) corroborated the ESR results by pointing to a rapid buildup of aggregates of $NaYF_4$:Yb,Er,Gd UCNPs in leaves and roots of the exposed plants. Confocal microscopy also confirmed translocation of Gd_2O_3:Yb,Er,Zn and $Y_3Al_5O_{12}$:Yb,Er,Gd UCNPs from the directly exposed roots to the further located leaves.

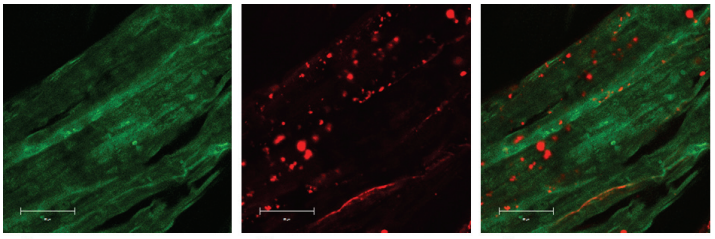

Figure 3. Confocal microscopy images of the fragment of roots of *Vallisneria spiralis* exposed to waterborne UCNPs, $NaYdF_4$:Yb,Er,Gd (40% of Gd). (**a**) The autofluorescence image under excitation with λ_{ex} = 488 nm (emission λ_{em}= 508-585 nm); (**b**) The image obtained under excitation with NIR light, λ_{ex} = 980 nm (UCL emission at λ_{em}= 650 nm); (**c**) Superposition of images shown in (a) and (b). Scale bar 200 μm.

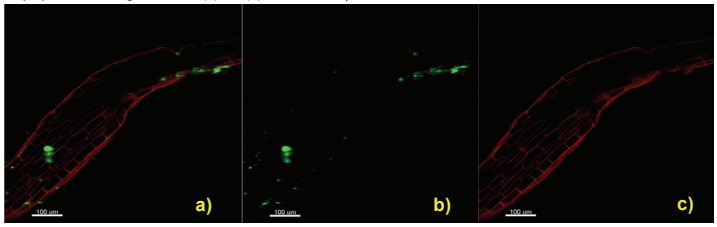

Figure 4. Two-photon microscopy images of the root fragment of *Vallisneria spiralis* exposed to waterborne UCNPs, upconversion nanoparticles $NaYF_4$:Yb,Er,Gd (50% of Gd), under excitation with NIR light (λ_{ex}= 980 nm, laser power 46 mW). (**a**) Image overlay; (**b**) Image taken with green bandpass filter (λ_{em} = 500-550 nm); (**c**) Image taken with red bandpass filter (λ_{em} = 565-605 nm).

ESR detection of superparamagnetic and paramagnetic ENPs in plants

The uptake and internalization of superparamagnetic SPIONs (γ-Fe_2O_3) and paramagnetic UCNPs ($NaYF_4$:Yb,Er,Gd, $Y_3Al_5O_{12}$:Yb,Er,Gd and Gd_2O_3:Yb,Er,Zn) were followed by electron spin resonance (ESR). In particular, ESR revealed a very fast uptake and a significant extent of biomagnification of waterborne SPIONs by leaves of both plants. Concomitantly, ESR also confirmed the translocation of $NaYF_4$:Yb,Er,Gd nanoparticles from the directly exposed plant roots towards the further located leaves of *Vallisneria spiralis*.

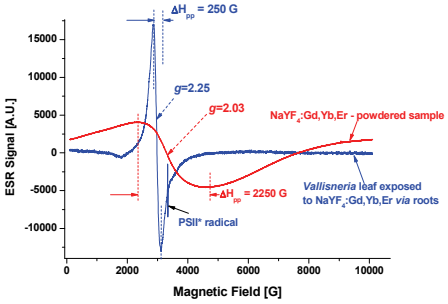

Figure 5. Comparison of ESR spectra measured for the powdered sample of $NaYF_4$:Yb,Er,Gd (40% of Gd) nanoparticles (red trace) and for a leaf fragment of *Vallisneria spiralis* exposed to waterborne $NaYF_4$:Yb,Er,Gd nanoparticles *via* roots (blue trace).

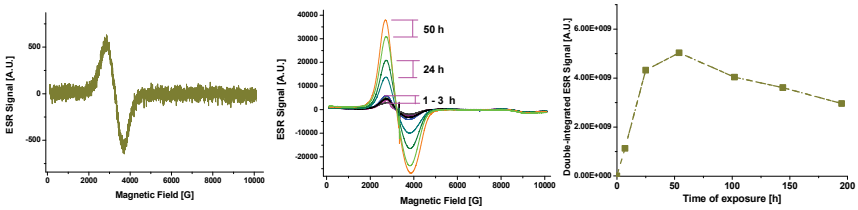

Figure 6. ESR tracking of the uptake and biomagnification of waterborne SPIONs by *Egeria densa*. (**a**) ESR spectrum of the strongly diluted SPIONs in the tap water at the beginning of exposure. The ESR signal intensity corresponds to ~6.0×10^{13} of SPIONs particles per 1 mL, *i.e.* ~ 2 mg of iron per 1 liter, thus roughly 3 times more than the median iron concentration in environmental waters. (**b**) Typical time evolution of ESR signals of SPIONs accumulated by the leaves of *Egeria densa*. (**c**) Typical plot of the ESR signal intensity of SPIONs accumulated by the leaves of *Egeria densa* as a function of exposure time.
The highest concentrations of SPIONs in *Egeria densa* leaves were observed after *ca.* 2 days of exposure. We detected ~8×10^{13} SPIONs particles per 1 mg dry mass. This points to a rapid and large accumulation (biomagnification) of waterborne SPIONs by leaves of *Egeria densa*.

Conclusions

Our findings point to a rapid accumulation of waterborne SPIONs in leaves of *Egeria densa*, *i.e.* to biomagnification. Moreover, we also demonstrate that ESR in combination with multifunctional paramagnetic / NIR-sensitive up-conversion UCNPs and confocal/two-photon microscopy techniques open new avenues for studying the uptake, internalization and fate of ENPs in plants.

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