Influence of two types of organic matter on interaction of CeO$_2$ nanoparticles with plants in hydroponic culture

Franziska Schwabe [a], Rainer Schulin [a], Ludwig K. Limbach [b], Wendelin Stark [b], Diane Bürge [c], Bernd Nowack [d],*

[a] Soil Protection, Institute of Terrestrial Ecosystems, ETH-Zurich, Universitaetstrasse 16, CH-8092 Zurich, Switzerland
[b] Institute for Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH-Zurich, CH-8093 Zurich, Switzerland
[c] Analytical Chemistry, Research Station Agroscope Reckenholz-Tänikon ART, CH-8046 Zürich, Switzerland
[d] Empa-Swiss Federal Laboratories for Materials Science and Technology, Technology & Society Laboratory, Lerchenfeldstrasse 5, CH-9014 St. Gallen, Switzerland

HIGHLIGHTS

▶ Pumpkin and wheat were exposed to CeO$_2$ nanoparticles in hydroponics at 100 mg L^{-1}.
▶ Treatments included pure, gum arabic- and fulvic acid-containing nutrient solutions.
▶ None of the plants exhibited reduced growth or any toxic response.
▶ CeO$_2$–NPs translocated into pumpkin shoots but not into wheat.
▶ The presence of organic matter affected the amount of CeO$_2$ associated with roots.

GRAPHICAL ABSTRACT
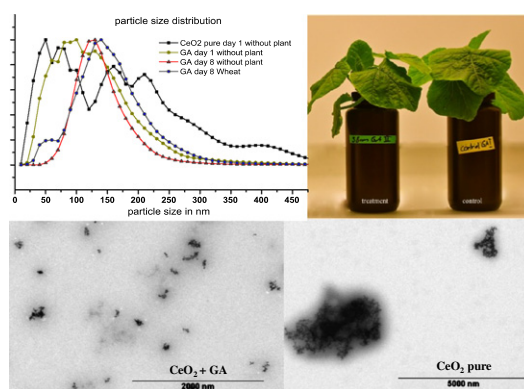
ARTICLE INFO

ABSTRACT

An important aspect in risk assessment of nanoparticles (NPs) is to understand their environmental interactions. We used hydroponic plant cultures to study nanoparticle–plant-root interaction and translocation and exposed wheat and pumpkin to suspensions of uncoated CeO$_2$–NP for 8 d (primary particle size 17–100 nm, 100 mg L^{-1}) in the absence and presence of fulvic acid (FA) and gum arabic (GA) as representatives of different types of natural organic matter. The behavior of CeO$_2$–NPs in the hydroponic solution was monitored regarding agglomeration, sedimentation, particle size distribution, surface charge, amounts of root association, and translocation into shoots. NP-dispersions were stable over 8 d in the presence of FA or GA, but with growing plants, changes in pH, particle agglomeration rate, and hydrodynamic diameter were observed. None of the plants exhibited reduced growth or any toxic response during the experiment. We found that CeO$_2$–NPs translocated into pumpkin shoots, whereas this did not occur in wheat plants. The presence of FA and GA affected the amount of CeO$_2$ associated with roots (pure > FA > GA) but did not affect the translocation factor. Additionally, we could confirm via TEM and SEM that CeO$_2$–NPs adhered strongly to root surfaces of both plant species.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Engineered nanomaterials (ENMs) are used in many commercial products and applications, and it is certain that the large quantity of ENM-production and use will result in exposure of these

* Corresponding author. Tel.: +41 (0)58 765 76 92; fax: +41 (0)58 765 76 82.
E-mail address: nowack@empa.ch (B. Nowack).

materials to the environment (Gottschalk and Nowack, 2011). Exposure modeling has shown that soils are important sinks for ENM, especially in countries where sludge application on agricultural land is a standard procedure (Mueller and Nowack, 2008; Gottschalk et al., 2009). As a result, ENM will inevitably interact with plants growing in these soils.

Contrasting results were published so far about uptake of NP by plants. Birbaum et al. (2010) could not find translocation of 38 nm CeO_2 in soil-irrigated or directly aerosol-exposed maize plants. Also, 20 nm TiO_2 particles were not translocated into wheat shoots although they were found in roots (Du et al., 2011). Other studies observed uptake on NP: 7 nm CeO_2 particles were taken up into seedlings of cucumber, alfalfa, tomato, and corn at concentrations up to 4000 mg L^{-1} (Lopez-Moreno et al., 2010). 7 and 25 nm CeO_2 particles were shown to be translocated to the shoots of cucumber (Zhang et al., 2011). Also for TiO_2 and CuO, it was shown that cucumber and maize were able to translocate the particles from the roots to the shoots (Servin et al., 2012; Wang et al., 2012).

Various factors influence uptake and translocation of particles within the plant, mainly size, agglomeration state, shape, dissolution rate, and surface chemistry (Stark, 2011). Differences between plant species are also important. It was demonstrated that Fe_3O_4-particles (min. size 20 nm) were translocated from the roots to the leaves of pumpkin plants but not of lima beans (Zhu et al., 2008). 10, 30, and 50 nm Ag particles were taken up by tobacco but not by wheat (Judy et al., 2012).

So far, the influence of the presence of organic matter in the medium on ENM uptake into plants has not been studied. The uptake of C_{60} and CNT by rice plants in the presence of NOM was investigated (Lin et al., 2009), but this study did not include a NOM-free control, and thus, the effect of NOM could not be elucidated. What has been done so far in several studies was that NP coated with organic matter were added to the medium. For example, Ag-NP coated with tannic or citric acid (Judy et al., 2012) or gum arabic-coated Ag-NP (Yin et al., 2011) were exposed to plants. Uptake of CNTs coated with gum arabic or fulvic acid into wheat or rape was compared (Larue et al., 2012). Also, citrate-coated and bare Ni-hydroxide particles were compared (Parsons et al., 2010). In all these experiments, the coated NP were added to the nutrient solution, but no additional organic matter was present in solution.

Organic matter is a ubiquitous component of soils. High molecular-weight NOM favor the formation of large aggregates with ENMs, whereas low molecular-weight NOM stabilize dispersed particles (Navarro et al., 2008). Furthermore, the surface charge of ENM might be altered by NOM interactions. Thus, stability of ENM dispersions might be improved due to increased electrostatic or steric repulsion (Domingos et al., 2009). It has been shown that stabilization of CeO_2–NP dispersion with polyacrylic acid is due to higher steric repulsion (Sehgal et al., 2005; Limbach et al., 2008). Polyacrylic acid has very similar chemical properties to fulvic acid (Navarro et al., 2008). The detailed characterization of form and state of ENMs during the experiment are the key aspects for identifying impact and risk of ENMs (Powers et al., 2007; Montes-Burgos et al., 2010; Scown et al., 2010; Stone et al., 2010; Handy et al., 2012).

The aim of this study was to characterize the effects initiated by plant root growth and natural organic matter on CeO_2–NP behavior in a controlled hydroponic system. We studied two economically important plants, spring-wheat that after corn and rice is the most important crop plant and pumpkin because of its large xylem sizes. CeO_2–NPs are industrially used in semiconductor manufacturing, as fuel additives and for polishing lenses (Limbach et al., 2008). They combine a low background signal in plants with insolubility under environmental conditions and remain unaltered after uptake by plant roots (Birbaum et al., 2010). For higher environmental relevance, we used two different forms of NOM, FA and GA (Street

and Anderson, 1983). Additionally, both served as stabilizers for NP-dispersions. Particle size, agglomeration state, and surface charge were monitored over the entire time of the experiments. Furthermore, plant growth parameters: relative chlorophyll-content of leaves, biomass development, root/shoot ratio, and phenotypic detection of chlorosis were documented to estimate possible phytotoxicity.

2. Materials and methods

2.1. Materials

The CeO_2-particles used here were produced by flame-spray-synthesis as described and characterized before (Grass and Stark, 2006; Limbach et al., 2008). In short, NPs were uncoated 99.9% pure CeO_2 with particle-sizes (measured as average diameter in TEM images) between 17 and 100 nm. XRD (X-ray diffraction) revealed a primary particle size of $\varnothing$ 38 nm and DLS (dynamic light scattering) a hydrodynamic diameter in pure water of $\varnothing$ 110 nm. The specific surface area was calculated from BET to be $22.3 \pm 0.16 \, m^2 \, g^{-1}$.

2.2. Hydroponic system

The plants were grown in Hoagland medium as described by Tandy et al. (2006). For wheat, a 10% strength medium was used, resulting in 3.2 mM ionic strength; for pumpkin, 20% strength Hoagland was utilized due to the higher nutrient demand of this plant. The media were adjusted to pH 5.6 by 2 M KOH and buffered with 5.1 mM MES (2-N-morpholino-ethanesulfonic acid).

The NPs were dispersed at a concentration of 2 g L^{-1} CeO_2 in Hoagland medium by ultrasonication (Ultralab 4000, B. Braun, D) at 180 W for 12 min and diluted to a final concentration of 100 mg L^{-1} CeO_2–NPs. The Ce-content of the 100 mg L^{-1} CeO_2–NP solution was 81.4 mg L^{-1}. As dispersing agent, either 10 mg L^{-1} gum arabic (GA) (Sigma Aldrich) or 15 mg L^{-1} fulvic acid (FA) (Suwannee River Fulvic Acid Standard I; IHSS) were added to the media. These concentrations are representative for organic matter in soil systems in all soil depth (Kalbitz et al., 2000). Four different treatments were prepared:

(1) Control (medium of 10% for wheat or 20% for pumpkin Hoagland solution).
(2) CeO_2 pure (medium + 100 mg L^{-1} CeO_2–NPs).
(3) CeO_2 + FA (medium + 100 mg L^{-1} CeO_2–NPs + 15 mg L^{-1} fulvic acid).
(4) CeO_2 + GA (medium + 100 mg L^{-1} CeO_2–NPs + 10 mg L^{-1} gum arabic).

2.3. Solubility of CeO_2–NPs and nutrients

To determine the solubility of CeO_2–NPs and to quantify nutrient ions in the medium, samples were filtered through 30 kDa cellulose membranes in 15 mL tubes (Millipore, Billerica, MA, USA) and analyzed for Ce and other elements (P, Ca, Fe, Mg, Mn, Na). Nutrient-concentrations in mg L^{-1} (ppm) were measured via ICP-OES (ICP-OES-MPX, Varian, CH) in the different Hoagland solutions after incubation of 20 d without plant growth. Solutions as described before, in addition: FA = 10% Hoagland + 15 mg L^{-1} FA; GA = 10% Hoagland + 10 mg L^{-1} GA, were analyzed.

2.4. Plants and growth conditions

Wheat (*Triticum aestivum* var. Sella) and pumpkin (*Cucurbita maxima*, var. Gelber Zentner) seeds were purchased from UFA-

Samen (Fenaco Genossenschaft, Winterthur, CH). The seeds were surface sterilized with 6% NaOCl solution for 10 and 15 min, respectively. Afterward, they were rinsed 5 times in Millipore water and put on germination paper. Wheat seeds were kept at room temperature (RT) for 4 d in the dark and than 1 d in light, irrigated with Millipore water, before transplanted to the hydroponic system. Pumpkin seeds were kept 5 d in the dark at 28 °C and then 2 d in light at RT, wetted with Millipore water, before transplanted to the hydroponic system. Each plant was grown in an individual 1 L bottle with solution aeration.

Plants were grown in an airflow-climate-control-chamber (Kälte 3000, Landquart, CH) in 14.5 h daylight cycles (see Table S1, Supplementary material for details). Wheat and pumpkin were grown under control conditions for 21 d on 1 L 20% Hoagland control-medium (replaced every 7 d). Plants were then exposed to the above-described treatments for 8 d in 1 L bottles (for further details on plant growth see Supplementary material). Each day samples of 2 mL were taken from the supernatant, avoiding resuspension of CeO_2 attached to roots and container walls, for analysis of suspended CeO_2 and for particle characterization, wheat plants evapotranspired 50 mL and pumpkin plant around 200 mL during the treatment time, and these losses were considered in measurements of concentration. On day 8 of treatment, the plants were removed from the bottles and divided into roots and shoots. The shoots were weighed and oven dried at 70 °C. The roots were rinsed 10 times in DI water, dried with tissues, weighed, and dried at 70 °C.

2.5. Particle size and surface charge of CeO_2

During each day of treatment, the particle size distribution of the NP was recorded via NTA (nanoparticle tracking analysis) using a NanoSight LM20, (NanoSight Ltd., Wiltshire, UK). Each sample was analyzed 3 times, and the count values for each nm between 1 and 500 were summed up and converted into relative intensity. On treatment-day 1 and 8, the hydrodynamic diameter and the surface charge of NPs in the media were measured with a Zetasizer 3000 (Malvern, Worcestershire, UK) in three replicates per sample, 10 measurements each.

2.6. Quantification of cerium in particles, suspension and plant material

Quantification of Ce was carried out after a 2-step acid digestion with conc. HNO_3 for 120 min and in a 2nd step with an addition of 38% H_2O_2 in a microwave oven (lavis Ethos EM-2, MLS GmbH, Leutkirch, D), with a maximum temperature of 165 °C. After digestion, the samples were diluted to 25 mL with DI water. Ce concentrations in the supernatant of hydroponic bottles were measured via ICP-OES-MPX after the described 2-step acid digestion, following (Limbach et al., 2008). Shoot-Ce-content was measured via ICP-MS (ICP-MS-920, Varian, CH) from 200 mg of dried plant material after the described 2-step acid digestion.

2.7. Imaging of particles via transmission electron microscopy (TEM)

Nanoparticle imaging was carried out on a CM 12 transmission electron microscope (FEI, Eindhofen, NL) operated at 100 kV. Prior to the measurements, media samples were diluted 1:1000 using Millipore water. Preparation was done by placing carbon-coated copper grids that were glow-discharged for 45 s (Emitech K100X, GB) on holders for centrifugal tubes. 3 mL of diluted sample were added, and NPs were centrifuged with 3000 rpm for 180 min in a 90° angle onto the grid. After centrifugation, the grids were briefly dipped in Millipore water, and the excess moisture was drained along the periphery using a piece of filter paper.

2.8. Imaging of particles on root surfaces via scanning electron microscopy (SEM)

For preparation of SEM samples, the roots were gently rinsed in double distilled water. The preparation of samples and analysis by SEM is described in the Supplementary material.

3. Results

3.1. CeO_2–NP concentration in Hoagland media

Fig. 1a shows that the total Ce-concentration in supernatants of 10% Hoagland media in hydroponic bottles remained constant over a time of 8 d after dispersion with FA or GA, with no difference between stabilizers. In 20% Hoagland medium, dispersed particles remained stable over 5 d before the concentration decreased slightly when FA or GA were present (data not shown). In contrast, all particles sedimented within 3 d when pure CeO_2 was dispersed in 10% or 20% Hoagland media.

Ce concentrations in the supernatant changed in the presence of plants (Fig. 1b and c). The Ce-concentration in CeO_2 + FA and CeO_2 + GA suspensions was reduced when compared to initial concentrations by 36% and 18% in wheat media and 36% and 26% in pumpkin media, respectively. In the pure CeO_2 treatment of wheat and pumpkin, particle behavior was the same as without plants. Within each experiment (without plant, wheat-medium, pumpkin-medium), the concentrations did not significantly differ between treatments on day 1. From day 2 on, the concentrations were always significantly lower ($p < 0.01$) in the CeO_2 treatment than in the FA and GA treatments, while the FA and GA treated media showed no significant concentration difference ($p < 0.01$) in any of the treatments during the entire experiment.

### 3.2. Nutrients and CeO_2 solubility in Hoagland media

To test the solubility of 100 mg L^{-1} CeO_2-particles, a filter-experiment with the four different growth media was carried out. After filtration of all treatment and control media, we could not detect any Ce via ICP-OES, with a detection limit for Ce of 0.1 mg L^{-1}. Therefore, less than 0.1% of the CeO_2 was dissolved. The concentrations of nutrients in the growth media are shown in Table S2 (Supplementary material). In control Hoagland medium, we could detect 92–108% of the added nutrients after filtration of 20 d old samples. This was also true for Ca, Fe, Mg, Mn and Na in the 20 d-incubated CeO_2-dispersions. Only the P-concentration was reduced by 17%, 6%, and 20% for pure, FA, and GA CeO_2-dispersions compared to NP-free control media and initial concentration of 100 μM KH_2PO_4. Wheat plants increased the pH from the initial 5.6 to 6.1, whereas pumpkin plants decreased the pH down to 5.2–5.0 (Table 1).

3.3. Zeta potential

The zeta potential of 100 mg L^{-1} CeO_2–NPs in Millipore water was +21.3 mV. When the particles were dispersed in Hoagland solution with pH adjusted to 5.6, the zeta potential changed to negative counts (Table 1) with values from −12.3 mV (pure CeO_2) to −22.5 mV (CeO_2 + FA). After 8 d, in the presence of plants, the values were higher, −10.7 mV in pure CeO_2 medium of wheat and −16.5 mV in CeO_2 + GA medium of wheat and pumpkin.

3.4. Particle size distribution in media

Particle size distributions in the Hoagland medium in the absence and presence of plants are shown in Fig. 2. After dispersion of pure CeO_2, a range of 17–400 nm was observed which reflects single
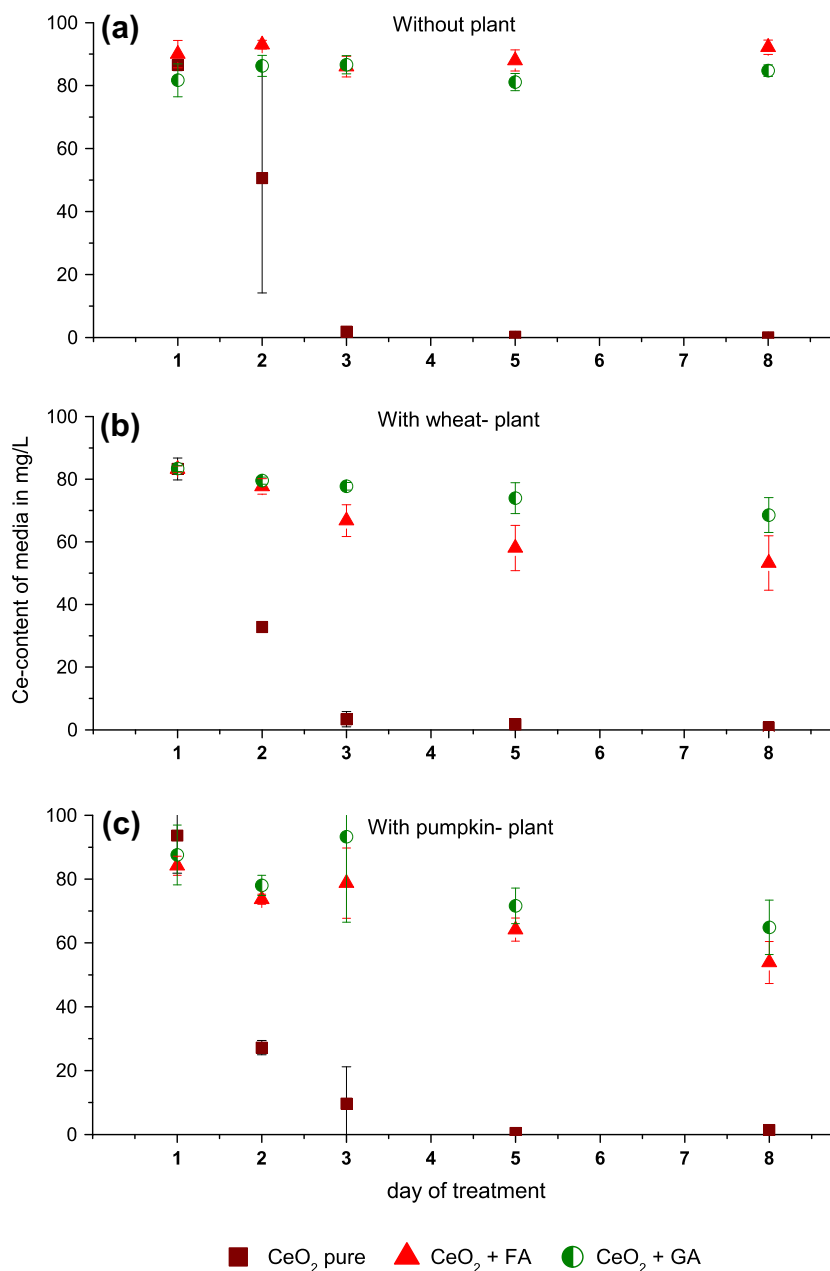
Fig. 1. Ce-content in Hoagland media over exposure time of 8 d (mean of 3 replicates), 100 mg L^{-1} CeO$_2$–NPs suspended in Hoagland media in the absence or presence of FA (15 mg L^{-1}) and GA (10 mg L^{-1}). (a) Without plants in 10% Hoagland, (b) in presence of wheat in 10% Hoagland and (c) in presence of pumpkin in 20% Hoagland.

Table 1
Zeta potential (ZP) in mV and pH of CeO2–NP-suspensions in the absence (day 1 and 8) and presence of wheat and pumpkin (day 8). Measurements were conducted with a Zetasizer 3000 by Malvern, STDV (±) are directly calculated over 3 × 10 measurements per dispersion.

Medium of CeO$_2$–NPs	Day 1		Day 8		
	ZP (mV)	pH	ZP (mV)		pH
			Wheat	Pumpkin	
in Millipore water	+21.3 ± 0.8	6.9	n.a.		n.a.
in Millipore water + 15 mg L^{-1} fulvic acid	−34.3 ± 1.1	5.6	n.a.		n.a.
in Millipore water + 10 mg L^{-1} gum arabic	−24.7 ± 5.9	6.7	n.a.		n.a.
in 10% Hoagland	−12.3 ± 0.6	5.6	−10.7 ± 0.6		6.1
in 10% Hoagland + 15 mg L^{-1} fulvic acid	−22.5 ± 0.5	5.6	−13.8 ± 0.9		6.1
in 10% Hoagland + 10 mg L^{-1} gum arabic	−18.2 ± 0.9	5.6	−16.5 ± 0.1		6.1
in 20% Hoagland	−8.6 ± 0.1	5.6		−12.3 ± 0.6	5.2
in 20% Hoagland + 15 mg L^{-1} fulvic acid	−18.3 ± 0.3	5.6		−16.3 ± 0.9	5.0
in 20% Hoagland + 12.5 mg L^{-1} gum arabic	−16.4 ± 0.7	5.6		−16.5 ± 0.1	5.2
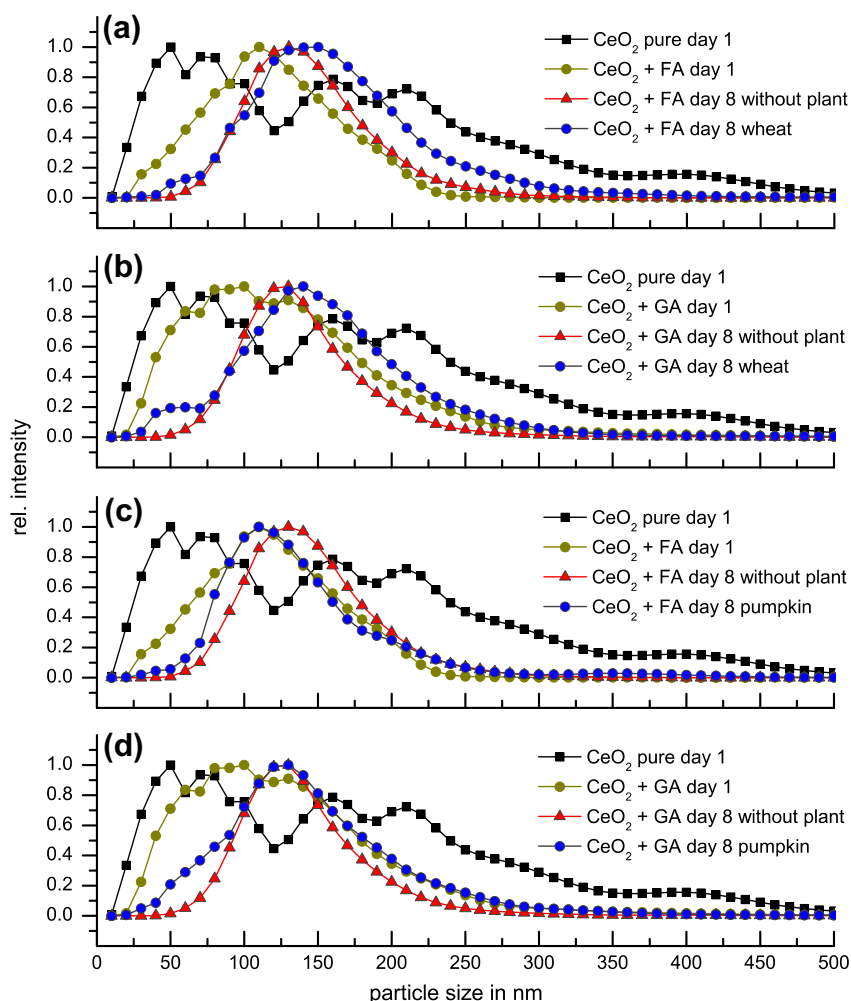
Fig. 2. Particle size distribution measured via NTA at day 1 and day 8 of treatment in the absence and presence of plants (mean of 3 independent measurements). (a) Fulvic acid-stabilized system with/without wheat, (b) gum arabic stabilized system with/without wheat, (c) fulvic acid-stabilized system with/without pumpkin and (d) gum arabic stabilized system with/without pumpkin. STDV are not given for clarity reasons.

primary particles as well as bigger agglomerates and aggregates. When FA or GA was present, the size range narrowed to 25–250 nm, mirroring that both stabilizers prevented the formation of agglomerates and aggregates in the size range of 200–400 nm. When particles were incubated over 8 d in media without plants, the size shifted slightly to higher values, that is, the main peak for FA shifted from 110 nm to 130 nm, for GA from 100 nm to 130 nm.

DLS measurements on day 8 confirmed these small shifts. For FA, the main DLS peak was at 170 nm and for GA at 165 nm (data not shown). The measurements by DLS and NTA for CeO_2 pure treatment after more than 24 h gave non-valid data most likely because of particle sizes of $\geqslant 1$ µm and fast sedimentation of NPs during analysis.

When wheat plants were grown on the media the size shifted to a main peak at 150 nm on day 8. With pumpkin growth, another feature occurred. Particle size remained the same as without plants when GA was present. When FA was present, particle size was slightly reduced over time of plant treatment. Sizes of around 100 nm were measured on day 8 compared to 130 nm on day 1 and 130 nm without plants on day 8.

3.5. Interaction of CeO₂–NPs with roots

The amounts of cerium associated with roots are displayed in Fig. 3. The highest amounts were detected for wheat and pumpkin in the CeO_2 pure treatment with 130.3 and 91.9 mg kg^{-1},

respectively. The amount of NPs associated with pumpkin roots was significantly ($p < 0.001$) lower in the presence of FA (32.2 mg kg^{-1}), while for wheat, the reduction was not significant. In the presence of GA, the amount of NP associated with the roots was significantly ($p < 0.001$) lower for both pumpkin and wheat (21.8 and 13.2 mg kg^{-1}). The FA and GA treatments were also significantly ($p < 0.001$) different from each other. The interaction of CeO_2 with the roots was therefore reduced almost sevenfold for pumpkin roots and sixfold for wheat in the presence of FA and GA. Since wheat had a smaller biomass production (0.18 g of dry root/plant) than pumpkin (0.41 g dry root/plant), there is a biomass-dilution-effect that has an influence on the values in Fig. 3. When Ce-values are converted to Ce/plant, pumpkin shows a higher amount of cerium associated with the roots in CeO_2 pure and CeO_2 + GA treatment than wheat (Table 2).

3.6. Translocation of CeO₂–NPs into plant shoots

The Ce-values of all control plants were below limits of quantification; thus, data are only shown for treated plants (Fig. 3). Also, the wheat plants did not contain any measurable Ce. Pumpkin plants contained Ce in all three NP-treatments (CeO_2 pure > FA > GA). Although the amount of NPs attached to the roots in CeO_2 pure treatment was 7 times more than in the CeO_2 + GA treatment, only 5 times more Cerium was found in the CeO_2 treated pumpkin
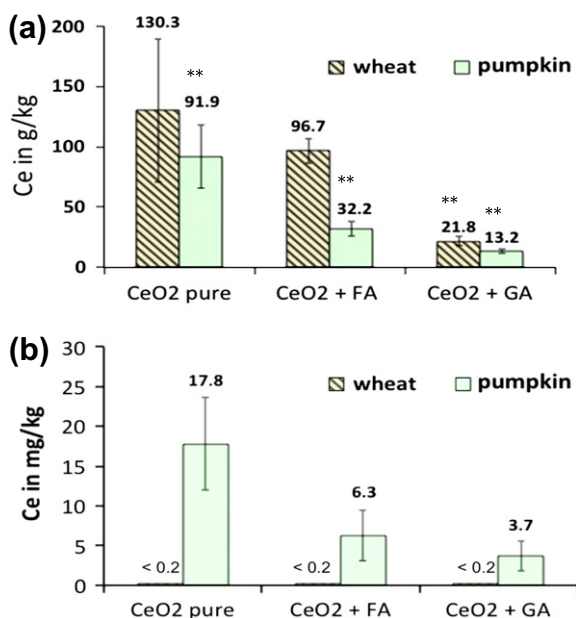
Fig. 3. Ce-concentration of plant material after 8 d of treatment with 100 mg L^{-1} CeO$_2$ NP in Hoagland medium (mean of 3 treatments, error bars indicate STDV). (a) Ce in g kg^{-1} of plant roots ** indicates p-value < 0.001 (values of control plants were below LOD therefore values are not shown). (b) Ce in mg kg^{-1} of plant shoots (values of control plants and for wheat treatments were below LOD therefore values are not shown).

Table 2

Relative distribution of cerium in the system after 8 d of plant treatment, measured distribution in % of initial amount Ce in the hydroponic system: dispersion, adhered to the roots of plant, translocated into the shoot, rest (accounting for precipitation and losses during washing process).

Treatment	Dispersion in %	Root in %	Shoot in %	Rest in %
Wheat				
CeO$_2$ pure	0	27.8	0	72
CeO$_2$ + FA	63.3	20.7	0	16
CeO$_2$ + GA	81	4.6	0	13.8
Pumpkin				
CeO$_2$ pure	0	45	<0.1	55
CeO$_2$ + FA	64	16	<0.1	20
CeO$_2$ + GA	77.5	6	<0.1	16.5

shoots. Translocation factors of Ce (defined as Ce-content ratio of shoot to root) were 0.00019 in CeO$_2$ pure and FA treatment and 0.00028 in the GA treatment. This was far below the described translocation factors reported for natural occurring cerium from soils (0.04–0.09) (Tyler, 2004).

3.7. Overall CeO$_2$–NP distribution

In the overall distribution of NPs after 8 d of treatment strong differences could be measured between FA and GA-stabilized dispersions compared to pure CeO$_2$ (Table 2). Most of the NPs could still be found in dispersion when FA or GA was added. In the non-stabilized dispersions, the highest amount had disappeared, implying sedimentation. High amounts of NPs were associated with the roots in the CeO$_2$ pure treatment, 27.8% in wheat and 45% in pumpkin. The translocation to pumpkin shoots accounted for less than 0.1 wt.% of CeO$_2$.

3.8. TEM and SEM imaging of particles and root surfaces

The TEM pictures in Fig. 4 illustrate the difference between agglomerates of CeO$_2$–NPs in pure treatment and the FA and GA

treatments. Fig. 4a shows the CeO$_2$ particles at high magnification. Fig. 4b shows large and very dense agglomerates of CeO$_2$ pure treatment, 4 h after dispersion. It was possible to confirm the agglomerate-size of >1 μm in CeO$_2$ pure medium via the TEM-method, explaining the non-valid measurements using NTA or DLS. In Fig. 4c the CeO$_2$–NPs stabilized with FA are depicted 1 h after dispersion and in Fig. 4d after 8 d of interaction with pumpkin roots. The pictures illustrate that there was no great change in size of agglomerates over time. However, smaller and larger agglomerates exist and are distributed all over the grid. The same was true for NPs from the GA treatment (Fig. 4e and f). It was distinguishable that the GA-stabilized particles, with pumpkin root interaction, were the smallest of all treatments. The TEM pictures and the results from the particle size analysis by NTA agree with each in that there is a wide range of particle sizes even in the presence of GA and FA.

In Fig. S1 (Supplementary material), the SEM image of the root surface of a control wheat plant is compared to the CeO$_2$ pure treatment, where big aggregates are scattered over the root surface. The identification of CeO$_2$ on the root surface was carried out via EDX-determination (Fig. S3, Supplementary material). The EDX-spectra clearly showed that the lighter spots on the SEM-images represent CeO$_2$–NPs attached to the root surface. Identical behavior was found for pumpkin roots (images not shown). TEM-studies of wheat-root surfaces showed a quite uniform dispersal of NPs from CeO$_2$ + GA and CeO$_2$ + FA treatment (a and b), in contrast to the CeO$_2$ pure treatment (Fig. S2, Supplementary material).

3.9. Toxicity

Neither wheat nor pumpkin showed any phenotypic response to CeO$_2$ exposure. The monitored physiological parameters are given in Tables S3–S6, Supplementary material. The relative chlorophyll-content of the leaves, the biomass development including root/shoot ratio, and visual observations did not reveal any impact of the NPs. Hence, we conclude that there was no direct toxic effect induced by CeO$_2$–NPs at a concentration of 100 mg L^{-1}. The low toxicity of CeO$_2$–NP to plants is already known (Ma et al., 2010). In the literature, indirect toxic effects of NPs have also been described, e.g. starvation caused by nutrient binding to NP-surfaces (van Hoecke et al., 2009). We demonstrated in filter-experiments of CeO$_2$ dispersions that the available phosphate concentration was reduced to 85%, whereas all other nutrients showed no reduction in availability (Table S2, Supplementary material). The removal of phosphate was therefore not enough to cause any indirect starvation effect.

4. Discussion

4.1. Behavior of CeO$_2$–NPs in growth medium and influence of NOM

The CeO$_2$–NP behavior was altered when fulvic acid or gum arabic was added to dispersions. In the presence of both compounds, the particles remained in dispersion longer compared to the uncoated and non-functionalized basic material. Agglomeration of ENMs is strongly dependent on surface charge, free ions, and interaction of electric forces between particles or other solids (Limbach et al., 2005, 2008). The two main reasons for this are van der Waals attraction and electrostatic interaction (Hotze et al., 2010). The ionic composition of Hoagland medium altered the surface charge of NPs measured as zeta potential and resulted in a rapid formation of dense and large agglomerates of uncoated and non-functionalized CeO$_2$–NPs (Limbach et al., 2009). In general, zeta potential values between −20 and +20 mV favor agglomeration. As a result, particles agglomerate and reach sizes of >1 μm, followed by rapid
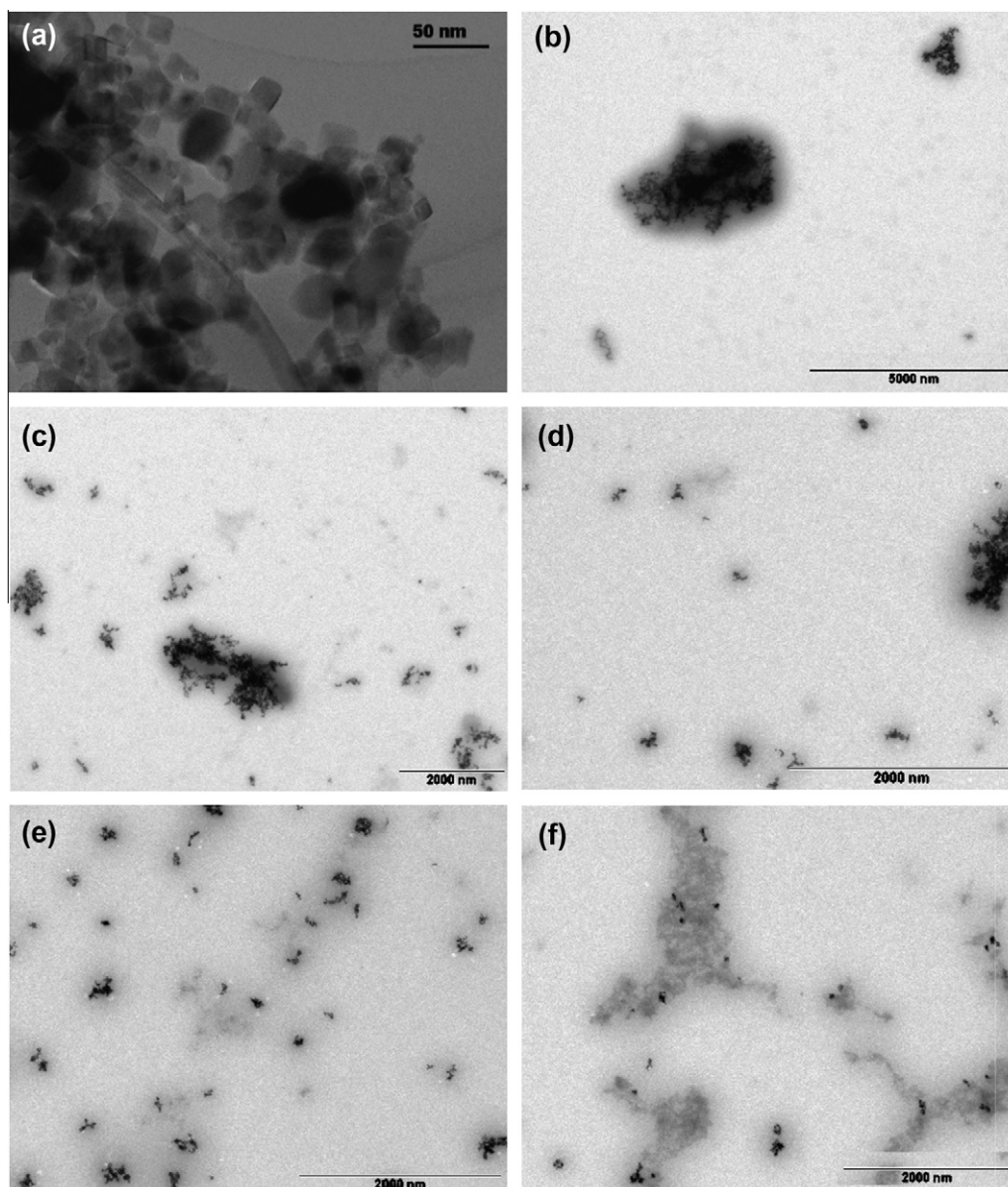
Fig. 4. Transmission electron micrographs of CeO$_2$-particles in the different media: (a) 1 h after ultrasonic dispersion with acryl polymer in Millipore water, (b) 4 h after dispersion in 20% Hoagland solution, (c) 1 h after dispersion in 20% Hoagland solution with addition of 15 mg L^{-1} fulvic acid, (d) as in c but after 8 d in presence of pumpkin, (e) 1 h after dispersion in 20% Hoagland solution with addition of 10 mg L^{-1} gum arabic and (f) as in e but after 8 d in presence of pumpkin.

sedimentation due to low electrostatic repulsion (Limbach et al., 2005). Due to electrostatic repulsion, particle suspensions are the more stable the more the zeta potential differs from the point of zero charge. The zeta potential of CeO$_2$ in Hoagland medium was more or less similar in all dispersions and remained constant over time with values in general less negative than −20 mV (−10.7, −13.8, and −16.5 mV for pure, FA and GA, respectively). In all treatments, the CeO$_2$ particles were therefore in a non-stabile electrostatic condition. This explained why particles in the CeO$_2$ pure treatment formed large agglomerates and sedimented within 2 d. But FA- and GA-stabilized NPs were maintained in dispersion over 8 d, and therefore, we conclude that the higher stability of particles in the presence FA or GA was not of an electrostatic nature (only small difference in zeta potential of pure and FA) but due to steric repulsion (Domingos et al., 2009; Hotze et al., 2010).

In the presence of plants, only small changes in particle size distribution were detected. The observed differences were in good agreement with changes in pH and in line with expected changes

in electrostatic repulsion between particles. Plant roots exudate protons and organic acids that change the chemical composition of the surrounding medium (Rovira, 1969; Jones and Darrah, 1994). In the wheat treatment, the pH increased and the zeta potential decreased. Because the NPs surface charge was closer to the point of zero charge (pzc: in medium at pH 7), the agglomeration increased (Limbach et al., 2009). Pumpkin plants reduced the pH, resulting in constant (CeO$_2$ + GA) or slightly decreased (CeO$_2$ + FA) particle sizes in media, in agreement with the higher stability of CeO$_2$ at lower pH.

4.2. Interaction of plant root with CeO$_2$ and translocation

We showed via ICP-OES, SEM and TEM analysis that a high amount of CeO$_2$–NPs adhered to the root surface of wheat and pumpkin. Considering the root biomass and the amount of available NPs in suspension, there was no considerable difference in root adherence between the two species. Nevertheless, the

addition of NOM had a large influence on the amount of CeO$_2$ associated with the roots because the adsorption of particles to the roots stabilized with organic matter was strongly reduced. FA and GA therefore did not only slow down agglomeration between particles but likewise inhibited the sorption of NPs to roots. Similarly, they reduced the uptake of NPs into pumpkin shoots. Since we could not detect any dissolved Ce-ions in the liquid media, we conclude that all Ce found inside shoots are most likely derived from translocated CeO$_2$–NPs. However, the translocation factors (ratio of shoot/root concentration) were the same in the CeO$_2$-pure and FA-treatments and not much higher for GA (0.00028 instead of 0.00019). The effect of FA and GA on uptake of CeO$_2$ was therefore an indirect one by reducing the amount of root-associated CeO$_2$ that was available for translocation. A slight effect of surface charge (in the case of GA) cannot be excluded, but the fact that the pure and FA-treated particles had the same relative translocation indicated that once on the root surface, the aqueous surface speciation of the particles no longer played a major role in determining the translocation of the particles inside the plant.

Our work showed that CeO$_2$–NPs with a size range of 17 nm to >1 μm are at least partially available for uptake by pumpkin (*Cucurbita maxima*). This is in accordance with Zhu et al. (2008) who found that Fe-NPs with a particle size of 20 nm translocated to pumpkin leaves in hydroponic solution. Another study (Zhang et al., 2011) obtained similar results for cucumber, a related species. Even 50 nm Au-particles were shown to be taken up by plants (Judy et al., 2012).

In contrast to pumpkin we could not detect any translocation of Ce into the shoots of wheat. It has already been reported before that monocots are less likely to take up NP, e.g. (*Zea mays*) (Birbaum et al., 2010; Lopez-Moreno et al., 2010). Also, 10, 30, and 50 nm Au-NP were taken up by tobacco but not wheat (Judy et al., 2012). The differences in CeO$_2$-accumulation between wheat and pumpkin plants might be explained by specific alterations in root structures and the physical and chemical interactions between NPs and root exudates in the rhizosphere (Rico et al., 2011; Judy et al., 2012). For plants in a hydroponic system, the Casparian strip may be missing in some cells of the root exodermis (Zimmermann et al., 2000). This would result in a lower hydraulic conductivity of roots and may create an easy passage to vascular bundles of the endodermis. However, the mechanism by which NP are taken up by plants is not well understood, but they were shown to be present in xylem and phloem sap and to be mobile within the plant (Wang et al., 2012).

A very obvious difference between wheat and pumpkin is their water uptake capacity. Regardless of apoplastic or symplastic uptake pathways, the translocation of NPs is most likely to be driven by water flow. Since pumpkin transpired far more water (200 mL) than wheat (50 mL) during the 8 d of treatment, it would take much longer for wheat to accumulate detectable amounts of Ce in the shoots. Another difference deriving from the high water uptake capacity of pumpkin is larger sieving pores that may act as larger pathways compared to the wheat endoderm. The characteristics of pumpkin to take up larger insoluble organic molecules are already known and makes it a successful phytoremediator (Lunney et al., 2004; Gent et al., 2007). Yet, no explanation was found for how this uptake is realized. We suggest that the pathway of particle uptake and incorporation of insoluble organic molecules may be the same.

Acknowledgements

Appendix A. Supplementary material

Details of plant growth and system build up, as well values of plant toxicology. Images of particles on the root surfaces, via transmission electron microscopy (TEM) and Scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX). Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.12.025.

References

Birbaum, K., Brogioli, R., Schellenberg, M., Martinoia, E., Stark, W.J., Gunther, D., Limbach, L.K., 2010. No evidence for cerium dioxide nanoparticle translocation in maize plants. Environ. Sci. Technol. 44, 8718–8723.

Domingos, R.F., Tufenkji, N., Wilkinson, K.J., 2009. Aggregation of titanium dioxide nanoparticles: role of a fulvic acid. Environ. Sci. Technol. 43, 1282–1286.

Du, W.C., Sun, Y.Y., Ji, R., Zhu, J.G., Wu, J.C., Guo, H.Y., 2011. TiO$_2$ and ZnO nanoparticles negatively affect wheat growth and soil enzyme activities in agricultural soil. J. Environ. Monit. 13, 822–828.

Gent, M.P., White, J.C., Parrish, Z.D., Isleyen, M., Eitzer, B.D., Mattina, M.I., 2007. Uptake and translocation of p,p′-dichlorodiphenyldichloroethylene supplied in hydroponics solution to Cucurbita. Environ. Toxicol. Chem. 26, 2467–2475.

Gottschalk, F., Nowack, B., 2011. The release of engineered nanomaterials to the environment. J. Environ. Monit. 13, 1145–1155.

Gottschalk, F., Sonderer, T., Scholz, R.W., Nowack, B., 2009. Modeled environmental concentrations of engineered nanomaterials (TiO$_2$, ZnO, Ag, CNT, fullerenes) for different regions. Environ. Sci. Technol. 43, 9216–9222.

Grass, R.N., Stark, W.J., 2006. Flame spray synthesis under a non-oxidizing atmosphere: preparation of metallic bismuth nanoparticles and nanocrystalline bulk bismuth metal. J. Nanopart. Res. 8, 729–736.

Handy, R.D., Cornelis, G., Fernandes, T., Tsyusko, O., Decho, A., Sabo-Attwood, T., Metcalfe, C., Steevens, J.A., Klaine, S.J., Koelmans, A.A., Horne, N., 2012. Ecotoxicity test methods for engineered nanomaterials: practical experiences and recommendations from the bench. Environ. Toxicol. Chem. 31, 15–31.

Hotze, E.M., Phenrat, T., Lowry, G.V., 2010. Nanoparticle aggregation: challenges to understanding transport and reactivity in the environment. J. Environ. Qual. 39, 1909–1924.

Jones, D.L., Darrah, P.R., 1994. Role of root derived organic-acids in the mobilization of nutrients from the rhizosphere. Plant Soil 166, 247–257.

Judy, J.D., Unrine, J.M., Rao, W., Wirick, S., Bertsch, P.M., 2012. Bioavailability of gold nanomaterials to plants: importance of particle size and surface coating. Environ. Sci. Technol. 46, 8467–8474.

Kalbitz, K., Solinger, S., Park, J.H., Michalzik, B., Matzner, E., 2000. Controls on the dynamics of dissolved organic matter in soils: a review. Soil Sci. 165, 277–304.

Larue, C., Pinault, M., Czarny, B., Georgin, D., Jaillard, D., Bendiab, N., Mayne-L'Hermite, M., Taran, F., Dive, V., Carrière, M., 2012. Quantitative evaluation of multi-walled carbon nanotube uptake in wheat and rapeseed. J. Hazard. Mater. 227–228, 155–163.

Limbach, L.K., Bereiter, R., Müller, E., Krebs, R., Gälli, R., Stark, W.J., 2008. Removal of oxide nanoparticles in a model wastewater treatment plant: influence of agglomeration and surfactants on clearing efficiency. Environ. Sci. Technol. 42, 5828–5833.

Limbach, L.K., Grass, R.N., Stark, W.J., 2009. Physico-chemical differences between particle- and molecule-derived toxicity: can we make inherently safe nanoparticles? Chimia 63, 38–43.

Limbach, L.K., Li, Y., Grass, R.N., Brunner, T.J., Hintermann, M.A., Muller, M., Gunther, D., Stark, W.J., 2005. Oxide nanoparticle uptake in human lung fibroblasts: effects of particle size, agglomeration, and diffusion at low concentrations. Environ. Sci. Technol. 39, 9370–9376.

Lin, S.J., Reppert, J., Hu, Q., Hudson, J.S., Reid, M.L., Ratnikova, T.A., Rao, A.M., Luo, H., Ke, P.C., 2009. Uptake, translocation, and transmission of carbon nanomaterials in rice plants. Small 5, 1128–1132.

Lopez-Moreno, M.L., de la Rosa, G., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2010. X-ray absorption spectroscopy (XAS) corroboration of the uptake and storage of CeO$_2$ nanoparticles and assessment of their differential toxicity in four edible plant species. J. Agric. Food Chem. 58, 3689–3693.

Lunney, A.I., Zeeb, B.A., Reimer, K.J., 2004. Uptake of weathered DDT in vascular plants: potential for phytoremediation. Environ. Sci. Technol. 38, 6147–6154.

Ma, Y., Kuang, L., He, X., Bai, W., Ding, Y., Zhang, Z., Zhao, Y., Chai, Z., 2010. Effects of rare earth oxide nanoparticles on root elongation of plants. Chemosphere 78, 273–279.

Montes-Burgos, I., Walczyk, D., Hole, P., Smith, J., Lynch, I., Dawson, K., 2010. Characterisation of nanoparticle size and state prior to nanotoxicological studies. J. Nanopart. Res. 12, 47–53.

Mueller, N.C., Nowack, B., 2008. Exposure modeling of engineered nanoparticles in the environment. Environ. Sci. Technol. 42, 4447–4453.

Navarro, E., Baun, A., Behra, R., Hartmann, N.B., Filser, J., Miao, A.J., Quigg, A., Santschi, P.H., Sigg, L., 2008. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology 17, 372–386.

Parsons, J.G., Lopez, M.L., Gonzalez, C.M., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2010. Toxicity and biotransformation of uncoated and coated nickel hydroxide nanoparticles on mesquite plants. Environ. Toxicol. Chem. 29, 1146–1154.

Powers, K.W., Palazuelos, M., Moudgil, B.M., Roberts, S.M., 2007. Characterization of the size, shape, and state of dispersion of nanoparticles for toxicological studies. Nanotoxicology 1, 42–51.

Rico, C.M., Majumdar, S., Duarte-Gardea, M., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2011. Interaction of nanoparticles with edible plants and their possible implications in the food chain. J. Agric. Food Chem. 59, 3485–3498.

Rovira, A.D., 1969. Plant root exudates. Bot. Rev. 35, 35.

Scown, T.M., van Aerle, R., Tyler, C.R., 2010. Review: do engineered nanoparticles pose a significant threat to the aquatic environment? Crit. Rev. Toxicol. 40, 653–670.

Sehgal, A., Lalatonne, Y., Berret, J.F., Morvan, M., 2005. Precipitation–redispersion of cerium oxide nanoparticles with poly(acrylic acid): toward stable dispersions. Langmuir 21, 9359–9364.

Servin, A.D., Castillo-Michel, H., Hernandez-Viezcas, J.A., Diaz, B.C., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2012. Synchrotron Micro-XRF and Micro-XANES confirmation of the uptake and translocation of TiO_2 nanoparticles in Cucumber (*Cucumis sativus*) Plants. Environ. Sci. Technol. 46, 7637–7643.

Stark, W.J., 2011. Nanoparticles in biological systems. Angewandte Chemie-International Edition 50, 1242–1258.

Stone, V., Nowack, B., Baun, A., van den Brink, N., von der Kammer, F., Dusinska, M., Handy, R., Hankin, S., Hassellov, M., Joner, E., Fernandes, T.F., 2010. Nanomaterials for environmental studies: classification, reference material issues, and strategies for physico-chemical characterisation. Sci. Total Environ. 408, 1745–1754.

Street, C.A., Anderson, D.M.W., 1983. Refinement of structures previously proposed for gum arabic and other acacia gum exudates. Talanta 30, 887–893.

Tandy, S., Schulin, R., Nowack, B., 2006. The influence of EDDS on the uptake of heavy metals in hydroponically grown sunflowers. Chemosphere 62, 1454–1463.

Tyler, G., 2004. Rare earth elements in soil and plant systems – a review. Plant Soil 267, 191–206.

van Hoecke, K., Quik, J.T.K., Mankiewicz-Boczek, J., De Schamphelaere, K.A.C., Elsaesser, A., Van der Meeren, P., Barnes, C., McKerr, G., Howard, C.V., Van De Meent, D., Rydzyński, K., Dawson, K.A., Salvati, A., Lesniak, A., Lynch, I., Silversmit, G., De Samber, B., Vincze, L., Janssen, C.R., 2009. Fate and effects of CeO_2 nanoparticles in aquatic ecotoxicity tests. Environ. Sci. Technol. 43, 4537–4546.

Wang, Z.Y., Xie, X.Y., Zhao, J., Liu, X.Y., Feng, W.Q., White, J.C., Xing, B.S., 2012. Xylem- and phloem-based transport of CuO nanoparticles in maize (*Zea mays* L.). Environ. Sci. Technol. 46, 4434–4441.

Yin, L.Y., Cheng, Y.W., Espinasse, B., Colman, B.P., Auffan, M., Wiesner, M., Rose, J., Liu, J., Bernhardt, E.S., 2011. More than the ions: the effects of silver nanoparticles on *Lolium multiflorum*. Environ. Sci. Technol. 45, 2360–2367.

Zhang, Z.Y., He, X., Zhang, H.F., Ma, Y.H., Zhang, P., Ding, Y.Y., Zhao, Y.L., 2011. Uptake and distribution of ceria nanoparticles in cucumber plants. Metallomics 3, 816–822.

Zhu, H., Han, J., Xiao, J.Q., Jin, Y., 2008. Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. J. Environ. Monit. 10, 713–717.

Zimmermann, H.M., Hartmann, K., Schreiber, L., Steudle, E., 2000. Chemical composition of apoplastic transport barriers in relation to radial hydraulic conductivity of corn roots (*Zea mays* L.). Planta 210, 302–311.