Distribution, Bioaccumulation, Trophic Transfer, and Influences of CeO₂ Nanoparticles in a Constructed Aquatic Food Web

Xingchen Zhao,^{†,‡} Miao Yu,^{†,‡} Dan Xu,^{†,‡} Aifeng Liu,^{†,‡} Xingwang Hou,^{†,‡} Fang Hao,^{†,‡} Yanmin Long,^{†,§} Qunfang Zhou,^{*,†,‡} and Guibin Jiang^{†,‡}

[†]State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, P.R. China

[‡]College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100049, P.R. China

[§]Institute of Environment and Health, Jiangnan University, Wuhan 430000, P. R. China

S Supporting Information

ABSTRACT: In view of the final destination of nanomaterials, the water system would be an important sink. However, the environmental behavior of nanomaterials is rather confusing due to the complexity of the real environment. In this study, a freshwater ecosystem, including water, sediment, water lettuce, water silk, Asian clams, snails, water fleas, Japanese medaka, and Yamato shrimp, was constructed to study the distribution, bioaccumulation, and potential impacts of CeO₂ nanoparticles (CeO₂ NPs) via long-term exposure. The results demonstrated most of the CeO₂ NPs deposited in the sediment (88.7%) when the partition approached to the constant 30 days later. The bioaccumulated Ce in six tested biota species was negatively correlated with its trophic level, showing no biomagnification of CeO₂ NPs through this food web. CeO₂ NP exposure induced visual abnormalities in hydrophytes, including chlorophyll loss in water silk and water lettuce, ultrastructural changes in pyrenoids of water silk, and root elongation in water lettuce. The generation of hydroxyl radical ($\cdot\text{OH}$) and cell-wall loosening induced by CeO₂ NP exposure might mediate the root growth in water lettuce. The findings on the environmental behavior of CeO₂ NPs in water system have provided useful information on the risk assessment of nanomaterials.

INTRODUCTION

The development and application of engineered nanomaterials (ENMs) have rapidly increased in diverse fields during the past decade. Like other emerging chemicals, concerns have concomitantly arisen on their potential hazards ever since the appearance of ENMs. As the ultimate sink for all pollutants, the aquatic ecosystem plays important roles in the full life cycle of ENMs due to their intentional and unintentional release.^{1,2} The behavior of ENMs in the aquatic ecosystem has thus become an urgent issue in view of their risk assessment.

Nanoparticles (NPs) may be accidentally or intentionally released to the aquatic system via (1) industrial discharges or domestic waste, (2) disposal of effluents, (3) indirect surface runoff from soils, and (4) precipitation-carrying NPs.^{3–6} Once released into the environment, manufactured nanomaterials can be transported or migrate in the ambient media. Due to the different properties of the media, like water, sediment, and biota, ENMs can be redistributed and transformed biotically or abiotically, thus causing changes in the physical and chemical characteristics of the particles. Laboratory simulative experiments are widely used to investigate the aquatic toxicology of NPs. It has been reported that metal oxide nanomaterials can cause mechanical cell damage to algae through particle exposure and metal ion release.^{7–9} In contrast to the

biomagnification of certain nanomaterials in terrestrial food chains,^{10,11} the aquatic animals can directly take up and transfer ENMs in food chains with relatively lower concentrations of nanomaterials in higher trophic organisms in comparison with those in lower trophic ones.^{12–14} The estuarine mesocosms were constructed to study the fates and bioavailabilities of gold nanorods with different charges in natural system, but the duration only lasted for 12 days, which did not necessarily capture the chronic influences occurring in the natural environment.^{15,16} Therefore, a better understanding of the transport and effects of nanomaterials from long-term exposure is imperative.

Cerium oxide nanoparticles (CeO₂ NPs) have become one of the most popular nanomaterials in the past several years and are currently being utilized in various fields as a catalyst, cell electrolyte, semiconductor, antioxidant, coating, and polishing chemical.^{17–20} Its wide use would eventually cause the emerging exposure issue in real environmental scenario like some other metal oxide nanoparticles, e.g., TiO₂ NPs.²¹ The

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increasing concerns have been raised, regarding their potential adverse impacts. Currently available data shows that CeO₂ NPs are more toxic than bulk CeO₂ and may induce cell death, oxidative stress, and DNA damage.^{22–24} Mesocosms were also introduced to assess the impact of CeO₂ NPs in aquatic ecosystem, and it was found that CeO₂ NPs were readily removed from the water column and partitioned between different organisms.²⁵ The distribution and accumulation characteristics of CeO₂ NPs in various aquatic organisms were different.²⁶ The valence conversion from Ce (IV) to Ce (III) occurred in the digestive gland of benthic organisms for both bare and coated CeO₂ NPs.²⁷ The size and surface modifications of CeO₂ NPs obviously influenced the temporal partition behavior of nanoparticles in aquatic systems and their bioavailability and toxicity to the biota species.^{22,23,25} Nevertheless, previous studies on food chains containing no more than three species could not fully represent the situation of a complete ecosystem or mimic the real environmental scenario. It was thus of importance to study the long-term effect of CeO₂ NP exposure in a water ecosystem constituted by multiple abiotic and biotic components to clarify their environmental fate and potential impacts.

In this study, we conducted 10 month investigations on CeO₂ NPs in a lab-constructed microcosm simulating real environmental scenarios. CeO₂ NPs were spiked in the simulated fresh water ecosystem, and their distribution among water column, sediments, water lettuce (*Pistia stratiotes*), water silk (*Spirogyra borealis*), Asian clams (*Corbicula fluminea*), snails (*Physa acuta*), water fleas (*Daphnia magna*), Yamato shrimp (*Caridina japonica*), and Japanese medaka (*Oryzias latipes*) was monitored for 10 months. The carbon sources and trophic levels of the organisms were analyzed by the determination of stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$). Using trophic transfer factor calculations, the bioaccumulation and biomagnification behaviors of Ce in the tested food web were subsequently assessed. The biological hazardous effects were specifically addressed on two tested hydrophytes. This study has provided fundamental information on understanding the transport and fate of CeO₂ NPs in aquatic environment, which helps the enactment of the related environmental management policies to reduce the potential negative impact.

MATERIALS AND METHODS

Reagents. Bare CeO₂ NPs with diameters of around 50 nm were purchased from Sigma (St. Louis, MO). The working suspension (200 mg/mL) was freshly prepared by directly dispersing the NPs (10 g) in 50 mL of the ultrapure water (18.2 M Ω ·cm, Millipore, Billerica, MA). *N*-Benzylidene-*tert*-butylamine-*N*-oxid (Sigma, St. Louis, MO) was used in the radical generation analysis. 37% HCl was bought from Merck (Darmstadt, Germany). All the other chemicals were obtained from Sinopharm Co., Ltd. (Beijing, China).

Experimental Setup and Exposure Design. The simulative aquatic system, including three main components (water, sediment, and biota), was established to compose the experimental microcosm in the glass tanks (40 cm in length $\times$ 20 cm in width $\times$ 25 cm in height). In each tank, about 9 L water and 3 kg of sediment were added, respectively. A total of three replicates was designed for both control and CeO₂ NPs exposure groups. The charcoal-filtered tap water was supplemented every week to keep the constant volume (i.e., 9 L) of the established aquatic system. The temperature ($^{\circ}\text{C}$),

light intensity (lux), and pH of the water system were monitored at around 12 a.m. every week, and the data are shown in Figures S1, S2, and S3, respectively. The light–dark cycle was constantly kept at 16 h/8 h.

These ecosystems were constructed by natural water and sediment collected from the dragon-shaped river of the Olympic Park, Beijing. The basal concentrations of Ce in the sediment (below ng/g) were negligible. The microcosms were allowed to equilibrate for 2 months prior to the start of CeO₂ NP exposure. The species, including water lettuce (*P. stratiotes*), water silk (*S. borealis*), Asian clams (*C. fluminea*), snails (*P. acuta*), and water fleas (*D. magna*), were commonly found in lakes or rivers in China. The Japanese medaka (*O. latipes*) was bred in our lab for generations, and the Yamato shrimp (*C. japonica*) were bought from the local aquarium market. These organisms comprise a food web naturally occurring in Asia.

The microcosm system in exposure group was gently spiked with 50 mL of 200 mg/mL CeO₂ NP solution by stirring water for 10 s for good dispersion. The spiking was performed carefully to ensure the upper surface of the floating water lettuce leaves free from NP solution splash. The exposure lasted for 10 months, and the volume of the water (9 L) and the amount of soil (3 kg) were approximately kept the same during the whole study. All of the samples, including water, sediments, *P. stratiotes*, *S. borealis*, *C. fluminea*, *P. acuta*, *D. magna*, *C. japonica*, and *O. latipes* were harvested when the exposure was terminated. Partial sampling was also performed for two hydrophytes (i.e., *P. stratiotes* and *S. borealis*) after 9 months of exposure for chlorophyll measurement, pathological observation, and hydroxyl radical analysis.

CeO₂ NP and Ce Analysis. A JEOL 2100F microscopy (JEOL, Tokyo, Japan) equipped with Oxford INCA energy dispersive X-ray spectroscopy suite was used to measure the morphology and dimension of the CeO₂ NPs. X-ray photoelectron spectroscopy (XPS) was measured using an X-ray photoelectron spectrometer (Thermo Escalab 250Xi) with a monochromatic X-ray source of Al K α . The spectra were collected at the pass energy of 20 eV in the fixed analyzer transmission mode.

For Ce concentration analysis, the samples, including sediments, *P. stratiotes*, *S. borealis*, *C. fluminea*, *P. acuta*, *D. magna*, *C. japonica*, and *O. latipes* were collected, rinsed with ultrapure water more than five times, and freeze-dried for 4 days, after which they were ground to powder. The digestion protocol for the biota (0.2 g) and water (1 mL) samples were performed using a mixture of HNO₃ and H₂O₂ (2 mL; 3:2, v/v) at 95 $^{\circ}\text{C}$ in 15 mL Teflon tubes for 4 h. The digestion protocol for the sediment samples (0.2 g) was similar except that the acid mixture of HNO₃, H₂O₂, and HF (2 mL; 1:1:1, v/v/v) was used. The resultant solutions were evaporated to about 0.5 mL. The residue solutions were then diluted to 10 mL using ultrapure water. Ce concentrations in the sample solutions were measured on an Agilent 8800 inductively coupled plasma mass spectrometer (ICP-MS).

Trophic Level and Food-Source Descriptors. To determine the food web structure and trophic levels of the organisms tested in the present study, we applied the classic stable-carbon ($\delta^{13}\text{C}$) and stable-nitrogen ($\delta^{15}\text{N}$) isotope methods.^{28–32} The stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopic ratios of all samples were determined at Chinese Academy of Forestry (Beijing, China) using a flash 2000 EA-HT elemental analyzer interfaced with a DELTA V advantage isotope ratio mass spectrometer (Thermo Fisher Scientific Inc.,

Figure 1. (A) Vertical view of the microcosm constructed in the lab. (B) Time-dependent Ce concentration variations in water and sediment of the simulated microcosm after the initial spiking. (C) TEM images of raw CeO_2 NPs (C1) and the NP aggregates in sediment after 10 months of exposure (C2). The enlarged views of CeO_2 NP aggregates were photographed using light field (C3) and HAADF-STEM (C4). (D) The absorption distribution maps of elements O (K-edge absorption, O-K), S (K-edge absorption, S-K), and Ce (L-edge absorption, Ce-L) in CeO_2 NP aggregates and the corresponding STEM-EDX spectrum.

Waltham, MA). The isotope ratio was standardized against atmospheric nitrogen or Pee Dee Belemnite (National Institute of Standards & Technology, Gaithersburg, MD) using $\delta^{13}\text{C}_{\text{sample}}$ or $\delta^{15}\text{N}_{\text{sample}} = (R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}} \times 1000\text{‰}$, where R is the ratio of ^{15}N to ^{14}N or ^{13}C to ^{12}C . Trophic levels (TLs) were determined based on the results of $\delta^{15}\text{N}$ using eq 1:

$$\text{TL}_{\text{consumer}} = 2 + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{plankton}})/\Delta\text{N} \quad (1)$$

where ΔN is the trophic enrichment factor (3.4 ‰). Trophic levels were assigned relative to the zooplankton (*D. magna*), which was assumed to occupy trophic level 2. The trophic magnification factors (TMFs) based on the entire food chain were derived from the slope of the plots of natural log concentrations (lipid normalized) versus TL:

$$\log[\text{concentrations}] = a + b\text{TL} \quad (2)$$

$$\text{TMF} = e^b \quad (3)$$

TMF > 1 indicates that the NPs are biomagnified, whereas TMF < 1 shows that the NPs are metabolized or biodiluted. The lipid content of the samples was normalized by gravimetry. The sample powder was mixed with Na_2SO_4 and eluted with *n*-hexane. The resultant extract was concentrated and evaporated to dryness. The remaining residue (lipid) was weighed.

Bioaccumulation factors (BAFs) were defined as the ratios of the lipid-normalized tissue concentrations of Ce (C_{biota} , μg/kg) to the dissolved concentration in water (C_{water} , μg/L):³³

$$\text{BAF} = \frac{C_{\text{biota}}}{C_{\text{water}}} \quad (4)$$

Chlorophyll Content Analysis. The contents of chlorophyll a, b, and the total chlorophyll of aquatic plants (*S. borealis* and the leaves of *P. stratiotes*) were determined using the classic Arnon assay.³⁴ Generally, 0.1 g leaves of the tested hydrophytes were collected after 9 months of exposure and rinsed using ultrapure water. They were cut into small segments and homogenized in a quartz mortar using 80% acetone as grinding medium. The extracts were filtered and diluted to 25 mL. Chlorophyll contents were finally measured spectrophotometrically and calculated by

$$\text{chlorophyll a (mg/g, FW)} = 1.454A_{665} - 0.296A_{649} \quad (5)$$

$$\text{chlorophyll b (mg/g, FW)} = 2.514A_{649} - 0.648A_{665} \quad (6)$$

$$\text{total chlorophyll (mg/g, FW)} = 0.806A_{665} + 2.215A_{649} \quad (7)$$

Morphological Observation of *S. borealis*. The fresh tissue slices were made for *S. borealis* from both the control and CeO_2 NP exposure groups. The microscopic images were photographed by Olympus BX41 (Japan).

Root Abnormality Detection for *P. stratiotes*. The formation of $\cdot\text{OH}$ was detected by electron spin resonance (ESR) with *N*-benzylidene-*tert*-butylamine-*N*-oxid (PBN) as the spin trapping agent. Generally, 1.0 g root sample was subjected

Table 1. Distribution of Ce in the Simulated Microcosms after 10 Months of Exposure ($n = 3$)

species	mass (g, wet weight)	Ce concentration ($\mu\text{g/kg}$, wet weight)	BAF	percentage of total NPs (%)
<i>O. latipes</i>	0.72 ± 0.10	333 ± 32	3.30 ± 0.41	0.0026 ± 0.0003
<i>C. japonica</i>	2.50 ± 0.17	648 ± 52	6.42 ± 0.34	0.019 ± 0.002
<i>C. fluminea</i>	5.24 ± 0.26	342 ± 49	3.39 ± 0.28	0.022 ± 0.001
<i>P. acuta</i>	3.26 ± 0.52	310 ± 28	3.07 ± 0.15	0.012 ± 0.0007
<i>D. magna</i>	0.74 ± 0.14	322 ± 51	3.19 ± 0.57	0.0029 ± 0.0002
<i>P. stratiotes</i>	10.20 ± 1.82	568 ± 101	5.62 ± 0.45	0.070 ± 0.006
<i>S. borealis</i>	15.52 ± 1.22	1546 ± 142	15.31 ± 1.10	0.29 ± 0.03
water	9033 ± 263	101 ± 12	1.00 ± 0.06	10.9 ± 0.8
sediment	3128 ± 219	2360 ± 104	N/A ^a	88.7 ± 9.5

^aN/A = not applicable.

to 1 mL of 100 mM PBN DMPO solution and allowed to homogenize. The mixture was incubated at 37.0 °C, after which it was centrifuged at 4500 rpm at 4 °C for 3 min. The extraction process was repeated, and the supernatant was utilized for ESR analysis. The spectra were recorded on a Bruker Biospin EMX-plus CW spectrometer (Bruker, Billerica MA) operated at 9.8 GHz.

As for the preparation of TEM observation specimens, the 1 mm³ cubic root samples were fixed overnight in 3% glutaraldehyde solution. The samples were then washed with phosphate buffered saline and transferred to 2% OsO₄ solution for postfixation (2 h). The gradient dehydration was carried out by a series of ethanol with gradient concentrations. The samples were embedded with Spurr resin at 70 °C for 12 h. The resin specimens were sectioned and observed on a Hitachi H-7650 TEM (Hitachi, Tokyo, Japan) operating at 80 kV. CeO₂ NP observation was conducted on a FEI Tecnai G2 F20 TEM (FEI, Hillsboro, OR) by loading NPs on the carbon-coated copper nets directly.

Statistical Analysis. The results are expressed as means $\pm$ standard deviations ($n = 3$). Student's *t* test was performed using Microsoft Excel 2013. Statistical significance was based on a probability of $p \leq 0.05$.

RESULTS AND DISCUSSION

Partition of CeO₂ NPs. All of the CeO₂ NPs were spiked into the simulated microcosm (Figure 1A) at one time. The 10 month exposure of CeO₂ NPs did not significantly affect the water quality, and the living condition of the aquatic system was kept constant, based on the monitoring of the parameters like temperature, light intensity, and pH (Figures S1–S3). The monitoring of Ce concentration in water column showed the NPs suspended in water gradually fell down due to sedimentation (Figure 1B). Correspondingly, Ce concentration in the sediment increased as exposure time prolonged. According to the data depicted in the subgraph of Figure 1B, it cost about 44 and 67 h for the NPs to reach half equilibrium in water (about 500 $\mu\text{g/kg}$) and sediment (about 1200 $\mu\text{g/kg}$), respectively. Approximately, 80% of the NPs settled down to the sediments 1 week after the initial spiking, which was in accordance with previous findings.²⁷ The final partition of CeO₂ NPs between the water and the sediment approached to the constant 30 days post-exposure (Figure 1B). When compared to the settling behavior of gold NPs in water (the equilibrium of sedimentation was reached after 5 h of exposure),¹⁵ the sedimentation of CeO₂ NPs was relatively slow, probably due to the differences in their densities (7.215 g/cm³ for CeO₂ and 19.32 g/cm³ for gold) and surface charges (−2.4 mV for bare CeO₂ NPs and 20–30 mV for gold NPs).

After 10 months of exposure, the distribution of Ce in the constructed microcosm was studied, and the data is shown in Table 1. The mass balance analysis showed total Ce finally recovered from the microcosm was 83.3% of the spiked dose, indicating that no significant loss of Ce happened in the constructed system during long-term exposure. Apparently, sediment is deemed as the most important reservoir for NP sedimentation, in which Ce amount accounted for $88.7 \pm 9.5\%$ of the total recovered NP mass. This is consistent with the sedimentation obtained in previous study.²⁷ Based on the characterization by TEM, CeO₂ NPs in the sediment after 1 day and 10 month exposures formed large aggregates with fuzzy edges (Figures S4 and 1C2–4), which were distinct from the original well-dispersed ones (Figure 1C1). This finding was consistent with what was reported previously, wherein TiO₂, ZnO, CeO₂, and Cu NPs may precipitate and aggregate in water by naturally existing organic matters.^{35,36} When STEM–EDX spectra obtained from the agglomerated CeO₂ NPs in the sludge matrix after 10 months of exposure (Figure 1D) were compared with that obtained after 1 day of exposure (Figure S4C), S signal intensity was found to be substantially elevated after long-term exposure. The close correlation between Ce and S (Figure 1D) in the agglomerated CeO₂ NPs shows the potential mineralization of the NPs by abundant elemental S in the sediment matrix. The consistent distribution of Ce and S provided the solid evidence for the good diffusion network of elemental S infiltrating through the NP aggregates, and the Ce–S bonds were formed in CeO₂ NP aggregates during long-term exposure, which was confirmed by XPS analysis (Figure S5). A similar finding was reported for CeO₂ NPs in the digestive gland of the snail.²⁷ The sulfuration was also observed for some other metallic nanoparticles like copper,³⁷ silver,³⁸ etc. The sulfuration rate of CeO₂ NPs calculated from XPS data was 19.0%, indicating the incomplete sulfuration in the agglomerated CeO₂ NPs. This was further confirmed by the consistent distribution of elemental O with that of S and Ce (Figure 1D).

In view of Ce concentrations in the other tested organisms based on wet weight (Table 1), the order is *S. borealis* > *C. japonica* > *P. stratiotes* > *C. fluminea* > *O. latipes* > *D. magna* > *P. acuta*. The BAF, which is the ratio of the concentration of a chemical in the organism to that in the surrounding environment, accordingly follow the same order. Obviously, *S. borealis* showed the highest bioaccumulation capability for Ce after long-term exposure, and its BAF was 15.31. It is well-known that submerged plants such as algae, have high capabilities to accumulate heavy metals, and they are widely employed in heavy metal removal techniques for water pollution treatment.^{39,40} As one of the typical aquatic submerged plants, *S. borealis* has large specific surface area,

which makes it possible for cell surface sorption and precipitation, and intracellular accumulation of the pollutants in water.^{39,40} This might explain why this species contained the highest level of Ce among the tested organisms in this study. *C. japonica* showed the second highest ability to accumulate aqueous Ce with BAF of 6.42. As we know, shrimp mainly feeds on deposits and the root of *P. stratiotes*. The high concentration of Ce (4.1 mg/kg) in the sediment due to CeO₂ NP settlement may provide the main source for the considerably high Ce accumulation in this species. Similar to *S. borealis*, *P. stratiotes* biomass, as a kind of floating plants in the aquatic system, is capable of biosorbing heavy metals like lead and chromium.⁴¹ The relatively high Ce concentration in *P. stratiotes* (568 µg/kg) confirmed that this plant had efficient metal removal ability in the aquatic environment. *C. fluminea*, as a filter feeder, is commonly believed to be able to take up and accumulate more pollutants than do other animals at the similar taxonomic hierarchy.¹⁵ Nevertheless, the result in Table 1 shows that Ce concentration in this species (342 µg/kg) is not very high, potentially indicating that it may have high metabolism capability for Ce elimination. Similar to *C. fluminea*, the other tested species including *O. latipes*, *D. magna*, and *P. acuta* contained relatively low Ce concentrations with BAF in the range of 3.07–3.30, showing their relatively low ability for NP bioaccumulation in water system.

Trophic Dilution of CeO₂ NPs. The isotope ratios of ¹⁵N-to-¹⁴N or ¹³C-to-¹²C provide information on the structure of food web (i.e., trophic levels, TLs) and diet sources. The prey sources are commonly traced by stable carbon and nitrogen isotope values in many studies.^{28,30,42} NP distribution-related parameters, like TMF, can thereby be estimated. As shown in Figure 2A, $\delta^{13}\text{C}$ values of biota ranged from −34.60‰ (*P. stratiotes*) to −13.69‰ (*P. acuta*) in the microcosm constructed in this study. Apparently, most of the biota species are distributed in the vicinity of mean value of $\delta^{13}\text{C}$ (i.e., the vertical dashed line in Figure 2A, −22.5‰) with the exception of *P. stratiotes* and *P. acuta*, suggesting they may have diverse diets. *P. stratiotes*, as a submerged plant, captures CO₂-originated carbon in the air, which endues this species with the lowest $\delta^{13}\text{C}$ value of −34.60‰. The benthic organism *C. fluminea* possesses $\delta^{13}\text{C}$ value of −17.54‰, which is close to that of the sediment (−15.71‰). This may be explained by the fact that *C. fluminea* assimilates foods from sediments. As for the other organisms, their nutrition is mainly from the water phase. It should be noted that *C. japonica* has the second lowest $\delta^{13}\text{C}$ value of −27.22‰ among all the tested biota because this species feed on hydrophytes. The analysis of N isotope showed relatively high $\delta^{15}\text{N}$ in *C. fluminea* (Figure 2A), which was contributed by the sediment with high $\delta^{15}\text{N}$ in the simulated microcosm, as *C. fluminea* fed on sediment. $\delta^{15}\text{N}$ values of the other organisms were reasonably distributed because their food were commonly from the water phase. Thus, *C. fluminea* was excluded in the TL calculation in this study. The food chain and the TLs of the other biota in this constructed ecosystem are depicted in Figure S6, showing the relatively simple and clear trophic relationships between different biota species.

TMF is commonly used to evaluate whether the chemical concentration increases along the food chain, and a TMF statistically greater than 1 is generally recognized as the occurrence of biomagnification.^{43,44} Based on the regression relationship between the lipid-normalized Ce concentrations in the biota samples and their trophic levels, TMF was estimated for Ce biomagnification in the simulated microcosm. The result

Figure 2. (A) Relationship between stable isotope ratio $\delta^{15}\text{N}$ and C/N adjusted $\delta^{13}\text{C}$ values of samples collected from the microcosms. Each dot stands for the mean value of three samples from one tank. The vertical dashed line represents the mean value of $\delta^{13}\text{C}$ for all seven tested species (−22.5‰). (B) Relationship between Ce concentration normalized by lipid content and the trophic level of each species. Each dot stands for the mean value of three samples from one tank. Green dashed lines stand for 95% confidence intervals. r is the correlation coefficient, and TMF is the biomagnification factor.

in Figure 2B shows that the TMF value for Ce is 0.5786, manifesting that CeO₂ NPs cannot be biomagnified in this freshwater food chain. This result is different from the previous finding that CeO₂ NPs were obviously biomagnified in terrestrial food chains.¹¹ In contrast to the limited information on biomagnification of nanomaterials in diverse food chains, TMF has been widely discussed in view of persistent organic pollutants (POPs). For example, both DDE and 2,2-bis-(chlorophenyl)-1-chloroethylene (DDMU) were biomagnified with TMF values of 3.26 and 3.70, respectively, in a marine aquatic food web from Bohai Bay, Northeast China, while 4-nonylphenol (4-NP) was biodiluted in the same food web.²⁹ Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs and DFs) were found to be trophically diluted in the aquatic food chains of Bohai Bay³¹ but biomagnified in those of Tokyo Bay⁴⁵ and Northern Baltic.³² β -Hexachlorocyclohexanes (β -HCHs) showed no biomagnification in piscivorous food web; however, they exhibited high biomagnification in terrestrial food web and marine mammalian food webs.⁴⁶ These findings

implied the complexity in the biomagnifications of POPs due to their versatile chemical and physical characteristics, as well as diverse food web structures. Accordingly, the biomagnification behavior of nanomaterials could be complicated and vary specifically with multiple factors from the food web and nanomaterial per se.

Abnormalities in Hydrophytes Due to Long-Term CeO₂ NP Exposure. Visual examination of the microcosms throughout 10 months of exposure did not show any obvious symptoms of adverse effects or abnormalities in the species including *C. japonica*, *C. fluminea*, *O. latipes*, *D. magna*, and *P. acuta*. In contrast to the aquatic animals tested in this study, long-term exposure of CeO₂ NPs did cause obvious changes in the appearance of the hydrophytes, i.e., *S. borealis* and *P. stratiotes*. As indicated in Figures 3A and 4A, the color of these

Figure 3. (A) The representative pictures of *S. borealis* in the control (left) and CeO₂ NP exposure groups (right). Light-microscopic images of *S. borealis* from the control (B) and CeO₂ NP exposure groups (C), respectively (400 $\times$). The ball-like organelles coupled with ribbon-like chlorophylls are pyrenoids. Samples were collected after 9 months of exposure.

two hydrophytes turned yellowish-green in the exposure groups. It is well-known that the leaf color of plants is directly related with the chlorophyll contents therein. The determination of both chlorophyll a and b showed that 9 month CeO₂ NP exposure caused significant decrease in chlorophyll concentrations of *S. borealis* and the leaves from *P. stratiotes* (Table 2), which was different from necrotic lesions found in nanomaterial-exposed tobacco (*Nicotiana xanthi*).⁴⁷ Although this chlorotic disorder in both plants visually appeared after about 90 days of exposure, the quantitative analysis was only performed right before the termination of the exposure experiments to avoid introducing the possible disturbance to the simulated microcosm in the early stage. Quantitatively, CeO₂ NP-induced losses of total chlorophyll were 52.9% in *S. borealis* and 41.3% in *P. stratiotes*, respectively. Chlorophyll a, as the principal photosynthetic pigment, is essential for oxygenic photosynthesis.^{48,49} It reflects green and yellow light, thus contributing the observed green color of most plants. Chlorophyll b helps in photosynthesis by absorbing light energy. It primarily absorbs blue light, thus showing yellow. Reduction in both chlorophyll a and b means low efficiency in

capturing light energy and transferring it to the reaction center of the cell, which may eventually impede sugar synthesis.⁵⁰ As we know, the chlorophyll a and b pigments adsorb at different spectra. If the plants dominantly contain chlorophyll a, the increment of chlorophyll b enhances the light absorption efficiency for energy production.⁵⁰ Adjustment of the chlorophyll a-to-b ratio was reported to be an integral feature of acclimation to high-light conditions and low N availability.⁵¹ Nevertheless, the ratio of chlorophyll a to b approximately remained unchanged upon CeO₂ NP treatment, manifesting such that no adjustment between photosystem I and photosystem II was stimulated in chloroplasts to compensate for the decreased photosynthesis efficiency due to the loss in chlorophyll contents. Similar findings were reported in the leaves of wheat and rice treated with CeO₂ NPs.⁵² The mechanisms were likely to be associated with the agglomeration as well as disturbance in chloroplast structures and the disorganization of thylakoid in leaf mesophyll cells.^{52,53} In contrast to CeO₂ NPs, another metallic oxide nanomaterial, TiO₂ NP, which has many chemical and physical properties alike, was reported to exert the opposite effects. Chlorophyll content in cucumber leaves was increased by almost 50% when the plant was treated with 750 $\mu\text{g/mL}$ TiO₂ NPs.⁵⁴ The increment of the chlorophyll formation and photosynthesis in spinach and green alga were also reported upon TiO₂ NP treatments.⁵⁵ The biological effects on chlorophyll content may rely on the plant species and the specific NPs used. The underlying mechanism for metallic oxide NP-induced phytochrome alterations still need to be further revealed and interpreted.

Microscopic observation of *S. borealis* showed that in the control group, the pyrenoids were abundant and orderly arranged in the filament. Their structures were clear and intact (Figure 3B). Comparatively, the morphology of pyrenoids in CeO₂ NP-treated groups were apparently damaged based on microscopic observation, and the structures were fuzzy and disorderly (Figure 3C). Pyrenoids are subcellular microcompartments in chloroplasts of many algae and associated with the operation of a carbon-concentrating mechanism.⁵⁶ Their main function is to act as center of carbon dioxide (CO₂) fixation by generating and maintaining a CO₂-rich environment to avoid the limitation of algal photosynthesis due to the lack of CO₂ availability.⁵⁷ The morphological changes in pyrenoids could definitely compromise the biological function in photosynthesis of this alga. The changes in protein contents both on the surface and inside the matrix of pyrenoids were also reported in the study of wheat treated with TiO₂ NPs, where higher grain protein contents were found in wheat with the treatments of 100 and 400 mg/kg NPs.⁵³ The apparent morphological defects observed in *S. borealis* could be related with the high burden of Ce in this species.

Aside from chlorophyll loss, another intriguing phenotype for CeO₂ NP-treated *P. stratiotes* was root elongation. This change became visually apparent after 3 months of exposure. As indicated in Figure 4A, the average initial root lengths from both groups were similar on Day 0 (about 6 cm). After 9 months of exposure of CeO₂ NPs, the plant roots significantly increased to 17.9 ± 2.5 cm, while the control kept unchanged. Apparently, CeO₂ NP exposure promoted the root growth of *P. stratiotes*. According to the distribution of Ce in the plants, relatively higher Ce concentration was found in the root (839 ± 234 $\mu\text{g/kg}$) than that in the leaves (296 ± 65 $\mu\text{g/kg}$) due to the direct exposure of the root in NP suspension. The increased

Figure 4. (A) Morphological changes in *P. stratiotes* after 9 months of exposure of CeO₂ NPs. The inset panel is the root length comparison between the control and CeO₂ NP exposure groups. (B) ESR spectra of *P. stratiotes* in the control (upper) and CeO₂ NP exposure (lower) groups. (C) The TEM images for the outer cell wall of *P. stratiotes* roots from control (left) and CeO₂ NP exposure (middle) groups. The cell wall width of *P. stratiotes* was significantly decreased by CeO₂ NP exposure (right panel; **, $p < 0.05$).

Table 2. Chlorophyll Concentrations in *S. borealis* and the Leaves of *P. stratiotes* after 9 Months of Exposure of CeO₂ NPs ($n = 3$)

	chlorophyll a ($\mu\text{g/g}$, FW)		chlorophyll b ($\mu\text{g/g}$, FW)		total chlorophyll ($\mu\text{g/g}$, FW)		chlorophyll a/b ratio	
	control	CeO ₂ NPs	control	CeO ₂ NPs	control	CeO ₂ NPs	control	CeO ₂ NPs
<i>P. stratiotes</i>	224 $\pm$ 13	131 $\pm$ 21 ^a	0.631 $\pm$ 0.62	0.411 $\pm$ 0.34 ^a	225 $\pm$ 21	132 $\pm$ 20 ^a	355 $\pm$ 17	319 $\pm$ 16 ^a
<i>S. borealis</i>	562 $\pm$ 17	277 $\pm$ 47 ^a	343 $\pm$ 8	149 $\pm$ 11 ^a	905 $\pm$ 26	426 $\pm$ 33 ^a	1.64 $\pm$ 0.06	1.86 $\pm$ 0.12 ^a

^aThe differences in chlorophyll concentrations between the control and CeO₂ NP exposure groups were all significant for both hydrophytes ($p \leq 0.05$).

root growth could be related with Ce exposure, as this element was also reported to significantly increase the lengths of primary roots in *Arabidopsis thaliana*.⁵⁸

Oxidative stress in the root was studied to clarify the CeO₂ NP exposure-induced effects on the root growth of *P. stratiotes*. Figure 4B shows that hydroxyl radical ($\cdot\text{OH}$) levels in CeO₂ NP-treated group (lower) were significantly higher than those in the control (upper). It was reported that the generation of $\cdot\text{OH}$ radicals could induce cell-wall loosening because $\cdot\text{OH}$ radicals could cleave polysaccharides, a main component of cell walls.⁵⁹ Loosening of the cell wall without destroying microfibrils hoops could reduce root cell-wall thickness (Figure 4C), promoting endocytosis and attenuating exocytosis.^{60,61} Therefore, CeO₂ NP exposure-caused root elongation was mediated by the generation of $\cdot\text{OH}$ radicals and cell-wall loosening in the roots. Similar findings were observed in which the exposure of CeO₂ NPs (less than 0.5 mg/mL) caused significant root elongation, and 0.5 mg/mL CeO₂ NP exposure

particularly increased root biomass by 63% in *Arabidopsis thaliana*.⁶² In a separate study performed by Gardea-Torresdey et al., the elongation effect exhibited in a species-dependent manner. That was, CeO₂ NPs (1 mg/mL) greatly promoted the root elongation of alfalfa but significantly decreased that of tomato.⁶³ Nevertheless, some other metal ions, such as Cd²⁺ and Al³⁺, were reported to significantly cause the inhibition of root elongation accompanied by the induction of oxidative stress and lipid peroxidation.^{64,65} Accordingly, the influences on root elongation might vary with multiple factors, like plant species, metal species, speciation etc.

Environmental Implications. Unlike toxicity studies, which have been widely performed, the environmental behavior and fate of NPs are still in their infancy. Studies on the interaction between this kind of emerging chemicals and environmental organisms of interest are confoundedly needed, and reliable experiments based on the simulated microcosms to test the status of nanomaterials in the environment remain

challenging. In this study, we constructed a fresh water ecosystem and studied the distribution, bioaccumulation, biomagnification, and impacts of CeO₂ NPs via long-term exposure. The results demonstrated that Ce was absorbed and accumulated by the tested biota and biodiluted in the constructed food web, as manifested by negative relationship between trophic levels and lipid-normalized Ce concentrations. CeO₂ NPs induced obvious morphological abnormalities in aquatic hydrophytes due to their unique chemical or physical properties, potentially causing the irreversible disturbance in the sustainability of the aquatic system. The findings provided useful information on the potential risks from the unintentional exposure of NPs in aquatic system. Comprehensive researches regarding the chemical and physical changes of NPs, like redox and aggregation in their full life cycles, are still necessary for the reasonable evaluation of benefits and risks from their application.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b05875.

Figures showing temperature, light intensity, and pH variations of the ecosystem; TEM images of CeO₂ NPs in sediment; EDS analysis; fitting curves; and a trophic relationship description among the biota. (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone and fax: +86-10-62849334; e-mail: zhouqf@rcees.ac.cn.

ORCID

Qunfang Zhou: 0000-0003-2521-100X

Notes

The authors declare no competing financial interest.

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