Environmental Pollution 212 (2016) 584–591

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Fate of engineered cerium oxide nanoparticles in an aquatic environment and their toxicity toward 14 ciliated protist species☆

Wei Zhang [a, b, c], Zhichao Pu [c], Songyan Du [b], Yongsheng Chen [b, *], Lin Jiang [c, **]

[a] State Environmental Protection Key Laboratory of Environmental Risk Assessment and Control on Chemical Process, School of Resource and Environmental Engineering, East China University of Science and Technology, Shanghai 200237, China
[b] School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta 30332, USA
[c] School of Biology, Georgia Institute of Technology, Atlanta 30332, USA

ARTICLE INFO

Article history:
Received 22 December 2015
Received in revised form
29 February 2016
Accepted 3 March 2016
Available online xxx

Keywords:
Acute toxicity
CeO_2 NPs
Ciliated protist
Environmental fate
Phylogenetic conservatism

ABSTRACT

The potential environmental impacts of engineered cerium oxide nanoparticles (CeO_2 NPs) on aquatic organisms have remained largely unknown. Therefore, the laboratory study featured herein was performed to determine the fate of CeO_2 NPs in an aquatic environment and their toxicity towards 14 different ciliated protist species at a specified population level. An investigation of 48 h aggregation kinetics in the Dryl's solution showed the CeO_2 NPs to be relatively stable. The pH values in three test medium were too far away from PZC, which explained the stability of CeO_2 NPs. CeO_2 NPs generally elicited more toxicity with increasing NP concentration, following certain dose-response relationships. Nano-CeO_2 resulted in greater toxicity in a particle state than when added as bulk material. LC_{50} values showed a negative correlation with the surface-to-volume ratio for these protists, suggesting that surface adsorption of CeO_2 NPs might contribute to the observed toxicity. Additionally, acute toxic responses of 14 ciliated protist species to CeO_2 NPs were not significantly phylogenetically conserved. The results of these observations provide a better insight into the potential risks of CeO_2 NPs in an aquatic environment.

1. Introduction

Nanotechnology utilizes nanoparticles (NPs) that are unique not only due to their minute size (smaller than 100 nm) but also because of their size-dependent characteristics. NPs are being spotlighted as a center stage phenomenon for future industry (Arnaldi, 2014). The production and number of applications of engineered NPs are increasing rapidly worldwide. Current applications include the use of NPs in consumer products, construction materials, medical and pharmaceutical industries, agriculture, and information technology (Karen et al., 2009). Metal oxide NPs are an important category of manufactured NPs, accounting for about one-third of the consumer products nanotechnology market. For instance, cerium oxide (CeO_2) NPs are increasingly used as a catalyst in the automotive industry (Zhao et al., 2012; Zhang et al., 2014). Consequently, CeO_2 NPs are expected to enter environmental water samples via waste streams from industries that synthesize or use CeO_2 (Hu et al., 2012; Auffan et al., 2013). CeO_2 NPs are on the Organization for Economic Co-operation and Development (OECD) list of priority nanomaterials for immediate testing and it is imperative to perform the risk assessment of their potential eco-toxicological effects (OECD, 2010a b). To date, the studies on the CeO_2 NPs toxicity conducted with aquatic organisms are rather limited and their environmental fate and impacts have remained largely unknown.

NP dispersion in aqueous media for toxicity studies remains a challenge because of the tendency for NPs to aggregate. Aggregation will cause the decrease of exposed surface area (Dhawan et al., 2009), changing oxidative damage induced by the self-quench of reactive oxygen species (Hotze et al., 2010). Previous studies have shown that the problems are exacerbated in culture media due to the presence of salts and other compounds (Fubini et al., 2010). Solution pH is an essential factor governing NP aggregation. Aggregation can be prevented by adjusting the solution pH away from

☆ This paper has been recommended for acceptance by Sarah Michele Harmon.

* Corresponding author. School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta 30332, USA.
** Corresponding author. School of Biology, Georgia Institute of Technology, Atlanta 30332, USA.
E-mail addresses: yongsheng.chen@ce.gatech.edu (Y. Chen), lin.jiang@biology.gatech.edu (L. Jiang).

http://dx.doi.org/10.1016/j.envpol.2016.03.011
0269-7491/

the NP point of zero charge (PZC), thereby conferring a net negative or positive charge to cerium oxide surfaces. This results in an increased interparticle repulsion, decreasing the rate of aggregation (Hotze et al., 2010). The extent to which aggregation of CeO_2 NPs in different types of solutions influences NP dispersion needs to be addressed before conducting the toxicity study. Additionally, CeO_2 NPs dissolution could play an important role in toxicity evaluation. During the incubation the core metal Ce could be released by oxidative and photolytic conditions from the NPs, which might be a key factor in determining its toxicity to aquatic organisms (Dahle et al., 2015; Zhao et al., 2012).

Previous tests on environmental toxicity of NPs in freshwater usually focused on conventional model organisms such as bacteria, algae (single-celled with cell walls), Daphnia (multicellular organisms), and zebrafish (Zhang et al., 2012). Additionally, the factors (and related underlying mechanism) that influence environmental toxicity among different species remain largely unknown. A variety of other organisms in the aquatic environment are important to maintain the balance of ecological systems, and the toxicity of NPs against these organisms has not been extensively investigated.

Freshwater protist species provide a good model for study on how environmental toxicity differs among species because of their ubiquitous global distribution and special sensitivity to environmental contaminants. Protozoa are single cell organisms that do not possess a protective cell wall. As a consequence, NPs could enter protozoan cells more easily than bacterial and algal cells and interact directly with the cellular structures and organelles (Mortimer et al., 2014; Zou et al., 2013). The ciliated protists have been widely studied by eco-toxicologists not only because of their role in the regulation of microbial populations through the ingestion and digestion of bacteria but also because of their high sensitivity to chemical materials; hence, they are often used as indicator species of environmental pollution (Bick, 1972).

Species phylogenetic histories often place a constraint on their trait evolution, such that more closely related species tend to exhibit more similar traits, a phenomenon termed phylogenetic trait conservatism (Harvey and Pagel, 1991; Bello et al., 2009). On the other hand, convergent evolution could result in distantly related species that are similar in their traits; this could serve to decouple the linkage between species phylogenetic relatedness and trait similarity (Losos et al., 2003).

The objectives of this research were 1) to assess the environmental fate of CeO_2 NPs and their impacts on 14 freshwater ciliated protist species by conducting acute toxicity tests 2) to further evaluate whether acute toxicological responses of these ciliated protist species to CeO_2 NPs are phylogenetically conserved. Towards these goals, the fate of CeO_2 NPs in test media was first determined via the kinetic study of hydrodynamic size and zeta potentials. LC_{50} values of CeO_2 NPs derived from acute toxicity tests for 14 different protist species were then estimated, and the phylogenetic signal of LC_{50} for each tested protist species was analyzed. Our findings establish a useful scientific basis for CeO_2 NPs ecological risk assessment.

2. Materials and methods

2.1. Chemicals

Aqueous cerium (IV) oxide NPs and bulk were purchased from Sigma-Aldrich (St. Louis, MO, USA), and CeO_2 NPs stock suspension was made with the concentration of 100 g/L. All the solvents used in the experiments were of analytical grade.

2.2. Ciliated protozoans

A total of 14 common ciliated protist species in an aquatic environment were used in the experiment. These species include *Blepharisma americanum* (*B. americanum*), *Colpidium kleini* (*Colpidium kleini*), *Colpidium striatum* (*Colpidium striatum*), *Glaucoma scintillans* (*G. scintillans*), *Loxocephalus* sp. (*L. sp.*), *Paramecium aurelia* (*P. aurelia*), *Paramecium bursaria* (*P. bursaria*), *Paramecium multimicronucleatum* (*P. multimicronucleatu*), *Paramecium tetraurelia* (*P. tetraurelia*), *Spirostomum teres* (*S. teres*), *Spirostomum ambiguum* (*S. ambiguum*), *Tetrahymena pyriformis* (*Tetrahymena pyriformis*), *Tetrahymena thermophila* (*Tetrahymena thermophila*), and *Tetrahymena vorax* (*Tetrahymena vorax*). These species were isolated from local ponds or obtained from the Carolina Biological Supply Company (Burlington, NC, USA).

2.3. Dryl's solution

The Dryl's solution was prepared and autoclaved according to the following recipe: 1 mM NaH_2PO_4-monobasic (Fisher Biotech, Pittsburgh, PA), 1 mM Na_2HPO_4-dibasic (Fisher Chemical, Pittsburgh, PA), 2 mM trisodium citrate dehydrate (Fisher Scientific, Pittsburgh, PA), and 1.5 mM $CaCl_2$ (Fisher Scientific, Pittsburgh, PA). The Dryl's solution was autoclaved before use, with $CaCl_2$ solution autoclaved separately.

2.4. Protozoan pellet medium (PPM)

The growth medium was made by dissolving protozoan pellets (concentration: 0.55 g pellet/L) in deionized (DI) water. To provide bacterial food for the protists, we inoculated three bacterial species (*Bacillus cereus*, *Bacillus subtillis*, and *Serratia marcescens*) into the autoclave-sterilized medium. After 24 h of bacterial incubation, we dispensed the medium into individual microcosms. Each microcosm also received two autoclaved wheat seeds that provided additional carbon. All the microcosms and stock cultures were stored in an incubator maintained at room temperature (22 °C).

All 14 protozoans are filter feeders, which feed on bacteria and other small particles. These ciliates are small to medium sized, with generation times ranging from a few hours to no more than two days. These species were separately cultivated in stock cultures and these cultures were periodically renewed by a spot of solution transfer. When inoculating the protists into microcosms, we always used 2-week-old stock cultures to minimize the differences in the physiological conditions of species.

2.5. Particle size distribution (PSD) and zeta potential determination

PSD was determined on a Zetasizer Nano ZS instrument (Malvern Instruments, Malvern, UK) by filling 1.5 mL CeO_2 NPs solution into a clean cuvette. The light scattering detector was positioned at a scattering angle of 173° from the incident laser beam, and the autocorrelation function automatically accumulated for at least 10 runs for each sample.

Zeta potential was also measured by a Zetasizer Nano ZS instrument. Four measurements were repeated for each test condition.

2.6. Aggregation kinetics test

To determine the stability of spiked CeO_2 NPs in the Dryl's solution, which was used for the set-up of the short-term toxicity test, two different concentrations (10 and 1000 mg/L) of CeO_2 NPs were prepared by adding corresponding amounts of CeO_2 stock solution