Ecotoxicology and Environmental Safety 84 (2012) 70–77

Contents lists available at SciVerse ScienceDirect

Ecotoxicology and Environmental Safety

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

A temporal study on fate of Al_2O_3 nanoparticles in a fresh water microcosm at environmentally relevant low concentrations

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ARTICLE INFO

Article history:
Received 3 May 2012
Received in revised form
20 June 2012
Accepted 21 June 2012
Available online 21 July 2012

Keywords:
Microcosm
Aluminum oxide
Nanoparticle
Environmental concentration
Long term exposure
Ionic effect

ABSTRACT

This study on a microcosm, brings out the temporal changes in physico–chemical behavior of aluminum oxide nanoparticles (for a period of 210 days), at environmentally relevant concentrations (1000 μg/L and below). The dynamics of particle behavior in terms of mean hydrodynamic diameter, specific surface area and dissolution of soluble aluminum and, their possible ecological implications have been presented in this study. A thorough statistical analysis brings out nanoparticle behavior, where a rapid aggregation of particles (79 ± 13 nm at 0 h to 1464 ± 80 nm at 48 h), with a decrease in specific surface area (32 m^2/g at 0 h to 1.7 m^2/g at 48 h) was observed. Ion release profile indicated a significant increase in soluble aluminum concentration only after 36 h (277 ± 15 μg/L at 0 h to 462 ± 3 μg/L at 36 h) which reduced over a period of 60 days (279 ± 20 μg/L). A differential response at 1000 μg/L concentration was observed, short term exposure (5 days) showed an immediate effect on the resident algal population (~25% decreased viability) and the long term (7 months/210 days) exposure showed a gradual recovery. Thus, nanomaterials may not have the stipulated toxic response, at low concentration and longer standing period, presumably owing to the complexity of the natural systems.

1. Introduction

Recently there has been a huge increase in the production and use of engineered nanomaterials (ENM) (Darlington et al., 2009; Dunphy et al., 2006; Elsaesser and Howard, 2011). These EMNs are being widely used in sporting goods, tires, stain-resistant clothing, cosmetics, and electronics industry (Nel et al., 2006). Al_2O_3 nanoparticles are used in a number of applications, including energetic, alloys, explosive, coatings and sensors (Darlington et al., 2009; Schrand et al., 2010; Jiang et al., 2009). Because of their widespread use, it is inevitable that ENPs will find their way into the environments where their possible toxicological behavior are till now not understood properly (Nowack and Bucheli, 2007; Navarro et al., 2008; Elsaesser and Howard, 2011).

Nanomaterials behavior largely influenced by particle size, shape, surface and core chemistry, agglomeration state, crystallinity, redox potential, purity, catalytic activity, surface charge, and porosity (Elsaesser and Howard, 2011; Nel et al., 2006). The natural variation in environmental condition leads to changes in the property of nanomaterials. Thus to determine the risk associated with release of nanoparticles one must clearly define the changes in property of nanomaterials with time once they interact with environment (Navarro et al., 2008).

Scientific literatures have confirmed the difference in toxicity of nano- and micro-sized nanoparticles (Kahru and Dubourguier, 2010). A laboratory based study on resident algal population (*Scenedesmus* sp. and *Chlorella* sp.) recorded bulk alumina to be less toxic than nano-sized alumina (Sadiq et al., 2011). The toxicity of oxide nanoparticle has also been reported for mammalian cell lines (Brunner et al., 2006) and plants (Lin and Xing, 2008). Yang and Watts (2005) proposed that algal growth is related to the Al_2O_3 nanoparticles size rather than their chemical composition. Most scientific literature suggest the large surface area per given mass as main factor for the increased toxicity of nanoparticles as compared to bulk (micro-sized) particles (Karakoti et al., 2006). However, composition and phase of the material is still considered to be determining factor than size or surface area (Sayes et al., 2006; Gojova et al., 2007).

According to the model proposed by Gottschalk et al. (2009) the environmental concentrations of nanomaterials (TiO_2, ZnO, Ag, CNT and fullerenes) was suggested to be less than 1 μg/mL. Hence, to evaluate the environmental behavior of these nanoparticles, it is necessary to study their behavior at a low concentration to analyze the impact (if any). This influenced us to assess the fate and behavior of Al_2O_3 nanoparticle at environmental concentrations of 0.05 μg/mL–1 μg/mL.

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0147-6513/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ecoenv.2012.06.015

In spite of huge research on toxicity of nanoparticles for years, till now the risk and the ultimate fate of nanoparticles in natural environment are largely undetermined (Elsaesser and Howard, 2011). To date very few literature records could be found for long term study of the variation in nanomaterial property in natural environment. Short term studies have been done on several photo-catalytic nanoparticles but no record could be found for Al_2O_3 nanoparticles. Huang et al. (2005) studied the short-term toxicity of photo-catalytic TiO_2 nanoparticles on three aquatic organisms: bacteria, algae, and zooplankton. Most of the ecotoxicity study of nanomaterials has been done at laboratory set up and till date there is no comprehensive report on physico–chemical behavior influencing ecotoxicity of Al_2O_3 nanoparticles or other nanomaterials also in aquatic microcosms left in natural environment, without external interferences.

The incorporation of the complex microcosm system helps in predicting both the direct nanoparticle toxicity as well as indirect toxicity derived from physical restraints as ENPs. To study the nanotoxicity effect, organisms which interacts strongly with the immediate environment becomes very important. According to Kahru and Dubourguier, algae are one of the most sensitive environmentally relevant species for study of synthetic nanoparticles. In aquatic ecosystem algae plays an important role by acting as the first level of the trophic chain producing biomass and thus balancing the whole food web (Ji et al., 2011; Sadiq et al., 2011). Hence they can truly depict the effect of toxicity of nanoparticles on the whole aquatic food web as well as ecosystem. In addition their sensitivity to toxicants, ready availability, and fast growth rates makes them good test models for toxicity evaluation.

To the best of our knowledge the current study is first of its kind dealing with analysis of particle behavior in terms of aggregation, changes in specific surface area and ion release in a microcosm system under natural environmental conditions over a period of 210 days (7 months). This study provides an overview of physico–chemical behavior of nanoparticles at low concentrations (1000 μg/L and less), over the test period in terms of hydrodynamic diameter, specific surface area and release of soluble aluminum and a brief report on impact and response of biological flora and their implications on the ecosystem.

2. Materials and methods

2.1. Site details

The study was performed at a natural water lake at VIT, Vellore, India. Vellore is located between 12–15° and 13–15° Northern Latitude and between 78–20° and 79–50° Eastern Longitude. It has an average elevation of 216 m. The area has a typical Mediterranean climate with mild winter and dry summer. Temperature ranges from as low as 15 °C in the winter months of December–February to even 43 °C in the summer months of April–June. It is essentially a dry climate, rainy and humid only at the times of the two monsoons: June–August and October–December. The average annual rainfall ranges from 950 to 1550 mm.

2.2. Establishment of microcosm

The experiment was carried out in 10 L capacity tanks, in which the lake water ecosystem was replicated. The principal consideration was to create an isolated experimental set up with smaller working volume which can be manipulated with ease and will not contaminate the natural environment. The tanks were placed under normal weathering conditions in a garden adjacent to our laboratory to facilitate continuous monitoring, with no control over rainfall, temperature and exposure to sunlight (Fig. 1).

The soil and the water were collected from the lake and left to acclimatize. Biological oxygen demand (BOD) and pH, total dissolved solids content (TDS) monitored at every seven days to ensure true replication of a representative lake water ecosystem. After 6 weeks acclimatization, the microcosm was found to be comparable to the lake water ecosystem where BOD was found to be 3600 ± 80 μg/L, pH of 7.8 ± 0.9 and TDS was 1380 ± 40 μg/L.

Fig. 1. Photograph showing microcosm setup in the garden denoting the experimental conditions under natural conditions.

After establishment, Al_2O_3 nanoparticles were introduced to the system to maintain a final exposure concentration of 50 μg/L, 500 μg/L and 1000 μg/L. Tanks were replenished with required volume of lake water to compensate evaporation on alternate days.

2.3. Preliminary characterization of as received nanoparticle

Dry alumina nanoparticles were procured from Aldrich (St. Louis, Missouri; CAS Number 1344-28-1). The supplier's data can be summarized as follows: gamma phase alumina nanopowder, particle size < 50 nm, surface area 35–43 m^2/g.

2.3.1. X-ray diffraction analysis

The crystalline properties of the Al_2O_3 nanoparticles were characterized by powdered XRD (D8 Advanced X-ray Diffractometer, Burker, Germany) scan with 2.2 kW Cu anode radiations at wavelength 1.54 Å produced by a Ceramic X-ray tube. The scanning was done in 10°–100° range. 250 mg of dry powdered Al_2O_3 nanoparticle was used for the analysis. The crystalline nature was determined from the diffraction pattern.

2.3.2. Transmission electron microscopic analysis

Size and shape of the Al_2O_3 nanoparticles were characterized by transmission electron microscopy. Al_2O_3 nanoparticles were subjected to ultrasonication for 15 min with a frequency of 20 kHz and output energy of 130 W [ultrasonic processor, Sonics Corp., USA] in Millipore water. Aliquots of nanoparticles dispersion were placed on the thin glass slides and dried in hot air oven at 60 °C for 30 min; the slides were subjected to transmission electron microscopic analysis (FEI Sirion, Eindhoven, Netherlands).

2.3.3. Dynamic light scattering analysis

A stock dispersion of 10,000 μg/L was prepared using dry Al_2O_3 nanoparticles in Millipore filtered water and subsequent sonication using an ultrasonic processor (Sonics, USA). From the stock dispersion, a working dispersion of 1000 μg/L was prepared in Millipore filtered as well as in filtered lake water. For both of the dispersions, the hydrodynamic size distribution and mean hydrodynamic diameter of the particles or particle agglomerates were determined through dynamic light scattering using 90 Plus Particle Size Analyzer with zeta option (Brookhaven Instruments Corp, USA).

2.4. Aggregation of particles in lake water

Required amounts of aliquots from a stock dispersion of 10,000 μg/L were added to the test systems to obtain an exposure dose of 50 μg/L, 500 μg/L and 1000 μg/L. About 20 mL of sample was collected and filtered serially through a series of coarse to fine pored filters (20 μm mesh–Whatman no.1 filter paper–Whatman no. 42 filter paper) and were subjected to particle size analysis through dynamic light scattering at 6 h interval for a period of 48 h. Specific surface area corresponding to the mean hydrodynamic diameter at each time interval was calculated from Eq. (1) assuming spherical shape of particles. Where, d is mean diameter of the particle, ρ is density of Al_2O_3.

$$SSA = \frac{\pi \times d_m^2}{\rho \times (\pi \times d_m^3/6)}. \quad (1)$$