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RESEARCH ARTICLE

# Toxicity, uptake, and accumulation of nano and bulk cerium oxide particles in *Artemia salina*

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**Abstract** Although the toxicological impact of metal oxide nanoparticles has been studied for the last few decades on aquatic organisms, the exact mechanism of action is still unclear. The fate, behavior, and biological activity of nanoparticles are dependent on physicochemical factors like size, shape, surface area, and stability in the medium. This study deals with the effect of nano and bulk $CeO_2$ particles on marine microcrustacean, *Artemia salina*. The primary size was found to be $15 \pm 3.5$ and $582 \pm 50$ nm for nano and bulk $CeO_2$ (TEM), respectively. The colloidal stability and sedimentation assays showed rapid aggregation of bulk particles in seawater. Both the sizes of $CeO_2$ particles inhibited the hatching rate of brine shrimp cyst. Nano $CeO_2$ was found to be more toxic to *A. salina* (48 h $LC_{50}$ 38.0 mg/L) when compared to bulk $CeO_2$ (48 h $LC_{50}$ 92.2 mg/L). Nano $CeO_2$-treated *A. salina* showed higher oxidative stress (ROS) than those treated with the bulk form. The reduction in the antioxidant activity indicated an increase in oxidative stress in the cells. Higher acetylcholinesterase activity (AChE) was observed upon exposure to nano and bulk $CeO_2$ particles. The uptake and accumulation of $CeO_2$ particles were increased with respect to the concentration and particle size. Thus, the above results revealed that nano $CeO_2$ was more lethal to *A. salina* as compared to bulk particles.

## Introduction

The global production rate and usage of metal oxide nanoparticles (NPs) has been increased drastically over few decades. Among the engineered nanoparticles, cerium dioxide ($CeO_2$) is used extensively for many commercial and industrial applications (Keller et al. 2010). Cerium nanoparticles are used in the production of fuel cells, glass, pigments, fuel additives and as a catalyst in the chemical process (Dahle and Arai 2015; The Project of Emerging Nanotechnologies, 2015 catalyst). It is certain that these nanoparticles can be inevitably released into the aquatic environment and cause serious health implications for living organisms and environment (Rogers et al. 2010). Nanoparticles can persist in the natural aquatic environment for a long time due to their aggregation, sedimentation, slow degradation rate, and this could increase their uptake, accumulation, and bio magnification in the food chain (García 2011).

The toxicity of $CeO_2$ nanoparticles was studied over few decades on different test organisms. Cerium oxide particles are shown to cause membrane damage and ROS generation in bacterial and algal cells due to the cellular uptake/adsorption of particles. In crustaceans, the toxicity of $CeO_2$ is due to NP ingestion, accumulation, and adhesion. The recent reports on ecotoxicity of $CeO_2$ are mentioned in Table 1.

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**Table 1** Ecotoxicity of CeO$_2$ on aquatic organisms

| CeO$_2$ particle | Test organism | Significant findings | References |
|---|---|---|---|
| Nano (35 ± 11 nm) | N. europaea | Cell membrane disruption, Charge based interaction of NPs and bacterial cells, Internalization of NP | Yu et al. 2016 |
| Nano (238 ± 77 nm 377 ± 63 nm) Bulk (5 μm) | E.coli | Size dependent toxicity, Light illumination and ROS generation, Cell membrane damage | Leung et al. 2015 |
| Nano (20 nm) | E. coli B. subtilis S. typhimurium E. faecalis | MIC: 16 mg/L, 4 mg/L and 8 mg/L Release of Ce$^{3+}$ and Ce$^{4+}$ ions, Intracellular ROS generation, Cell membrane damage | Krishnamoorthy et al. 2014 |
| Nano (7 and 25 nm) Bulk (>200 nm) | E.coli | Size dependent toxicity, Intracellular ROS generation, Internalization of Ce Cell membrane damage | Kuang et al. 2011 |
| Nano particle 28 to 126 nm | E.coli S.oneidensi B.subtilis | Size-dependent antibacterial activity, Intracellular ROS generation, Metabolic changes | Pelletier et al. 2010 |
| Nano (75 nm) Bulk (1.5 μm) Ionic Ce | A. thaliana | Phytotoxicity, Uptake of Np's, Antioxidant response, Changes in photosynthesis, Subcellular ]localization of Ce (ions or Nps's) | Yang et al. 2017 |
| Nano: (82-135 nm) Bulk (1.4 μm) | P. subcapitata | Size dependent toxicity, Dissolution of Ce, Sorption of CeO$_2$ on algae, IC$_{50}$: 7.6–28 mg/L and 52 mg/L Intracellular ROS generation, | Angel et al. 2015 |
| Nano (53 nm) Bulk | C.elegans D. rerio | Size dependent toxicity, ROS generation, Subcellular localization of Ce (ion or Nps's) | Arnold et al. 2013 |
| Nano (8 nm) | D. similis D. pulex | NPs ingestion, accumulation and adhesion on daphnia sorface | Artells et al. 2013 |
| Nano (3 nm) | D. pulex | Uptake, accumulation, Changes in the metabolism and feeding behavior | Auffan et al.2013 |
| Nano CeO$_2$ (8 nm) | G. roeseli D. polymorpha | Bio accumulation, Bio concentration, ROS generation, CAT activity | Garaud et al. 2015 |
| Nano (25 nm) | D. rerio O. mykiss | Toxicity, Uptake, Dissolution, | Johnston et al. 2010 |
| Nano (50–105 nm) | A.salina | No mortality, Decreased swimming speed, Decrease in Choline esterase and GST activities. Increase in catalase activity. | Gambardella et al. 2014 |
| Nano 10–15 nm | D. rerio | Mortality, Changes in body length, Uptake of Nps's | Jemec et al. 2015 |
| Nano 5–80 nm & 300 nm Cerium salt | E.fetida | EC$_{50}$ (317.8, 294.6 and 41–10,000 mg Ce kg$^{-1}$) Survival rate, Reproduction, Accumulation and histopathological changes seen | Lahive et al. 2014 |
| nCeO$_2$ (800 nm) | M.galloprovincialis I. galbana Algae | Toxicity, Trophic transfer, Accumulation, Dissolution | Conway et al. 2014 |

*Artemia salina* is widely used as a food source in aquaculture industries due to its ease of availability, convenience, method of culturing, and lack of labor intensiveness. *A. salina* (pelagic organisms) has been considered as an important test organism to study the eco-toxicological implications of nanoparticles (Gambardella et al. 2014). The bioavailability of NPs and their accumulation in the food chain are important parameters to assess the risk of nanoparticles on aquatic organisms (Wang and Wang 2014). Despite of many reports on the toxicity of CeO$_2$ on aquatic and terrestrial organisms, their mechanism of toxicity is still not clear. Besides, the previous report on changes in the enzymatic activity of CeO$_2$ NPs by Gambardella et al. (2014) on *A. salina*, the current study is the first of its kind to quantify the uptake and accumulation of cerium oxide particles on *A. salina*.

The major objective of this study was to investigate the nano and bulk CeO$_2$ toxicity on *A. salina* in natural seawater system. The dynamic behavior (sedimentation and stability) of CeO$_2$ in seawater and its effect on the hatching rate of *Artemia* cyst were studied. The changes in the enzyme activities like CAT, GST, SOD, and AChE and the morphological characteristics of *A. salina* nauplii after exposure to nano and bulk CeO$_2$ were investigated. Nevertheless, the current study would bring a better understanding of the eco toxicological implications (toxicity, accumulation, depuration, and changes in biochemical markers) of nano and bulk CeO$_2$ particles on *A. salina*, a representative toxicity testing organism of the marine environment.

## Materials and methods

### Chemicals

Nano $CeO_2$ particles (particle size < 50 nm, purity 99.8%) and bulk $CeO_2$ (particle size > 5 μm, purity 99%) were procured from Sigma-Aldrich, India. All the chemicals used in the studies were of analytical grade.

### Experimental medium

Natural seawater was obtained from Central Institute of Brackish water Aquaculture (CIBA, Chennai, Tamil Nadu, India). Natural seawater was utalized as an experimental medium for all the toxicity studies. Initially, seawater was filtered using Whatman no. 1 paper and further sterilized to remove the biological contaminants present. The physicochemical characterizations of seawater medium as reported in our previous study (Bhuvaneshwari et al. 2016) are as follows, pH (8.0), conductivity 14.65 mS/cm, TDS 12,000 mg/L, TOC 8.56 mg/L, and salinity 37 ppt. The presence of heavy metals was measured to be 0.020 (Ce), 0.140 ± 0.01 (Zn), 1.84 ± 0.02 (Fe), 0.145 ± 0.04 (Cu), 0.513 ± 0.03 (Al), 0.108 ± 0.02 (Ti), 0.189 (Mn), and 0.030 mg/L (Cr).

### Primary characterization of nanoparticles

The stock of 100 mg/L of nano and bulk $CeO_2$ was prepared in Milli-Q water and sonicated using the probe sonicator (Sonics, USA) at 130 W for 20 min. The hydrodynamic diameter of nano and bulk $CeO_2$ (100 mg/L) in seawater medium was measured by a particle size analyzer (90 Plus Particle Size Analyzer, Brookhaven Instruments Corp., USA) using the dynamic light scattering method. The particle size measurement by Dynamic light scattering (DLS) method was based on the time-dependent variations on the scattering of laser light by the nanoparticles in Brownian motion. The scattered light is collected at 90° angle and directed to the multichannel correlator, which calculates the diffusion coefficient of the nanoparticles. The mean hydrodynamic size was calculated from the Stokes–Einstein equation, which correlates the diffusion coefficient calculated from the light scattering measurements with that of the particle size (Kaszuba et al. 2008).

The primary particle size and surface morphology of procured nano and bulk cerium oxide particles were imaged using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). For TEM, the nano and bulk $CeO_2$ particles were coated on the copper gird and subjected to imaging (TEM, Philips, CM12, Netherlands). For SEM imaging, the stock concentration was further diluted to 10 mg/L in Milli-Q water and coated on a glass side under dust-free environment. Further, this was subjected to gold sputtering and observed using a scanning electron microscope (EV018 Carl Zeiss, Germany). The average size of nano and bulk $CeO_2$ particles were calculated by measuring 100 particles randomly from the TEM and SEM images with the help of an imaging software, Image J, Version 1.49. The crystallinity and purity of nano and bulk cerium oxide particles were analyzed using an X-Ray diffractometer (D8 Advanced X-ray Diffractometer, Bruker, Germany) with CuKα radiation of wavelength λ = 1.5406 in the 2θ scan range of 20–80°.

### Colloidal stability of nano and bulk $CeO_2$ in seawater medium

The mean hydrodynamic diameter of different concentrations of nano (0.3, 3, 9, 18, 25, and 32 mg/L) and bulk (0.7, 7, 14, 28, 56, and 100 mg/L) cerium oxide particles at different time intervals of 0, 1, 2, 4, 8, 12, 24, and 48 h was measured by dynamic light scattering technique using a particle size analyzer. The stock of 200 mg/L of nano and bulk $CeO_2$ was prepared in Milli-Q water and sonicated for 20 min using ultrasonics probe sonicator. For 0 h, the mean hydrodynamic diameter was measured immediately after the dilutions were made.

### Sedimentation assay

The sedimentation of $CeO_2$ particles was measured by the change in absorbance of the test medium at 345 nm using a UV–Visible spectrophotometer (U-2910, Hitachi, Japan). The nano and bulk cerium oxide particles of different concentrations were prepared in seawater medium and loaded into the new 50-mL falcon tubes (Gupta et al. 2016). One milliliter of samples were withdrawn from the top, bottom, and middle layer of the tubes at 0, 1, 2, 4, 8, 12, 24, and 48 h of time intervals using a pasture pipette. The change in absorbance of $CeO_2$ particles was measured in the samples withdrawn from the different layers of the tube.

### Dissolution of nano and bulk $CeO_2$ in seawater medium

The dissolution of different concentrations of nano (0.3, 3, 9, 18, 25, and 32 mg/L) and bulk (0.7, 7, 14, 28, 56, and 100 mg/L) cerium oxide particles was measured after 48 h of interaction in seawater using ICP-OES (Inductively coupled plasma atomic emission spectroscopy, Perkin Elmer Optima 5300). After 48 h of interaction, the nano and bulk suspensions were centrifuged at 15,000 g for 30 min at a temperature of 4 °C (Bhuvaneshwari et al. 2017). The supernatant was filtered using a 0.1-μm filter, followed by a 5-kDa filtration (Ultra filtration disc, Millipore). The filtrate containing only the Ce ions was used to measure the dissolution of nano and bulk $CeO_2$ in seawater.

## Toxicity assessment

### Hatching rate of brine shrimp

The effect of nano and bulk $CeO_2$ particles on the hatching of brine shrimp cysts was measured according to OECD guidelines 236 (OECD 2013). Nano and bulk $CeO_2$ concentrations of 10, 25, 50, 75, and 100 mg/L and control (devoid of $CeO_2$ particles) were prepared in seawater. Ten brine shrimp cysts were added to different concentrations of $CeO_2$ particle suspension taken in 12-well micro well plates. The rate of hatching at 48 h of incubation was noted. Hatching rate of brine shrimp cysts exposed to nano and bulk cerium particles was calculated according to the equation.

$$H = N/C + N^*(100)$$

$H$ corresponds to the hatching percentage, $N$ is the number of hatched cysts, including the hatched organism in umbrella stage, and $C$ is the decapsulated full cysts (Arulvasu et al. 2014).

### Acute toxicity assessment

Acute toxicity tests were performed on both nano and bulk $CeO_2$ particles according to the Organization for Economic Cooperation and Development (202) testing guidelines (OECD 2004). The brine shrimp cysts were allowed to hatch in the seawater with sufficient aeration at a temperature of 20 °C and 16:8-h light: dark condition. After 24 h, the first instar larvae (nauplii) were taken for toxicity studies. Nano and bulk $CeO_2$ of different concentrations, 10, 20, 40, 80, 160, and 320 mg/L, were interacted with 24-h-old nauplii. The control sample containing only seawater was maintained. The number of live *Artemia* nauplii was counted after 48 h of interaction. The lethal concentrations ($LC_{50}$) of nano and bulk $CeO_2$ toxicity on *A. salina* nauplii were calculated.

The toxicity of dissolved Ce ions was measured according to Yang et al. (2017). After 48 h of interaction, the nano and bulk suspensions were centrifuged at 15,000g at 4 °C for 30 min twice using an ultracentrifuge. The obtained supernatant was filtered through a 0.1-μm filter, followed by a 5-kDa filtration, and the suspension containing Ce ions was exposed to the 24-h-old *A. salina* for 48 h. The number of live nauplii was counted after 48 h of interaction.

## Biochemical changes

Depending on the median lethal ($LC_{50}$) concentrations, the range of test concentrations of nano (0.3, 3, 9, 18, 25, and 32 mg/L) and bulk (0.7, 7, 14, 28, 56, and 100 mg/L) cerium

oxide particles were chosen for further toxicity studies such as biochemical changes. The concentration of nano and bulk $CeO_2$ mentioned above was interacted with the first instar *A. salina* nauplii for 48-h interaction. The interacted nauplii were harvested and washed twice with Milli-Q water. To the washed nauplii, 1 mL of 100 mM potassium phosphate buffer was added and sonicated for 3 min using a probe sonicator (Sonics, USA) in ice. After sonication, the homogenate was centrifuged at 15,000g for 15 min, and the supernatant was collected. The sample supernatant was further used for the following biochemical assays.

### Estimation of total protein content

The difference in the total protein content of control and nanoparticle-treated samples was measured using a Bradford assay (Bradford 1976). About 10 μL of the sample was added to the 96-well plates, to which 200 μL of Bradford reagent was added under dark condition. The samples were incubated at room temperature for 5 min, and the absorbance was measured at 595 nm using the UV–vis spectrophotometer (Hitachi U2910).

### Reactive oxygen species generation (ROS)

The reactive oxygen species (ROS) generation was determined using a non-fluorescent dye 2′,7′-dichloro dihydrofluorescein diacetate (DCFH-DA) according to Ulm et al. (2015). *A. salina* nauplii interacted with nano and bulk $CeO_2$ particles were washed with Milli-Q water. A total of 10 washed nauplii were transferred to 24-well plates containing 10 μM DCFH-DA dye and incubated for 30 min at 37 °C. After incubation, the nauplii were washed twice using PBS of pH 7.4 and homogenized using a probe sonicator. The fluorescence intensity (DCF) was measured at an excitation wavelength of 485 nm and an emission wavelength of 535 nm using a fluorescence spectrophotometer (SL174, ELICO, India). The ROS generation was further confirmed by visualizing the control and treated nauplii incubated with DCFH-DA dye using fluorescence microscopy (DM2500, Leica Microsystems, Germany).

### Catalase assay (CAT)

The decomposition of hydrogen peroxide ($H_2O_2$) to $H_2O$ was measured at 240 nm according to the protocol adopted by Jemec et al. (2008). Fifty microliters of protein supernatant was added to 950 μL of hydrogen peroxide solution (10.8 mM) prepared in 50 mM of potassium phosphate buffer (pH 7.0). The absorbance was measured for 3-min interval at 240 nm using the UV- Visible spectrophotometer.

*Superoxide dismutase assay (SOD)*

Superoxide dismutase activity was measured according to method reported by Kono et al. (1979). The reaction mixture contained 1.3 mL sodium carbonate buffer, 500 μL (4-Nitro Blue tetrazolium chloride) NBT, and 100 μL Triton X-100, to which 100 μL hydroxylamine hydrochloride was added to initiate the reaction. After 2 min, 70 μL of the sample extract was added. The decrease in absorbance at 540 nm is a measure of NBT reduction by the SOD enzyme.

*Acetylcholinesterase assay (AChE)*

Acetylcholinesterase (AChE) is an enzyme involved in the hydrolysis of the neurotransmitter acetylcholine (ACh) to acetate and choline (Ellman et al. 1961). Ellman's reagent was prepared without substrate (100 mM phosphate buffer, 500 μM 5, 5′-Dithiobis-(2-nitrobenzoic acid)). 10 μL of sample supernatant was taken in a 96-well plate to which 180 μL of Ellman's reagent was added and incubated for 30 min. After incubation, 10 μL of the substrate (acetylthiocholine iodide 20 mM) was dispensed to the above cocktail, and the absorbance difference was measured for 3 min at 405 nm using the UV–vis spectrophotometer.

*Glutathione S-transferase (GST)*

Glutathione S-transferase activity was measured according to the protocol developed by Mannervik and Nise (1969). One milliliter of enzyme cocktail was prepared using 980 μL of PBS, 10 μL of 100 mM (1-chloro-2,4-dinitrobenzene) CDNB, and 10 μL of 100 mM glutathione. Nine hundred microliters of above enzyme cocktail and 100 μL of sample supernatant was taken in 1 ml cuvette and the absorbance was measured at 340 nm using UV–vis spectroscopy.

**Uptake and accumulation of nano and bulk $CeO_2$**

Twenty four-h-old nauplii were exposed to different concentrations of nano and bulk $CeO_2$ particles for 48 h (Ates et al. 2013). After 48 h of interaction, 10 nauplii were washed thoroughly with Milli-Q water and transferred to pre-weighted tubes. The washed nauplii were dried at 80 °C in a hot-air oven, after which, the dry mass of the nauplii was measured. To determine the uptake of nano and bulk $CeO_2$, the dried nauplii were acid digested with an excess of concentrated $HNO_3$ acid. The samples were analyzed for Ce content using ICP-OES (Perkin Elmer Optima 5300), and the sensitivity of this instrument is about 0.004 mg/L. The ingested particles were allowed to depurate for 48 h in fresh medium. The nauplii were then separated and dried in the pre-weighted tubes. Further, it was acid digested using nitric acid, and the

samples were subjected for measurement of the accumulated Ce concentration present after depuration.

**Statistical analysis**

All experiments were carried out in the triplicates and expressed as the mean with the corresponding standard deviation (SD). Significance in the difference between the treatment groups of nanoparticles was calculated using two-way ANOVA (Bonferroni post-test) by Graph Pad Prism (Version 5) software. The lethal concentration ($LC_{50}$) values were calculated using a nonlinear regression model. The statistical significance between the control and test group was accepted at a level of $p < 0.05$.
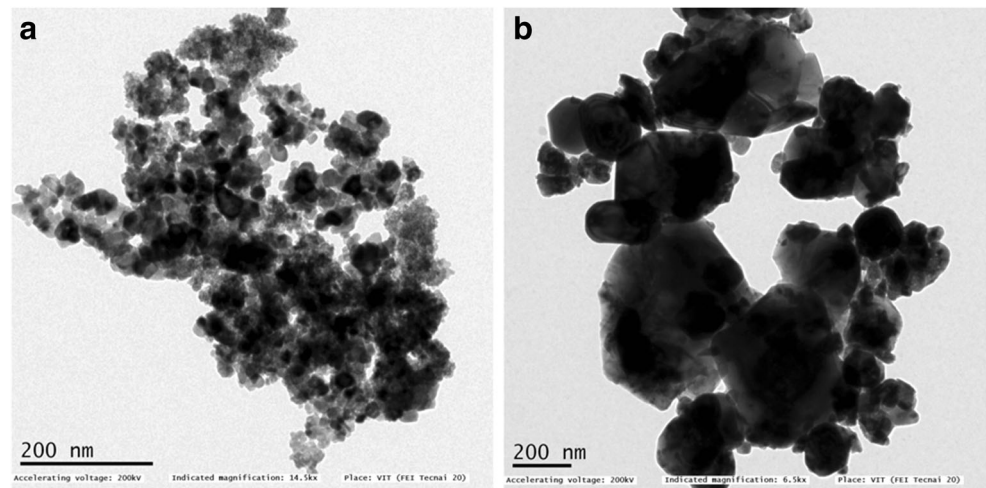
# Results

**Characterization of nanoparticles**

The primary size and morphology of nano and bulk $CeO_2$ particles were determined using TEM and SEM. The primary particle size measured using TEM was found to be $15 \pm 3.5$ and $582 \pm 50$ nm for nano and bulk $CeO_2$, respectively (Fig. 1a, b). SEM micrograph showed the average size of nano and bulk $CeO_2$ as $130 \pm 20$ nm and $530 \pm 60$ nm, respectively (Fig. S1 A and B). The mean hydrodynamic diameter of nano and bulk $CeO_2$ particles at 100 mg/L in seawater medium was measured to be $510 \pm 80$ and $1092 \pm 14$ nm at 0 h, respectively. The purity and crystalline structure of procured $CeO_2$ particles were analyzed from the X-ray diffraction pattern (Fig. S2). The progressive scan range from 20 to 80° showed the polycrystalline nature of particles with intense peaks. The sharp high peak at 28.53° corresponding to the 111 crystalline plane confirmed the presence of $CeO_2$ in the sample. The other sharp and intense peak at 33.09°, 47.5°, and 56.26° corresponded to 200, 220, and 311 crystalline planes, respectively. The crystalline size was measured to be 49 and 119 nm for nano and bulk $CeO_2$, respectively. The obtained crystalline planes were in a match with JCPDS (Joint Committee on Powder Diffraction Standards) No: 34-0394 for cerium oxide particles. All the crystalline planes correspond to the pure form and cubic fluorite structure of cerium oxide particles.

Dissolution of ions from the nano and bulk $CeO_2$ in seawater medium was measured using ICP OES, and the results are represented in Fig. 2a, b. The dissolution of $CeO_2$ particles was found to be dependent on the exposure concentrations ($p < 0.05$). The dissolution of Ce ions from the lower exposure concentrations of 0.3 mg/L (nano $CeO_2$) and 0.7 mg/L (bulk $CeO_2$) were found below the detection limit (BDL). The maximum concentration of dissolved Ce was found to be $2.3 \pm 0.2$ and $4.6 \pm 0.5$ mg/L from 32 and 100 mg/L of nano and bulk $CeO_2$, respectively. Further, the toxicity of dissolved Ce ions

**Fig. 1** Transmission electron micrographs of nano CeO$_2$ (**a**) and bulk CeO$_2$ particles (**b**)



from nano and bulk CeO$_2$ on *A. salina* was studied, and the results are represented in Fig. 2c, d.

## Stability of nano and bulk CeO$_2$ in seawater

The colloidal stability of nano and bulk cerium oxide particles in seawater medium was measured at different concentrations and time intervals (0, 1, 2, 4, 8, 12, 24, and 48 h) using the dynamic light scattering method (Fig. 3). The concentration-dependent increase in the mean hydrodynamic diameter of nano CeO$_2$ in seawater medium was observed for all kinetic intervals of exposure ($p < 0.001$). At 0 h, the mean hydrodynamic diameter of 0.3 mg/L nano CeO$_2$ was found to be $244 \pm 3.1$ nm, and it increased to $281 \pm 11$, $381 \pm 10$,

$318 \pm 15$, $420 \pm 35$, $450 \pm 9$, and $460 \pm 10$, and $480 \pm 21$ nm at 1, 2, 4, 8, 12, 24, and 48 h of interaction, respectively. At higher concentration (32 mg/L), the mean hydrodynamic diameter increased from initial size of $492 \pm 5.0$ to $759 \pm 5.2$, and $998 \pm 20$ nm at 0, 4, and 48 h of interaction, respectively.

In contrast to nano CeO$_2$, the bulk particles showed a significant increase in the mean hydrodynamic diameter for all the concentrations at all kinetic exposure intervals ($p < 0.001$). The mean hydrodynamic diameter of bulk CeO$_2$ (0.7 mg/L) was found to be $689 \pm 38$, $767 \pm 20$, $967 \pm 22$, $901 \pm 28$, $980 \pm 31$, $1065 \pm 50$, $1186.4 \pm 49$, and $1303.1 \pm 80$ nm at 0, 1, 2, 4, 8, 12, 24, and 48 h of interaction, respectively. The mean hydrodynamic diameter of particles at 100 mg/L bulk CeO$_2$ was found to be increasing from $1053 \pm 30$ (0 h) to $3055 \pm 87$

**Fig. 2** Dissolution of nano and bulk CeO$_2$ particles in seawater medium (**a** and **b**). Toxicity of dissolved Ce ions on *Artemia salina* (**c** and **d**). The symbols "$\delta$" ($p < 0.001$) represents the level of significance between different time intervals
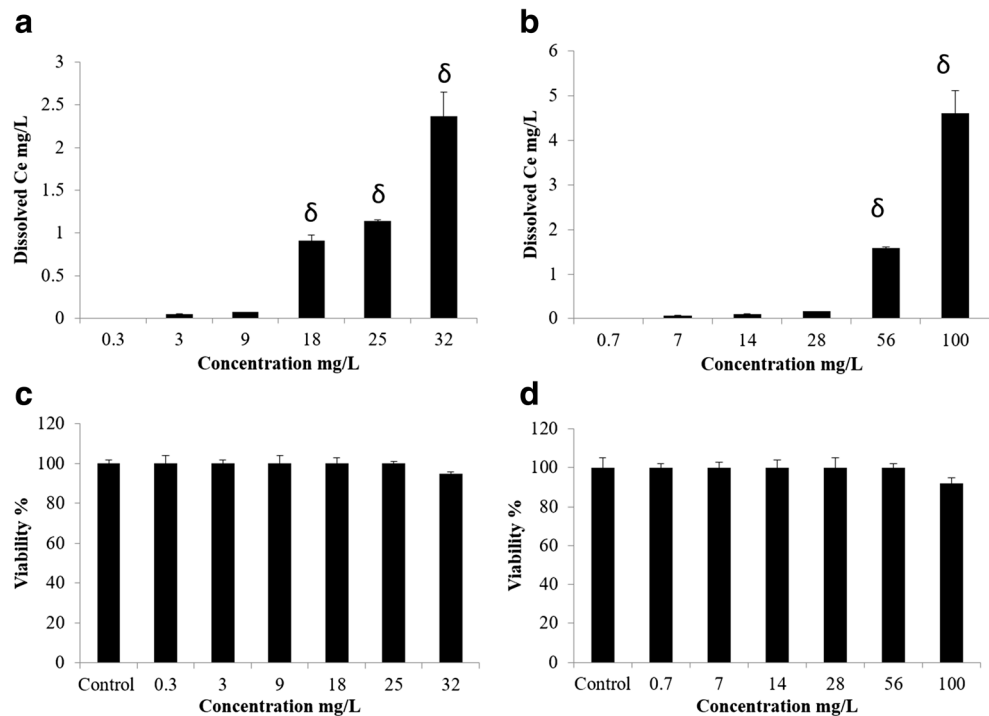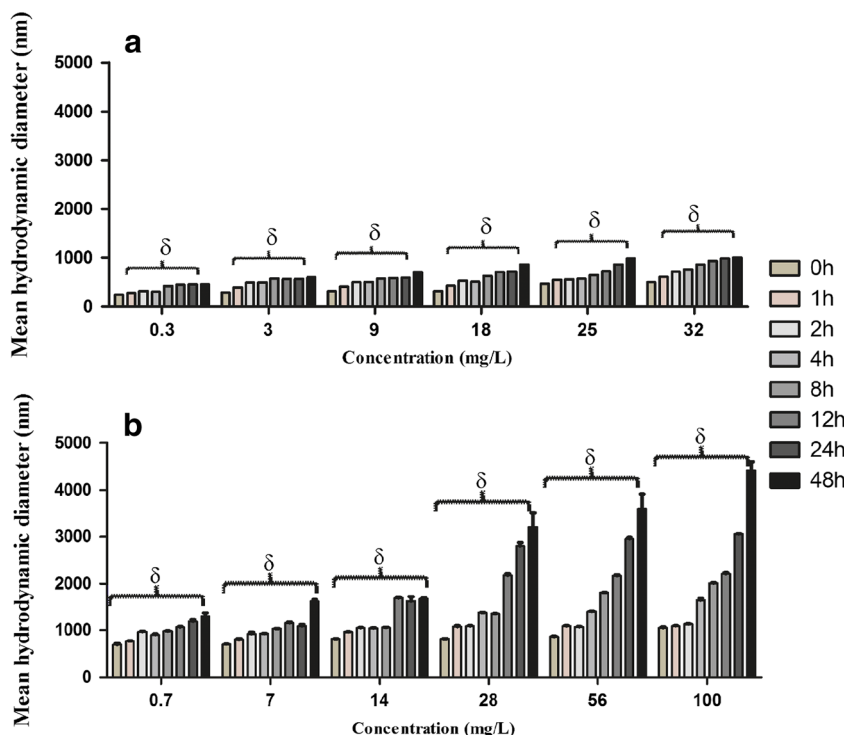
**Fig. 3** Hydrodynamic diameter of different concentrations of nano (**a**) and bulk (**b**) $CeO_2$ particles in seawater medium at different time intervals (0, 1, 2, 4, 8, 12, 24, and 48 h). The data are presented as mean ± SD, n-3. The symbols "δ" ($p < 0.001$) represents the level of significance between different time intervals



and 4413.1 ± 200 nm at 12 and 48 h of interaction, respectively. As the interaction time increases, the mean hydrodynamic diameter increased significantly ($p < 0.001$) for all concentrations. At 0 h of exposure, bulk $CeO_2$ (28 mg/L) particles reached micron size in seawater medium. Nano $CeO_2$ was observed to be in submicron (< 1 μm) size till 48 h of interaction at all the exposure concentrations, except 32 mg/L.

### Sedimentation of nano and bulk $CeO_2$ in seawater

The sedimentation profile of nano and bulk $CeO_2$ in seawater medium is represented in Fig. S3 A and B. The absorbance of nano and bulk $CeO_2$ particles at three different layers of the tube (upper, middle, and lower) was measured at different kinetic intervals (0, 1, 2, 4, 8, 12, 24, and 48 h). A concentration-dependent increase in the absorbance ($p < 0.001$) was observed for nano $CeO_2$ till 8 h of interaction, with no significant difference between all three layers ($p > 0.05$). At 12, 24, and 48 h of interaction, no change in the absorbance was noticed for all three layers of 0.3, 3, 9, and 18 mg/L concentrations. However, the absorbance of upper and middle layers decreased for 25 and 32 mg/L concentrations. Thus, the sedimentation of bulk $CeO_2$ was faster in seawater as compared to the nano $CeO_2$.
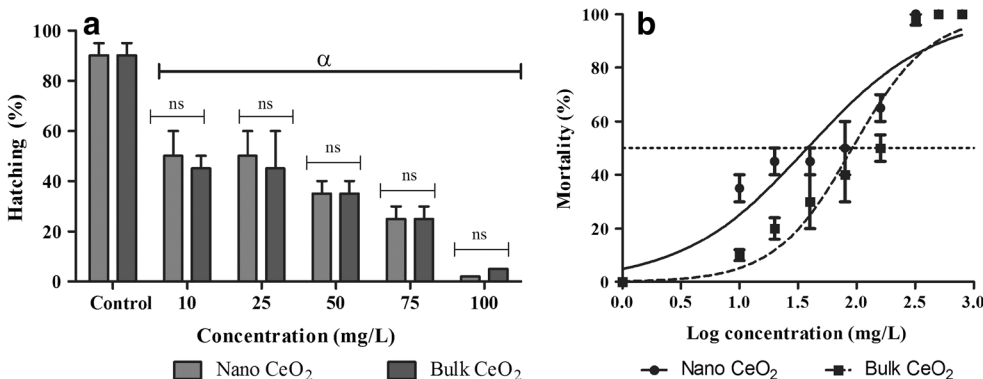


**Fig. 4** **a** Hatching rate of *Artemia salina* brine shrimp cysts exposed to nano and bulk $CeO_2$ particles in seawater medium. The level of significance between control and $CeO_2$ particle-treated group are represented as "α", corresponding to $p < 0.001$***. Symbol "ns" corresponds to $p > 0.05$ between nano and bulk $CeO_2$ treatment. The data are presented as mean ± SD, $n = 3$. **b** Acute toxicity ($LC_{50}$) of nano and bulk $CeO_2$ particles on *Artemia salina* in seawater medium. The concentrations of $CeO_2$ are expressed in log concentration. The data are presented as mean ± SD, $n = 3$

**Fig. 5** ROS generation in *Artemia salina* treated with nano (**a**) and bulk (**b**) CeO$_2$. SOD activity of *Artemia salina* treated with nano and bulk CeO$_2$ (**c** and **d**). The level of significance between control and CeO$_2$ particle-treated group are symbolized by $p < 0.05^*$, $p < 0.01^{**}$, and $p < 0.001^{***}$. The data are presented as mean ± SD, $n = 3$
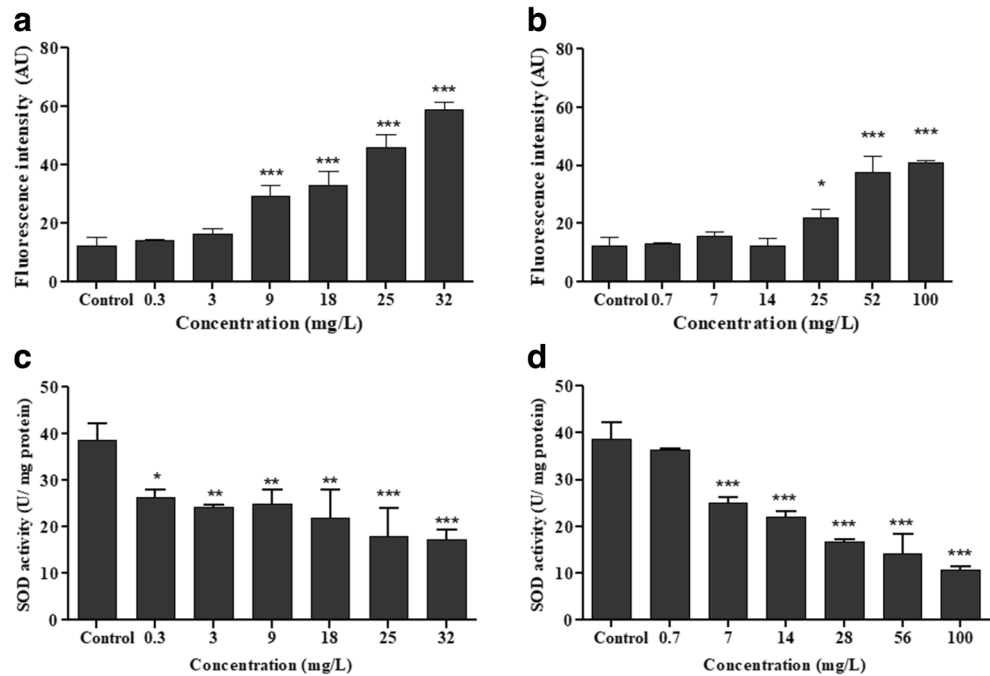


## Toxicity assessment

### Effect of nano and bulk CeO$_2$ on hatching of brine shrimp cysts

The toxic effect of nano and bulk cerium oxide particles on hatching of brine shrimp cysts in seawater was studied (Fig. 4a). The control group showed (devoid of nanoparticles) 80–90% hatching of cyst. As the concentration increased, the hatching of brine shrimp cysts decreased significantly ($p < 0.001$) at all exposure concentrations. The complete hatching inhibition was observed at 100 mg/L of both nano and bulk CeO$_2$ particles. There was no significant difference observed between nano and bulk cerium particles on the hatching of brine shrimp *Artemia* cysts ($p > 0.05$).

### Acute toxicity of nano and bulk CeO$_2$ A. salina nauplii

The mortality of *A. salina* nauplii interacted with nano and bulk cerium oxide particles are shown in Fig. 4b. No sign of mortality was observed in the control (only seawater) group. Both the nano and bulk cerium oxide showed a concentration-dependent increase in the toxicity for the 24-h-old nauplii. Nano CeO$_2$ showed significantly higher toxicity as compared to that of bulk particles ($p < 0.01$). The mortality of 24-h-old nauplii was observed to be 35 ± 5, 45 ± 15, 45 ± 5, 50 ± 2, 65 ± 5, and 100 ± 2% upon interaction with various concentrations (10, 20, 40, 80, 160 and 320 mg/L) of nano CeO$_2$. Similarly, the mortality was found to be 10 ± 2, 20 ± 4, 30 ± 10, 40 ± 10, 50 ± 5, and 98 ± 2% for the bulk CeO$_2$ concentrations of 10, 20, 40, 80,

160, and 320 mg/L, respectively. All the treated concentrations of nano and bulk cerium oxide particles were lethal to nauplii. The LC$_{50}$ value of nano and bulk CeO$_2$ was found to be 38.0 mg/L (with 95% CI of 27.32 to 52.85 mg/L) and 92.26 mg/L (with 95% CI of 73.82 to 115.3 mg/L), respectively. The median lethal concentration (LC$_{50}$) of nano CeO$_2$ on 24-h-old nauplii was observed to be significantly lower as compared to the bulk particles ($p < 0.001$). Dissolved ions from nano (32 mg/L) and bulk (100 mg/L) CeO$_2$ caused only 10% mortality on *A. salina* (Fig. 2b, c).

## Biochemical changes

### Assessment of reactive oxygen species generation

ROS generation in nauplii exposed to nano and bulk CeO$_2$ are represented in Fig. 5a, b. The fluorescence intensity was found to be significantly increased from 9 mg/L for nano CeO$_2$ with respect to the control ($p < 0.001$). Bulk CeO$_2$ particles of 0.7, 7, and 14 mg/L did not show any significant increase in the fluorescence intensity with respect to the control ($p > 0.05$). On other hand, nano CeO$_2$ showed significantly higher ROS generation as compared to the bulk particles, though the concentration of exposed bulk CeO$_2$ was higher. Further, the ROS generation was confirmed by staining the nauplii with DCFH-DA (Fig. S4), which shows the in vivo reactive oxygen species generation. The nano CeO$_2$ (25 and 32 mg/L) and bulk CeO$_2$ treatment (28 and 100 mg/L) showed green fluorescence due to the production of intracellular reactive oxygen species.
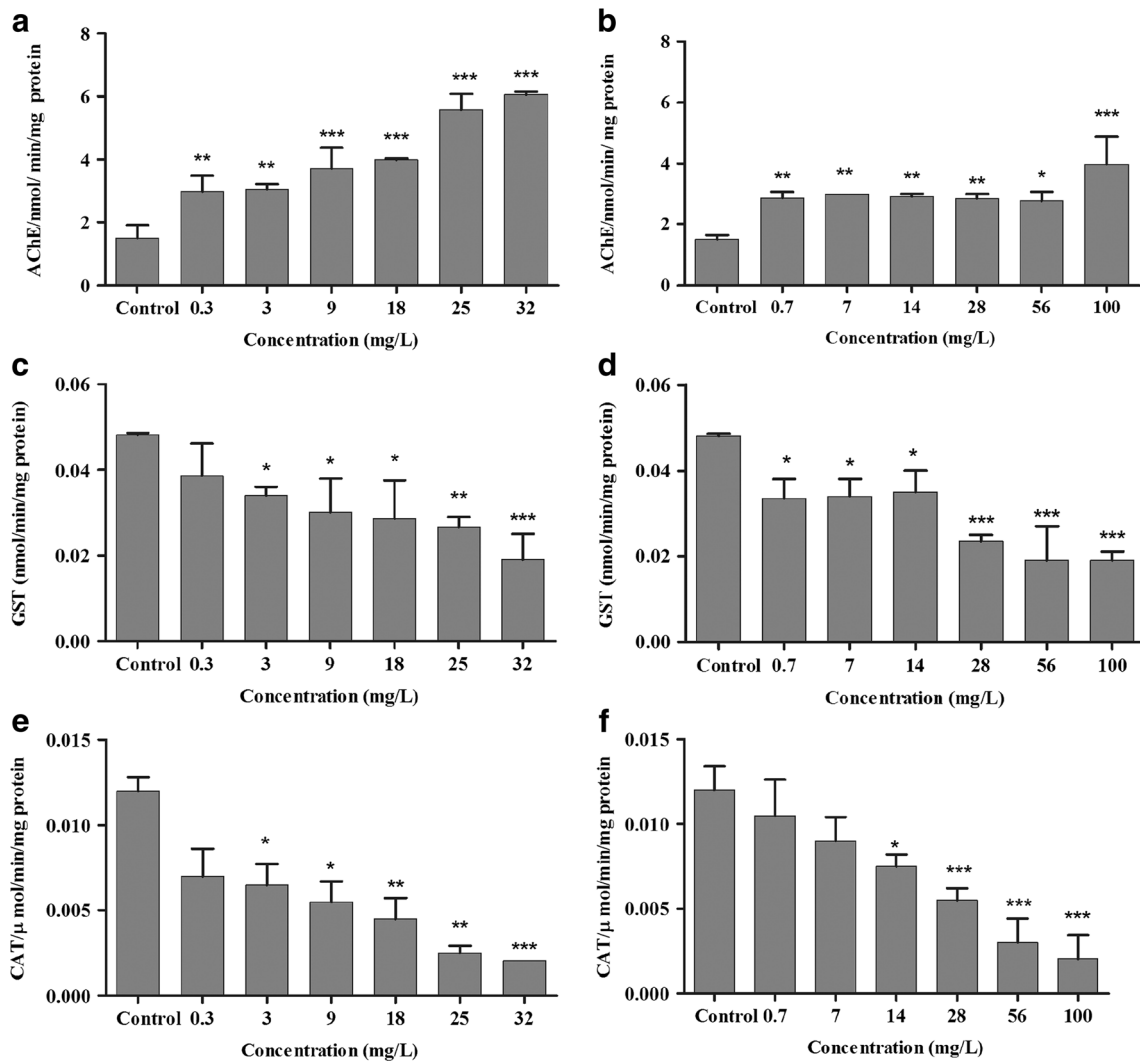
**Fig. 6** AChE activity of *Artemia salina* treated with nano and bulk CeO₂ (**a** and **b**), GST activity of *Artemia salina* treated with nano and bulk CeO₂ (**c** and **d**), CAT activity of *Artemia salina* treated with nano and bulk CeO₂ (**e** and **f**). The level of significance between control and CeO₂ particle-treated group is symbolized by $p < 0.05*$, $p < 0.01**$, and $p < 0.001***$. The data are presented as mean ± SD, $n = 3$

*Superoxide dismutase activity*

As the nano and bulk CeO₂ concentration increases, the antioxidant enzyme activity (SOD) was found to be decreased significantly, as compared to control. However, there was no significant difference ($p > 0.05$) seen between nano and bulk CeO₂ (Fig. 5c, d). Higher exposure concentrations of nano (32 mg/L) and bulk CeO₂ (100 mg/L) showed similar SOD activity at 48 h of interaction, despite the differences in the concentration of bulk CeO₂ as compared to nano CeO₂.

*Acetylcholinesterase activity*

The acetylcholinesterase activity in *A. salina* nauplii exposed to nano and bulk cerium oxide particles are represented in Fig. 6a, b. The concentration-dependent increase in acetylcholinesterase activity was observed upon exposure to nano and bulk CeO₂ particles ($p < 0.01$) as compared to the control group. A significant difference ($p < 0.05$) in the activity of AChE in nauplii treated with nano and bulk cerium particles was observed. The nano CeO₂ treated samples showed significantly higher acetylcholinesterase activity as compared to the bulk particle treatment ($p < 0.05$). Even though the bulk CeO₂ exposure concentrations were higher than nano CeO₂ particles, AChE activity was found to be higher in nauplii treated with nano CeO₂.

*Glutathione S-transferase activity*

GST is an antioxidant enzyme involved in metabolic inactivation of toxic substances that enters the cell. The concentration-dependent inhibition of GST activity was observed after

exposure to both nano and bulk $CeO_2$ particles (Fig. 6c, d). As compared to the control group, a significant decrease in GST activity was observed in all the treated concentrations of nano and bulk particle ($p < 0.001$), except for 0.3 mg/L ($P > 0.05$). There were no significant difference in GST activity observed between nano and bulk $CeO_2$ particle treatment ($P > 0.05$).

### Catalase activity

As compared to the control group, a significant decrease in CAT activity ($p < 0.001$) was observed for both nano and bulk cerium oxide-treated naupliis (Fig. 6e, f). However, lower concentrations of nano (0.3 mg/L) and bulk (0.7 and 7 mg/L) $CeO_2$ particle-treated nauplii showed an insignificant decrease in the CAT activity when compared to the control. Nano $CeO_2$-treated samples showed significantly lesser CAT activity as compared to the bulk particle treatment ($p < 0.05$). No significant difference was observed between higher exposure concentrations of nano 32 mg/L and bulk 100 mg/L ($P > 0.05$).

### Uptake and accumulation of nano and bulk $CeO_2$

The uptake and accumulation of different concentrations of nano and bulk $CeO_2$ exposed to *A. salina* nauplii are represented in Fig. 7a, b. The uptake and accumulation of nano and bulk $CeO_2$ were quantified based on the dry weight of *A. salina* nauplii after 48 h of interaction. Uptake of both nano and bulk $CeO_2$ into *A. salina* was increased significantly with respect to the concentrations ($p < 0.001$). *A. salina* nauplii treated with nano $CeO_2$ showed significantly higher uptake as compared to bulk particles ($p < 0.05$).

The accumulated nano and bulk $CeO_2$ was quantified after 48 h of depuration as illustrated in Fig. 7a, b. Concentration-dependent increase in accumulation was observed for nano $CeO_2$. On other hand, lower exposure concentrations of bulk $CeO_2$ did not show any significant increase in accumualtion (7, 14, and 28 mg/L), except at 52 and 100 mg/L ($p < 0.01$). Despite the higher concentration of bulk $CeO_2$, the uptake and accumulation was found to be higher in nauplii treated with nano $CeO_2$.
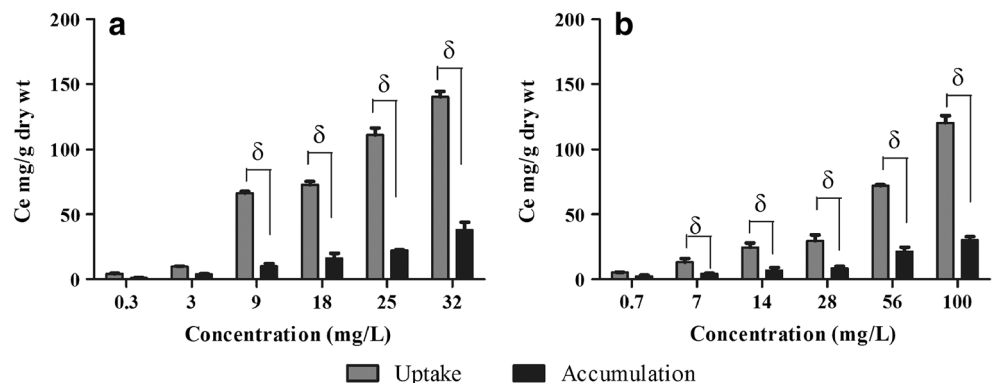
## Discussion

### Primary size of nano and bulk $CeO_2$

The TEM images revealed that the particle size of nano $CeO_2$ was significantly lesser than its bulk particles. Smaller sized nano $CeO_2$ particles exhibit higher reactivity as compared to its bulk counterpart due to their higher surface area to volume ratio. The hydrodynamic diameter of nano ($510 \pm 80$ nm) and bulk ($1053 \pm 14$ nm) $CeO_2$ at 100 mg/L showed significant size differences in seawater medium.

### Stability and sedimentation of nano and bulk $CeO_2$ in seawater

Nanoparticles tend to aggregate once it is exposed to the natural system (Adam et al. 2015) and their colloidal stability play a significant role in the toxicity (Pauluhn 2009). The stability and aggregation of nano and bulk $CeO_2$ was influenced by the physicochemical characteristics, such as surface charge, shape, particle size, and chemical reactivity in seawater medium. In this study, it was seen that as the interaction time increased, the hydrodynamic diameter showed a significant increase ($p < 0.001$) at all exposure concentrations for both nano and bulk $CeO_2$ particles. The particle size of nano $CeO_2$ was observed to be in the range of 460–750 nm till 4 h and reached the submicron size range of 800–900 nm at 24 h for all exposure concentrations. After 48 h, nano $CeO_2$ reached a micron size of 998 nm on exposure to 32 mg/L, whereas lower exposure concentrations remained in the submicron size range of 800–900 nm. In contrast to the nano $CeO_2$, the bulk $CeO_2$ was in the submicron size (700–850 nm) at 0 h for the concentrations of 0.7 to 52 mg/L and micron size (1053 nm) for 100 mg/L. As the interaction time increased, the size of bulk particles showed a significant increase to >1 μm at 1 h, 2 μm at 8 h, and reached 4 μm at 48 h, respectively.

In our study, both nano and bulk particles formed distinctive aggregates and sizes in seawater medium. The results also revealed faster aggregation of bulk particles in seawater medium as compared to nano cerium. Surface-area-to-unit-

**Fig. 7** Uptake and accumulation of nano $CeO_2$ exposed to *Artemia salina* (**a**), uptake and accumulation of bulk $CeO_2$ exposed to *Artemia salina* (**b**). The symbol "δ" ($p < 0.001$) represents the level of significance between uptake and accumulation. The data are presented as mean ± SD, $n = 3$

volume ratio decreases due to aggregation; hence, larger size particles are formed. Aggregation could be due to the direct adsorption of NOM (natural organic matters) and humic acid present in the natural water (Chinnapongse et al. 2011). Wang et al. (2009a) reported the aggregation of Ag and $TiO_2$ NPs particles due to higher salinity of seawater medium, which reduces the electrostatic repulsion between the particles. Higher electrolyte concentrations of monovalent $Na^+$ and $K^+$ and divalent ions $Ca^{2+}$ and $Mg^{2+}$ in the seawater facilitate the aggregation of metal oxide nanoparticles (Badawy et al. 2010; Stebounova et al. 2011). These electrolytes induce the aggregation of nanoparticles by reducing the electrostatic force of the electric double layer present between the nanoparticles (Zhang et al. 2008).

Aggregation can enhance the gravitational settling of particles in the experimental medium. Most of the pelagic and benthic organisms are highly exposed to sediment particles; hence, it is necessary to study the sedimentation profile of $CeO_2$. Sedimentation of both nano and bulk particles increased with the exposure concentration. Bulk $CeO_2$ was found to be sedimented within 2 h of interaction in the experimental medium. The rate of sedimentation increased with respect to time. Keller et al. (2010) reported the higher rate of sedimentation of $CeO_2$ nanoparticles due to lower TOC (Total Organic Carbon) and higher ionic strength in seawater. It is important to consider the aggregation of particles in test medium because the direct uptake/ ingestion and accmulation of particles have significant impact on the survival of the organism.

## Uptake and accumulation of $CeO_2$ into *A. salina*

Ingestion is considered as the primary route of uptake of nanoparticles and the leading cause of toxicity in most of the aquatic organisms like fishes, micro and macro-crustaceans, and zooplanktons (Zhu et al. 2010, Ward and Kach 2009). Being the filter-feeding pelagic organism, *A. salina* can ingest particles in the size range of 7 to 50 μm (Hund-Rinke and Simon 2006). Stability results show the submicron (< 1 μm) size of nano cerium till 48 h in seawater, whereas the initial size (0 h) of bulk particles was in the micron size range. Despite the higher exposure concentrations of bulk $CeO_2$, the uptake was found to be more for nano form at all the exposure concentrations. Due to smaller particle size, the ingested nano $CeO_2$ can be localized in the midgut epithelial cells, gastrointestinal tract, and exoskeleton of *A. salina*. Arnold et al. (2013) reported that the ingested $CeO_2$ particles could translocate to the epithelial cells and cross the intestinal barriers. The ingested polystyrene nanoparticles (Rosenkranz et al. 2009) and QD (Feswick et al. 2013) was reported to be translocated into the lipid-storage droplets of *Daphnia magna*, suggesting their penetration into the gut epithelial cells depending on the particle size. Also, the smaller particles are reported to

have longer gut retention time than the larger particle aggregates, indicating their transportation to the digestive gland (Ward and Kach 2009). These factors could facilitate higher uptake and accumulation of nano $CeO_2$ into *A. salina*. In case of bulk particles, the subcellular localization may not be possible due to larger aggregates. Apart from the size of the particle aggregates, there are other exogenous factors like concentration, physicochemical properties, fate and behavior of particles, (Moiseenko and Kudryavtseva 2001) that can influence the uptake of $CeO_2$ particles (Garaud et al. 2015). The accumulation of ingested particles after depuration was found to correlate with the exposure concentration. The uptake of both nano and bulk $CeO_2$ particles was higher than the accumulation of particles, which signifies the depuration of ingested particles by *A. salina*. The uptake of nano and bulk $CeO_2$ was found to be 3.5 and 4.0 fold higher than particle accumulation and were concurrent with the acute toxicity results of both the particles. Further, the ingestion of aggregated $CeO_2$ particles into the gut of *A. salina* was confirmed using microscopic studies (Fig. S5).

## Acute toxicity of nano and bulk $CeO_2$ on nauplii and cyst

Acute toxicity of nano and bulk $CeO_2$ particles on *Artemia salina* nauplii was evaluated. The observed $LC_{50}$ for nano $CeO_2$ (38.0 mg/L) was much lower than that of the bulk $CeO_2$ (92.26 mg/L). Bulk $CeO_2$ showed 50% mortality on *A. salina* only when its concentration increased to 3 fold as compared to nano $CeO_2$. The toxicity was found to be dependent on the particle size and exposure concentration. Acute toxicity results suggested that the interaction of nano sized $CeO_2$ particles occurs predominantly with *A. salina* than bulk particles. Similar to our study, the toxicity of nano $TiO_2$ on nematode *Caenorhabditis elegans* (Wang et al. 2009a) and to *D. magna* (Zhu et al. 2009) was reported to be significantly higher than the toxicity of bulk $TiO_2$. Further clarity in the mechanism of toxicity was sought by studying the effect of dissolved ions on the toxicity to *A. salina* (Fig. 2b, c). The dissolution percentage was in the range of 0.8–4% for nano and bulk particles in the concentration range of 32–100 mg/L. The obtained results indicated the poor dissolution properties of $CeO_2$ particles in the experimental system. Similarly, few studies reported that the dissolved Ce (III) ions do not exert any toxic effect on the algae, *P. subcapitata* (Pulido-Reyes et al. 2015; Rogers et al. 2010). Röhder et al. (2014) suggested that the low intrinsic toxicity of Ce ions does not account for the observed toxicity of nano $CeO_2$ on *Chlamydomonas reinhardtii*. Another report showed the $CeO_2$ dissolution into ions has not been shown to play a significant role in its toxicity on earthworm *Eisenia fetida* (Lahive et al. 2014).

The effect of nano and bulk $CeO_2$ on the hatching of brine shrimp cyst was studied. Both nano and bulk particles showed

similar trend and a concentration-dependent inhibition in the hatching of cysts Nanoparticles can inhibit the hatching of cyst by direct adsorption onto the outer chorion layer of the cysts and interfere in the oxygen intake and development of embryos (Bai et al. 2010; Sano et al. 2008; Arulvasu et al. 2014). Casals et al. (2010) stated that the nanoparticles could cause inactivation of proteins like metalloproteinase resulting in the inhibition of the hatching process. Therefore, from the study findings, it can be assumed that the higher toxicity of nano $CeO_2$ as compared to its bulk counterpart was due to particle size rather than the dissolved ions.

## ROS generation and antioxidant enzymes

Ingested (up taken and accumulated) particles could bring out deleterious changes in the physiological process of *A. salina*. One of the major mechanisms proposed for metal oxide nanoparticle toxicity was the ion-mediated toxicity due to the ROS generation in the cells. From the ionic toxicity results (Fig. 2b, c), it is evident that Ce ions are not likely to be responsible for the toxicity in *A. salina*. Park et al. (2008) reported that the $CeO_2$ particles can cause oxidative damage to cells or organisms. Similarly, our results showed a significant increase in the ROS generation with respect to exposure concentration and particle size of nano and bulk $CeO_2$. Potential redox cycling of $CeO_2$ particles with peroxide are reported to generate the damaging oxygen radicals (Heckert et al. 2008) in cells. The reduction of Ce (IV) to Ce (III) attains oxygen vacancy on $CeO_2$ particle surface, which is the most possible active site for ROS generation (Lin et al. 2006). The higher ROS generation by nano $CeO_2$ was responsible for the higher toxicity than that of its bulk particles.

The primary antioxidant enzymes like SOD and CAT act as defensive barriers to protect the cells against oxidative stress. Higher levels of ROS radicals ($O_2$ and $H_2O_2$) can inhibit the human extracellular SOD activities (Gottfredsen et al. 2013). In this study, SOD activity decreased with increasing $CeO_2$ concentration. Hao et al. (2009) also reported the inhibition of SOD activity in the gills, liver, and brain of carp exposed to 100 and 200 mg/L of $nTiO_2$ as an indication of oxidative stress. The minimum concentration of bulk $CeO_2$ (100 mg/L) required to cause a significant decrease in the SOD and CAT activities was 3 folds higher than the concentration of nano $CeO_2$ (32 mg/L) tested in this study. On other hand, the inhibition of CAT and SOD activities by a variety of toxicant and consequent oxidative stress have been reported in few studies (Bagnyukova et al. 2005, Vander et al. 2003). The exposure of Ni (nickel) NPs to fish (*Oreochromis mossambicus*) showed a concentration-dependent decrease in SOD activity, which contributes to the oxidative stress condition (Jayaseelan et al. 2014). Afifi et al. (2016) and Saddick et al. (2015) also suggested the inhibition of CAT and SOD activities in fish cells (*Oreochromis niloticus* and *Tilapia zillii*) exposed to Zn and Ag NPs. Such inhibition of SOD and CAT activities upon exposure to $CeO_2$ would enhance

the generation of ROS (Fig. 5a, b) and produce serious oxidative damage to tissues. Further, the decrease in GST activity was found to be dependent on the concentration of nano and bulk $CeO_2$. Our results can be corroborated with the study by Ali and Ali (2015), wherein they have reported the inhibition of GST activity in a freshwater snail, *Lymnea luteola*, upon exposure to CuO NPs as a result of the oxidative stress in the cells. Thus, the exposure of $CeO_2$ particles induces significant changes in the antioxidant defense system of *A. salina* in response to the uptake and accumulation of these particles in body.

## AChE activity

AChE activity is used as a biomarker of nanoparticle toxicity (Wang et al. 2009b). The ingestion and accumulation of $CeO_2$ particles could have caused internal damage to tissues, and as a result, this might have direct influence on the biochemical enzymes. Our results showed the concentration- and particle size-dependent increase in AChE activity. An apparent increase in the AChE activity was detected in *D. magna* (Ulm et al. 2015) and Rainbow trout (Johari et al. 2013), following exposure to Ag NPs. They reported that the elevated AChE activity upon NP exposure could deplete the neurotransmitter level, which interacts with the acetylcholine receptor. Similarly, scallops (*Chlamys farreri*) exposed to $TiO_2$ NPs had elevated AChE activity in the gills and digestive gland (Xia et al. 2017). Gamberdella et al. 2013 reported a dose-dependent increase in the AChE activity in larvae of sea urchin. The increase in AChE activity led to tissue damage upon exposure to $CeO_2$ particles. Impairment of AChE activity can cause both behavioral and physiological consequences on *A. salina*. Behavioral response is the ability of organisms to manage with its surrounding environment to reproduce and survive (Engenheiro et al. 2005). Increase in AChE activity causes alteration in swimming behavior of *A. salina* (Gamberdella et al. 2014). Xuereb et al. (2009) reported that the impairment of feeding rate and locomotion bevaiour of *Gammarus fossarum* directly correlates with the AChE activity. Changes in feeding behavior as a result of AChE activity was observed by Baird et al. 2007. These effects can have direct consequences on the organism. Based on our results, it can be concluded that nano and bulk $CeO_2$ exerted size- and dose-based toxicity response in *A. salina*. Further confirmation at the molecular level would shed light on the detailed mechanism of toxicity. These findings provide a fundamental understanding of the behavior and toxicity of $CeO_2$ particles in the marine environment.

## Conclusion

This study aims to assess the toxic effect of nano and bulk cerium oxide particles towards *A. salina*. The primary particle size (TEM and SEM) and stability studies clearly revealed that the size of nano $CeO_2$ was lesser than the bulk particles. Higher uptake and

accumulation of nano CeO$_2$ induced deleterious changes to the physiological process of *A. salina*. ROS generation was observed as the major toxicity mechanism exhibited by both nano and bulk CeO$_2$ particles. Higher cholinesterase expression in *Artemia* tissues exposed to cerium particles was seen. As a result, higher toxicity of nano CeO$_2$ was observed on *A. salina* as compared to the bulk form of particles. This study adds knowledge on the differential effect of nano and bulk CeO$_2$ on *A. salina* based on stability and exposure concentration. The observed results indicated that the toxicity was found to be dependent on the concentration of CeO$_2$ particles and its stability in seawater. Future studies will focus on the effect of cerium oxide particles on different stages of *A. salina* and trophic transfer potential in the ecosystem.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no competing interests.

## References

Adam N, Leroux F, Knapen D, Bals S, Blust R (2015) The uptake and elimination of ZnO and CuO nanoparticles in Daphnia magna under chronic exposure scenarios. Water Res 68:249–261

Afifi M, Saddick S, Zinada OAA (2016): Toxicity of silver nanoparticles on the brain of Oreochromis niloticus and Tilapia zillii. Saudi J Biol Sci 23:754–760

Ali D, Ali H (2015) Susceptibility of the freshwater pulmonate snail Lymnea luteola L. to copper oxide nanoparticle. Toxicol Environ Chem 97:576–587

Angel BM, Vallotton P, Apte SC (2015) On the mechanism of nanoparticulate CeO2 toxicity to freshwater algae. Aquat Toxicol 168:90–97

Arnold M, Badireddy A, Wiesner M, Di Giulio R, Meyer J (2013) Cerium oxide nanoparticles are more toxic than equimolar bulk cerium oxide in Caenorhabditis elegans. Arch Environ Contam Toxicol 65:224–233

Artells E, Issartel J, Auffan M, Borschneck D, Thill A, Tella M, Brousset L, Rose J, Bottero J-Y, Thiéry A, Shah V (2013) Exposure to Cerium Dioxide Nanoparticles Differently Affect Swimming Performance and Survival in Two Daphnid Species. PLoS ONE 8(8):e71260

Arulvasu C, Jennifer SM, Prabhu D, Chandhirasekar D (2014) Toxicity effect of silver nanoparticles in brine shrimp Artemia. Sci World J 2014 1–10 (e256919)

Ates M, Daniels J, Arslan Z, Farah IO, Rivera HF (2013) Comparative evaluation of impact of Zn and ZnO nanoparticles on brine shrimp (Artemia salina) larvae: effects of particle size and solubility on toxicity. Environ Sci Process Impacts 15:225–233

Auffan M, Bertin D, Chaurand P, Pailles C, Dominici C, Rose J, Bottero J-Y, Thiery A (2013) Role of molting on the biodistribution of CeO 2 nanoparticles within Daphnia pulex. Water Res 47:3921–3930

Badawy AME, Luxton TP, Silva RG, Scheckel KG, Suidan MT, Tolaymat TM (2010) Impact of environmental conditions (pH, ionic strength, and electrolyte type) on the surface charge and aggregation of silver nanoparticles suspensions. Environ Sci Technol 44:1260–1266

Bagnyukova TV, Storey KB, Lushchak VI (2005) Adaptive response of antioxidant enzymes to catalase inhibition by aminotriazole in

goldfish liver and kidney. Comp Biochem Physiol B: Biochem Mol Biol 142:335–341

Bai W, Zhang Z, Tian W, He X, Ma Y, Zhao Y, Chai Z (2010) Toxicity of zinc oxide nanoparticles to zebrafish embryo: a physicochemical study of toxicity mechanism. J Nanopart Res 12:1645–1654

Baird DJ, Brown SS, Lagadic L, Liess M, Maltby L, Moreira-Santos M, Schulz R, Scott GI (2007) In situ-based effects measures: determining the ecological relevance of measured responses. Integr Environ Assess Manag 3:259–267

Bhuvaneshwari M, Iswarya V, Nagarajan R, Chandrasekaran N, Mukherjee A (2016) Acute toxicity and accumulation of ZnO NPs in Ceriodaphnia Dubia: relative contributions of dissolved ions and particles. Aquat Toxicol 177:494–502

Bhuvaneshwari M, Sagar B, Doshi S, Chandrasekaran N, Mukherjee A (2017) Comparative study on toxicity of ZnO and TiO2 nanoparticles on Artemia salina: effect of pre-UV-A and visible light irradiation. Environ Sci Pollut Res 24:5633–5646

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72(1-2):248–254

Casals E, Pfaller T, Duschl A, Oostingh GJ, Puntes V (2010) Time evolution of the nanoparticle protein corona. ACS Nano 4:3623–3632

Chinnapongse SL, MacCuspie RI, Hackley VA (2011) Persistence of singly dispersed silver nanoparticles in natural freshwaters, synthetic seawater, and simulated estuarine waters. Sci Total Environ 409:2443–2450

Conway JR, Hanna SK, Lenihan HS, Keller AA (2014) Effects and implications of trophic transfer and accumulation of CeO2 nanoparticles in a marine mussel. Environ Sci Technol 48:1517–1524

Dahle JT, Arai Y (2015) Environmental geochemistry of cerium: applications and toxicology of cerium oxide nanoparticles. Int J Environ Res Public Health 12:1253–1278

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95

Engenheiro EL, Hankard PK, Sousa JP, Lemos MF, Weeks JM, Soares AM (2005) Influence of dimethoate on acetylcholinesterase activity and locomotor function in terrestrial isopods. Environ Toxicol Chem 24:603–609

Feswick A, Griffitt RJ, Siebein K, Barber D (2013) Uptake, retention and internalization of quantum dots in Daphnia is influenced by particle surface functionalization. Aquat Toxicol 130:210–218

Gambardella C, Aluigi MG, Ferrando S, Gallus L, Ramoino P, Gatti AM, Rottigni M, Falugi C (2013) Developmental abnormalities and changes in cholinesterase activity in sea urchin embryos and larvae from sperm exposed to engineered nanoparticles. Aquat Toxicol 130:77–85

Gambardella C, Mesarič T, Milivojević T, Sepčić K, Gallus L, Carbone S, Ferrando S, Faimali M (2014) Effects of selected metal oxide nanoparticles on Artemia salina larvae: evaluation of mortality and behavioural and biochemical responses. Environ Monit Assess 186:4249–4259

Garaud M, Trapp J, Devin S, Cossu-Leguille C, Pain-Devin S, Felten V, Giamberini L (2015) Multibiomarker assessment of cerium dioxide nanoparticle (nCeO 2) sublethal effects on two freshwater invertebrates, Dreissena polymorpha and Gammarus roeseli. Aquat Toxicol 158:63–74

García MA (2011) Surface plasmons in metallic nanoparticles: fundamentals and applications. J Phys D Appl Phys 44:283001

Gottfredsen RH, Larsen UG, Enghild JJ, Petersen SV (2013) Hydrogen peroxide induce modifications of human extracellular superoxide dismutase that results in enzyme inhibition. Redox Biol 1:24–31

Gupta GS, Kumar A, Shanker R, Dhawan A (2016) Assessment of agglomeration, co-sedimentation and trophic transfer of titanium dioxide nanoparticles in a laboratory-scale predator-prey model system. Sci Rep 6:31422

Heckert EG, Seal S, Self WT (2008) Fenton-like reaction catalyzed by the rare earth inner transition metal cerium. Environmental science & technology 42:5014–5019

Hund-Rinke K, Simon M (2006) Ecotoxic Effect of Photocatalytic Active Nanoparticles (TiO2) on Algae and Daphnids (8 pp). Environ Sci Pollut Res Int 13(4):225–232

Jayaseelan C, Rahuman AA, Ramkumar R, Perumal P, Rajakumar G, Kirthi AV, Santhoshkumar T, Marimuthu S (2014) Effect of sub-acute exposure to nickel nanoparticles on oxidative stress and histopathological changes in Mozambique tilapia, Oreochromis mossambicus. Ecotoxicol Environ Saf 107:220–228

Jemec A, Tišler T, Drobne D, Sepčić K, Jamnik P, Roš M (2008) Biochemical biomarkers in chronically metal-stressed daphnids. Comp Biochem Physiol C Toxicol Pharmacol 147:61–68

Jemec A, Djinović P, Črnivec IGO, Pintar A (2015) The hazard assessment of nanostructured CeO2-based mixed oxides on the zebrafish Danio rerio under environmentally relevant UV-A exposure. Sci Total Environ 506-507:272–278

Johari S, Kalbassi M, Soltani M, Yu I (2013) Toxicity comparison of colloidal silver nanoparticles in various life stages of rainbow trout (Oncorhynchus mykiss). Iran J Fish Sci 12:76–95

Johnston BD, Scown TM, Moger J, Cumberland SA, Baalousha M, Linge K, van Aerle R, Jarvis K, Lead JR, Tyler CR (2010) Bioavailability of nanoscale metal oxides TiO2, CeO2, and ZnO to fish. Environ Sci Technol 44:1144–1151

Kaszuba M, McKnight D, Connah MT, McNeil-Watson FK, Nobbmann U (2008) Measuring sub nanometre sizes using dynamic light scattering. J Nanopart Res 10:823–829

Keller AA, Wang H, Zhou D, Lenihan HS, Cherr G, Cardinale BJ, Miller R, Ji Z (2010) Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. Environ Sci Technol 44:1962–1967

Kono Y, M-a T, Asada K (1979) Superoxide dismutases from kidney bean leaves. Plant Cell Physiol 20:1229–1235

Krishnamoorthy K, Veerapandian M, Zhang L-H, Yun K, Kim SJ (2014) Surface chemistry of cerium oxide nanocubes: Toxicity against pathogenic bacteria and their mechanistic study. J Ind Eng Chem 20(5): 3513–3517

Kuang Y, He X, Zhang Z, Li Y, Zhang H, Ma Y, Wu Z, Chai Z (2011) Comparison Study on the Antibacterial Activity of Nano- or Bulk-Cerium Oxide. J Nanosci Nanotechnol 11(5):4103–4108

Lahive E, Jurkschat K, Shaw BJ, Handy RD, Spurgeon DJ, Svendsen C (2014) Toxicity of cerium oxide nanoparticles to the earthworm Eisenia fetida: subtle effects. Environ Chem 11:268–278

Leung YH, Yung MM, Ng AM, Ma AP, Wong SW, Chan CM, Ng YH, Djurišić AB, Guo M, Wong MT (2015): Toxicity of CeO 2 nanoparticles–the effect of nanoparticle properties. Journal of Photochemistry and Photobiology B: Biology 145: 48–59

Lin W, Y-w H, Zhou X-D, Ma Y (2006) Toxicity of cerium oxide nanoparticles in human lung cancer cells. Int J Toxicol 25:451–457

Mannervik B, Nise G (1969) Synthesis and some reactions of the pantetheine-glutathione mixed disulfide. Arch Biochem Biophys 134:90–94

Moiseenko T, Kudryavtseva L (2001) Trace metal accumulation and fish pathologies in areas affected by mining and metallurgical enterprises in the kola region, Russia. Environ Pollut 114:285–297

OECD (2013) Guideline for testing of chemicals, 236. In: Fish embryo acute toxicity(FET) test. France, OECD, Paris Available at: http://www.oecd.org

OECD 202 (2004) Guideline for testing of chemicals. 'Daphnia sp., acute immobilisation test. OECD, Paris

Park E-J, Choi J, Park Y-K, Park K (2008) Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells. Toxicology 245:90–100

Pauluhn J (2009) Pulmonary toxicity and fate of agglomerated 10 nm and 40 nm aluminum oxyhydroxides (AlOOH) following 4-week inhalation exposure of rats: toxic effects are determined by agglomerated, not primary particle size. Toxicol Sci 109:152–67

Pelletier DA, Suresh AK, Holton GA, McKeown CK, Wang W, Gu B, Mortensen NP, Allison DP, Joy DC, Allison MR, Brown SD, Phelps TJ, Doktycz MJ (2010) Effects of Engineered Cerium Oxide Nanoparticles on Bacterial Growth and Viability. Appl Environ Microbiol 76(24):7981–7989

Pulido-Reyes G, Rodea-Palomares I, Das S, Sakthivel TS, Leganes F, Rosal R, Seal S, Fernández-Piñas F (2015) Untangling the biological effects of cerium oxide nanoparticles: the role of surface valence states. Sci Rep 5

Rogers NJ, Franklin NM, Apte SC, Batley GE, Angel BM, Lead JR, Baalousha M (2010) Physico-chemical behaviour and algal toxicity of nanoparticulate CeO2 in freshwater. Environ Chem 7:50–60

Röhder LA, Brandt T, Sigg L, Behra R (2014) Influence of agglomeration of cerium oxide nanoparticles and speciation of cerium (III) on short term effects to the green algae Chlamydomonas reinhardtii. Aquat Toxicol 152:121–130

Rosenkranz P, Chaudhry Q, Stone V, Fernandes TF (2009) A comparison of nanoparticle and fine particle uptake by Daphnia magna. Environ Toxicol Chem 28:2142–2149

Saddick S, Afifi M, Zinada OAA (2015): Effect of zinc nanoparticles on oxidative stress-related genes and antioxidant enzymes activity in the brain of Oreochromis niloticus and Tilapia zillii. Saudi J Biol Sci. https://doi.org/10.1016/j.sjbs.2015.10.021

Sano K, Inohaya K, Kawaguchi M, Yoshizaki N, Iuchi I, Yasumasu S (2008) Purification and characterization of zebrafish hatching enzyme—an evolutionary aspect of the mechanism of egg envelope digestion. FEBS J 275:5934–5946

Stebounova LV, Guio E, Grassian VH (2011) Silver nanoparticles in simulated biological media: a study of aggregation, sedimentation, and dissolution. J Nanopart Res 13:233–244

Ulm L, Krivohlavek A, Jurašin D, Ljubojević M, Šinko G, Crnković T, Žuntar I, Šikić S, Vrček IV (2015) Response of biochemical biomarkers in the aquatic crustacean Daphnia magna exposed to silver nanoparticles. Environ Sci Pollut Res 22:19990–19999

Van der Oost R, Beyer J, Vermeulen NP (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13:57–149

Wang H, Wick RL, Xing B (2009a) Toxicity of nanoparticulate and bulk ZnO, Al 2 O 3 and TiO 2 to the nematode Caenorhabditis elegans. Environ Pollut 157:1171–1177

Wang J, Wang W-X (2014) Low bioavailability of silver nanoparticles presents trophic toxicity to marine medaka (Oryzias melastigma). Environ Sci Technol 48:8152–8161

Wang Z, Zhao J, Li F, Gao D, Xing B (2009b) Adsorption and inhibition of acetylcholinesterase by different nanoparticles. Chemosphere 77:67–73

Ward JE, Kach DJ (2009) Marine aggregates facilitate ingestion of nanoparticles by suspension-feeding bivalves. Mar Environ Res 68:137–142

Xia B, Zhu L, Han Q, Sun X, Chen B, Qu K (2017) Effects of TiO 2 nanoparticles at predicted environmental relevant concentration on the marine scallop Chlamys farreri: an integrated biomarker approach. Environ Toxicol Pharmacol 50:128–135

Xuereb B, Lefèvre E, Garric J, Geffard O (2009) Acetylcholinesterase activity in Gammarus fossarum (Crustacea Amphipoda): linking AChE inhibition and behavioural alteration. Aquat Toxicol 94:114–122

Yang X, Pan H, Wang P, Zhao F-J (2017) Particle-specific toxicity and bioavailability of cerium oxide (CeO 2) nanoparticles to Arabidopsis thaliana. J Hazard Mater 322:292–300

Yu R, Wu J, Liu M, Zhu G, Chen L, Chang Y, Lu H (2016) Toxicity of binary mixtures of metal oxide nanoparticles to Nitrosomonas europaea. Chemosphere 153:187–197

Zhang Y, Chen Y, Westerhoff P, Hristovski K, Crittenden JC (2008) Stability of commercial metal oxide nanoparticles in water. Water Res 42:2204–2212

Zhu X, Wang J, Zhang X, Chang Y, Chen Y (2009) The impact of ZnO nanoparticle aggregates on the embryonic development of zebrafish (Danio rerio). Nanotechnology 20:195103

Zhu X, Wang J, Zhang X, Chang Y, Chen Y (2010) Trophic transfer of TiO 2 nanoparticles from daphnia to zebrafish in a simplified freshwater food chain. Chemosphere 79:928–933