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Toxicity of α-Fe_2O_3 nanoparticles to *Artemia salina* cysts and three stages of larvae

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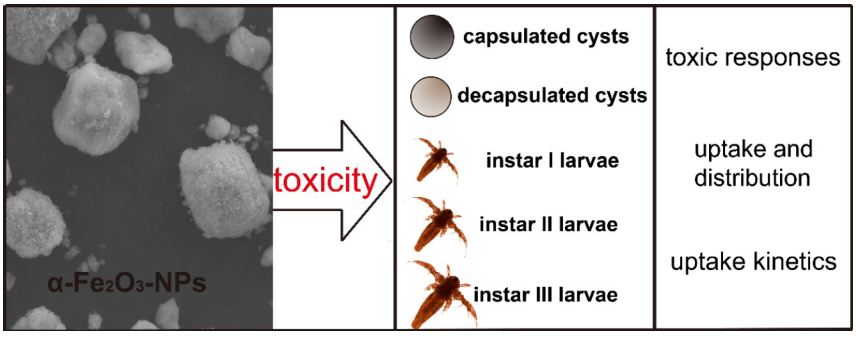
HIGHLIGHTS

- Significant effects on hatchability, mortality, and other end-points
- Effects are accounted for α-Fe_2O_3-NPs and mediated by oxidative stress.
- Instar II larvae show the greatest sensitivity to α-Fe_2O_3-NPs.
- NPs were distributed in nephridial duct, primary body cavity and intestine.
- The uptake kinetics was shown.

GRAPHICAL ABSTRACT



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ABSTRACT

Artemia salina cysts (capsulated and decapsulated) and larvae (instar I, II and III) were exposed to α-Fe_2O_3 nanoparticles (α-Fe_2O_3-NPs) to evaluate the effects on marine ecosystems. Hatchability, mortality and a number of ethological, morphological and biochemical parameters were selected as end-points to define the toxic responses. Results indicate that the hatchability of capsulated and decapsulated cysts was significantly decreased ($p < 0.01$) following exposure to 600 mg/L at 12, 18, 24 and 36 h. Both increases of mortality and decreases of swimming speed were shown concentration-dependent manners. The LC_{50} values for instar II and III were 177.424 and 235.495 mg/L, respectively (not calculable for instar I), the EC_{50} values for instar I, II and III were 259.956, 99.064 and 129.088 mg/L, respectively. Instar II larvae show the greatest sensitive to α-Fe_2O_3-NPs, and followed by instar III, instar I, decapsulated cysts and capsulated cysts. Body lengths and individual dry weight of instar I, II and III larvae were decreased following exposure. α-Fe_2O_3-NPs attached onto the gills and body surface of larvae, resulting in irreversible damages. All of malondialdehyde content, total antioxidant capacity, reactive oxygen species and antioxidant enzymes activities were substantially increased in dose-dependent manners after exposure to α-Fe_2O_3-NPs suspensions, indicating that toxic effects were mediated by oxidative stress. Finally, the uptake result indicated that α-Fe_2O_3-NPs were ingested and distributed in the nephridial duct, primary body cavity and intestine of *A. salina*. Moreover, the uptake kinetics data show that the maximum α-Fe_2O_3-NPs content (8.818 mg/g) was reached at 36 h, and a steady state was reached after 60 h. The combined results indicate that α-Fe_2O_3-NPs have the potential to affect aquatic life when released into the marine ecosystems.

1. Introduction

With the rapid development of nanotechnology, tremendous interest has arisen in the field of nano-materials. Due to their distinctive

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characteristics, nanomaterials have broad applications in various fields (Aragay and Merkoçi, 2012; Giersig and Khomutov, 2008; Xu et al., 2012). As one of the most important magnetic nanomaterials, α-Fe_2O_3 nanoparticles (α-Fe_2O_3-NPs) are being used in broad areas, such as sewage treatment, biomedical, electrochemical and photocatalytic applications (Alagiri and Hamid, 2014; Gao et al., 2009; Predescu and Nicolae, 2012; Wu et al., 2008). According to a previous report, the global market for magnetic nanoparticles in electronic, magnetic, and optoelectronic applications was exceeded $1.7 billion by 2012 (http://www.nanotechwire.com/news.asp?nid=5395).

With the rapidly increasing production and application of α-Fe_2O_3-NPs worldwide, they will be likely released into the environment at significant levels. Considerable α-Fe_2O_3-NPs release could occur during their applications, such as wastewater treatment (Predescu and Nicolae, 2012), biomedical and architectural application (Basilevsky and Shamov, 2003; Khoshakhlagh et al., 2012). In addition, α-Fe_2O_3-NPs could be introduced into the environment with the discharge of wastewater from the production processes. Eventually, most of the released nanoparticles will enter into the aquatic environment, especially into marine environment (Chen et al., 2012; Scown et al., 2010). Therefore, the subsequent impacts of α-Fe_2O_3-NPs to marine ecosystem have drawn significant attentions.

In recent years, using aquatic invertebrates as models to assess toxicological effects of environmental contaminants has become prevalent (Hu et al., 2012). *Artemia salina* (*A. salina*) is an invertebrate zooplankton found in various marine ecosystems. As one of the most popular live foods for fish larvae, *A. salina* plays a pivotal role in the energy flow of the food chains (Nunes et al., 2006). *A. salina* is a non-selective filter feeder, and filters a lot of water per hour. Therefore, it has significant interactions with aquatic environment, causing it faces a higher risk exposure to environmental contaminants compared with other aquatic species (Ates et al., 2015; Nunes et al., 2006). The intrinsic features of *A. salina* turn it into a suitable organism for studies in toxicology. For example, according to the differences in tissue differentiation and morphological characteristics, many stages are divided along the development process of *A. salina*. Previous studies showed that *A. salina* larvae exhibit discrepant sensitivity to pollutants in relation to the stages (Barahona and Sánchezfortún, 1996; Caldwell et al., 2003; Sorgeloos et al., 1979). Besides, varied end-points can be selected as criterions for toxicological evaluation, such as hatchability, mortality, and a number of ethological, morphological and biochemical parameters (Ates et al., 2016; Caldwell et al., 2003). For aquatic organisms, swimming represents an ethological response determinant that can be directly affected by physiological status (Gambardella et al., 2014). Biochemical parameters of oxidative stress have been proposed for the evaluation of potential toxic effects of NPs, such as reactive oxygen species (ROS), malondialdehyde (MDA) and antioxidant enzymes activities (Ates et al., 2013b; Gambardella et al., 2014). In recent years, an increasing number of studies have investigated the effects of nanoparticles (e.g., TiO_2, Al_2O_3 and NiO NPs) on *A. salina* (Ates et al., 2013a, 2016, 2015). Nevertheless, the related information in concerning the effects of α-Fe_2O_3-NPs on *A. salina* is currently limited.

The present study was conducted to evaluate the acute toxicity of α-Fe_2O_3-NPs on both cysts (capsulated and decapsulated) and larvae (instar I, II and III) of *A. salina*. Hatchability, mortality, and a number of ethological, morphological and biochemical parameters were selected as end-points to define the toxic responses. Microscope and transmission electron microscope (TEM) were used to observe the uptake and distribution of α-Fe_2O_3-NPs in *A. salina*. Moreover, the uptake kinetics of α-Fe_2O_3-NPs in *A. salina* was assessed.

2. Materials and methods

2.1. Preparation and characterization of α-Fe_2O_3-NPs

The α-Fe_2O_3-NPs were purchased from Beijing Dk Nano technology Co., Ltd. (Beijing, China), and the structural parameters are listed in Table S1. Scanning electron microscopy (SEM) analysis was carried out on a Hitachi S-4800 electron microscope (Japan) with an accelerating voltage of 15 kV. TEM observations were made on a JEM-1200EX electron microscope (Japan) operating at 80–100 kV. Fourier transform infrared (FTIR) spectra were recorded from 400 to 800 cm^{-1} with a Bruker Vetex70 spectrophotometer (Germany) using KBr pellet technique (Wang et al., 1998). X-ray diffraction (XRD) analysis was performed on a Bruker D8 Advance diffractometer (Germany) with CuKα radiation (λ = 1.54060 Å), and the sample was scanned from 10° to 80° (2θ) with a scanning rate of 1° min^{-1}. Diffraction peaks were compared with those of standard compounds reported in the JCPDS data file. α-Fe_2O_3-NPs were suspended in filtered natural seawater (FNSW; 30‰ m/v; pH 8.6) to create suspensions with concentration as required. To assess Fe^{3+} released from α-Fe_2O_3-NPs, the suspensions were centrifuged at 12,000 rpm for 30 min to pellet α-Fe_2O_3-NPs. The Fe^{3+} content in supernatants were then determined using inductively coupled plasma mass spectrometry (ICP-MS, Jarrell-Ash, MA). A dynamic light scattering (DLS, Brookhaven BI-200SM, USA) was used to estimate the hydrodynamic size distribution of α-Fe_2O_3-NPs in FNSW.

2.2. Model organism

The *A. salina* cysts were purchased from Binzhou Haifa Biological Technology Co., Ltd. (Shandong, China). The dehydrated cysts were first hydrated in distilled water at 4 °C for 12 h, and the sunken cysts were collected on a Buchner funnel. In order to acquire decapsulated cysts, a solution of NaOCl, NaOH and water was used as described by Sorgeloos et al. (1986). Approximately 2 g of cysts were incubated in 1 L FNSW in a hatcher at 28 °C, with a continuous 1300 lx light regime and strong aeration. Instar I, II and III larvae were obtained by using the procedure described by Sorgeloos et al. (1979). Briefly, to obtain a population consisting only of instar I, the larvae were separated from the unhatched cysts within 2 h after the first free-swimming larva was observed. One-third of the population was used immediately for the tests on the instar I larvae, and the other larvae were maintained for another 24 and 48 h to obtain instar II and III larvae, respectively.

2.3. Hatching assay

The capsulated and decapsulated cysts were cultivated in α-Fe_2O_3-NPs suspensions (0, 25, 50, 100, 200, 400 and 600 mg/L) to study the effects of α-Fe_2O_3-NPs on the hatchability. In order to evaluate the influence of Fe^{3+} released from α-Fe_2O_3-NPs on the hatchability, the α-Fe_2O_3-NPs suspensions were centrifuged at 12,000 rpm for 30 min and the capsulated and decapsulated cysts were cultivated in the supernatants. Hatching assay was performed in 24-well plates, and each well contained 1 mL test solution. Ten capsulated/decapsulated cysts were introduced into each well, and each treatment was taken out in octuplicate. All plates were incubated under a continuous illumination with shaking at 28 °C. The hatchability was detected using a microscope (Olympus Optical Co., Ltd., Tokyo, Japan) at 12, 18, 24 and 36 h.

2.4. Acute toxicity test

The acute toxicity test was performed by adding 10 larvae (instar I, II and III) to each well of 24-well plates that contained 1 mL of α-Fe_2O_3-NPs suspensions (0, 25, 50, 100, 200, 400 and 600 mg/L) or supernatants. The plates were incubated at 28 °C with shaking under a 16:8 h light/dark cycle. The larvae were not fed during the test. All of the tests were taken out in octuplicate. After 24 h, the numbers of dead larvae (completely motionless) were counted using a microscope (Olympus Optical Co., Ltd., Tokyo, Japan).

Larvae (instar I, II and III) were also randomly distributed into beakers (approximately 1000 larvae in each beaker) containing 100 mL of α-Fe_2O_3-NPs suspensions, and cultured as described above. After 24 h, the larvae were randomly sampled immediately prepared for