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Toxicity of lead on *Ceriodaphnia dubia* in the presence of nano-CeO_2 and nano-TiO_2

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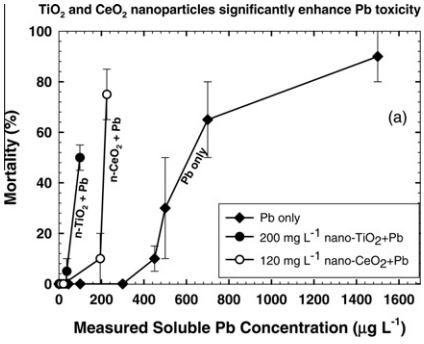
HIGHLIGHTS

- Pb is significantly accumulated on the surface of CeO_2 and TiO_2 nanoparticles (NPs).
- NPs are significantly ingested in the gastrointestinal tract of *Ceriodaphnia dubia*.
- The ingestion of Pb-loaded NPs enhances the overall toxicity of Pb.
- Reducing pH enhances the overall toxicity of Pb, with or without NPs.

GRAPHICAL ABSTRACT



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ABSTRACT

Although engineered nanoparticles (NPs) could negatively impact environmental organisms, the synergistic effect of NPs and other toxic substances, which could be more significant than that of NP alone, have seldom been examined. The effect of two common NPs, nano-CeO_2 and nano-TiO_2, on the toxicity of Pb was evaluated using *Ceriodaphnia dubia* (*C. dubia*) as the model organism. Standard EPA procedures were followed in the toxicity evaluation. The toxicity of bare NPs (without Pb) was first evaluated and safe doses (levels without causing lethal effect) of NPs were used in the synergistic studies. It was found that the overall toxicity of Pb in the system containing NPs was greater than that of Pb alone, as indicted by the reduced median lethal concentration (LC_{50}) of soluble Pb. The sorption of Pb onto the NP, and the uptake of NPs in the gastrointestinal tract of *C. dubia* were validated. Therefore, the uptake of Pb-loaded NPs increased the exposure of *C. dubia* to Pb, resulting in the enhanced toxicity. Reducing the solution pH could shift Pb speciation and enhance the overall toxicity of Pb, with or without the presence of NPs.

1. Introduction

Lead (Pb) is regulated by US EPA with an action level of 15 μg L^{-1} in its drinking water regulations (USEPA, 2007). Different concentrations of Pb were reported in water (Hu et al., 2010). The toxicity of Pb on different organisms including *Ceriodaphnia dubia* (*C. dubia*) has been well documented in the literature (Cooper et al., 2009). Similar to other heavy metals, the aquatic chemistry (e.g., pH) and concentrations of natural organic matters impact Pb toxicity (Schubauer-Berigan et al., 1993; Ryan et al., 2004). Metal chelates and solids reduced Pb toxicity when the total metal concentration was used as the dosage measure (Kim et al., 2001; Ma et al., 2002), and the toxic effect of heavy metals including Pb was highly correlated to the free metal ion concentration (Kim et al., 1999).

Because of the rapid development of nanotechnology, the release of engineered nanoparticles (NPs) is expected to increase significantly in the near future. The health concerns about NPs arise due to their characteristic properties (Oberdörster et al., 2005). As a new class of emerging contaminants, NPs could be present in the natural environment or sewage systems (Brar et al., 2010). However, the toxicity of many NPs to aqueous species is not clear due to the lack of information, although limited publications have

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indicated a case-by-case toxic response from bare NPs (Oberdörster, 2010). Different results have been reported, even for the same type of NP (Lam et al., 2006; Ren et al., 2010). In the realistic environment, different types of toxic substances, such as heavy metals, are present at various concentrations. The interactions of heavy metals with NPs could play an important role in overall toxicity. The significant differences of NPs from their larger-sized counterparts are their specific aggregation/agglomeration behavior and surface characteristics, including reactivity, sorption capacity, and larger specific surface area (Oberdörster et al., 2005). NPs could enter the gastrointestinal tract of different organisms as a fake food or by attaching onto the body surface. As a result, the toxicity of heavy metals in the presence of NPs is much more complex. Interactions of organisms occur with not only soluble metal ions but also NPs that carry sorbed heavy metals on the surface. For example, our previous research indicated that the toxicity of a representative toxic anion, As(V), to *C. dubia* could be significantly enhanced in the presence of specific amounts of nano-TiO_2 (Wang et al., 2011a), nano-Al_2O_3 (Wang et al., 2011b), and nano-Fe_2O_3(magnetic) (Hu et al., 2012).

The objective of this research was to explore the synergistic effect between Pb and selected NPs (i.e., nano-CeO_2 and nano-TiO_2) on the overall toxicity to *C. dubia*. The toxicity of soluble Pb alone and bare NPs on *C. dubia* was also investigated to provide as background information for toxicity comparisons.

2. Materials and methods

2.1. Chemicals and NPs

Chemicals used to prepare the culture medium and the Pb stock solution including $CaSO_4{\cdot}2H_2O$ (98%), KCl (99%), Na_2SeO_4 (99%), $CuCl_2{\cdot}2H_2O$, $NaHCO_3$ (100.2%, Pb < 5.0 mg kg^{-1}), $MgSO_4$ (Pb < 0.001%), $Pb(NO_3)_2$, and HNO_3 (67% in purity) were purchased from Fisher Scientific (Pittsburgh, PA). A Pb standard solution (1000 ± 2 mg L^{-1}) was purchased from PerkinElmer Inc. (Waltham, MA). Nano-CeO_2 (99.9%) and nano-TiO_2 (anatase, 99%) particles (see Table 1 for characterization data) were purchased from Skyspring Nanomaterials Inc. (Houston, TX, USA). Millipore water (>18.2 MΩ cm at 25 °C) for preparation of the culture medium and the Pb stock solution was generated using a Millipore Synergy® ultrapure water system (Billerica, MA).

2.2. Culturing of C. dubia and toxicity test

Commercial starter *C. dubia* was purchased from MBL Aquaculture (Sarasota, FL, USA). *C. dubia* foods YTC (Yeast, Trout chow, and Cereal leaves) and algae (*Selenastrum*) were purchased from ABS Inc. (Fort Collins, CO, USA). In order to limit the interference from airborne particles, a laminar flow hood (SVC-6AX, Streamline® laboratory products, Fort Myers, FL, USA) installed in a temperature control chamber at 25 °C was used for mass culture, individual culture, and toxicity tests. The culture medium was prepared by dissolving appropriate amounts of $NaHCO_3$, $CaSO_4.2H_2O$, $MgSO_4$, KCl, and Na_2SeO_4 into the Millipore water per the EPA standard method (USEPA, 2002). The culture medium was moderately hard synthetic water with a pH of 7.8 ± 0.2 (natural pH) and a hardness of 85 ± 5 mg L^{-1} as $CaCO_3$.

For the toxicity test, 20 healthy neonates aged less than 24 h after a 7-d (three-brood) culture were used at each concentration. Five 30-mL medicine cups (from Fisher) were used as toxicity test reactors. Four neonates were transferred into each after adding 15 mL of culture medium containing toxicants of interest. The EPA standard method (USEPA, 2002) was adapted to examine the static acute toxic effect. QC check was performed by periodically conducting the toxicity test with $CuSO_4$. When the $CuSO_4$ toxicity test yielded expected results, QC was satisfied. In addition, the survival rate of all controls in our toxicity tests exceeded 90%, which guaranteed the validity of results. QC was satisfied by a periodic checking with a $CuSO_4$ solution and larger than 90% survival (negative control only). Median lethal concentration (LC_{50}) and other parameters were calculated using ToxCalc Software from Tidepool Scientific, LLC. (v5.0, McKinleyville, CA).

2.3. Sorption of Pb onto NPs

The interactions of Pb with NPs were first characterized by performing the traditional sorption experiment. A stock solution of Pb(II) (150 mg L^{-1}) was prepared by dissolving a certain amount of $Pb(NO_3)_2$ into one liter of Millipore water, and then acidified by using 0.2% nitric acid to maintain a pH of less than 4.0. Working Pb(II) solutions at different concentrations (normally less than 1.5 mg L^{-1} due to the low solubility at an alkaline pH) were prepared by diluting the stock solution with the culture medium into 50 mL polypropylene conical tubes (Fisher Scientific). After dilution, Pb(II) solutions were mixed in the shaker for 10 min. The measured pH values of working Pb(II) solutions were in the range of 7.6–7.8. Weighed dry NPs (nano-CeO_2 or nano-TiO_2) were added into each tube to reach a concentration of 200 ± 5 mg L^{-1}. The mixed solutions were then shaken for 24 h to achieve the sorption equilibrium. The supernatants were then collected for residual Pb(II) analysis. The accumulation of Pb on the NP surface was characterized by estimating the surface concentration of Pb(II) and energy dispersive spectroscopy (EDS) analysis using scanning electron microscopy (SEM).

2.4. Toxicity of Pb or bare NPs

For the toxicity of Pb, 100 mL of solutions at several concentrations were prepared by diluting the Pb(II) stock solution with the culture medium in six plastic bottles (125 mL, Nalgene). The solutions were mixed and added with foods before performing the toxicity test. The increase in volume for each bottle after the addition of foods was about 1 mL (600 μL algae and 420 μL YTC). Therefore, the change in Pb(II) concentration caused by the food addition was only approximately 1%. Seventy-five millilitre of each solution was used for the toxicity test, which contained five toxicity test reactors, and 15 mL of the solution was distributed to each reactor. The remaining solution was used to analyze the soluble Pb(II) concentration.

For the toxicity of NPs (nano-CeO_2 or nano-TiO_2), solutions at different NP concentrations (50, 100, 200, 500, and 1000 mg L^{-1}) were prepared by adding appropriate amounts of NPs into five bottles (125 mL, Nalgene), each containing 100 mL of the culture medium. A control bottle (without NP) was also included. After adding foods, the solutions were mixed and the pH values were

Table 1
Characterization of interested NPs.

NPs	Nano-TiO_2	Nano-CeO_2
Purity (%)	99.5	99.99
Primary size (nm)	5–10	10–30
Color	White	Yellow green
Hydrodynamic size in culture medium (nm)	30–600	>1000
Preparation method	Sol-gel	Supercritical fluid
Zeta potential in culture medium (mV)	~16	~8
Crystal structure	Anatase	N/A
Specific surface area (m^2 g^{-1})	50	30–50
pH_{zpc}	9.1–9.5	9.3–9.8