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Impact of surface modification on the toxicity of zerovalent iron nanoparticles in aquatic and terrestrial organisms

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ABSTRACT

Nanoscale zerovalent iron (nZVI)-based materials are increasingly being applied in environmental remediation, thereby lead to their exposure to aquatic and terrestrial biota. However, little is known regarding the toxic effects of surface-modified nZVI on multiple species in the ecosystem. In this study, we systematically compared the toxicities of different forms of nZVIs, such as bare nZVI, carboxymethyl cellulose (CMC)-stabilized nZVI, tetrapolyphosphate (TPP)-coated nZVI and bismuth (Bi)-doped nZVI, on a range of aquatic and terrestrial organisms, including bacteria (*Escherichia coli* and *Bacillus subtilis*), plant (*Arabidopsis thaliana*), water flea (*Daphnia magna*) and earthworm (*Eisenia fetida*). The Bi- and CMC-nZVI induced adverse biological responses across all the test systems, except *E. fetida*, varying from cell death in *E. coli* and *B. subtilis* to inhibition of the physiological states in *D. magna* and *A. thaliana*. The particle characterization under exposure conditions indicated that the surface modification of nZVI played a significant role in their toxicities by changing their physicochemical properties. The underlying mechanisms by which nZVI induces toxicity might be a combination of oxidative stress and another mechanism such as cell membrane disruption, chlorosis and hypoxia. Overall, our findings could provide important implications for the development of environment-friendly nanomaterials and direct further ecotoxicological researches regarding interspecies exploration.

1. Introduction

Nanoscale zerovalent iron (nZVI) is the only commercially available engineered nanoparticle that can be injected into the contaminated soil and groundwater on a large scale. Ample literature exists regarding the splendid application prospects of nZVI for the removal of various contaminants. These beneficial properties have led to a rapid increase of site remediation with nZVI in the USA and Europe (Mueller et al., 2012).

The wide range of applications of nZVI in in situ remediation has led to extensive work on nZVI surface modification due to its low transportability and rapid oxidation. Various modification strategies, e.g., secondary metal deposition, sulfidation and polymer coating, have been reported in the literature to overcome the shortcomings of bare nZVI (Kim et al., 2011; Liu et al., 2014; Zhao et al., 2011). Therefore, one-third of the sites were applied the surface-modified nZVIs for the real contaminated soil and groundwater remediation (Karn et al., 2009). However, conformational changes in nZVI can affect particle mobility and reactivity, subsequently altering the fate of nZVI in biological systems and potentially posing risk. Despite the increasing use of surface-modified nZVIs in the field and the intense interest in nanomaterial safety, only a few studies have addressed the ecotoxicological impact of surface-modified nZVIs. Several studies demonstrated that certain surface coatings with carboxymethyl cellulose (CMC) or humic acid alleviated nZVI toxicity toward bacteria by limiting particle adhesion to cells (Li et al., 2010). In contrast, bimetallic nZVI in oxygenic water enhanced the production of reactive oxygen species (ROS) and thus its cytotoxicity (Kim et al., 2014a).

The toxic action of nanomaterials varies considerably depending on the test organisms in addition to their physicochemical properties and concentrations (Nel et al., 2009). Thus far, most of the studies have been focused on the toxicity of nZVI on each species in the ecosystem. In addition, compared to the amount of literature on bacteria, available data on the adverse biological impact on a range of aquatic and terrestrial organisms is limited. It was found that the toxic effects on medaka fish were noticeable at the nZVI concentrations of 50–100 mg L^{-1} (Chen et al., 2012), whereas higher order organisms, i.e., plant and earthworm, required relatively higher nZVI

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concentrations for induction of observable toxicities. According to El-Temsah and Joner, the median effective concentrations estimated from the avoidance data of earthworm species were > 500 mg nZVI kg^{-1} (El-Temsah and Joner, 2012a). The phytotoxicity of nZVI was assessed in *Lepidium sativum* and *Sinapis alba*, with no significant inhibition on seed germination at approximately 300 mg L^{-1} (Libralato et al., 2016). Extrapolating effects of toxicants from a single test species to the ecosystem as a whole is an essential part of environmental risk assessment. However, the limited number of test species can't take into account the interactions between the toxicants. nZVI can be transferred from injection sites to soil and groundwater streams via waterborne transportation (Karn et al., 2009; Mueller et al., 2012), their toxic effects should be considered at multiple levels of organization along a potential exposure route to provide a more realistic risk assessment.

In the present study, the toxicities of surface-modified nZVIs in various aquatic and terrestrial organisms (bacteria, water flea, plant and earthworm) were systematically compared with bare nZVI through various ecotoxicity tests. The test species and endpoints were chosen under the worst assumption that nZVI has spread to aquatic and terrestrial environments at high concentrations. Three different types of surface-modified nZVIs [CMC-stabilized nZVI (CMC-nZVI), tetrapolyphosphate-coated nZVI (TPP-nZVI) and bismuth-iron bimetallic nanoparticle (Bi-nZVI)] were employed because of their effectiveness and attentions, as reported in previous studies (Bokare et al., 2010; Gong et al., 2015; Kim et al., 2015a). Additionally, we aimed to determine how the toxicity relates to the test species and the physicochemical properties of each surface-modified nZVIs.

2. Materials and methods

2.1. Preparation of nZVIs

Four different nanomaterials were used in this study. Bare nZVI (Nanofer STAR) was commercially obtained from NANOIRON (Czech Republic). CMC-, TPP- and Bi-nZVIs were prepared using the same procedure as described in previous works (Bokare et al., 2010; Gong et al., 2015; Kim et al., 2015a). CMC- and Bi-nZVI were produced through the reduction of a Fe(II)–CMC mixture ([CMC] / [Fe(II)] = 0.2) and Bi-Fe mixture ([Bi(III)] / [Fe(II)] = 0.07) with $NaBH_4$, respectively. TPP-nZVI was prepared by mixing an aliquot of nZVI with TPP in a pH-adjusted solution.

2.2. Particle characterization

The particle size, morphology and elemental analysis of the products were analyzed using high-resolution transmission electron microscopy (HR-TEM, JEM-2010, Jeol Ltd) coupled with electron energy loss spectroscopy (EELS). Hydrodynamic diameter and ζ-potential measurements of nZVIs dispersed in deionized (DI) water or media were performed on a zetasizer (Nano ZS90, Malvern Instruments). Surface area analysis was conducted using the Brunauer-Emmett-Teller (BET) method with a micropore physisorption analyzer (ASAP-2020 M, Micrometrics). The dissolved iron concentration of each sample was measured with an inductively coupled plasma-optical emission spectrometer (ICP-OES, Optima 7000 DV, PerkinElmer).

2.3. Test organism and toxicity assessment

The model organisms used in the present study were two bacterial strains (Gram-positive *Bacillus subtilis* and Gram-negative *Escherichia coli*), a water flea (*Daphnia magna*), a plant (*Arabidopsis thaliana*) and an earthworm *(Eisenia fetida*).

2.3.1. Bacteria

E. coli and *B. subtilis* (from the Korean Agricultural Culture Collection) were grown aerobically at 37 °C and 30 °C, respectively. The bacterial toxicity was examined in terms of cell viability, intracellular ROS and cellular damage. Cell viability was assessed using Fluorescein Diacetate (FDA). The bacterial suspensions were treated with various concentrations of nZVIs (1–500 mg L^{-1}) in distilled water and incubated for 24 h in a shaking incubator at 150 rpm. Control and nZVIs-treated bacteria suspensions were washed, and then FDA was added to the suspensions to a final concentration of 10 μM. The samples were incubated for 30 min and the fluorescent signal was measured at excitation wavelength of 485 nm and emission wavelength of 530 nm using Fluorescence spectrometer (Fluoromax 4, Horiba). The cell viability was calculated by comparing between the control group and nZVIs-treated bacteria after 24 h of incubation. The equation for the cell viability (%) for Fig. 1:

$$Cell\ viability\ inhibition\,(\%) = \left(1 - \frac{[FDA]_{sample}}{[FDA]_{control}}\right) \times 100$$

The intracellular ROS in nZVIs-treated cells were measured using 2', 7'-dichlorofluorescein diacetate (DCF-DA). The experimental method was same as the FDA method, but different incubation time (10 min) and dye concentration (2 μM) were applied. The fluorescent signal was measured at an excitation wavelength of 488 nm. To check the cellular damage by nZVIs, Fourier transform infrared spectroscopy (FTIR) analysis has been used. The bacterial suspensions were treated with 100 mg L^{-1} of nZVIs in distilled water. After 24 h of treatment, the suspensions were centrifuged to collect the pellets. The bacterial pellets were dried overnight at 60 °C in the vacuum oven and analyzed using FTIR (iS50, Thermo) in 800–3000 cm^{-1} range. For TEM imaging, the nZVI treated and control cells were fixed in formaldehyde and glutaraldehyde, dehydrated in ethanol, and embedded into EMBed 812 resin and propylene oxide. After sample was sectioned by an ultratome, analysis of bacterial sections was carried out using the TEM (H-7600, Hitachi).

2.3.2. Plant

The seeds of *A. thaliana* ecotype Columbia were sterilized using 70% ethanol with 20% Clorox and washed five times with distilled water. Seeds were germinated in hydroponics or soil for 30 days in a plant growth chamber (DS-330DHL, Daewon Sci., Korea) at 22–24 °C and 60% humidity with a 16:8 h (light: dark) photoperiod. In the hydroponic culture, the nZVI slurry was mixed with 1/2 Murashige and Skoog medium (MS; Duchefa Biochemie, Netherlands) at pH 5.8 (adjusted with 0.1 N KOH, Sigma-Aldrich, USA). In the soil culture, the slurry of nZVI was added to the test soil (purchased from Hungnong Co., Korea) to make the concentration 500 mg kg^{-1}. The toxicity in *A. thaliana* was assessed based on the following endpoints: seed germination (%), root lengths (mm), relative biomass (%), H_2O_2 imaging, and iron accumulation. After 30 days of growth, weights of the harvested plants were quantified. The weight measurements were conducted on the shoots except roots. Average weights of three groups consisting of ten individual plants were measured. For H_2O_2 detection, segments of roots and leaves were incubated in 25 μM DCF-DA for 30 min in the dark and imaged using a confocal microscope (FV 1000, Olympus) in 485–530 nm wavelength. To determine the iron accumulation in tissues, freeze-dried plant samples were dissolved in 60% HNO_3 at 120 °C overnight. After diluting the sample, the element contents were measured using an ICP-OES.

2.3.3. Water flea

D. magna was cultured in M4 medium prepared according to the Organization for Economic Co-operation and Development (OECD) test guideline (pH; 7.8 ± 0.1, hardness; 250 ± 25 mg $CaCO_3$ L^{-1}) at 20 ± 1 °C with a 16:8 h (light:dark) photoperiod. The green algae, *Pseudokirchneriella subcapitata* and a mixture (yeast and CEROPHYLL®) were provided as food on a daily basis. The acute toxicity tests for *D. magna* were conducted in accordance with the OECD Test Guidelines