Research Article

Preparation, Characterization, and Biotoxicity of Nanosized Doped ZnO Photocatalyst

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Metal-doped nanosized ZnO (nZnO) photocatalyst has been widely used for its typical properties and has thus gained considerable attention. In this study, five types of nZnO (nondoped nZnO, iron- (Fe-) doped nZnO, cobalt- (Co-) doped nZnO, nickel- (Ni-) doped nZnO, and manganese- (Mn-) doped nZnO) materials were prepared through a wet chemical method and then exposed to *Daphnia magna* (*D. magna*) at low and high concentrations (50 and 250 $\mu\text{g L}^{-1}$). Results showed that the different metal-doped nZnOs had a variety of shapes and sizes and exhibited diverse dissolubility. After exposure tests, Zn accumulation, metallothionein (MT), and malondialdehyde (MDA) contents in *D. magna* under 250 $\mu\text{g L}^{-1}$ were significantly higher than those in the 50 $\mu\text{g L}^{-1}$ groups. Compared with nondoped nZnO, Co-doped and Ni-doped nZnO enhanced Zn accumulation in *D. magna* whereas Mn-doped nZnO reduced such accumulation. MT and MDA contents in metal-doped nZnO (except Ni-nZnO) treatments were lower than those in nondoped nZnO. Zn accumulation showed a negative relationship with dissolved Zn percentage, which can be explained by the swallowing of nZnO particles as an important pathway of *D. magna* ingestion. Sizes, dissolubility, and physiological functions of doping metals were the influencing factors on metal-doped nZnO biotoxicity to *D. magna*.

1. Introduction

ZnO is an excellent photocatalyst because of its high electrical conductivity and optical transmittance in the solar spectrum. Particularly in a nanometer scale, nanosized ZnO (nZnO) exhibits numerous novel characteristics, such as high ratio of surface area to volume, high electron conductivity, and transmission capability, which provides more opportunities for potential applications in the areas of paint industry, biomedicine, and environmental engineering [1, 2].

However, the photocatalytic activity of nZnO is highly dependent on its crystallite size, specific surface area, morphologies, and UV irradiation wavelengths [3, 4]. Numerous studies had proven that modified nZnOs with other elements improve photocatalytic properties. N-containing ZnO showed higher visible-light photocatalytic activity than pure ZnO [5]. ZnO-coated steel mesh has been repeatedly used for over 10 cycles without significant loss of catalyst

mass [6]. The photocatalytic properties of the Co-doped ZnO had also been found to have improved significantly, and the degradation ratio of an organic dye (methyl orange) reached 78% when the doping concentration was 3% [7]. Mn-doped ZnO bleached methylene blue considerably faster than nondoped ZnO upon exposure to visible light [8].

Widespread use has caused these doped nZnO photocatalysts to enter the environment inevitably, thus resulting in intentional and unintentional exposures and giving rise to environmental and health risks [9]. Zn^{2+} ion dissolution, nanoscale size, and oxidative damage comprise the widely accepted potential mechanism of nZnO toxicity [10]. To the best of our knowledge, only the toxicity of Fe-doped nZnO was evaluated by Xia et al. [11], who revealed that Fe doping reduced nZnO dissolution, thus preventing its toxicity to animals and the environment. However, these findings are insufficient to facilitate understanding of the effect of doping on nZnO toxicity.

In this study, we synthesized five doped nZnO photocatalysts (nondoped, Fe-doped, Co-doped, Ni-doped, and Mn-doped) through a wet chemical method. The morphology and dissolubility of these photocatalysts in natural water were characterized. Zn accumulation, metallothionein, and lipid peroxidation contents of *Daphnia magna* (*D. magna*) in response to five nZnO exposures were quantified to evaluate the biotoxicity of nZnO photocatalysts. The influence and relationships of metal-doped nZnO, as well as the toxic effects to *D. magna*, were analyzed and discussed. These results facilitated understanding of the effects of element doping on nZnO photocatalyst toxicity.

2. Materials and Methods

2.1. Preparation of Doped nZnO Photocatalysts. The metal-doped nZnO (M-nZnO) powders were obtained by using a wet chemical method in aqueous solutions as reported previously [12]. First, zinc nitrate hexahydrate [$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$] and hexamethylenetetramine ($\text{C}_6\text{H}_{12}\text{N}_4$) with a certain concentration of Fe (Co, Ni, and Mn) nitrate hexahydrate were separately dissolved in 100 mL of deionized water. The concentrations of each metal dopant were 5% in molarity. Subsequently, the mixture was placed into glass vials and heated at 95°C for several hours to grow metal-doped nZnO. Thereafter, the containers were taken out and cooled naturally to room temperature. The samples were rinsed thoroughly with deionized water and dried at 100°C for 2 h. Finally, metal-doped nZnO (Fe, Co, Ni, and Mn) powders were obtained. The nondoped nZnO samples were synthesized through the same process without adding other metals.

2.2. Characterization of nZnO Photocatalysts. The morphology of five types of nZnO (nondoped nZnO, Fe-nZnO, Co-nZnO, Ni-nZnO, and Mn-nZnO) particles in water was observed by using a JEOL transmission electron microscope (TEM) (JEOL, JEM-2100F), which was operated at 100 kV. The nZnO stocks were under ultrasonic treatment for better dispersion before making TEM samples. Several droplets of nZnO stock solution were injected into the Formvar-coated (Electron Microscopy Sciences, Fort Washington, PA, USA) copper grids using a capillary tube. After drying at room temperature, the samples were placed in the TEM for imaging.

The dissolubility of the five types of nZnO in natural water was investigated through dissolution tests. The nZnOs were dissolved in pH 8.5 natural water contained in 100 mL Erlenmeyer flasks. The concentration of nZnO was $100 \mu\text{g L}^{-1}$. Three replicates were set up for each kind of nZnO. After rapid mixing, the flasks were placed on a shaker at 150 rpm. Then, a 10 mL aliquot was removed at 30, 60, 120, 180, and 240 min. These samples were centrifuged at 12 000 rpm for 10 min with a versatile compact centrifuge (Himac CF 16RX, Hitachi, Tokyo, Japan) to separate nZnO particles. The Zn^{2+} concentration in the supernatant was determined by using inductively coupled plasma mass spectrometry (ICP-MS) and then used to calculate the quantity of dissolved nZnO in the water. The water that was used in both the dissolubility and

toxicity experiments was collected near Huo Qi Ying Bridge ($116^\circ 16' 732 \text{ E}$, $39^\circ 58' 401 \text{ N}$).

2.3. Biotoxicity Test of Doped nZnO Photocatalysts

2.3.1. Toxicity Exposure of *D. magna* to nZnO Photocatalysts. The test organism was *D. magna*, an ecologically important freshwater zooplankton widely used in toxicity tests. nZnO stock suspension was prepared with natural water and dispersed evenly through ultrasonic treatment. nZnO concentrations of stocks were determined according to the metal concentrations of Zn, Fe, Co, Ni, and Mn in the suspension by utilizing inductively coupled plasma atomic emission spectrometry (ICP-AES). The suspension was then diluted with natural water, and two treatment concentrations (50 and $250 \mu\text{g L}^{-1}$) of each nZnO were used in the experiments. The natural water sample without nZnO was used as the control. Two replicates were set up for each treatment, each containing 50 daphnids (1 individual/10 mL) aged 14 d to 21 d. The daphnids were not fed, and the water was not changed during exposure.

2.3.2. Determination of Toxic Effects in *D. magna*. After 48 h of exposure, living daphnids were collected from each treatment and treated similar to the treatment in our previous study [13]. Ten to fifteen daphnids were subsequently dried at 80°C to a constant weight and then digested in 68% nitric acid (HNO_3 , Aristar grade) at 110°C. The digestion solution was used to determine Zn accumulation in *D. magna* by using ICP-MS. The remaining 15 to 20 daphnids were immediately weighed on a wet basis. The daphnid tissues were homogenized through ultrasonication in 0.5 mL of sucrose buffer (0.25 mol L^{-1} sucrose, 0.1 mol L^{-1} Tris-HCl, and pH 8.6) and then centrifuged at $12\,000 \times g$ in a refrigerated centrifuge for 20 min. The supernatant was collected and diluted with a homogenate of 1.5 mL. The 0.5 mL of diluted supernatant fluid was mixed with Ag^+ solution for MT measurement according to the modified silver saturation method [14]. Thereafter, 0.2 mL of the aforementioned diluted supernatant fluid was used to measure MDA by using a spectrophotometer according to the manufacturer's instructions. MDA contents in *D. magna* were assayed using commercially available kits according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, China). The analysis of MT and MDA contents was completed within 3 d after 48 h of exposure.

3. Results and Discussion

3.1. Characterization of Five nZnO Photocatalysts

3.1.1. Morphology of Five nZnO Photocatalysts. Images of nZnO dispersion in water were characterized by TEM, as shown in Figure 1. The Mn-nZnO was a rod with nano-sized diameter and micro-sized length. The remaining nZnO materials were particles. Fe-nZnO had the smallest particle size (approximately 20 nm) with a uniform shape. The sizes of Co-nZnO, Ni-nZnO, and nondoped nZnO were between

FIGURE 1: TEM images of different doped nZnO photocatalysts. (a) Nondoped nZnO, (b) Fe-nZnO, (c) Co-nZnO, (d) Ni-nZnO, and (e) Mn-nZnO.

40 and 100 nm. All the five types of nZnO materials tended to aggregate in water, especially for Mn-nZnO. The table of ionic radius showed that the radius of Zn^{2+} was 0.074 nm, that of Fe^{3+} was 0.0645 nm, that of Co^{2+} was 0.074 nm, that of Ni^{2+} was 0.072 nm, and that of Mn^{2+} was 0.08 nm. These results may explain why Fe-nZnO had the smallest particle size, whereas Mn-nZnO had the largest. Xia et al. [11] also proved that Fe doping can reduce nZnO size.

3.1.2. Dissolubility of Five nZnO Photocatalysts in Natural Water. Figure 2 shows the percentage content of dissolved Zn^{2+} in the supernatant of each natural water sample, which indicated that nZnO exhibited certain solubility in water. However, the dissolved Fe, Co, Ni, and Mn were not detected because their contents were under the detection limit of ICP-MS. When the nZnO concentration was $100 \mu\text{g L}^{-1}$, the dissolved Zn^{2+} content of each sample was lower than 2% at 240 min. This solubility was considerably lower than that in Franklin's study, which determined the solubility of nZnO in natural water at 100 mg L^{-1} in 72 h [15]. The solubility of nZnO might be influenced by nominal nZnO concentration and testing period. Moreover, the solubility of Mn-nZnO and Fe-nZnO was significantly higher than that of other nZnO. Nevertheless, the solubility of Ni-nZnO was similar to that of the nondoped nZnO. In addition, the solubility of Co-nZnO

FIGURE 2: Dissolution of different doped nZnO photocatalysts in natural water. Mean $\pm$ standard deviation ($n = 2$).

was lower than 1%, which was the lowest among the five kinds of nZnO. These results indicate that different doped metals changed the solubility of nZnO. However, the solubility of Fe-doped nZnO from this study was different from that in the

FIGURE 3: Zn accumulation in *D. magna* after exposure to different doped nZnO photocatalysts. Mean $\pm$ standard deviation ($n = 2$).

study of Xia et al. [11]. This condition might be attributed to the different synthesis nZnO methods. The synthesis method of Xia et al. was flame spray pyrolysis, whereas our method was a wet chemical method. Miao's study also indicated that several physicochemical factors have to be considered in nZnO dissolution behavior.

3.2. Biototoxicity of Five nZnO Photocatalysts to *D. magna*

3.2.1. Bioaccumulation of Zn in *D. magna*. Zn accumulation in *D. magna* after exposure to five different nZnO photocatalysts was measured, as shown in Figure 3. The high-concentration exposure groups of all types of nZnO had higher Zn accumulation than the low-concentration exposure groups. Zn accumulation was 156 $\mu\text{g/g}$ dry wt in nonexposure *D. magna*, significantly lower than that in the nZnO exposure groups. No apparent difference was observed in the Zn accumulation in *D. magna* after exposure to nondoped nZnO, Fe-nZnO, and Mn-nZnO (400–700 $\mu\text{g/g}$ dry wt). While exposed to Co-nZnO and Ni-nZnO, the Zn accumulation in *D. magna* was higher than 1,000 $\mu\text{g/g}$ dry wt. *D. magna* is a filter feeding organism that can swallow a range of sizes of nanoparticle clusters [17]. Nano-ZnO, especially Mn-nZnO rods, tended to aggregate in water. Mn-nZnO, which had a larger size and poorer dispersion, more easily sank to the bottom of the container and was not conducive for *D. magna* to adsorb, which might be the reason why Mn-nZnO treatment exhibited the least accumulation in daphnids after 48 h of exposure. Zn accumulation in Co-doped exposure groups was significantly higher than that in the other groups because of the large intestinal particle aggregation, which was difficult to excrete (Figure 4(c)).

To explore the states of existence of nZnO in *D. magna*, intestinal slices of daphnids exposed to nZnO particles were observed by SEM (JSM-6380) with an EDAX-Genesis-2000 energy X-ray dispersive spectrometer. *D. magna* was prepared before dissection according to the method described

by Tervonen et al. [18]. As shown in Figure 4, the existing particles at the root of the *D. magna* intestinal villi were observed. Energy dispersive X-ray spectroscopy (EDS) elemental analysis showed that these particles are the exposed ZnO nanoparticles. The doping elements Fe, Co, and Mn were identified in the corresponding intestinal slice samples. In the intestinal slice sample of Ni-nZnO treatment, Fe was detected instead of Ni because the proportion of Ni was low at the detected point and the Fe content was high in air and natural water.

3.2.2. Induction of Metallothioneins by Five nZnOs. MT is a cysteine-rich metal binding protein with low molecular weight, which can combine some essential trace metals, such as Zn and Cu, as well as nonessential toxic metals, such as Cd, Hg, and Ag in cells [19]. Therefore, MT regulates the intracellular metal concentration and protects cells from toxic effects. As a biomarker, the MT contents of *D. magna*, in response to nZnO exposure, are shown in Figure 5. MT inductions were between 30 and 40 $\mu\text{g/g}$ wet wt in the low-concentration exposure groups and 40 and 50 $\mu\text{g/g}$ wet wt in the high-concentration exposure groups. A minor difference was observed between the two concentration groups, which was similar to the results obtained by Wong et al. [20]. Comparing five different nZnOs, MT inductions caused by Ni-nZnO were slightly higher than those by nondoped nZnO exposure groups, whereas Fe-, Co-, and Mn-doped nZnO decreased such inductions in the daphnids to a small degree. Binding to the excess metal ions was usually believed to be a protection mechanism of MT during metal exposure. Low dissolubility of the five types of nZnO might be a reason for the lack of a significant difference in MT content.

3.2.3. MDA Levels in *D. magna* after Exposure to Five nZnOs. MDA is a product of lipid oxidation caused by reactive oxygen species (ROS) attacking unsaturated fatty acids (PUFA) in biofilms [21]. The amount of MDA reflected the body lipid peroxidation levels, thus indirectly reflecting the degree of cell damage [22]. MDA contents of *D. magna* without nanomaterials exposure were measured to be approximately 10 nmol/mgprot. Figure 6 shows the MDA contents of *D. magna* after exposure to nZnO particles. The MDA contents were between 20 and 30 nmol/mgprot in 50 $\mu\text{g L}^{-1}$ exposure groups (i.e., twice to thrice that of the control) and between 40 and 50 nmol/mgprot in 250 $\mu\text{g L}^{-1}$ exposure groups (i.e., four to five times that of the control). That is, the MDA contents of the high-concentration groups were 1.5 to two times that of the low-concentration groups after nZnO exposure. These results indicated that these five types of nZnO can cause serious lipid peroxidation, and this extent of damage expanded rapidly with increasing exposure concentration. At the same concentration, the Ni-doped nZnO exposure group has similar MDA levels to the nondoped nZnO exposure group, whereas the doping of Fe, Co, and Mn reduced MDA contents. This finding indicates that the doping elements changed the bioeffects of nZnO. Previous studies found that doping of Co [23] and Mn [24] in ZnO influenced the antibacterial property of nZnO.

FIGURE 4: Continued.

FIGURE 4: SEM images and microanalysis by an energy dispersive spectrometer of accumulated nZnO in the intestines of *D. magna* after 2 d exposure. (A, a) Nondoped nZnO, (B, b) Fe-nZnO, (C, c) Co-nZnO, (D, d) Ni-nZnO, and (E, e) Mn-nZnO.

FIGURE 5: MT induction of *D. magna* after exposure to different doped nZnO photocatalysts. Mean $\pm$ standard deviation ($n = 2$).

3.3. Relationship of the Dissolubility of Five nZnO Photocatalysts with the Biotoxicity to *D. magna*. As previously discussed, five nZnO (nondoped nZnO, Fe-nZnO, Co-nZnO, Ni-nZnO, and Mn-nZnO) photocatalysts showed varying dissolubility in natural water and toxic effects to *D. magna*, and their relationships were investigated in Figure 7. A negative relationship was observed between dissolved Zn percentage and Zn accumulation in *D. magna*, as shown in Figure 7(a). This finding indicates that the ingestion of Zn^{2+} from aqueous phase was not the main uptake behavior in this study. Zn accumulation was distinctive under different metal-doped nZnO, which might be because of the dissolved Zn^{2+} , different aggregation clusters of M-nZnO, and particularities of doping metals. As for soluble Ag nanoparticles, the results of the uptake experiments showed that water drinking was an important pathway to obtain AgNPs in the medium for *D.*

FIGURE 6: MDA activities of *D. magna* after exposure to different doped nZnO photocatalysts. Mean $\pm$ standard deviation ($n = 2$).

magna [25]. On the contrary, MT and MDA contents were independent of the dissolved Zn percentage in the medium (Figures 7(b) and 7(c)). Among all the metals in this study, only Zn can induce the generation of MT. Thus, the MT contents were correlated with the concentration of free Zn^{2+} in the body instead of external mediums. One important toxicity mechanism of nZnO photocatalysts was oxidative damage by ROS. Meanwhile, MDA reflected the degree of lipid peroxidation and was influenced by many factors. In this study, physiological functions of doping metals might alleviate the biotoxicity of M-nZnO photocatalysts to various extents. Fe, Co, Zn, and Mn are essential elements that have important effects on the growth of organisms [26]. During the experiment process, parts of the doped Fe, Co, and Mn dissolved into the medium despite being undetected. The release of trace metals can supply nutrition for *D. magna* under short-term exposure.

FIGURE 7: Relationships between dissolubility of five nZnO photocatalysts and toxic effects in *D. magna*. (a) Zn accumulation, (b) MT concentration, and (c) MDA activity.

4. Conclusions

Metal-doped nZnO (Fe-nZnO, Co-nZnO, Ni-nZnO, and Mn-nZnO) and nondoped nZnO photocatalysts were prepared through hydrothermal method of zinc nitrate, hexamethylenetetramine, and various metal ions. The morphologies and dissolubility of five nZnO photocatalysts were diverse because of the doping metals. Moreover, metal-doped nZnO photocatalysts changed toxic effects in *D. magna* compared with nondoped nZnO. Co-doped and Ni-doped nZnO enhanced Zn accumulation in daphnids, whereas Mn-doped nZnO reduced such accumulation. Biomarkers, such as MT and MDA contents, in metal-doped nZnO treatments, except for Ni-nZnO, were lower than those in nondoped nZnO. Zn accumulation showed a negative relationship with dissolved Zn percentage, which indicated that Zn^{2+} was not the only pathway of *D. magna* ingestion. By contrast, MT and MDA contents in organisms were dependent on the dose of nZnO

exposure rather than on dissolubility. When considering the biotoxicity of metal-doped nZnO photocatalysts, physiological functions of doping metals should not be ignored.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Both Lingling Liu and XiangRui Wang contributed equally to this work.

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