



The joint effects on *Photobacterium phosphoreum* of metal oxide nanoparticles and their most likely coexisting chemicals in the environment



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ABSTRACT

Metal oxide nanoparticles (NPs) have been used increasingly and are likely to accumulate in natural water bodies, where they encounter and interact with other environmental chemicals. These interactions result in joint effects on biological systems and the environment. However, compared with the intensive research examining the toxicities of individual NPs, the toxicities of NP mixtures remain relatively unexplored. In this work, we studied the joint effects of NPs and their most likely coexisting chemicals in the environment, including NPs with different compositions, humic substances, and surfactants. Our results indicate that the joint effects of NP mixtures were usually simple addition, which is commonly adopted in real risk assessment. However, the joint effects obtained for mixtures that contained ZnO were exclusively associated with antagonism. In addition, the mixtures of NPs and surfactants resulted in complex joint effects, i.e., antagonistic, additive, and synergistic effects were found for the mixtures with ZnO, NiO, and Fe-oxide, respectively. Our study suggests that the assessments of the ecological risk of NPs, particularly ZnO NPs, should consider the impact of their coexisting chemicals in the environment.

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1. Introduction

Metal oxide nanoparticles (NPs) are produced on a large scale for widespread applications such as plastics, personal care items, textiles, catalysts, semiconductors, and drug carriers. Because of the toxic potential of NPs, there have been serious concerns over their health and environmental risks (Brayner, 2008; Nel et al., 2006). A large number of studies have indicated that NPs can be toxic to plants (Ma et al., 2010), algae (Navarro et al., 2008), bacteria (Xu et al., 2010), fishes (Baek and An, 2011), and human cells (Wong et al., 2010). However, one problem with these studies is that they are generally based on individual NPs; therefore, they may not reflect the toxic effects of NPs in the real environment, where the released NPs are likely to encounter and interact with both natural

and anthropogenic chemicals. Moreover, the interaction between NPs and their coexisting chemicals can cause complex and substantial changes in the NP properties and consequently induce either increased or decreased effects. Therefore, it is important and necessary to investigate both the individual and combined toxicity of NPs to obtain a comprehensive understanding of the adverse effects caused by NPs.

The joint effects of NPs and their coexisting chemicals in the environment have been reported in some published studies, most of which focused on humic substances. Because humic substances are a fairly common component of natural organic matter (NOM) in aquatic environments, the NPs accumulated in natural waters will inevitably encounter humic substances and coexist with them as mixtures in the environment. The interaction between NPs and humic substances can have dramatic impacts on NP properties, resulting in altered fate and transport as well as bioavailability and toxicity. For example, the NPs can adsorb humic substances onto their surfaces, which causes the NPs to acquire negative surface charge and thus reduces their propensity to aggregate (Gao et al., 2012; Yang et al., 2013; Zhang et al., 2009). The dissolution of the NPs is also affected by humic substances, leading to either decreased (Liu and Hurt, 2010) or increased (Bian et al., 2011) metal

Abbreviations: NPs, metal oxide nanoparticles; DTAC, dodecyl trimethyl ammonium chloride; NaHA, humic acid sodium salt.

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ion (aq) release. Moreover, the toxicity of the NPs can be remarkably influenced by humic substances. A decreased toxicity in the presence of humic substances has been observed with C_{60} (Li et al., 2008), Ag (Gao et al., 2012), Fe (Chen et al., 2011), TiO_2 (Klaine et al., 2008), ZnO (Li et al., 2010), and CuO NPs (Blinova et al., 2010). However, the results in other literature may suggest increased toxicity of NPs in the presence of humic substances (Yang et al., 2013). Other NOM, e.g., fulvic acid, was also found to increase the toxicity of NPs (Wang et al., 2011).

In addition to humic substances, surfactants are another example of the chemicals that most likely coexist with NPs in the environment. Due to the abundant use of surfactants during the preparation and modification of NPs (Antonietti, 2001; Franklin et al., 2007; John et al., 2002; Santra et al., 2001), the coexistence of the two chemicals is inevitable in the environment, particularly near places where products containing NPs are manufactured. However, the literature on the joint effects of NPs and surfactants is very limited. Sayes et al. (2006) showed that the toxicity of single-walled carbon nanotubes decreased in the presence of surfactants, perhaps as a result of surface modification by surfactants. Wallace et al. (2007) noted that NPs can adsorb surfactants onto the particle surfaces, conditioning the particle surfaces and affecting their in vitro expression of cytotoxicity or genotoxicity.

Despite these studies, the joint effects of NPs and their coexisting chemicals remain largely unexplored. There is still no unified conclusion on the joint effects of NPs and humic substances as well as surfactants, and the mechanisms through which humic substances and surfactants influence NP toxicity are not yet clear. Therefore, in this study, we investigated the joint effects of NPs and their coexisting chemicals in the environment, with sodium humate (NaHA) and dodecyl trimethyl ammonium chloride (DTAC) selected as the representatives of humic substances and surfactants, respectively. In addition, the joint effects of binary NP mixtures were also determined. *Photobacterium phosphoreum* was chosen as the test organism because the bioluminescence inhibition assay is a rapid, sensitive, and cost-effective method for acute toxicity estimation, and it has high experimental reproducibility, which can meet the requirement of the joint effects test (Lin et al., 2003; Zou et al., 2012).

The purposes of this study are as follows: (1) to investigate the individual toxicity of metal oxide NPs to *P. phosphoreum*; (2) to determine the joint effects of NPs and their coexisting chemicals, i.e., NPs with different compositions, humic substances, and surfactants; and (3) to propose possible mechanisms for the joint effects of NP mixtures. This study will provide an accurate and comprehensive understanding of the adverse effects caused by NPs in the real environment.

2. Materials and methods

2.1. Chemicals and test organism

The NPs and DTAC were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Eleven NPs were investigated in our study; see more details in Table 1. NaHA was supplied by Sigma-Aldrich Chemical Co. (Shanghai, China).

P. phosphoreum (T3 mutation) was obtained from the Institute of Soil Science of the Academy of Science of China (Nanjing, PRC). This organism was reconstituted and maintained on agar slants at 4°C.

2.2. Toxicity assessment

A preculture of *P. phosphoreum* was grown for 12 h (logarithmic growth period) in complete medium and was used as an inoculum.

Suspensions (or solutions) of the test chemicals, either individually or in a mixture, with different concentrations were prepared in 3% NaCl. The concentration gradients designed for the joint effects test are provided in Table S1 of the Supporting information. The prepared suspensions were then sonicated for 30 min to break up the agglomerates using an ultrasonic cleaner (DS-7510DT, Shanghai Shengxi Ultrasonic Instruments Co., Ltd., China). The chemical suspensions were then mixed with the inoculum. After exposure for 15 min at 20°C (Chinese Environmental Protection Agency, 1995), the bioluminescence was determined using a BHP (Model 9507) toxicity analyzer (Beijing Hamamatsu Co., Ltd., China).

The median effective concentration (EC_{50}), i.e., the concentration of a chemical that inhibits 50% of the bioluminescence, was chosen as an indicator of the individual toxicity. This measurement can be calculated based on the decrease in bioluminescence using a probit model (Lin et al., 2005). Equitoxic mixtures of the chemicals were then prepared based on their individual toxicities and evaluated to determine their joint effects. The joint effects are described by the sum of the toxic unit indexes as follows:

$$TU_x = \sum_{i=1}^n \frac{C_i}{ECx_i},$$

where C_i is the concentration of the i th component in an n -compound mixture and ECx_i denotes the concentration of substance i that provokes an effect equal to $x\%$ when applied individually. According to Broderius et al. (1995), a simple addition is characterized by $1.2 > TU > 0.8$, whereas $TU > 1.2$ represents antagonism, and $TU < 0.8$ indicates synergism.

2.3. Characterization of NPs

The NP suspensions (100 mg/L) were prepared in 3% NaCl, and the pH values were adjusted to 7.0. The hydrodynamic sizes were measured through dynamic light scattering (DLS) using a Zetasizer Nano Z instrument (Malvern Instruments Ltd., UK). The surface charge of the NPs was characterized by measuring the zeta potentials using the Zetasizer Nano Z instrument.

2.4. Metal ion concentration measurement in suspension

The individual and mixture suspensions of NPs were prepared in 3% NaCl and then centrifuged at 20,000 rpm for 15 min. The clear supernatant was carefully collected and acidified with 1% nitric acid. The ion concentrations were then determined through inductively coupled plasma (Agilent 720-ES).

3. Results

3.1. Single toxicity of NPs on *P. phosphoreum*

The NP suspensions with different compositions were found to be toxic to varying degrees: the EC_{50} values ranged from 0.34 to 3747 mg/L (4.25×10^{-6} – 1.6×10^{-2} mol/L), and the range of toxicity spanned three to four orders of magnitude (Table 1). The analysis of the results revealed that ZnO NPs exhibited the highest toxicity, and their EC_{50} values were only 0.34 mg/L (4.25×10^{-6} mol/L). The remaining NPs presented EC_{50} values exceeding 100 mg/L (2.2×10^{-4} – 1.6×10^{-2} mol/L), which indicates relatively lower toxicity. In particular, WO_3 had the lowest toxicity, with an EC_{50} of up to 3747 mg/L (1.6×10^{-2} mol/L). The dose–effect curves of the test NPs are shown in Fig. S1 (see Supporting information).

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