Comparison of toxicities from three metal oxide nanoparticles at environmental relevant concentrations in nematode Caenorhabditis elegans

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Comparison of toxicities from three metal oxide nanoparticles at environmental relevant concentrations in nematode Caenorhabditis elegans

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HIGHLIGHTS

► We compared toxicities of 30 nm metal oxide NPs at environmental relevant concentrations.
► Prolonged exposure (L1-larvae–adult) was performed for NPs toxicity assay in nematode.
► Adverse effects of Ti–NPs and Zn–NPs were detected at the concentration of 0.05 l g/L.
► In vivo toxicity order for the examined NPs was: Zn–NPs > TiO2–NPs > SiO2–NPs.
► Antioxidants treatment inhibited the adverse effects of Ti–NPs, Zn–NPs, and Si–NPs.

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ABSTRACT

Nematode Caenorhabditis elegans has been developed in a variety of environmental studies to address adverse effects of a wide range of toxicants. In the present study, we compared the toxicities of three metal oxide nanoparticles (NPs) including TiO2–NPs, ZnO–NPs, and SiO2–NPs with the same nanosize (30 nm) after prolonged exposure from L1–larvae to adult at environmental relevant concentrations. Our data indicated that the adverse effects were detected in nematodes exposed to TiO2–NPs and ZnO–NPs at concentrations more than 0.05 μg/L and SiO2–NPs at concentrations more than 5 μg/L with locomotion behavior and ROS production as endpoints. With growth, locomotion behavior, reproduction, and ROS production as endpoints, toxicity order for the examined metal oxide NPs was: ZnO–NPs > TiO2–NPs > SiO2–NPs. In nematodes exposed to the examined metal oxide NPs, ROS production was significantly correlated with lethality, growth, reproduction, and locomotion behavior. Moreover, treatment with antioxidants of ascorbate or NAC effectively inhibited the formation of oxidative stress and retrieved the adverse effects of TiO2–NPs, ZnO–NPs, and SiO2–NPs on survival, growth, reproduction and locomotion behaviors in nematodes. Our data demonstrated the subtle toxicity differences of different NPs exposure at environmental relevant concentrations in C. elegans.

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1. Introduction

Engineered nanoparticles (NPs) are defined as any intentionally produced particle that has a characteristic dimension from 1 to 100 nm (Nel et al., 2006). To date, the rapid expansion of nanotechnology has resulted in a vast array of NPs. Meanwhile, the potential environmental toxicity of NPs has received more and more attentions because of the possible entering of NPs contained in consumer products into the environment at some stage between production and disposal of consumer products (Behra and Krug, 2008). It was reported that Ag–NPs, TiO2–NPs, and carbon nanotubes were released into the air, soil and water through the life cycle of products in Switzerland (Mueller and Nowack, 2008). Nevertheless, now most of the NPs toxicity studies have still focused on human health, and studies on nanoecotoxicology have largely been unexplored (Behra and Krug, 2008).

Among the existed nanomaterials, there are substantial interests for metal and metal oxide NPs in commercial development and concerns surrounding their ecotoxicological effects (Auffan et al., 2009). For example, TiO2–NPs are often used as a pigment or additive for paints, paper, ceramics, plastics, foods, and other products, ZnO–NPs has a broad range of applications from chemical sensors to personal care products, and SiO2–NPs is used in industrial manufacturing, packaging, high-molecular composite.
materials and ceramics synthesis, disease labeling, drug delivery, cancer therapy and biosensor (Wang, 2004; Oberdorster et al., 2005; Yang et al., 2010). Because the chemical, size, and being not biodegradable properties of these NPs, they will rapidly distribute throughout the environment with largely unknown consequences (Wang et al., 2009). Some ecotoxicological studies have been performed for some metal oxide NPs. Acute toxicity assay of TiO2–NPs and ZnO–NPs using crustaceans Daphnia magna and Thamnocephalus platyurus and earthworm Eisenia fetida found the increased mortality and decreased reproduction after exposure (Lovern and Klaper, 2006; Heinlaan et al., 2008; Canas et al., 2011). Acute exposure to TiO2–NPs and ZnO–NPs caused elevated oxidative stress in liver tissues and biotoxicity to zebrafish (Xiong et al., 2011; Yu et al., 2011a). Acute exposure to ZnO–NPs killed embryos, retarded the embryo hatching, reduced the body length of larvae, and caused tail malformation in zebrafish (Bai et al., 2010). ZnO–NPs were more toxic towards algae than ZnO, but relatively less toxic towards crustaceans and fish (Wong et al., 2010). ZnO–NPs exposure increased mortality, negatively affected metamorphosis, and inhibited growth of Xenopus laevis (Nations et al., 2011). Exposure to TiO2–NPs resulted in gill injury and oxidative stress in rainbow trout (Federici et al., 2007). Chronic exposure to TiO2–NPs induced inhibition of growth, decrease of liver weight ratio, and histopathological changes of gills in zebrafish (Chen et al., 2011). Previous studies have compared the in vivo toxicities on zebrafish embryos and larvae from different NPs at relatively high concentrations; however, the toxicity comparison was not performed on NPs of the identical nanolevels (Zhu et al., 2008). Therefore, it is very necessary to perform the toxicity comparison of different NPs at environmental relevant concentrations with the identical nanosizes.

Caenorhabditis elegans, a free-living nematode mainly found in liquid phase of soils, has been developed in a variety of environmental studies to address adverse effects of a wide range of toxicants from molecular to individual levels (Khanna et al., 1997; Gerhardt et al., 2002; Boyd et al., 2007; Harada et al., 2007; Cai et al., 2008; Leung et al., 2008; Guo et al., 2009; Xing and Wang, 2009; Yu et al., 2011b). In nematodes, both lethal and sublethal endpoints such as development, reproduction, and locomotion behavior have been used for environmental and toxicological studies (Williams and Dusenberg, 1990; Donkin and Williams, 1995; Dhanaw et al., 2000; Roh et al., 2006; Shen et al., 2009; Xing et al., 2009; Helmcke et al., 2009; Wang et al., 2010; Wang and Xing, 2010; Ye et al., 2010; Wu et al., 2011a). Especially, C. elegans can be used to detect the possible toxicity of specific toxicant(s) after prolonged exposure (from L1–larvae to adults) or chronic exposure (from adult day 1 to day 10) at environmental relevant concentrations (Zhang et al., 2011; Wu et al., 2012). Recently, C. elegans has been further successfully used in evaluating the toxicity of metal oxide NPs, such as ZnO–NPs, SiO2–NPs, and TiO2–NPs, with lethality, growth, reproduction, movement, and gene expression as endpoints (Wang et al., 2009; Ma et al., 2009; Pluskota et al., 2009; Roh et al., 2010; Khare et al., 2011; Ma et al., 2011). It has been reported that acute exposure to Zn–NPs (1.5 nm, 20 nm, 60 nm), and TiO2–NPs (7 nm, 20 nm, 50 nm) at relatively high concentrations caused the increase of mortality, inhibition of growth, reduction of reproduction, decrease of movement, or alteration of gene expression patterns in C. elegans (Wang et al., 2009; Ma et al., 2009; Roh et al., 2010; Khare et al., 2011; Ma et al., 2011). In the present study, we compared the toxicities of three metal oxide NPs, including TiO2–NPs, ZnO–NPs, and SiO2–NPs, with the same nanosize (30 nm) after prolonged exposure from L1–larvae to adult at environmental relevant concentrations. Lethality, growth, locomotion behavior, reproduction, and ROS production were used as the endpoints. Our data demonstrated the important values of C. elegans in evaluating the subtle toxicity differences of different NPs exposure at environmental relevant concentrations.

2. Materials and methods

2.1. Reagents and preparation of NPs suspensions

The sizes of all used metal oxide NPs were 30 nm without any coating throughout this study. TiO2–NPs and ZnO–NPs were from Nano Applied Research Center of Nanjing University of Technology, and SiO2–NPs was from Wan Jing New Material Co. Ltd., (Hangzhou, Zhejiang, China). Purities of TiO2–NPs, ZnO–NPs, and SiO2–NPs were >99%, >98%, and >99.7%. Shapes of the used NPs were determined using a transmission electron microscope (TEM) (JEM-100CXII, JEOL, Ltd, Japan) (Fig. 1). Zeta-potentials of TiO2–NPs, ZnO–NPs, and SiO2–NPs were −13.1, −0.32, and −63.32 mV. Wide distributions of particle sizes of TiO2–NPs, ZnO–NPs, and SiO2–NPs were 405 ± 137, 627 ± 175, and 45.3 ± 10.7 nm, which were determined by a Nano-Zetasizer (1000 HS, Malvern Instrument Ltd., UK) using a dynamic light scattering (DLS) technique. Concentration-dependent dissolution of ZnO-NPs was determined by inductively coupled plasma mass spectrometer (Elan 6100, PerkinElmer, USA) (Supplementary Fig. 1). Prepared stock suspension concentrations for the used metal oxide NPs were 0.0005, 0.005, 0.05, 0.5, 5, 10, and 50 μg/L and selection of these stock suspension concentrations was referred to previous publications (Mueller and Nowack, 2008; Hasselöv et al., 2008; Tiede et al., 2009; Zhang et al., 2011). Series of stock suspensions of the used metal oxide NPs were prepared in a K-medium (0032 M KCl, 0051 M NaCl) and dispersed by probe sonication at 100 W and 40 kHz for 30 min to form homogeneous suspensions. During the testing periods, suspensions of the used metal oxide NPs were stable and uniform throughout the K-medium. All the other chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA).
2.2. Strain preparation

Nematodes used in the present study were wild-type N2, originally obtained from the Caenorhabditis Genetics Center (funded by the NIH National Center for Research Resource, USA), which were maintained on nematode growth medium (NGM) plates seeded with Escherichia coli OP50 at 20 °C as described (Brenner, 1974). Gravid nematodes were washed off the plates into centrifuge tubes, and were lysed with a bleaching mixture (0.45 M NaOH, 2% HOCl). Age synchronous populations of L1–larval nematodes were obtained by the collection as described (Donkin and Dusenbery, 1993). Exposures to different metal oxide NPs at the examined concentrations were performed from L1–larval stage in 12-well sterile tissue culture plates at 20 °C incubator in the presence of food, and the exposed nematodes were used for toxicity evaluation using lethality, growth, locomotion behavior, reproduction, and ROS production as endpoints when they developed into adults.

2.3. Lethality

Lethality was evaluated by the percentage of survival animals. Following exposure, the inactive nematodes were scored under a dissecting microscopy, and the animals were judged to be dead if they did not respond to stimulus using a small, metal wire. One hundred nematodes were examined per treatment.

2.4. Growth

Growth was assessed by the body length of nematodes. Body length was determined by measuring the flat surface area of nematodes using a Image-Pro Express software. 30 nematodes were examined per treatment.

2.5. Reproduction

Reproduction was assessed by the brood size. To assay the brood size, number of offspring at all stages beyond the egg was counted. 10 nematodes were examined per treatment.

2.6. Locomotion behavior

Locomotion behavior was assessed by head thrash, and body bend. To assay the head thrash, every examined nematode was transferred into a microtiter well containing 60 μL of K-medium on the top of agar, and head thrashes were counted for 1-min after a 1-min recovery period. A thrash was defined as a change in the direction of bending at the mid body. To assay the body bend, the examined nematodes were picked onto a second plate and scored for the number of body bends in an interval of 20 s. A body bend was counted as a change in the direction of the part of the nematodes corresponding to the posterior bulb of the pharynx along the y axis, assuming that the nematode was traveling along the x axis. 30 nematodes were examined per treatment.

2.7. Reactive oxygen species (ROS) production

To quantify whether the examined metal oxide NPs treatment activated the oxidative damage, ROS production was assayed. The examined nematodes were transferred to M9 buffer containing 1 μM CM-H2DCFDA to pre-incubate for 3 h at 20 °C, and then mounted on agar pads for examination with a laser scanning confocal microscope (Leica, TCS SP2, Bensheim, Germany) at 488 nm of excitation wavelength and 510 nm of emission filter. Relative fluorescence intensities of the intestines were semi-quantified. The semiquantified ROS was expressed as relative fluorescent units (RFU). Thirty nematodes were examined per treatment.

2.8. Pharmacological assay

The metal oxide NPs (50 μg/L) exposed (from L1–larvae to adult) nematodes were further treated with 10 mM ascorbate or 5 mM N-acetyl-L-cysteine (NAC) for 24 h (Huang and Lemire, 2009). Ascorbate and NAC are two antioxidants and used to treat mitochondrial dysfunction, and treatment with 10 mM ascorbate or 5 mM NAC did not influence survival of nematodes (Huang and Lemire, 2009). Graphs are representative of five trials.

2.9. Statistical analysis

All data were expressed as means ± standard error of the mean (S.E.M.). Statistical analysis was performed using SPSS 12.0 (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was used to determine the significance of differences between the groups. Probability levels of 0.05 and 0.01 were considered statistically significant. Associations of ROS production with lethality, growth, reproduction and locomotion behavior were assessed with linear regression analysis. The dependent variables were lethality, growth, reproduction and locomotion behavior, and the independent variable was ROS production.
3. Results

3.1. Adverse effects of different metal oxide NPs on survival of nematodes

Previous study demonstrated that *C. elegans* can be employed to assess the toxicity of nano-materials at environmental relevant concentrations by prolonged exposing the animals from L1–larvae to adults (Zhang et al., 2011). We first investigated the effects of prolonged exposure to different metal oxide NPs, including TiO$_2$–NPs, ZnO–NPs, and SiO$_2$–NPs, at environmental relevant concentrations on survival of nematodes. Nanosizes of the examined TiO$_2$–NPs, ZnO–NPs, and SiO$_2$–NPs were all 30 nm. Prolonged exposure to 0.0005–0.005 μg/L of the examined three metal oxide NPs did not obviously affect the survival of nematodes (data not shown). Prolonged exposure to 0.05–10 μg/L of the examined three metal oxide NPs and 50 μg/L of SiO$_2$–NPs also did not obviously influence the survival of nematodes (Fig. 2). In contrast, prolonged exposure to 50 μg/L of TiO$_2$–NPs and ZnO–NPs significantly ($P < 0.01$) increased the mortality of nematodes (Fig. 2). No significant toxicity differences were observed between TiO$_2$–NPs and ZnO–NPs as assessed by the endpoint of lethality.

3.2. Adverse effects of different metal oxide NPs on growth of nematodes

Growth of nematodes can be evaluated by the body length. Prolonged exposure to 0.0005–0.005 μg/L of the examined three metal oxide NPs did not obviously influence the body lengths of nematodes (data not shown). Similarly, as shown in Fig. 3, prolonged exposure to 0.05–10 μg/L of the examined three metal oxide NPs and 50 μg/L of SiO$_2$–NPs did not obviously affect the body lengths of nematodes; however, prolonged exposure to 50 μg/L of TiO$_2$–NPs and ZnO–NPs significantly ($P < 0.01$) reduced the body lengths of nematodes. The toxicity of ZnO–NPs was greater than TiO$_2$–NPs in nematodes as assessed by the endpoint of growth.

3.3. Adverse effects of different metal oxide NPs on reproduction of nematodes

We next examined the possible effects of different metal oxide NPs exposure on reproduction of nematodes with the aid of brood size as the endpoint. Prolonged exposure to 0.0005–0.005 μg/L of the examined three metal oxide NPs did not induce the alterations of brood size of nematodes (data not shown). Prolonged exposure to 0.05 μg/L of the examined three metal oxide NPs also did not
cause the alterations of brood size of nematodes (Fig. 4). In contrast, prolonged exposure to 0.5–50 \( \mu \text{g}/\text{L} \) of TiO\(_2\)–NPs and ZnO–NPs significantly (\( P < 0.01 \)) reduced the brood size of nematodes compared with control (Fig. 4). In addition, the significant (\( P < 0.05 \)) decrease of brood size was observed in 50 \( \mu \text{g}/\text{L} \) of SiO\(_2\)–NPs exposed nematodes. The reproductive toxicity of ZnO–NPs was greater than that of TiO\(_2\)–NPs in nematodes.

### 3.4. Adverse effects of different metal oxide NPs on locomotion behavior of nematodes

We further investigated the effects of different metal oxide NPs exposure on locomotion behavior by evaluating body bend and head thrash of nematodes. As shown in Fig. 5, prolonged exposure to TiO\(_2\)–NPs and ZnO–NPs at concentrations of 0.05–50 \( \mu \text{g}/\text{L} \) significantly (\( P < 0.01 \)) decreased both body bends and head threshes of nematodes compared with control. The neurotoxicity of ZnO–NPs was greater than that of TiO\(_2\)–NPs in nematodes. In contrast, the significant decreases of body bends and head threshes were only observed in 5–50 \( \mu \text{g}/\text{L} \) of SiO\(_2\)–NPs exposed nematodes compared with control. Prolonged exposure to 0.0005–0.005 \( \mu \text{g}/\text{L} \) of the examined three metal oxide NPs did not obviously influence the locomotion behaviors of nematodes (data not shown). These data indicate that both the locomotion behavior and the ROS production were relatively sensitive for evaluating the metal oxide NPs toxicity at environmental relevant concentrations in nematodes.

### 3.5. Adverse effects of different metal oxide NPs on oxidative stress of nematodes

Again, we investigated the effects of different metal oxide NPs exposure on oxidative stress as assessed by the ROS production. As shown in Fig. 6, prolonged exposure to TiO\(_2\)–NPs and ZnO–NPs at concentrations of 0.05–50 \( \mu \text{g}/\text{L} \) significantly (\( P < 0.01 \)) induced the ROS production in nematodes compared with control. The ROS productions in ZnO–NPs exposed nematodes were greater than those in TiO\(_2\)–NPs exposed nematodes. In contrast, the significant induction of ROS productions were only observed in 5–50 \( \mu \text{g}/\text{L} \) SiO\(_2\)–NPs exposed nematodes compared with control. Prolonged exposure to 0.0005–0.005 \( \mu \text{g}/\text{L} \) of the examined three metal oxide NPs did not obviously result in the significant induction of ROS production in nematodes (data not shown). These data indicate that both the locomotion behavior and the ROS production were relatively sensitive for evaluating the metal oxide NPs toxicity at environmental relevant concentrations in nematodes.

### 3.6. Associations of ROS production with lethality, growth, reproduction, and locomotion behavior in nematodes exposed to different metal oxide NPs

To examine the possible associations of ROS production with lethality, growth, reproduction, and locomotion behavior in nematodes exposed to different metal oxide NPs, the linear regression analysis was performed. The dependent variables were lethality,
growth, reproduction, and locomotion behavior, and the independent variable was ROS production. The results of linear regression analysis showed that, under our experimental conditions, ROS production was significantly correlated with lethality ($R^2 = 0.859$, $P < 0.01$), growth ($R^2 = 0.849$, $P < 0.01$), reproduction ($R^2 = 0.803$, $P < 0.05$), body bend ($R^2 = 0.701$, $P < 0.05$), and head thrash ($R^2 = 0.713$, $P < 0.05$) in ZnO–NPs exposed nematodes, ROS production was significantly correlated with lethality ($R^2 = 0.847$, $P < 0.01$), growth ($R^2 = 0.848$, $P < 0.01$), reproduction ($R^2 = 0.817$, $P < 0.01$), body bend ($R^2 = 0.743$, $P < 0.05$), and head thrash ($R^2 = 0.724$, $P < 0.05$) in TiO$_2$–NPs exposed nematodes, and ROS production was significantly correlated with lethality ($R^2 = 0.948$, $P < 0.01$), growth ($R^2 = 0.947$, $P < 0.01$), reproduction ($R^2 = 0.934$, $P < 0.01$), body bend ($R^2 = 0.895$, $P < 0.01$), and head thrash ($R^2 = 0.859$, $P < 0.01$) in SiO$_2$–NPs exposed nematodes (Supplementary Table 1). Therefore, the ROS production was significantly correlated with the lethality, growth, reproduction, and locomotion behavior in nematodes exposed to the examined metal oxide NPs.

3.7. Effects of antioxidants treatment on the toxicity formation in metal oxide NPs exposed nematodes

To further confirm the roles of oxidative stress in inducing the metal oxide NPs toxicity in nematodes at environmental concentrations, we finally investigated the effects of antioxidants treatment on the toxicity formation in metal oxide NPs exposed nematodes. Our data indicated that, after prolonged exposure to the examined three metal oxide NPs at the concentration of 50 μg/L, post-treatment with 10 mM ascorbate or 5 mM NAC effectively suppressed the significant increase in mortality, decrease in body length, reduction in brood size, decrease in locomotion behavior as reflected by body bend and head thrash, and induction of ROS production formed in TiO$_2$–NPs, ZnO–NPs, and SiO$_2$–NPs exposed nematodes (Fig. 7). Therefore, treatment with antioxidants of ascorbate or NAC can inhibit the formation of oxidative stress and retrieve the adverse effects on survival, growth, reproduction and locomotion behaviors from TiO$_2$–NPs, ZnO–NPs, and SiO$_2$–NPs in nematodes.

4. Discussion

In the present study, we compared the toxicities of three metal oxide NPs with the same small size (30 nm) using C. elegans as a bioindicator. Although many NPs exist, here we selected three metal NPs, including TiO$_2$–NPs, ZnO–NPs, and SiO$_2$–NPs, to compare their toxicities because they are already widely used in consumer products, and metal oxide NPs belong to the more realistic forms of NPs. In general, well-studied organisms such as Daphnia, fish and algae can be used as representatives of the major trophic levels (Behra and Krug, 2008). Besides these organisms, C. elegans is also a good animal model for the ecotoxicological study because of its abundance in soil ecosystem, its convenient handling in the laboratory, and its relative sensitivity to different kinds of stresses or toxicants (Leung et al., 2008). The reason to select the size of 30 nm was that metal oxide NPs larger than 30 nm usually do not show properties that would require regulatory scrutiny beyond that required for their bulk counterparts, and the possible adverse effects of metal oxide NPs typically result from their small size rather than a unique nanoscale property (Auffan et al., 2009).

Previous study assessed the concentrations of TiO$_2$–NPs in Swiss fresh water and the results suggest a possible risk in Swiss water bodies (Mueller and Nowack, 2008). In C. elegans, assays
on metal oxide NPs suggest that acute exposure to metal oxide NPs at relatively high concentrations would induce the elevated lethality (Wang et al., 2009; Ma et al., 2009; Roh et al., 2010; Khare et al., 2011; Ma et al., 2011; Wu et al., 2011b); however, it is still unclear whether the prolonged exposure (from L1–larvae to young adult) to TiO2–NPs, ZnO–NPs, and SiO2–NPs at environmental relevant concentrations will cause the adverse effects on animals. In the present study, our data indicated that the adverse effects were detected in nematodes exposed to 0.05 \( \mu \text{g/L} \) of TiO2–NPs and ZnO–NPs with locomotion behavior and ROS production as the endpoints (Figs. 5 and 6). With locomotion behavior and ROS production as the endpoints, the adverse effects were also observed in 5 \( \mu \text{g/L} \) of SiO2–NPs exposed nematodes (Figs. 5 and 6). In contrast, the endpoints of lethality, growth and reproduction were less sensitive than the locomotion behavior and the ROS production while assessing the possible adverse effects of the examined metal oxide NPs on nematodes. The predicted environmental concentrations in water for TiO2–NPs, ZnO–NPs, and SiO2–NPs are 16 or 24.5 \( \mu \text{g/L} \), 76 \( \mu \text{g/L} \), and 0.0007 \( \mu \text{g/L} \) (Mueller and Nowack, 2008; Tiede et al., 2009). Our data suggest the adverse effects on nematodes from exposure to 30 nm TiO2–NPs and ZnO–NPs at environmental relevant concentrations using brood size, locomotion behavior and ROS production as the endpoints. The metal oxide exposed (50 \( \mu \text{g/L} \)) adults from the L1–larvae to the young adult were treated with 10 mM ascorbate or 5 mM N-acetyl-L-cysteine (NAC) for 24 h. ND, not done. Bars represent mean ± S.E.M. *\( P < 0.01 \).*

In this study, our data demonstrate that the toxicity order for the examined three 30 nm metal oxide NPs at the environmental...
relevant concentrations was: ZnO–NPs > TiO$_2$–NPs > SiO$_2$–NPs. This toxicity order was consistent with previous studies for these metal oxide NPs at relatively high concentrations. For example, the cytotoxicity of ZnO–NPs to Gram-positive Bacillus subtilis and Gram-negative bacteria Vibrio fisheri or E. coli was greater than that for TiO$_2$–NPs (Adams et al., 2006; Heinlaan et al., 2008). In addition, Adams et al. observed very low cytotoxicity from Si–NPs (Adams et al., 2006). In C. elegans, acute exposure to TiO$_2$–NPs did not affect the lifespan of nematodes, but moderately induced the reduction of progeny production (Pluskota et al., 2009). In this study, we compared the toxicity of three metal oxide NPs with the same size and without further modification; however, NPs may vary in shape, charge, chemistry, coating and solubility in the environment, and may enter cells through different routes depending on their sizes, and surface modifications and the cell types (Behra and Krug, 2008). Based on the characterizations of the examined three metal oxide NPs, formation of the toxicity order may be not mainly due to the differences of aggregation sizes for the examined metal oxide NPs, because the order of aggregation size was not consistent with the order of toxicity for the examined metal oxide NPs in nematodes. Therefore, the observed toxicity order for the examined metal oxide NPs may be largely due to the differences of metal types of oxide.

We also provided several lines evidence to indicate the important role of oxidative stress in inducing the toxicity for the examined metal oxide NPs at the environmental relevant concentrations in nematodes. Firstly, with the alterations of locomotion behavior in nematodes exposed to 0.05–50 µg/L of TiO$_2$–NPs and ZnO–NPs, ROS productions were significantly altered by the exposure to 0.05–50 µg/L of TiO$_2$–NPs and ZnO–NPs (Figs. 4 and 5). Secondly, the linear regression analysis indicated the close association of induction of ROS production with alterations of other endpoints (Table 1). Thirdly, treatment with antioxidants of ascorbate or NAC effectively retrieved the toxicity of the examined metal oxide NPs on nematodes were closely associated to support such a notion that the observed adverse effects of induction of ROS production with alterations of other endpoints (Adams et al., 2006). In environments of antioxidants may be have adverse effects on nematodes NPs at the environmental relevant concentrations. Nevertheless, if TiO$_2$–NPs and ZnO–NPs at the environmental relevant concentrations in nematodes. Therefore, the observed toxicity order for the examined metal oxide NPs may be largely due to the differences of metal types of oxide.

5. Conclusion

In this study, we compared the toxicities of 30 nm TiO$_2$–NPs, ZnO–NPs, and SiO$_2$–NPs at the environmental relevant concentrations on C. elegans using lethality, growth, reproduction, locomotion behavior, and ROS production as endpoints. Our data demonstrated that, with the aid of reproduction, locomotion behavior, and ROS production as the endpoints, the adverse effects of TiO$_2$–NPs and ZnO–NPs at the environmental relevant concentrations on nematodes were detected. Moreover, we raised the evidence to support such a notion that the observed adverse effects of the examined metal oxide NPs on nematodes were closely associated with the induction of oxide stress, and treatment with antioxidant of ascorbate or NAC effectively retrieved the toxicity formed in nematodes exposed to the examined metal oxide NPs.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.09.019.

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