Environ Sci Pollut Res (2017) 24:2507–2514
DOI 10.1007/s11356-016-8001-6

RESEARCH ARTICLE

The biochemical and toxicological responses of earthworm (*Eisenia fetida*) following exposure to nanoscale zerovalent iron in a soil system

Jun Liang[1] · Xiaoqian Xia[1] · Wei Zhang[1] · Waqas Qamar Zaman[1] · Kuangfei Lin[1] · Shuangqing Hu[2] · Zhifen Lin[3]

Received: 19 October 2016 / Accepted: 25 October 2016 / Published online: 7 November 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract Nanomaterials have increasingly gained a great amount of interest due to their widespread applications, while their potential impacts on invertebrates in soil lack thorough investigation. This study is mainly aimed at determining the acute and subacute toxicity to the earthworm *Eisenia fetida*, induced by different levels of nanoscale zerovalent iron (nZVI) (100, 500, 1000 mg kg^{-1}) in natural soils. The results showed that compared to the controls, exposure to 500 and 1000 mg kg^{-1} of nZVI significantly ($P < 0.05$) inhibited growth and respiration and increased avoidance response in earthworms. The perturbations of antioxidant enzyme activities (superoxide dismutase—SOD and catalase—CAT), malondialdehyde (MDA) content, and reactive oxygen species (ROS) clearly revealed that oxidative stress was induced in *E. fetida* exposed to nZVI. Good correlations were observed in current results among the growth, respiration, MDA, and ROS ($R > 0.8$; $P < 0.05$), and that ROS was the most sensitive parameter in response to the stress caused by nZVI. Additionally, the histopathological examination of transverse sections of the exposed earthworms passing through the body wall illustrated that there was a serious injury in epidermal tissue after an exposure of 28 days. These findings will provide a comprehensive understanding of toxicological effects of nZVI in a soil-earthworm system.

Keywords Nanomaterial · Nanoscale zerovalent iron · *Eisenia fetida* · Toxicological effects

Responsible Editor: Thomas D. Bucheli

Electronic supplementary material The online version of this article (doi:10.1007/s11356-016-8001-6) contains supplementary material, which is available to authorized users.

✉ Wei Zhang
wzhang@ecust.edu.cn

✉ Shuangqing Hu
husq@saes.sh.cn

[1] State Environmental Protection Key Laboratory of Environmental Risk Assessment and Control on Chemical Process, School of Resource and Environmental Engineering, East China University of Science and Technology, Shanghai 200237, China

[2] Shanghai Academy of Environmental Sciences, Shanghai 200233, China

[3] College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China

Introduction

Nanoscale zerovalent iron (nZVI) has special properties such as larger specific surface area and higher surface reactivity in comparison to bulky particles. nZVI has been extensively used in in situ remediation (i.e., groundwater and soil) (Chowdhury et al. 2015; Tosco et al. 2014), and the removal efficiency of pollutants is regarded as considerable (Zhao et al. 2016). Quantitatively higher release of nZVI to the soils have raised great concerns on health and safety (Lankadurai et al. 2015; Nowack and Bucheli 2007), because most of the studies were performed on aquatic organisms (Chen et al. 2012; Marsalek et al. 2012) and not on edaphic species that is important for risk evaluation of nZVI application.

One of the important hazardous concerns is that how soil organisms are affected by high iron doses, particularly high reactive iron. nZVI was mainly intended to be used in the subsoil under saturated conditions. Furthermore, due to the aggregation and deposition characteristics (Phenrat et al. 2008), high concentrations of nZVI may possess the transportation ability towards unsaturated soil (i.e., surface layers). The highly redox-reactive nZVI and oxidized products may

then penetrate into animal and plant cells and thus induce toxic effects. Therefore, elucidating the ecological hazard of nZVI in the soil environment is urgently needed (Ma et al. 2013; Saccà et al. 2014).

Among soil organisms, the earthworm *Eisenia fetida* has the ability to accumulate large quantities of contaminants and is considered as a model organism for toxicity assessment (OECD 1984). A recent study has used earthworms to determine the toxicity and bioavailability of engineered nanoparticles (ENPs) (Kwak and An 2015), while to the best of our knowledge, nZVI has been barely assessed in detail with regard to *E. fetida*. In fact, nZVI could result in cellular damage by the formation of reactive oxygen species (ROS) (Chen et al. 2012). Therefore, the objectives of the present study are to determine the biochemical and toxicological effects of nZVI to earthworms. To this end, we investigated the survival, growth, avoidance, respiration, ROS, superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) content of *E. fetida* when subjected to nZVI exposure. Additionally, the skin damage of earthworms was also assessed based on histological examinations. The results and related findings would establish a more integrated understanding of potential ecological risk of nanomaterials in a soil system for its better diagnosis and remediation.

Materials and methods

Chemicals

nZVI was purchased from Chaowei Nanotechnology Co. Ltd., Shanghai, China. According to the manufacturer, nZVI was made of iron nanoparticles coated with 1-nm iron oxide shell. In addition to this, its specific surface area was 23 $m^2\ g^{-1}$, and the purity was 99.9%. All reagents used in the study were of analytical grade.

Characterization of nZVI

The characterization of nZVI was conducted by transmission electron microscopy (TEM, JEOL JEM-1400). The particle size of nZVI ranged from 50 to 100 nm, confirming the information provided by the manufacturer. X-ray diffraction (XRD) analysis of nZVI was obtained by RIGAKU D/MAX-2550 VB/PC at 40 kV and 100 mA. The detailed data is shown in Fig. S1.

Soil and earthworm collection

Soil samples were collected from the surface layer (0–20 cm) in the center of East China University of Science and Technology, China. The soil was air-dried and sieved to 2 mm. Soil properties were assessed according to Nelson and Sommers (1982) and can be summarized as follows: silty clay loam; pH, 7.3; organic matter, 6.5%. Variation of oxidation-reduction potential (ORP) was monitored in soils for indicated days. Additionally, the background level of total Fe was 47.62 mg kg^{-1} in pristine soil by atomic absorption spectrometer (AAS) (Agilent, USA).

Mature (clitellate) specimens of *E. fetida* (0.55 ± 0.12 g) were bought from Yonghe Earthworm Culture Farm (Shanghai, China) and cultured in natural soils with cattle manure at room temperature (20 ± 1 °C) for about 28 days.

Acute toxicity tests

The whole experiment outline is depicted in Fig. 1.

The toxicity of nZVI to earthworms was investigated based on filter paper contact and natural soil tests as recommended by OECD (1984) with slight modifications. The nZVI-containing solutions were prepared in an anaerobic glovebox.

As for the filter paper test, the experiments were conducted in 9-cm petri dishes, each of which contained a filter paper. Ultrapure water was exposed to nitrogen gas for 10 min. Stable suspensions of nZVI were prepared with ultrapure water (18.2 MΩ) by 30-min sonication in an ultrasonic bath to have a homogenous suspension. Then, 2-mL suspensions were added onto the filter paper. Five different treatments of nZVI were prepared as 100, 500, 1000, 1500, and 2000 μg cm^{-2}. As a control, 2 mL of deionized water was added to another batch of dishes. Twenty replicates were used for each concentration treatment of nZVI. Each replicate contained one depurated earthworm. The dishes were capped loosely with medical gauze. Tests were conducted under the conditions: 20 ± 1 °C, 12/12 h day/dark. Mortality was recorded after the incubation periods of 24, 48, and 72 h. Worms were considered dead when they did not respond to gentle mechanical stimulus.

As for the natural soil test, 500 g of soil amended with nZVI was filled into 800-mL glass beakers. Ultrapure water was exposed to nitrogen gas for 10 min. Stable suspensions of nZVI were prepared with ultrapure water (18.2 MΩ) by sonication for 30 min in an ultrasonic bath to have a homogenous

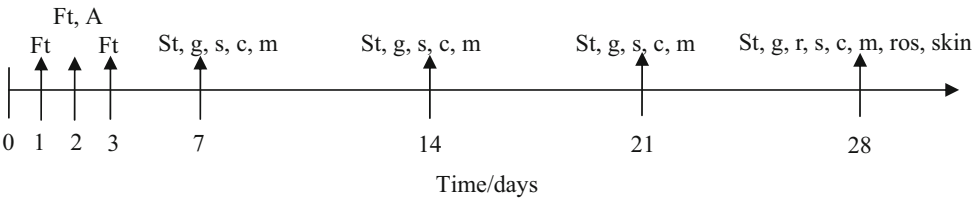

Fig. 1 Experimental outline

suspension. After that, the suspension was immediately hand mixed into a subsample of soil for each replicate with a glass rod. The concentrations were selected based on El-Temsah and Joner (2012), being 0, 100, 500, and 1000 mg kg^{-1} dry soil of nZVI. Three replicates were used for each dose of nZVI. Each replicate had ten depurated earthworms. The test was run at 20 ± 1 °C, 12/12 h day/dark. Water was added regularly to maintain the soil moisture to 65% of the total water holding capacity (WHC). According to Zhang (2003), nZVIs remain reactive for more than 1 month. Mortality was recorded after the incubation periods of 7, 14, 21, and 28 days. Worms were considered dead on being irresponsive to gentle mechanical stimulus.

Growth test

Experimental processing and concentration levels were referred to the present natural soil test. After 7, 14, 21, and 28 days of exposure, earthworms were removed from the substrate, washed in water, placed on moisture filter paper for 1 day to purge the guts, and weighed. Growth inhibition of earthworms after various exposure periods in each dose group was calculated according to the following equation:

$$GI_n = (W_0 - W_t)/W_0 \times 100\%$$

where GI_n is the growth inhibition for dose group n, W_0 is the weight on day 0, and W_t is the weight after t days of exposure.

Avoidance test

The avoidance test was performed in two-sectioned vessels following the description provided by the ISO guideline (ISO 2007). Briefly, one side with 500 g of soil spiked with different levels of nZVI and the other with 500 g of control soil was included. Three replicates for each treatment were performed, with ten adult earthworms in each replicate. In order to prevent the escape of earthworms, the boxes were covered by perforated medical gauze. The test was run at 20 ± 1 °C, 12/12 h day/dark. After 48 h of incubation, the number of earthworms in each vessel was recorded. The mean percentages of net response (NR) were calculated using the following equation:

$$NR = (C - T)/N \times 100\%$$

where C is the number of earthworms observed in the control soil, T is the number of earthworms observed in the test soil, and N is the total number of earthworms per replicate.

Respiratory test

The respiratory test was performed following a method described by Li et al. (2015) with slight modifications. Briefly, the test was conducted in a beaker (800 mL) filled with sterilized soils (100 g), and later, earthworms were exposed to the spiked soils for 4 weeks. The concentration levels were referred to above 28-day growth toxicity test. After the 28-day exposure, the earthworms were removed from the containers (two earthworms in a beaker), depurated and weighed, then placed in a flask, and the amount of CO_2 was determined after 16 h. The CO_2 was captured by a small beaker filled with 5 mL of 1 M NaOH solution that was determined by titrating with 0.1 M HCl on day 28. The final results were expressed as 10^{-5} mol CO_2 per g of earthworms' body weight per hour (Tang et al. 2016). Three replicates were set up for each of the four treatments.

Biochemical indices assay

The procedure implemented was that employed by Zhang et al. (2015a) with slight modifications. Experimental processing and concentration levels were referred to the above 28-day growth toxicity test. After 28 days of exposure, two depurated earthworms were homogenized using a glass homogenizer under ice-cold conditions in Tris–HCl buffer (1:4, *w*/*v*) and centrifuged at 10,000×*g* at 4 °C for 15 min. The supernatants were used to assess SOD and CAT activities as well as MDA contents by the commercial kits (Nanjing Jiancheng, Nanjing, China). Three runs of each treatment were conducted.

ROS detection

ROS detection was conducted using the DCFH-DA method according to Zhao et al. (2013) with slight modifications. After 28 days, two depurated earthworms were homogenized using a glass homogenizer under ice-cold conditions in potassium phosphate buffer (pH = 7.4) and centrifuged at 1000×*g* at 4 °C for 10 min. Then, the supernatants were re-centrifuged at 12,000×*g* at 4 °C for 20 min to obtain the mitochondrial protein, which was incubated at 37 °C with DCFH-DA solution for 30 min in a water bath. The fluorescence of samples was monitored at an excitation wavelength of 485 nm and an emission wavelength of 530 nm using a Varian Cary Eclipse fluorescent spectrophotometer.

Histology

The whole procedure was conducted based on the previous reports by Chen et al. (2011) and Zhang et al. (2015b) with slight modifications. Experimental processing and concentration levels were referred to the present natural soil test. After 28 days, the depurated earthworms were fixed with formalin, embedded in paraffin, and 7 μm transverse sections were stained with hematoxylin-eosin (HE) for light microscope observation (Nikon Eclipse 80i).

Data analysis

All data analysis was conducted using SPSS 16.0 (SPSS, Chicago, USA). Significant differences on growth, avoidance, respiration, ROS, SOD, and CAT activities and MDA contents were assessed by one-way analysis of variance followed by Duncan's post hoc test. Figures were completed using Origin 8.0 (Origin Lab, Northampton, MA, USA). Pearson's correlation analysis was performed to identify significant correlations between the measured variables.

Results and discussion

Acute toxicity

The acute toxic effects of nZVI on earthworms evaluated by the filter paper contact and soil tests are shown in Fig. S2. In the case of the filter paper contact test, the results showed that after 24-h incubation, the mortality (>10%) was observed in earthworms exposed to nZVI (>1000 μg cm^{-2}). Afterward, it was aggravated with increasing nZVI dosage and exposure time. In the natural soil assay, the mortality of earthworms was lower than 50% in different treated groups throughout the entire incubation. Seventy-nine percent of mortality was reported by El-Temsah and Joner (2012) for *E. fetida* exposed to 500 mg kg^{-1} nZVI in sandy loam soil after 14-day exposure. The discrepancy might be because nZVI toxicity was strongly affected by soil properties. Chen et al. (2012) reported that ORP was an absolutely crucial parameter to elucidate the toxicity mechanisms, and ORP values (Fig. S3) in the soil could verify the corresponding indicators in the present study. In addition, we noticed that the toxicity of nZVI was different from that observed in the filter paper contact assay, which could be due to the bioavailability of pollutants modified by soil physicochemical characteristics. Therefore, the exposure media should be taken into consideration when evaluating the toxicity of nanomaterials (Park et al. 2013).

Impacts on growth

Effects of nZVI on earthworms' growth are shown in Fig. 2. In general, the growth was induced (>5%) when earthworms were exposed to the control and 100 mg kg^{-1} nZVI groups. The inhibitory effects of nZVI (1000 mg kg^{-1}) appeared to be time-dependent, and the growth was inhibited by approximately 15% at the highest level of nZVI. It has been shown that the inhibition resulted from the depletions of glycogen and lipid contents and decrease in protein content (Liu et al. 2015; Ribeiro et al. 2001). In the present study, the reason might be the same, i.e., the declining energy reserves of the earthworms.

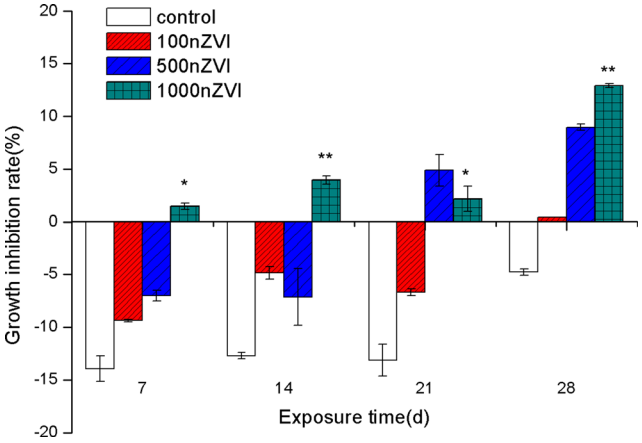

Fig. 2 Growth responses of *Eisenia fetida* exposed to various concentrations of nZVI for indicated days

Impacts on avoidance behavior

The results of the avoidance test are presented in Fig. 3. At the lowest nZVI concentration (100 mg kg^{-1}), the earthworms exhibited low avoidance behavior, i.e., their numbers were higher in the spiked soils than in the control samples. Similarly, Amorim and Scott-Fordsmand (2012) showed that Cu-NP exposure (30 mg kg^{-1}) resulted in low avoidance behavior. When nZVI concentration reached 1000 mg kg^{-1}, the avoidance response reached 40%. The NR values were less than 80%, which indicated that no obvious avoidance response occurred after 48-h exposure to nZVI. Nevertheless, the NR in the treatments was significantly higher ($P < 0.05$) than in the controls, illustrating that it could mean an advantage in case of spilling of nZVI in natural soils (Hu et al. 2014).

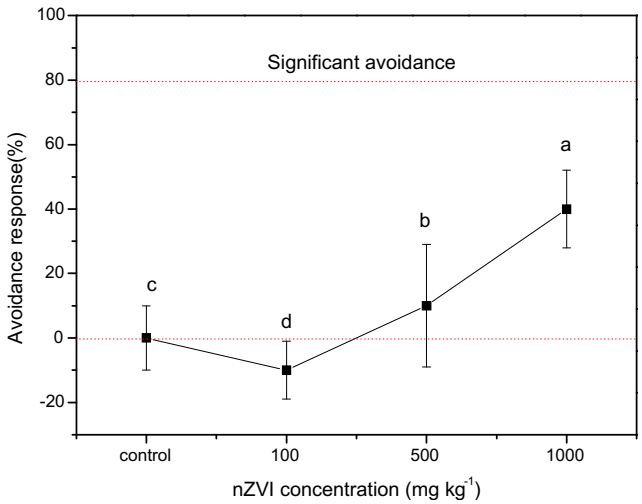

Fig. 3 Avoidance responses of *Eisenia fetida* exposed to various concentrations of nZVI after 48 h of incubation

Impacts on respiration

The effects of nZVI on the respiration of earthworms were determined based upon the principle of CO_2 production. As illustrated in Fig. 4, there were no significant ($P > 0.05$) changes when earthworms were exposed to 100 mg kg^{-1} nZVI. However, exposures to 500 and 1000 mg kg^{-1} nZVI significantly inhibited the respiratory activity ($P < 0.05$) with inhibition rates of 18.1 and 24.5%, respectively. Li et al. (2015) reported that the CO_2 production of earthworms was decreased after exposure to enrofloxacin-spiked soils, and it was correlated to the inhibition in earthworms' metabolism. In the present study, significant decreases in respiration after exposure to higher levels of nZVI suggest the inhibition in earthworms' metabolism probably leading to lower vital activity. Additionally, the result of respiration was well correlated with growth, which could be ascribed to energy reserves of soil invertebrates (Khalil 2015).

Impacts on antioxidant enzymes, MDA and ROS levels

The living organisms have the ability to protect themselves from ROS damage through the antioxidant enzymes (i.e., SOD and CAT) (Foyer and Shigeoka 2011; Hao et al. 2009). The impacts on SOD and CAT activities of *E. fetida* after 7, 14, 21, and 28 days of exposure are shown in Fig. 5. The activity of SOD was clearly ($P < 0.05$) induced after exposure to different levels of nZVI after a short-term incubation (7 days), with activation rates of 54.6, 29.4, and 50.0%, respectively, which could be considered as a protection mechanism to the chemical stress (Hao et al. 2009). After a long-term incubation (28 days), SOD activity was significantly ($P < 0.05$) inhibited in the low (100 mg kg^{-1} nZVI) and high (1000 mg kg^{-1} nZVI) tested groups, with inhibition rates of 24.0 and 20.6%, respectively. The reason might be due to the overwhelming effect on SOD by

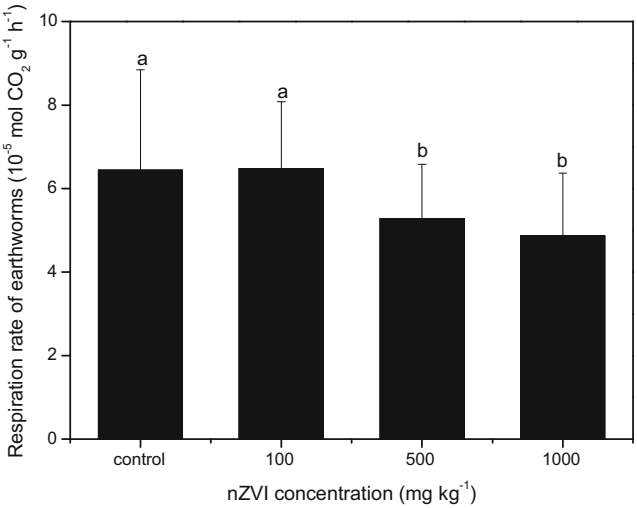

Fig. 4 Respiration responses of *Eisenia fetida* exposed to various concentrations of nZVI for 28 days

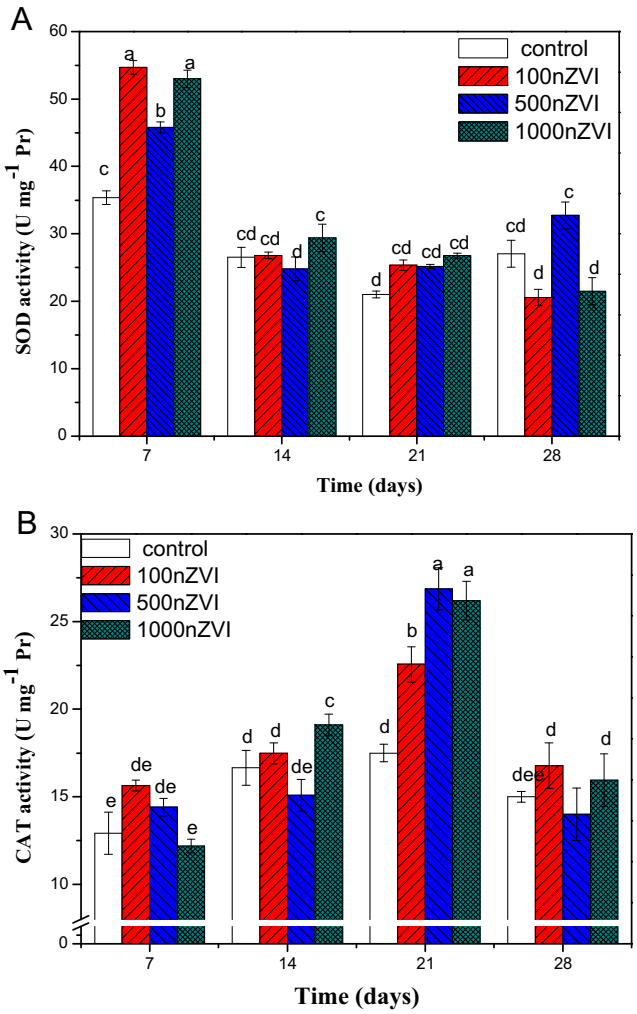

Fig. 5 SOD and CAT activities responses of *Eisenia fetida* exposed to various concentrations of nZVI for indicated days

the excess of reactive superoxide anion induced by nZVI (Foyer and Shigeoka 2011; Hu et al. 2016). On contrary to the SOD activity, CAT was significantly increased ($P < 0.05$) in only one treatment (1000 mg kg^{-1} nZVI) after short-term exposure (14 days), while it was significantly ($P < 0.05$) induced after a long-term exposure (21 days), indicating that the CAT has enough time to depose of excess H_2O_2. Apart from that, momentous enhancement of the CAT activity resulting from the early activation of SOD pointed out their complementary mechanism in earthworms.

Lipid peroxidation (LPO), regarded as the biomarker to assess the oxidative stress, was determined by evaluating earthworm's MDA content (Dotan et al. 2004). As depicted in Fig. 6, there was a clear dose- and time-response pattern of MDA content in earthworms during the whole incubation, and the peak was observed at 1000 mg kg^{-1} nZVI after 28 days of incubation, increasing by 18.9% relative to controls. Increases in the MDA contents in earthworms exposed to the pollutant would suggest that the organisms were attacked by excessive ROS (Zhao et al. 2013), which is evidenced from Fig. 7. It was

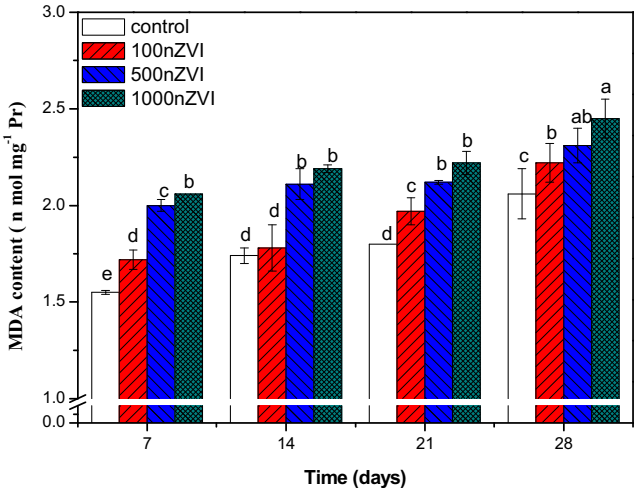

Fig. 6 MDA contents responses of *Eisenia fetida* exposed to various concentrations of nZVI for indicated days

demonstrated that there was a clear dose-dependent pattern of ROS after exposure to nZVI. In addition, the results obtained from this study showed that the generation of ROS might also cause oxidative perturbations in earthworms.

Previous studies have demonstrated that the measurement of biological indicators could be useful in environmental pollutant monitoring. Therefore, it is vital to establish that the specific responses were dose-responsive and sensitive to the pollutant of interest (Wang et al. 2016; Li et al. 2016). In this study, the correlation analysis was performed in order to compare the sensitivity of earthworm's biomarkers to nZVI toxic effects (Table 1). Growth, respiration, MDA, and ROS were well correlated with each other ($R > 0.8$; $P < 0.05$). Additionally, a negative correlation ($R = -0.974$; $P < 0.05$) was observed between CAT and SOD activity. We can conclude that increasing nZVI contents in soils toxically affected earthworms. This could lead to ROS generation and subsequent production of MDA ($R = 0.880$), which inhibited the

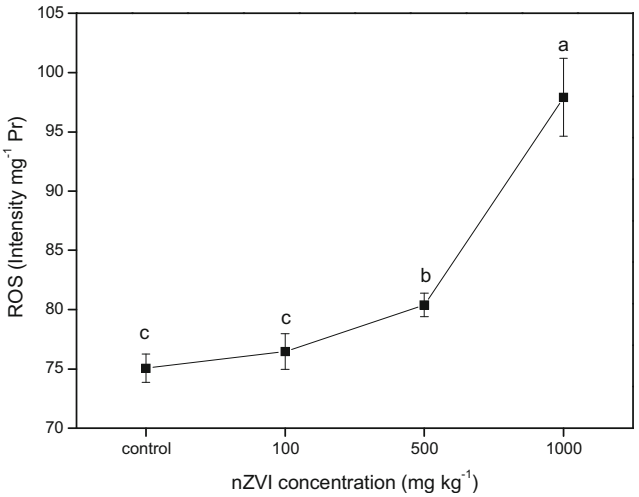

Fig. 7 ROS levels in *Eisenia fetida* exposed to nZVI for 28 days

Table 1 Pearson's correlation coefficients between pairs of parameters measured in earthworms after 28 days of exposure to nZVI in natural soils (*R* value)

Parameters	Growth	Respiration	SOD	CAT	MDA
Respiration	−0.960*				
SOD	0.030	−0.172			
CAT	−0.121	0.304	−0.974*		
MDA	0.975*	−0.895*	−0.186	0.101	
ROS	0.842*	−0.855*	−0.330	0.160	0.880**

SOD superoxide dismutase, *CAT* catalase, *MDA* malondialdehyde, *ROS* reactive oxygen species

*$P < 0.05$; **$P < 0.01$

growth ($R = 0.842$) and respiration ($R = 0.855$) of earthworms. Hence, ROS might be the most sensitive parameter.

According to the previous reports (Adeleye et al. 2016; Clark et al. 2003; Matheson and Tratnyek 1994), non-nano-ZVI particles have been proved non-toxic, indicating that they would not exert significant toxic effects on earthworms. Therefore, we could speculate that the observed results in the current study were mainly nano-specific. Furthermore, Lee et al. (2008) observed that Fe ions may have ability to enter the cells to induce oxidative stress and further damage cell membranes. The hypothesized mechanisms may be that nZVI attached to the surfaces of earthworms and transferred electrons to different biochemicals, leading to some undesired reactions.

Histological effects

The histopathological examination of transverse sections of *E. fetida* after the 28-day exposure is shown in Fig. 8. When the earthworms were exposed to the control group, normal skin structure including epidermal, circular, and longitudinal muscles were observed. The addition of 100 mg kg^{-1} nZVI barely instigated any distinctive change in earthworms. However, the aggravated structures of circular muscle layer and disintegration of longitudinal muscle layer were observed at 500 and 1000 mg kg^{-1} nZVI-treated groups. Similar conclusions were proved by SEM results (Fig. S4). Figure S4a–g illustrated the toxic impacts of different levels of nZVI on earthworms' skin. The intersegmental furrows or setae in earthworms in the control and 100 mg kg^{-1} nZVI tested groups showed normal architecture (Fig. S4a–c). However, various anomalies appeared in earthworms after being exposed to the higher nZVI-treated groups (500 and 1000 mg kg^{-1}) (Fig. S4d–g). For example, irregular folds, disorganized texture, dehydration symptoms, and lacerations were observed clearly in the intersegmental furrows or setae. The present study demonstrated that ROS was induced after

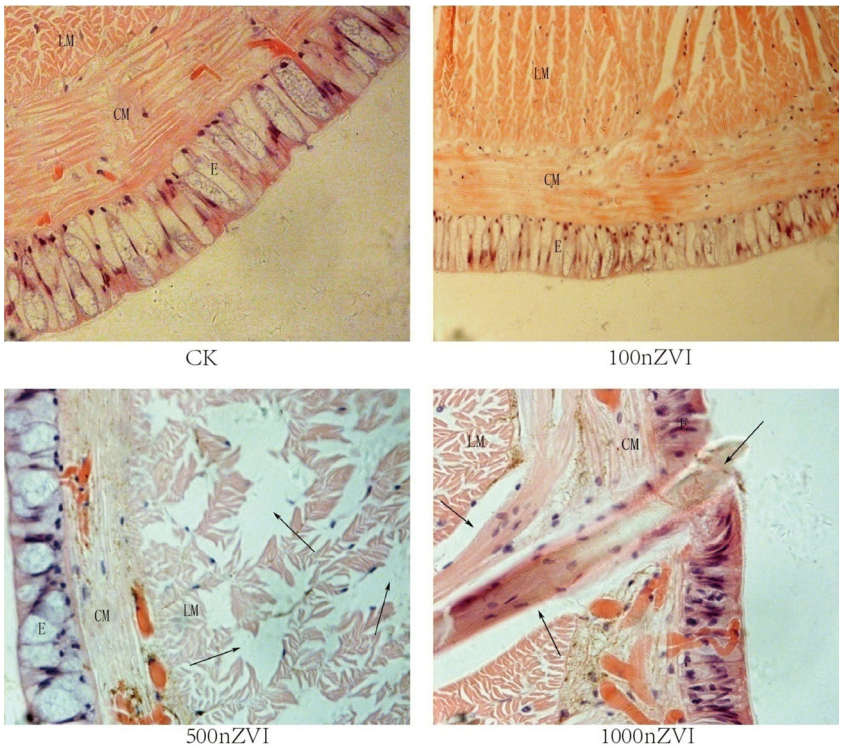

Fig. 8 Histopathological examination of *Eisenia fetida* exposed to nZVI for 28 days

exposure to nZVI, which may be responsible for the degeneration of skin in earthworms. Further investigations are needed to demonstrate the potential mechanisms.

Conclusions

In the present work, multiple biomarkers, including mortality, growth, respiration, avoidance behavior, enzymatic activities (SOD and CAT), MDA content, ROS intensity, and histopathological examination, were investigated to assess the potential toxicological effects of nZVI on earthworms. The adverse impacts of nZVI on the individual parameters of the earthworms, such as growth, respiration, and behavioral parameters, were clearly observed. The biochemical parameters, including SOD and CAT, clearly revealed that oxidative stress was induced by nZVI in earthworms. The accumulation of ROS was obviously induced by nZVI, followed by a significant increase in the MDA level. Furthermore, we observed that the biomarkers differed in their sensitivity to the nZVI exposure, and ROS seemed to be the most sensitive endpoint. The produced ROS might be responsible for the degeneration of skin cells, generating various anomalies in histopathological observations. These comprehensive biomarker responses will be useful in environmental monitoring and could be a useful approach in ecotoxicological studies.

Acknowledgments This research was supported by projects of the National Natural Science Foundation of China (41371467), the Shanghai Pujiang Program (15PJD013), and the National Key Research and Development Program (2016YFD0800405).

References

Adeleye AS, Stevenson LM, Su Y, Nisbet RM, Zhang Y, Keller AA (2016) Influence of phytoplankton on fate and effects of modified zerovalent iron nanoparticles. Environ Sci Technol 50:5597–5605

Amorim MJB, Scott-Fordsmand JJ (2012) Toxicity of copper nanoparticles and $CuCl_2$ salt to *Enchytraeus albidus* worms: survival, reproduction and avoidance responses. Environ Pollut 164:164–168

Chen PJ, Su CH, Tseng CY, Tan SW, Cheng CH (2011) Toxicity assessments of nanoscale zerovalent iron and its oxidation products in medaka (*Oryzias latipes*) fish. Mar Pollut Bull 63:339–346

Chen PJ, Tan SW, Wu WL (2012) Stabilization or oxidation of nanoscale zerovalent iron at environmentally relevant exposure changes bioavailability and toxicity in medaka fish. Environ Sci Technol 46: 8431–8439

Chowdhury AI, Krol MM, Kocur CM, Boparai HK, Weber KP, Sleep BE, O'Carroll DM (2015) nZVI injection into variably saturated soils: field and modeling study. J Contam Hydrol 183:16–28

Clark CJ II, Rao PSC, Annable MD (2003) Degradation of perchloroethylene in cosolvent solutions by zerovalent iron. J Hazard Mater 96: 65–78

Dotan Y, Lichtenberg D, Pinchuk I (2004) Lipid peroxidation cannot be used as a universal criterion of oxidative stress. Prog Lipid Res 43: 200–227

El-Temsah YS, Joner EJ (2012) Ecotoxicological effects on earthworms of fresh and aged nano-sized zerovalent iron (nZVI) in soil. Chemosphere 89:76–82

Foyer CH, Shigeoka S (2011) Understanding oxidative stress and antioxidant functions to enhance photosynthesis. Plant Physiol 155:93–100

Hao LH, Wang ZY, Xing BS (2009) Effect of sub-acute exposure to TiO_2 nanoparticles on oxidative stress and histopathological changes in juvenile carp (*Cyprinus carpio*). J Environ Sci 21:1459–1466

Hu C, Zhang L, Wang W, Cui Y, Li M (2014) Evaluation of the combined toxicity of multi-walled carbon nanotubes and sodium pentachlorophenate on the earthworm *Eisenia fetida* using avoidance bioassay and comet assay. Soil Biol Biochem 70:123–130

Hu S, Zhang W, Li J, Lin K, Ji R (2016) Antioxidant and gene expression responses of *Eisenia fetida* following repeated exposure to BDE209 and Pb in a soil-earthworm system. Sci Total Environ 556:163–168

ISO (2007) Soil quality-avoidance test for testing the quality of soils and effects of chemicals—part 1: test with earthworms (*Eisenia fetida* and *Eisenia andrei*) ISO Guideline 17512–1, Geneve, Switzerland

Khalil AM (2015) Neurotoxicity and biochemical responses in the earthworm *Pheretima hawayana* exposed to TiO_2 NPs. Ecotoxicol Environ Saf 122:455–461

Kwak JI, An YJ (2015) Ecotoxicological effects of nanomaterials on earthworms: a review. Human and Ecological Risk Assessment: An International Journal 21:1566–1575

Lankadurai BP, Nagato EG, Simpson AJ, Simpson MJ (2015) Analysis of *Eisenia fetida* earthworm responses to sub-lethal C_{60} nanoparticle exposure using ^{1}H-NMR based metabolomics. Ecotoxicol Environ Saf 120:48–58

Lee C, Kim JY, Lee WI, Nelson KL, Yoon J, Sedlak DL (2008) Bactericidal effect of zerovalent iron nanoparticles on *Escherichia coli*. Environ Sci Technol 42:4927–4933

Li Y, Hu Y, Ai X, Qiu J, Wang X (2015) Acute and sub-acute effects of enrofloxacin on the earthworm species *Eisenia fetida* in an artificial soil substrate. Eur J Soil Biol 66:19–23

Li Y, Tang H, Hu Y, Wang X, Ai X, Tang L, Matthew C, Cavanagh J, Qiu J (2016) Enrofloxacin at environmentally relevant concentrations enhances uptake and toxicity of cadmium in the earthworm *Eisenia fetida* in farm soils. J Hazard Mater 308:312–320

Liu J, Xiong K, Ye X, Zhang J, Yang Y, Ji L (2015) Toxicity and bioaccumulation of bromadiolone to earthworm *Eisenia fetida*. Chemosphere 135:250–256

Ma X, Gurung A, Deng Y (2013) Phytotoxicity and uptake of nanoscale zerovalent iron (nZVI) by two plant species. Sci Total Environ 443:844–849

Marsalek B, Jancula D, Marsalkova E, Mashlan M, Safarova K, Tucek J, Zboril R (2012) Multimodal action and selective toxicity of zerovalent iron nanoparticles against cyanobacteria. Environ Sci Technol 46:2316–2323

Matheson LJ, Tratnyek PG (1994) Reductive dehalogenation of chlorinated methanes by iron metal. Environ Sci Technol 28:2045–2053

Nelson DW, Sommers LE (1982) Carbon, organic carbon and organic matter. In: Page AL (ed) Methods of soil analysis, part 2: chemical and microbiological properties. Am Soc Agron, Madison, pp 539–580

Nowack B, Bucheli TD (2007) Occurrence, behavior and effects of nanoparticles in the environment. Environ Pollut 150:5–22

OECD (1984) OECD guideline for testing of chemicals. Earthworm acute toxicity. OECD, Paris. No. 207

Phenrat T, Long TC, Lowry GV, Veronesi B (2008) Partial oxidation ("aging") and surface modification decrease the toxicity of nanosized zerovalent iron. Environ Sci Technol 43:195–200

Park K, Tuttle G, Sinche F, Harper SL (2013) Stability of citrate-capped silver nanoparticles in exposure media and their effects on the development of embryonic zebrafish (*Danio rerio*). Arch Pharm Res 36:125–133

Ribeiro S, Sousa J, Nogueira A, Soares A (2001) Effect of endosulfan and parathion on energy reserves and physiological parameters of the terrestrial isopod *Porcellio dilatatus*. Ecotoxicol Environ Saf 49:131–138

Saccà ML, Fajardo C, Costa G, Lobo C, Nande M, Martin M (2014) Integrating classical and molecular approaches to evaluate the impact of nanosized zerovalent iron (nZVI) on soil organisms. Chemosphere 104:184–189

Tang H, Yan Q, Wang X, Ai X, Robin P, Matthew C, Qiu J, Li X, Li Y (2016) Earthworm (*Eisenia fetida*) behavioral and respiration responses to sublethal mercury concentrations in an artificial soil substrate. Appl Soil Ecol 104:48–53

Tosco T, Papini MP, Viggi CC, Sethi R (2014) Nanoscale zerovalent iron particles for groundwater remediation: a review. J Clean Prod 77:10–21

Wang Z, Cui Z, Liu L, Ma Q, Xu X (2016) Toxicological and biochemical responses of the earthworm *Eisenia fetida* exposed to contaminated soil: effects of arsenic species. Chemosphere 154:161–170

Zhang WX (2003) Nanoscale iron particles for environmental remediation: an overview. J Nanopart Res 5:323–332

Zhang W, Liu K, Li J, Chen L, Lin K (2015b) Uptake and depuration kinetics of lead (Pb) and biomarker responses in the earthworm *Eisenia fetida* after simultaneous exposure to decabromodiphenyl ether (BDE209). Ecotoxicol Environ Saf 113:45–51

Zhang W, Liu K, Li J, Liang J, Lin K (2015a) Impacts of BDE209 addition on Pb uptake, subcellular partitioning and gene toxicity in earthworm (*Eisenia fetida*). J Hazard Mater 300:737–744

Zhao X, Liu W, Cai Z, Han B, Qian T, Zhao D (2016) An overview of preparation and applications of stabilized zerovalent iron nanoparticles for soil and groundwater remediation. Water Res 100:245–266

Zhao X, Wang S, Wu Y, You H, Lv L (2013) Acute ZnO nanoparticles exposure induces developmental toxicity, oxidative stress and DNA damage in embryo-larval zebrafish. Aquat Toxicol 136:49–59