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Effects of silver nanoparticles with different dosing regimens and exposure media on artificial ecosystem

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ABSTRACT

Due to the wide use of silver nanoparticles (AgNPs) in various fields, it is crucial to explore the potential negative impacts on the aquatic environment of AgNPs entering into the environment in different ways. In this study, comparative experiments were conducted to investigate the toxicological impacts of polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNPs) with two kinds of dosing regimens, continuous and one-time pulsed dosing, in different exposure media (deionized water and XiangJiang River water). There were a number of quite different experimental results (including 100% mortality of zebrafish, decline in the activity of enzymes, and lowest number and length of adventitious roots) in the one-time pulsed dosing regimen at high PVP-AgNP concentration exposure (HOE) compared to the three other treatments. Meanwhile, we determined that the concentration of leached silver ions from PVP-AgNPs was too low to play a role in zebrafish death. Those results showed that HOE led to a range of dramatic ecosystem impacts which were more destructive than those of other treatments. Moreover, compared with the continuous dosing regimen, despite the fact that higher toxicity was observed for HOE, there was little difference in the removal of total silver from the aquatic environment for the different dosing regimens. No obvious differences in ecological impacts were observed between different water columns under low concentration exposure. Overall, this work highlighted the fact that the toxicity of AgNPs was impacted by different dosing regimens in different exposure media, which may be helpful for assessments of ecological impacts on aquatic environments.

Introduction

With the rapid development of nanotechnology, nanomaterials have already migrated from laboratory research into daily life. The increasing use of nanomaterials in consumer products has resulted in multiple effects on human health and the environment (Feng et al., 2010; Gong et al., 2009; Hu et al., 2016, 2017; Xu et al., 2012; Zhang et al., 2007; Zeng et al., 2013). Silver nanoparticles (AgNPs) are one of the most popular nanomaterials in the Consumer Products Inventory, being present in 435 products (24% of total products) (Vance et al., 2015). Commonly, AgNPs are applied

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in household antiseptic sprays, food packaging, clothes, and antimicrobial coatings of medical devices, because of their low manufacturing cost and broad-spectrum antimicrobial properties (Quadros and Marr, 2011; Faunce and Watal, 2010; Fabrega et al., 2011). The wide applications of AgNPs have inevitably released AgNPs into the aquatic environment, which has given rise to continuously increasing worldwide concern about their toxicological impacts. Hitherto, large numbers of reports have suggested that AgNPs exhibit harmful effects toward most organisms and even important ecosystem processes. For example, AgNPs show toxicity toward organisms including zooplankton (Das et al., 2013), algae (Das et al., 2014), fish (Farmen et al., 2012), and fungus (Huang et al., 2017). In addition, many studies have concluded that the toxicity of AgNPs toward organisms was not solely due to the nanoparticles themselves (Choi et al., 2017; Massarsky et al., 2013). AgNPs in aquatic environments could release silver ions (Ag^+) (Huang et al., 2018). Ag^+ is considered to be more toxic to most aquatic organisms compared to AgNPs (Massarsky et al., 2013).

Although the toxicity mechanism of AgNPs and Ag^+ on fish is well-studied, especially for zebrafish, it is quite hard to extrapolate their effects to an aquatic environment. The majority of AgNPs are released into aquatic ecosystems (Kaegi et al., 2013; Keller et al., 2013). Simulated natural environments have been widely used to understand the processes and impacts of pollutants in the aquatic environment. Hence, many researchers have chosen to study the impacts of AgNPs in simulated environments. In previous studies, Colman et al. (2014) investigated the impacts of AgNP size (12 *vs.* 49 nm) and Ag^+ concentration on a simulated aquatic ecosystem. *Potamogeton diversifolius* and *Egeria densa* in the microcosms (with and without sediments) reduced both the concentration and toxicity of AgNPs and Ag^+ in the water column (Bone et al., 2012). Lowry et al. (2012) simulated an emergent wetland environment to determine the long-term behavior of polyvinylpyrrolidone-coated AgNPs (PVP-AgNPs) in microcosms. Furtado et al. (2015) investigated the fate (persistence, transformations, and distribution) of PVP-AgNPs and citrate-coated AgNPs in boreal lake microcosms by dosing with either a chronic regimen or a one-time pulse at environmentally relevant dosing levels. It is well-known that the heavy use of AgNPs will increase the potential for accidental (uncontrolled leaching from consumer products or textiles) or incidental (untreated runoff) release into the environment. Therefore, it is worthwhile to explore the toxic impacts of AgNPs on natural environments for different ways of entry. Besides, compared to lab scenarios, which are conducted in clean water conditions or in simple media, experiments performed in conditions approaching a real environment will offer more meaningful information. In this work, we not only tested the impacts of PVP-AgNPs on an aquatic environment with two kinds of exposure media and different dosing regimens, but also explored the roles of PVP-AgNPs or released Ag^+ in zebrafish mortality.

To date, there have been a large number of articles on the toxicity mechanism of AgNPs. Currently, the best-developed mechanism of nanoparticle toxicity for eukaryotes is the generation of reactive oxygen species (ROS), which cause an oxidative stress response (Kahru et al., 2008). Cellular damage and death on exposure to AgNPs can be induced by oxidative stress, by means of ROS such as superoxide anions, hydrogen peroxide, and hydroxyl radicals (Grossklaus et al., 2013). To prevent oxidative damage, cells have developed indirect defense mechanisms (physical defense and cell repair systems) and direct defense systems (antioxidant enzymes and low-molecular-weight antioxidants). Superoxide dismutase (SOD) and hydrogen peroxidase (CAT) are used to relieve oxidative damage. The amount of malondiadehyde (MDA) in organisms indicates the degree of damage to cell membranes (Grossklaus et al., 2013). As a consequence, the activities of SOD, CAT and MDA were evaluated in an attempt to reflect the damage to the cell membrane as well as the levels of antioxidant enzymes, which protected them from the oxidative stress induced by AgNPs.

The objectives of this study were to (1) explore the ecological response and toxicity of AgNPs entering the environment in different ways; (2) compare the toxicity of AgNPs on organisms in different water column microcosms; (3) explore the roles of PVP-AgNPs or released Ag^+ in zebrafish mortality. Through these efforts, we hope that the results may help to elucidate differences in toxicity of AgNPs between different exposure media, and explore the ecological response to AgNPs entering the environment in different ways.

1. Materials and methods

1.1. *Preparation and characterization of AgNPs*

PVP-AgNPs were prepared as described in our previous work (Guo et al., 2016). In short, 5 mL of 3.4 mg/L silver nitrate and 12 mL of 5.67 mg/L sodium borohydride (>99% purity, Sigma Aldrich) were mixed in an ice bath with the presence of 0.3% polyvinyl pyrrolidone (PVP10) (molecular weight 10,000, Sigma Aldrich) for 3 min. Then, the solution was stirred at ambient temperature for 2 hr continuously. All prepared suspensions were purified by diafiltration using a 1 kDa regenerated cellulose membrane to remove excess PVP and Ag^+. The concentration of PVP-AgNPs was monitored by flame atomic absorption spectrometry (AAS700, PerkinElmer, USA). The obtained AgNP solutions were stored at 4°C in the dark. PVP-AgNP suspensions and $AgNO_3$ solutions were freshly prepared before use.

The morphology of PVP-AgNPs was characterized by transmission electron microscopy (TEM) on a JEOL JEM-3010 (JEM-3010, JEOL, Japan) at 100 kV. The mean hydrodynamic diameter of particles in the suspension of PVP-AgNPs was determined by dynamic light scattering analysis (including exposure in deionized water and natural water). The zeta potential of AgNPs (including exposure in deionized water and in natural water) was determined using a Zetasizer Nanoseries instrument (Nano-ZS, Malvern Instruments, UK). The ultraviolet (UV) absorption spectrum of the stock AgNP solution was obtained using ultraviolet–visible (UV–vis) light spectrophotometer (Model UV-2550, Shimadzu, Japan) in the wavelength range from 300 to 600 nm.

1.2. Water sample collection and determination of water chemistry

The natural water was taken from the XiangJiang River at 112.95 E and 28.18 N. Before experiments, the water samples were filtered using 0.45-μm filter membranes to remove excessive algae and zooplankton. No observable interfering concentrations of silver were detected in the pretreated water. The pH, temperature and redox potential of the water were recorded in the center of the microcosms. The total nitrogen and total phosphorus were also assayed. Dissolved organic carbon was analyzed for non-purgeable organic carbon using total organic carbon (TOC) analyzer. (TOC-V CPH, Shimadzu, Japan).

1.3. Experimental setup

1.3.1. Microcosm design

Matrices were constructed in 3000-mL beakers containing 900-g silica sand, 3000-mL water, and aquatic organisms (16 g of *E. densa* and 12 zebrafish). Quartz sand with particle size 10–20 mesh was purchased from the Changsha Shengyang Chemical Materials Limited Company. The microcosms were divided into two groups with different waters: (1) 3000-mL river water; (2) 3000-mL deionized water (DI). Other components were the same. Then, microcosms were incubated at 25°C with a 14:10 hr light/dark cycle under a fluorescent lamp. Four different environmental matrices were established, and all the treatments were conducted in triplicate for each concentration matrix.

Microcosms were designed with four environmental matrices as shown in Table 1, including two concentration categories: low concentration PVP-AgNP exposure (0.1 to 1 mg/L) and high concentration PVP-AgNP exposure (1.5 to 2 mg/L). The low concentration exposures included low concentrations of PVP-AgNPs in deionized water (LDW) and in natural River water (LRW). The high concentration PVP-AgNP exposures included high concentrations of PVP-AgNPs with one-time pulsed exposure (HOE) and continuous exposure (HCE).

1.3.2. Microcosm dosing

Dosing in the HCE was maintained for 15 days, and with 3 additions (every day) of 11.96, 14.52, and 16.24 mL of PVP-AgNP stock suspension to the microcosms to achieve nominal concentrations of 1.5, 1.8, and 2.0 mg/L, respectively. The PVP-AgNP stock suspension was added to the treatments for target nominal concentrations of 0.1, 0.3, and 0.5 mg/L in the LDW; 0.3, 0.5, and 1 mg/L in the LRW, and 1.5, 1.8, and 2.0 mg/L in the HOE treatment. The water column was mixed after each addition by vertical passes with a plastic disk to avoid disturbing the *E. densa* and fish.

1.3.3. Sampling time

During the 21-day exposure, water samples of LDW, LRW, and HOE were taken at specific time intervals (0.5, 1, 3, 6, and 24 hr; 3, 5, 10, 15 and 21 days). For HCE, water samples were taken every three days during the first 15 days, and then were taken every day for analysis during days 16–21.

1.4. Determination of silver concentration

1.4.1. Total Ag measurements

PVP-AgNP suspensions were digested with ultrapure HNO_3 and measured by flame atomic absorption spectrometry (AAS700, PerkinElmer, USA).

1.4.2. Silver ions

Silver ions released by PVP-AgNPs were separated by centrifugal ultrafiltration (Amicon Ultra-0.5 3K, Millipore, USA) using a membrane with a nominal molecular weight limit of 3 KDa. Suspensions were centrifuged at 3000 r/min for 30 min. Filtered solutions were acidified with 4% HNO_3 and measured by inductively coupled plasma optical emission spectrometry (ICP-OES, IRIS Intrepid II XSP, Thermo Electron Corporation, USA).

1.5. Biological assay

1.5.1. Cultivations of zebrafish and E. densa

Adult zebrafish were obtained from the Pearl River Fisheries Research Institute (mixed sex, 3–4 cm in length). *E. densa* was collected from a specialized plant breeding base. *E. densa* was placed in clean water and washed gently. Then zebrafish and *E. densa* were placed in a rectangular pool respectively, containing river water under a light and dark cycle (14 hr : 10 hr) for at least two weeks to adapt. A cool fluorescent lamp with a photosynthetic photon flux of 100 $\mu mol/(m \cdot sec)^2$ was placed above the rectangular pool. At the same time, an oxygen pump was placed in the pool to provide enough oxygen for the zebrafish every day before the formal experiment. The water was renewed every seven days to remove

Table 1 – Four environmental matrices (LDW, LRW, HOE and HCE) designed for the experiments, and the configurations of each matrix.

Category	Concentration of AgNPs		Dosing regimens		Exposure media	
	Low concentration	High concentration	Continuous dosing	One-time dosing	Deionized water	River water
LDW	+	–	–	+	+	–
LRW	+	–	–	+	–	+
HOE	–	+	–	+	–	+
HCE	–	+	+	–	–	+

AgNPs: silver nanoparticles; LDW: the experiment was conducted in deionized water at low concentration of AgNPs; LRW: in river water at low concentration of AgNPs; HOE: at high concentration of AgNPs with one-time pulse dosing regimen; HCE: at high concentration of AgNPs with continuous dosing regimen; + and – refer to having and having not the configuration, respectively.

waste during the acclimation period, while the water was not renewed in the formal experiment. Zebrafish were fed every two days at noon with dry flake food and brine shrimp during the acclimation period and formal experiment.

1.5.2. Enzyme assays

At the endpoint of exposure, zebrafish were frozen at −80°C until analysis. The *E. densa* and zebrafish were collected at the end of the 21th day. The whole fish bodies for the same treatment were ground and homogenized before analysis. All *E. densa* for the same treatment were ground and mixed. The subsequent steps followed methods appropriate to the different parameters.

The supernatant of SOD was centrifuged at 4000 r/min at 4°C for 15 min. SOD activity was assayed by the nitro-blue tetrazolium colorimetric method (Qiu et al., 2008), and the samples were read at 560 nm. For SOD, inhibition of 50% of the reaction was defined as one unit of enzyme and the enzyme activity was expressed as U/g fresh weight (U/g FW).

The CAT activity was evaluated according to the method reported by Qiu et al. (2008). The reaction mixture consisted of 1.5-mL phosphate buffer, 0.2-mL enzyme extract and 1.0-mL ultrapure water. The reaction was started by adding 0.3-mL hydrogen peroxide (34 mg/L), and a control assay was carried out in the absence of hydrogen peroxide. For CAT, the enzyme activities were expressed as U/g FW, and one unit of enzyme was defined as the increase (decrease) of 0.1 unit of absorbance at 240 nm (A_{240}). The CAT and SOD were monitored by a UV–vis light spectrophotometer (Model UV-2550, Shimadzu, Japan).

1.5.3. Determination of malondialdehyde

The lipid peroxidation level was expressed by the production of MDA. The reaction mixture consisted of 5-mL 5% thiobarbiturate and the zebrafish or *E. densa* extract. Then the reaction mixture was placed in boiling water for 10 min until the appearance of small bubbles, and cooled at room temperature. The mixture was centrifuged at 3500 r/min for 15 min, and the supernatant was extracted. The control sample only consisted of 5-mL 5% thiobarbiturate. The MDA was monitored by the absorbance at 450, 532, and 600 nm using a UV–vis spectrophotometer (Model UV-2550, Shimadzu, Japan) (Zeng et al., 2012).

1.5.4. Quantification of total silver in biota

The thawed fish were charred in crucibles on a hot plate first, followed by moving the crucibles to a muffle furnace at 550°C for 6 hr and cooled to room temperature, followed by digestion of the ashed samples. Ashed samples were digested with acid (concentrated nitric acid) using a Graphite digester. The digestion solutions were diluted to constant volume for analysis.

The *E. densa* was washed with deionized water to remove the adhered PVP-AgNPs. Then, the *E. densa* was stored individually in paper bags, and dried in an air oven at 70°C for 48 hr. All *E. densa* for the same treatment were weighed when the temperature naturally cooled to room temperature after drying. The subsequent processes followed the steps described for zebrafish.

The silver content of *E. densa* and zebrafish was determined for each subsample by flame atomic absorption spectrometry (AAS700, PerkinElmer, USA) after digestion.

1.6. Auxiliary experiments

1.6.1. Experiments for color change

The experimental conditions were with the same as for HOE, but the river water used in HOE was changed to deionized water.

1.6.2. Experiments on Ag^+ toxicity

The toxicity of Ag^+ toward zebrafish was assessed by concentration-response experiments, by exposing zebrafish to increasing concentrations of $AgNO_3$. $AgNO_3$ concentrations ranged from 5 to 40 μg/L, and the concentration-response experimental conditions were consistent with those for HOE.

1.7. Statistics analysis

Analysis of variance (ANOVA) was performed by using SPSS 19.0 (SPSS, USA) with Tukey's multiple comparisons. Significant differences were determined using one-way ANOVA ($p < 0.05$). All reported error bars represent one standard error of the arithmetic mean.

2. Results

2.1. AgNP characterization and water chemistry

The average diameters of PVP-AgNPs in DI water and river water were 23.67 ± 0.90 and 85.86 ± 5.6 nm, respectively, detected by dynamic light scattering. The shape of PVP-AgNPs was characterized by TEM, which showed that the PVP-AgNPs were spherical, without intense aggregation. The concentration of PVP-AgNPs was 25 mg/L. The UV absorption peak of the synthesized PVP-AgNPs was 392 nm, which corresponded to the results of Zuo et al. (2015). The zeta potential of the PVP-AgNPs in DI water was −35.3 ± 6.8 mV, and that in river water was −3.5 ± 0.9 mV. The temperature increased over the duration of the experiment from 25 to 28°C. The redox potential was −33 mV and the pH ranged from 7.23 to 7.48. Concentrations of TOC ranged between 9.0 and 10.9 mg/L. The concentrations of total nitrogen and total phosphorus were 6.14 and 0.06 mg/L, respectively.

2.2. Total silver concentration in water column

In general, the total Ag (TAg) concentration gradually decreased over time in the one-time pulsed microcosms (Fig. 1a, b, and c). The TAg concentration increased incrementally when PVP-AgNPs were added into HCE proportionally, and then a decline occurred after the 15th day because the addition of PVP-AgNPs was stopped (Fig. 1d). A common feature during the one-time addition was that the TAg concentration declined sharply within 24 hr, decreasing from 0.300 to 0.116 mg/L for LDW treatment, 0.500 to 0.289 mg/L for LRW treatment, and 2.00 to 1.06 mg/L for HOE treatment, respectively (Fig. 1a, b, and c). Similarly, the initial

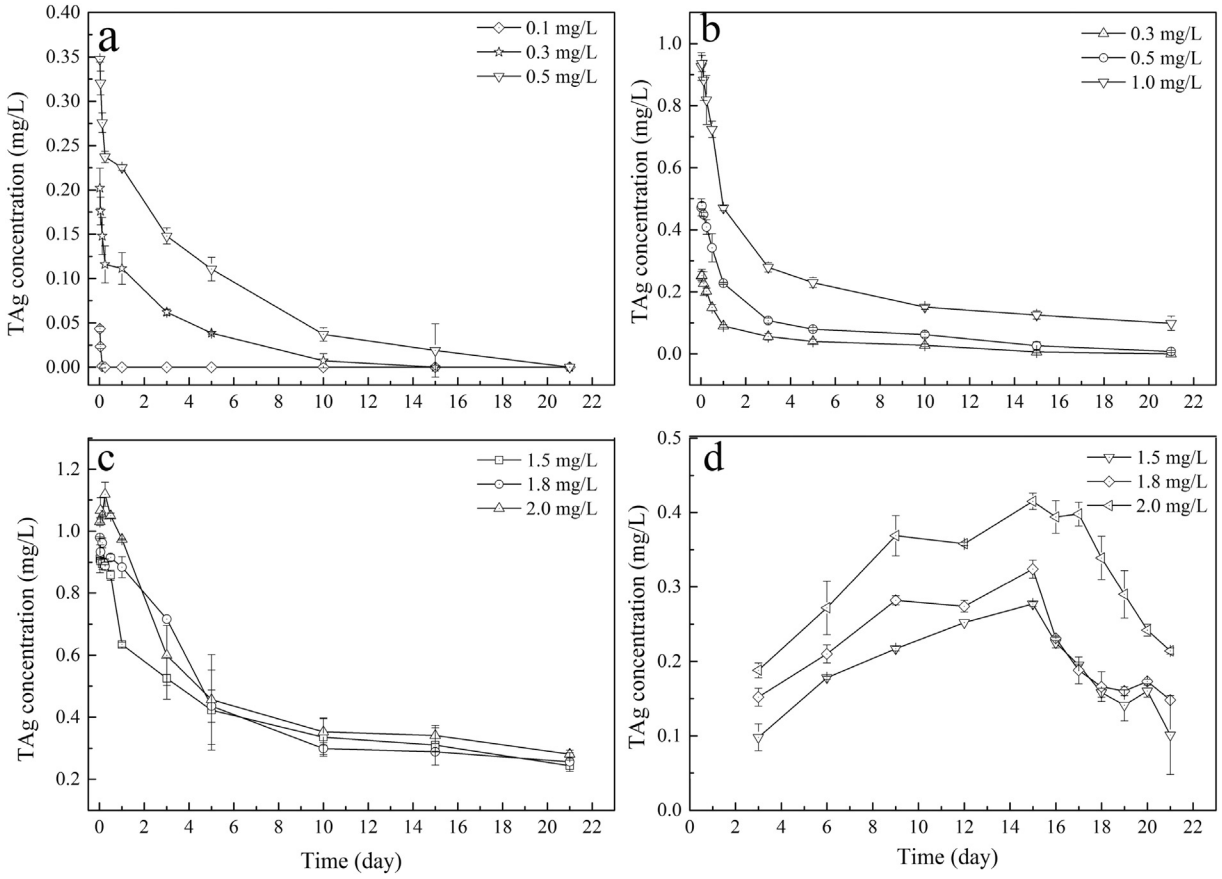

Fig. 1 – Variation of total Ag (TAg) concentration over time in the four treatments, containing low concentration of polyvinylpyrrolidone-coated Ag nanoparticles (PVP-AgNPs) in (a) deionized water and (b) in river water, and high concentration of PVP-AgNPs with (c) one-time pulsed exposure and (d) continuous exposure.

relatively rapid decline of TAg became slow in the LDW, LRW, and HOE treatments after the 5th day (Fig. 1a, b, and c). The TAg concentration in the HOE (nominal PVP-AgNP concentration 2.0 mg/L) was 0.281 mg/L after 21 days of exposure, while that in the HCE was about 0.214 mg/L, which was lower than the TAg concentration in the HOE treatment. The TAg concentration was below the detection limit after 7 days following exposure to 0.3 mg/L PVP-AgNPs. The HCE treatment was expected to reach the target concentration in 14 days, but the final concentration was approximately 19.1% of the target concentration at the end of addition. Different treatments showed a similar result, that the TAg concentration determined in the water column was always less than the concentration of Ag added (Fig. 1).

2.3. Effects of AgNPs on zebrafish

2.3.1. Mortality of zebrafish

The trend of mortality rates in LDW was similar to that in the LRW treatment. The mortality rates of zebrafish in both LDW and LRW increased with the elevation of PVP-AgNP concentration. In the LDW treatment, the mortality rates of zebrafish were 8.33%, 16.67%, and 25.00% at 0.1, 0.3, and 0.5 mg/L PVP-AgNP concentration, respectively. In the LRW treatment, the mortality rates of zebrafish were 19.44%, 30.55%, and 36.11% at PVP-AgNP concentrations of 0.3, 0.5, and 1 mg/L, respectively (Fig. 2). However, the mortality of the zebrafish in the HOE (100%) was significantly higher than that in the HCE treatment, with maximum and minimum mortality of 66.7% and 41.6%, respectively (ANOVA, $p < 0.05$).

2.3.2. Oxidative stress

To explore the impacts of oxidative damage on zebrafish (Fig. 3) and *E. densa* (Fig. 4) under AgNP stress, the activities of SOD and CAT in response to PVP-AgNP exposure were determined. In low-concentration treatments (LDW and LRW), there was no significant difference in the SOD activity of zebrafish ($p > 0.05$). The SOD activity of zebrafish in the HCE treatment showed an obvious decreasing trend, and the magnitude of the change was greater than that in low-concentration exposure (Fig. 3b). The CAT activity in zebrafish showed large variability for the LDW and LRW treatments, however, no significant difference was found between the LDW and LRW treatments ($p > 0.05$). The CAT activity in zebrafish decreased in the HCE ($p < 0.05$) and was lower than that of other treatments (Fig. 3a). The decreasing levels of enzymatic activity corresponded to increased oxidative stress. Similar results were observed by García-Sánchez et al. (2012).

Membrane damage in cells of zebrafish and *E. densa* was evaluated by quantifying the production of MDA after PVP-

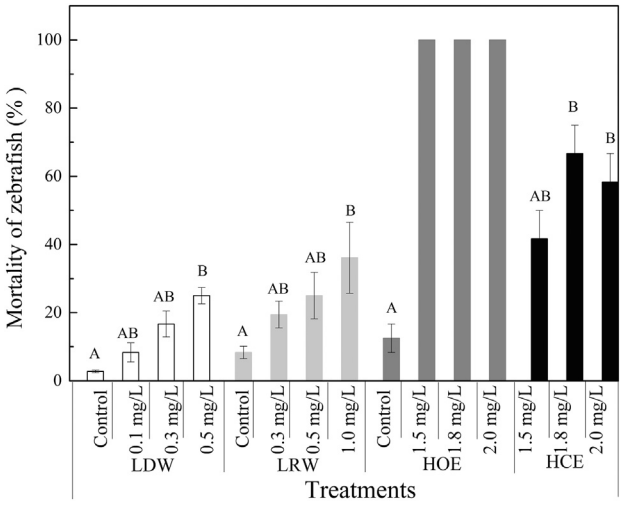

Fig. 2 – Mortality of zebrafish in the four treatments obtained at the end of the experiment. Different letters (A, AB and B) over the bars represented statistically different concentrations within treatments (LDW, LRW, HOE and HCE), respectively. LDW: in deionized water at low concentration of AgNPs; LRW: in river water at low concentration of AgNPs; HOE: at a high concentration of AgNPs with one-time pulse dosing regimen; HCE: at a high concentration of AgNPs with continuous dosing regimen.

AgNP exposure. MDA is a product of lipid peroxidation, and its release levels are indicative of membrane damage (Zeng et al., 2012). The results showed that the MDA content of zebrafish in the LDW was higher than that of control. However, no significant difference was detected between the LRW and LDW treatments. There was an ascendant trend of MDA in zebrafish (Fig. 3c), and no significant difference was observed in the HCE treatment ($p > 0.05$).

2.4. *Effects of Ag^+ on zebrafish*

Fig. 4a shows that the dissolution rate of PVP-AgNPs increased rapidly in the first 12 hr, and reached a plateau at about 48 hr. The amount of leached Ag^+ from PVP-AgNPs was low, less than 0.7%. As shown in Fig. 4a, the exposure concentrations of 1.5–2.0 mg/L PVP-AgNPs contributed amounts of Ag^+ ranging from 9 to 13 μg/L. Therefore, $AgNO_3$ concentrations were set at 5, 10, 20, 30, and 40 μg/L. Fig. 4b demonstrates that $AgNO_3$ concentrations less than 30 μg/L did not cause the death of zebrafish.

2.5. *Effects of AgNPs on E. densa*

2.5.1. *Oxidative stress*

E. densa maintained a good growth status in the low-concentration exposures. The SOD activity of *E. densa* displayed variability for LDW and LRW treatments, but no significant difference ($p > 0.05$) was observed among LDW and LRW treatments (Fig. 5b). When the nominal PVP-AgNP concentration was 2.0 mg/L, the SOD of *E. densa* in the high concentration treatments (the HOE and HCE) compared to

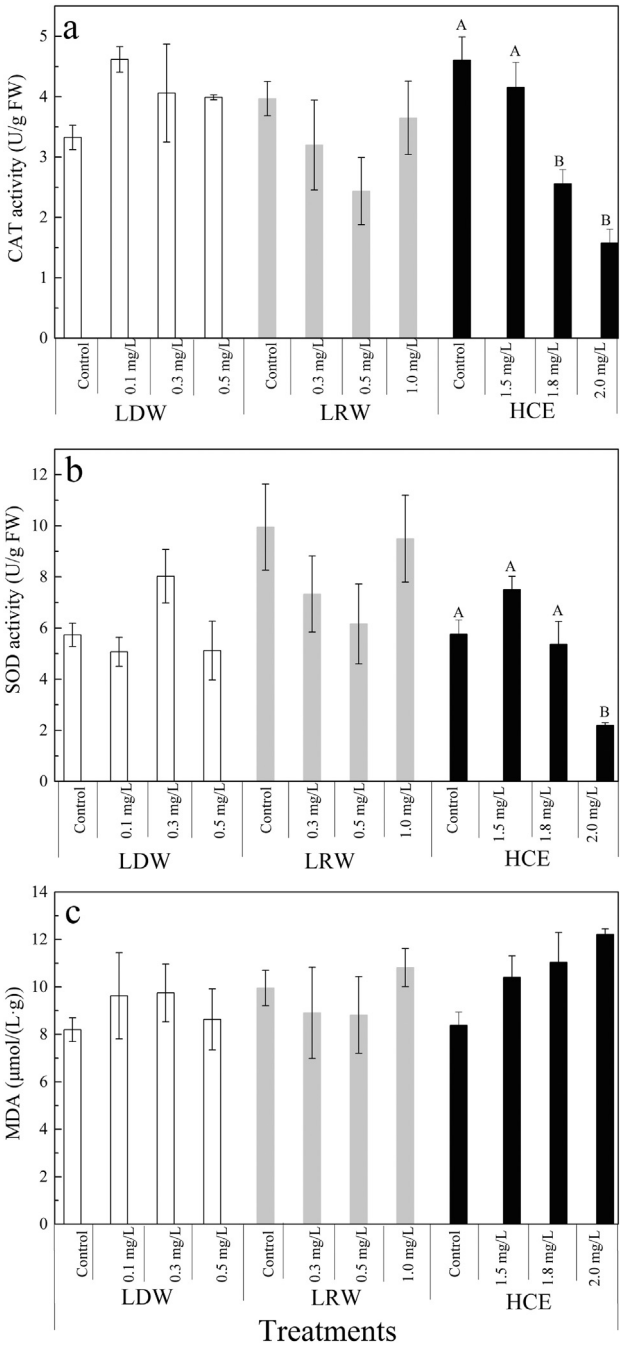

Fig. 3 – (a) Hydrogen peroxidase (CAT) activity, (b) superoxide dismutase (SOD) activity and (c) malondiadehyde (MDA) content of zebrafish in different exposure treatments. Data are not available for HOE due to 100% mortality of zebrafish. Treatments with no letters indicate $p > 0.05$ (ANOVA) within treatments (LDW, LRW and HCE). Different letters (A and B) over the bars represent statistically different concentration matrices within treatments (LDW, LRW and HCE). FW: fresh weight.

control (15.68 U/g FW) was significantly higher than those in the HOE and HCE treatments (Fig. 5b). Significant differences of SOD in *E. Densa* were detected among the HOE and HCE treatments ($p < 0.05$).

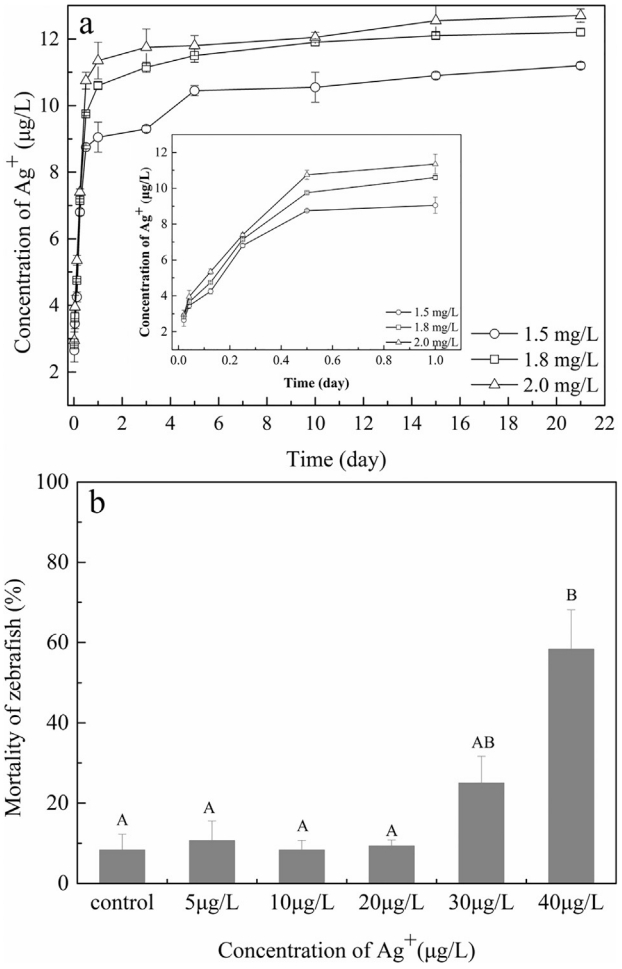

Fig. 4 – (a) Dissolved concentration of AgNPs in HOE and (b) mortality of zebrafish in concentration-response experiments. The concentrations of Ag^+ in (b) were according to the concentration of Ag^+ released by AgNPs. Different letters (A, AB and B) over the bars represent statistically different concentration matrices within treatments.

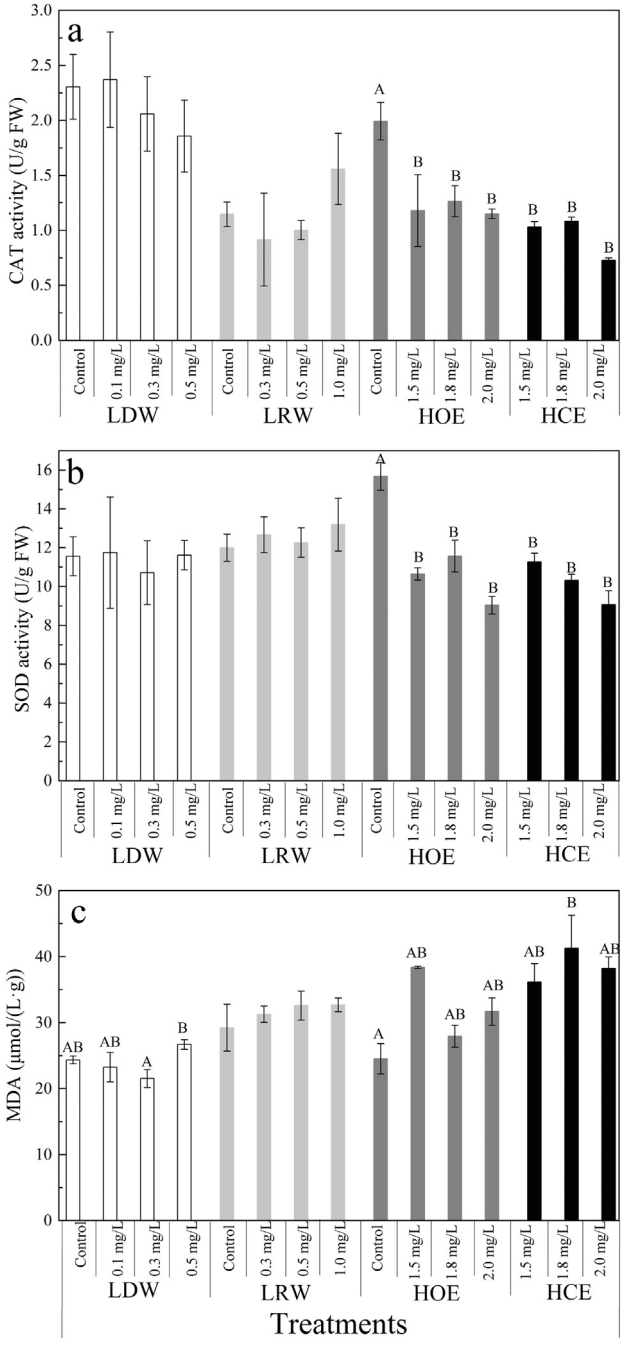

Fig. 5 – (a) CAT activity, (b) SOD activity and (c) MDA content of *Egeria densa* in four treatments. Treatments with no letters indicate $p > 0.05$ (ANOVA) within treatments (LDW, LRW, HOE and HCE). Different letters (A, AB and B) over the bars represent statistically different concentration matrices within treatments (LDW, LRW, HOE and HCE).

There was no observable difference in the CAT activity in *E. densa* among LDW and LRW treatments ($p > 0.05$), although the CAT activity showed variability in the LDW and LRW treatments. The CAT activity of *E. densa* was significantly lower ($p < 0.05$) in the HOE (1.15 U/g FW) and HCE (0.96 U/g FW) treatment than control (1.99 U/g FW) when the added concentration of AgNPs was 2.0 mg/L (Fig. 5a). An increase of MDA content for *E. densa* was observed in the LDW, HCE and HOE with elevation of the PVP-AgNP concentration (Fig. 5c).

2.5.2. Effect of AgNP toxicity on the growth of E. densa

The *E. densa* was green in the low-concentration exposure. In the HOE treatment, *E. densa* turned yellow, which suggested that the condition was unfavorable for growth. *E. densa* will grow adventitious roots to adapt to changing environments; therefore, growth of adventitious roots reflects the growth state of *E. densa* (Norie et al., 2003). In the LDW, the quantity of adventitious roots was higher in the blank control (11.33 ± 1.24) compared to the 0.1 mg/L (9.00 ± 1.60), 0.3 mg/L (8.33 ± 1.24) and 0.5 mg/L (8.67 ± 1.69) treatments, but the LDW treatments showed no significant differences among each other (Fig. 6a) ($p > 0.05$). The quantities of roots in the LRW treatment for the control (19.67 ± 2.60) and 0.3 mg/L treatment (20.33 ± 2.86) were significantly higher than those for 0.5 mg/L (12.44 ± 4.70) and 1 mg/L (14 ± 2.16) ($p < 0.05$) treatments. The quantity of roots in the LRW was higher than that in the LDW treatment. There was a slight decrease in root

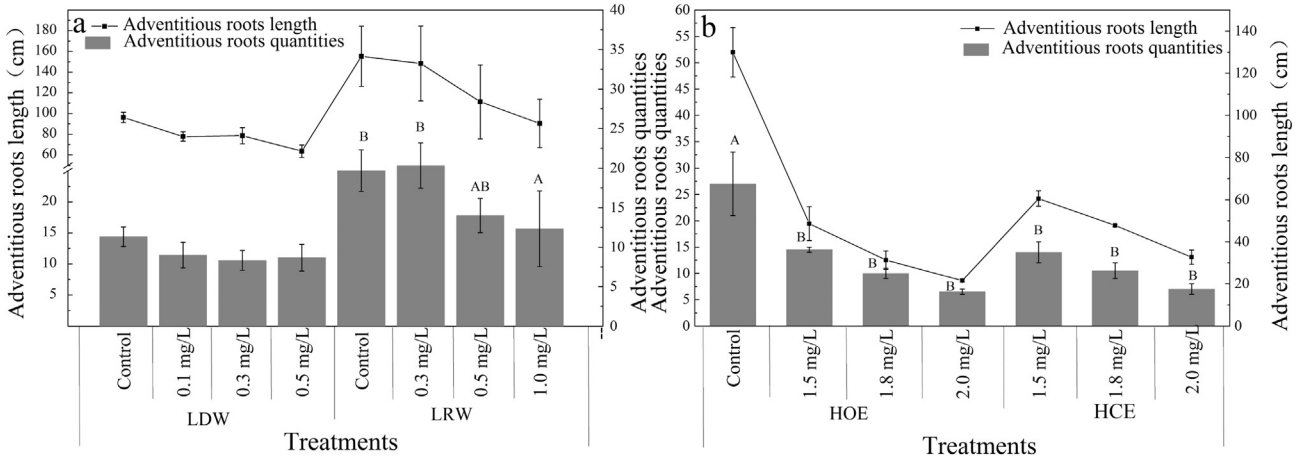

Fig. 6 – Length and quantities of *E. densa* roots as impacted by different concentrations of PVP-AgNPs under (a) low concentration exposure treatment and (b) high concentration exposure treatment. Different letters (A, AB and B) over the bars represent statistically different concentration matrices within treatments (LDW, LRW, HOE and HCE). Treatments with no letters indicate $p > 0.05$ within treatments (LDW, LRW, HOE and HCE).

quantity for the high concentration exposure treatments (the HOE and HCE treatments). The quantity and length of adventitious roots decreased with increasing PVP-AgNP concentration, and this was a common trend among the four treatments (Fig. 6).

2.6. Accumulation of Ag in *E. densa* and zebrafish

In all treatments, the silver content in *E. densa* and zebrafish exhibited an increasing trend with elevated exposure concentration. When exposed to AgNP concentrations of 0.3, 0.5, and 1 mg/L, zebrafish accumulated 1.31 ± 0.64, 10.7 ± 7.50, and 30.9 ± 1.10 μg Ag/g in LRW, respectively. In the LDW treatment, the silver contents of zebrafish were 3.03 ± 0.17 μg Ag/g (exposed to nominal concentration of 0.3 mg/L) and 5.50 ± 0.32 μg Ag/g (0.5 mg/L). No significant trend in body accumulation was found between different water columns ($p > 0.05$). In the HCE, there was an increasing trend ($p < 0.05$) with the elevation of exposure concentration (Fig. 7a).

E. densa accumulated 40 ± 2.8 μg Ag/g (nominal concentration of AgNPs was 0.3 mg/L), 67 ± 2.7 μg Ag/g (0.5 mg/L) and 226 ± 43.0 μg Ag/g (1 mg/L) in the LRW treatment. In the LDW treatment, the silver content in *E. densa* was 18.3 ± 2.6 μg Ag/g (0.1 mg/L), 46.9 ± 14.5 μg Ag/g (0.3 mg/L) and 74.8 ± 8.0 μg Ag/g (0.5 mg/L). Similar ascending trends of accumulation in *E. densa* were observed among all treatments ($p < 0.05$). Moreover, it was obvious that plants were more likely to accumulate silver than animals and that the tissue burden of Ag increased with elevation of PVP-AgNP concentrations in the aquatic environment (Fig. 7b).

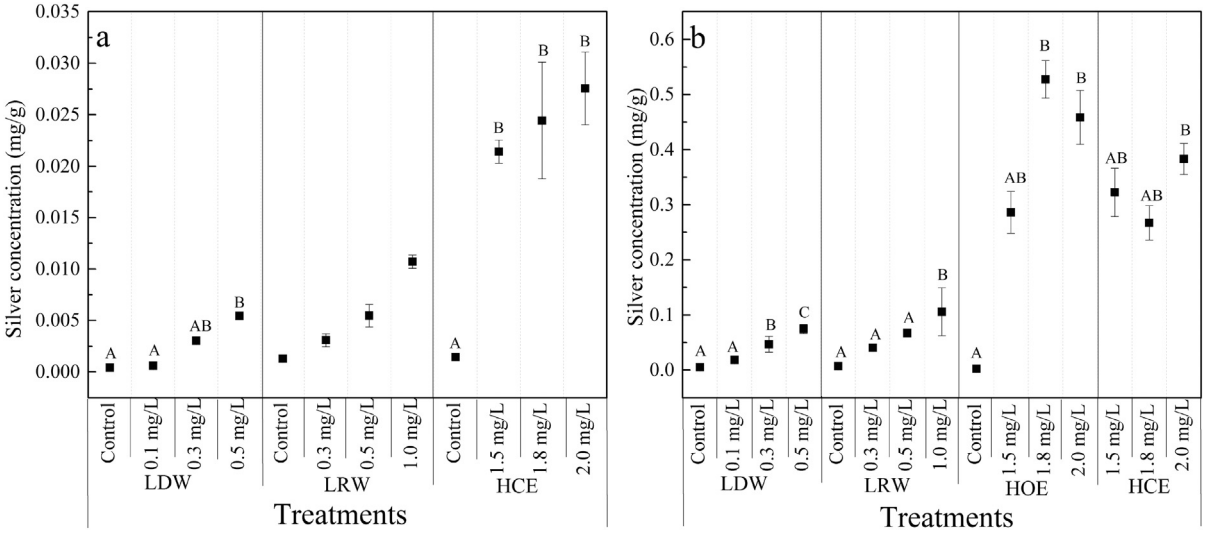

Fig. 7 – Silver content of (a) zebrafish and (b) *E. densa* in different treatments. Different letters (A, B, AB and C) over the bars represent statistically different concentration matrices within treatments (LDW, LRW, HOE and HCE).

3. Discussion

3.1. Removal of AgNPs from aquatic environment

AgNPs' inherent properties (*e.g.*, size and capping agents) and environmental factors affect the stability, transformation, and fate of AgNPs (Ramskov et al., 2015). Kennedy et al. (2012) reported that agglomeration increased with increasing AgNP concentration, and high concentrations of AgNPs could lead to more homoagglomeration. In this study, the exposure concentrations of AgNPs (0.1–2 mg/L) that we used were reasonable compared to values found in the actual environment, and lower magnitude than those in other studies expressing obvious effects. Although realistic exposure concentrations of AgNPs are likely to be low in the natural environment, their fate also can be affected by the complex and varied environmental media (Bernhardt et al., 2010). The enlargement of PVP-AgNPs' average diameter and increase in the zeta potential suggested that the stability of the PVP-AgNPs had been impaired in the river water column. Gottschalk et al. (2013) reported that heteroagglomeration may be a major factor impacting AgNP behavior as the concentrations approach those expected in natural water. It is known that many factors in natural waters affect the fate of AgNPs: (1) the appearance of organic ligands that may reduce Ag^{+} to Ag^{0}; (2) the presence of electrolytes (*e.g.*, S^{2-}, Cl^{-}) leading to precipitated Ag species (Levard et al., 2011); (3) the impact of agglomeration in blocking reaction sites (He et al., 2013); and (4) accumulation in biota. In our experiment, different treatments showed a similar trend whereby the TAg determined in the water column was always less than the concentration of Ag added (Fig. 1). This might be attributed to the fact that once the AgNPs are released into the water, the TAg concentration could be reduced after interaction of the nanoparticles with various factors in the aquatic environment. The accumulation of AgNPs in biota is possibly influenced by the exposure concentrations and media conditions, as well as the differences among species (Cong et al., 2011; Huang et al., 2008). As shown in Fig. 7, the *E. densa* accumulated more silver in tissue compared to zebrafish. In addition, the mass of AgNPs accumulated in zebrafish and *E. densa* was elevated with increasing exposure concentration. Thus, interaction with various factors in the natural environment and accumulation in biota were the dominant reasons for AgNP removal from the aquatic environment. In this study, the TAg concentration for nominal AgNP concentrations under 0.5 mg/L did not reach the detection limit, and the TAg concentration reached 14.0% of the target concentration in HOE and 10.7% in HCE at the end of the experiment after exposure to 2 mg/L PVP-AgNPs (Fig. 1). Undoubtedly, the TAg concentration declined over time. Those results suggested that AgNPs were ultimately removed from the water column, ending up in plants and influenced by many other factors in the actual water.

The mortality of zebrafish and the damage exhibited by antioxidant enzymes had similar magnitudes in the LDW and LRW treatments. Likewise, no significant difference of TAg concentration appeared in the LDW and LRW treatments. On the basis of these results, little difference was observed in the biotoxicity or environmental fate of AgNPs between the two kinds of water column. There were indications that the exposure concentrations of AgNPs, which approached realistic concentrations in the natural environment, caused minor harm in the actual ecosystem.

3.2. Effects of different dosing regimens

There was no color change in the water in HCE; in contrast, a visual color change from the initial yellow color to red was observed in HOE after about 24 hr. Meanwhile, to further investigate the color change, comparison experiments were implemented in DI water under the same experimental conditions as HOE. No color change was observed in the comparison experiment. Thus, the reason for the color change was related to the matters in river water. Specifically, the color change might be ascribed to the aggregation of AgNPs, which would cause a color change as reported by Guo et al. (2014). They found that AgNP heteroagglomeration increased as the concentration of Tween 20-AgNPs varied from 0.5 to 17 mg/L, and a change of color was also observed. In particular, the color was not changed while the concentration of Tween 20-AgNPs was as low as μg/L (Guo et al., 2014). In this experiment, the heteroagglomeration of PVP-AgNPs occurred by reaction with matters in the aquatic environment, causing color change in the one-time dosing regimen at high concentration. The reason for the lack of color change in the continuous dosing regimen treatment was that the initial concentration was not changed.

There were a number of quite different experimental phenomena in HOE compared to the other three treatments (including 100% mortality of zebrafish, *E. densa* becoming yellow, the edges of leaves gradually decaying and falling off, and the lowest numbers and lengths of adventitious roots). These phenomena showed that HOE was more toxic to the ecosystem compared with the other treatments. Several studies have researched AgNP toxicity *in vivo* in aquatic organisms (Handy et al., 2011; Baker et al., 2014). For fish, lethal and sublethal toxic concentrations were reached in the low (mg/L) or high (μg/L) range (Chae et al., 2009; Laban et al., 2010). Coincidentally, 100% mortality of zebrafish appeared in the one-time dosing regimen (Fig. 2). Naturally, our results suggested that those obvious detriments were caused after exposure to a high concentration of AgNPs. For the continuous dosing regimen, there were moderate impacts at the end of the experiment.

The TAg concentrations remaining at the end of the experiment were 0.281 and 0.214 mg/L for exposure to 2 mg/L PVP-AgNPs in the HOE and HCE treatments, respectively (Fig. 1). No significant trend of silver content in *E. densa* was found between the HOE and HCE treatments (Fig. 7). Furtado et al. (2015) found that various factors, such as the presence of electrolytes, high dissolved organic carbon (DOC) concentration, and the presence of biotic particles accelerating heteroagglomeration, may have alleviated the effects of continuous dosing on AgNP fate in a realistic aquatic environment. Likewise, our data proved that the continuous dosing regimen did not lead to longer persistence of the AgNPs in the aquatic environment. In brief, different dosing regimens would result in different magnitudes of damage to

the ecosystem, but had little influence on the removal of AgNPs from the aquatic environment.

3.3. Ecological responses

In the HOE treatment, there was a cascade of ecosystem effects. The initially green leaves turned yellow and senescence was observed for the macrophytes, together with a decline in the rate of leaf photosynthesis. Colman et al. (2014) reported that high concentration of PVP-AgNP prevented photosynthesis and caused a swift several-fold increase in DOC, CO_2, and Cl^-, and rapid depletion of O_2 in the water column. Some papers have demonstrated that the presence of aquatic plants would reduce the toxicity of AgNPs (Das et al., 2014). It should be pointed out that the photosynthesis of impaired plants was limited and the plants needed oxygen for respiration, leading to the depletion of O_2 in the aquatic environment.

Additionally, because AgNPs will release Ag^+ in an aquatic environment, whether dissolved Ag or AgNPs play the predominant role in the toxicity toward biota has always been controversial. In this work, the concentration of Ag^+ leached from AgNPs in HOE ranged from 9 to 13 μg/L. Subsequent experiments demonstrated that the concentration of Ag^+ (which was less than 30 μg/L) did not cause the zebrafish mortality (Massarsky et al., 2013). Consequently, the dissolution of AgNPs did not play a key role in zebrafish death. In fact, the zebrafish died of AgNP toxicity.

The biota will be prone to oxidative stress when exposed to heavy metal pollutants (García-Sánchez et al., 2012), and the antioxidant defense mechanism has been well-studied in recent years. Normally, SOD is regarded as the first line of defense against ROS (Colman et al., 2014). CAT plays an important role in this process, the inactivation of hydrogen peroxide. The activities of CAT and SOD were reduced (Fig. 5) for high concentrations of PVP-AgNPs in the HCE treatment. This might be due to long-term stress caused by PVP-AgNPs, with oxidative damage beyond the cell tolerance range. PVP-AgNPs would cause cell damage or even cause death when the defense systems were overloaded (Dazy et al., 2009), just as with the mortality of zebrafish in the HOE treatment. Different kinds of organisms have different tolerances for AgNPs. According to Figs. 3 and 5, zebrafish were more sensitive to AgNPs than *E. densa* in the aquatic ecosystem. Hence, the highly sensitive zebrafish can be a sentinel species to monitor environmental pollution, particularly heavy metal pollution.

The results in Fig. 7 revealed that the content of Ag in *E. densa* was increased with the elevation of PVP-AgNP concentration, and similar results were also observed for manufactured nanoparticles (*e.g.*, nano-TiO_2, nano-CeO_2, and nano-Au) (Wang and Wang, 2014). Undoubtedly, plants are an important sink for Ag, no matter whether due to uptake by plants, biofilms (Yang et al., 2010; Cheng et al., 2016), or sorption (Pietrobelli et al., 2009). Consequently, bioremediation using plants and other organisms has been often employed for removing toxic pollutants from the aquatic environment (Fan et al., 2008; Hu et al., 2011; Zeng et al., 2017).

This study shines light on the ecological response and toxicity of AgNPs with different dosing regimens in the natural aquatic environment. Our results revealed that the one-time dosing regimen presented more serious impacts than the continuous dosing regimen, and little difference was observed in the magnitude of these changes among different water columns under low concentration exposure to AgNPs. These results suggest that adoption of an intermittent discharge pattern may alleviate impairment of the environment when discharging wastewater containing AgNPs into the aquatic environment.

4. Conclusions

In summary, the HOE treatment caused a range of dramatic ecosystem impacts that were more severe than those in the other three treatments (LDW, LRW, and HCE). However, there was little difference in the removal of TAg from the aquatic environment among the different dosing regimens. Moreover, there were no obvious differences in ecological impacts between different water columns at close to realistic concentrations. Plants are the dominant biotic sink of silver in aquatic environments. Zebrafish are sensitive to AgNPs, and can be the sentinel species to monitor environmental pollution.

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REFERENCES

Baker, T.J., Tyler, C.R., Galloway, T.S., 2014. Impacts of metal and metal oxide nanoparticles on marine organisms. Environ. Pollut. 186, 257–271.

Bernhardt, E.S., Colman, B.P., Hochella Jr., M.F., Cardinale, B.J., Nisbet, R.M., Richardson, C.J., et al., 2010. An ecological perspective on nanomaterial impacts in the environment. J. Environ. Qual. 39 (6), 1–12.

Bone, A.J., Colman, B.P., Gondikas, A.P., Newton, K.M., Harrold, K. H., Cory, R.M., et al., 2012. Biotic and abiotic interactions in aquatic microcosms determine fate and toxicity of Ag nanoparticles: part 2-toxicity and Ag speciation. Environ. Sci. Technol. 46 (13), 6925–6933.

Chae, Y.J., Pham, C.H., Lee, J., Bae, E., Yi, J., Gu, M.B., 2009. Evaluation of the toxic impact of silver nanoparticles on Japanese medaka (*Oryzias latipes*). Aquat. Toxicol. 94 (4), 320–327.

Cheng, Y., He, H.J., Yang, C.P., Zeng, G.M., Li, X., Chen, H., et al., 2016. Challenges and solutions for biofiltration of hydrophobic volatile organic compounds. Biotechnol. Adv. 34 (6), 1091–1102.

Choi, Y., Kim, H., Kim, K.W., Lee, B., 2017. Comparative toxicity of silver nanoparticles and silver ions to *Escherichia coli*. J. Environ. Sci. https://doi.org/10.1016/j.jes.2017.04.028.

Colman, B.P., Espinasse, B., Richardson, C.J., Matson, C.W., Lowry, G.V., Hunt, D.E., et al., 2014. Emerging contaminant or an old toxin in disguise? Silver nanoparticle impacts on ecosystems. Environ. Sci. Technol. 48 (9), 5229–5236.

Cong, Y., Bantaa, G.T., Selcka, H., Berhanub, D., Jones, E.V., Forbes, V.E., 2011. Toxic effects and bioaccumulation of nano-, micron- and ionic-Ag in the polychaete, *Nereis diversicolor*. Aquat. Toxicol. 105 (3–4), 403–411.

Das, P., Xenopoulos, M.A., Metcalfe, C.D., 2013. Toxicity of silver and titanium dioxide nanoparticle suspensions to the aquatic invertebrate, *Daphnia magna*. Bull. Environ. Contam. Toxicol. 91 (1), 76–82.

Das, P., Metcalfe, C.D., Xenopoulos, M.A., 2014. Interactive effects of silver nanoparticles and phosphorus on phytoplankton growth in natural waters. Environ. Sci. Technol. 48 (8), 4573–4580.

Dazy, M., Masfaraud, J.F., Ferard, J.F., 2009. Introduction of oxidative stress biomarkers associated with heavy metal stress in *Fontinalis antipyretica* Hedw. Chemosphere 75 (3), 297–302.

Fabrega, J., Zhang, R., Renshaw, J.C., Liu, W.T., Lead, J.R., 2011. Impact of silver nanoparticles on natural marine biofilm bacteria. Chemosphere 85 (6), 961–966.

Fan, T., Liu, Y., Feng, B., Zeng, G., Yang, C., Zhou, M., et al., 2008. Biosorption of cadmium (II), zinc (II) and lead (II) by *Penicillium simplicissimum*: isotherms, kinetics and thermodynamics. J. Hazard. Mater. 160 (2–3), 655–661.

Farmen, E., Mikkelsen, H.N., Evensen, O., Einset, J., Heier, L.S., Rosseland, B.O., et al., 2012. Acute and sub-lethal effects in juvenile Atlantic salmon exposed to low μg/L concentrations of Ag nanoparticles. Aquat. Toxicol. 108, 78–84.

Faunce, T., Watal, A., 2010. Nanosilver and global public health: international regulatory issues. Nanomedicine 5 (4), 617–632.

Feng, Y., Gong, J.L., Zeng, G.M., Niu, Q.Y., Zhang, H.Y., Niu, C.G., et al., 2010. Adsorption of Cd (II) and Zn (II) from aqueous solutions using magnetic hydroxyapatite nanoparticles as adsorbents. Chem. Eng. J. 162 (2), 487–494.

Furtado, L.M., Norman, B., Xenopoulos, M., Frost, P.C., Metcalfe, C. D., Hintelmann, H., 2015. Environmental fate of silver nanoparticles in Boreal Lake ecosystems. Environ. Sci. Technol. 49 (14), 8441–8450.

García-Sánchez, M., Garrido, I., Casimiro, I.J., Casero, P.J., Espinosa, F., Aranda, I., et al., 2012. Defense response of tomato seedlings to oxidative stress induced by phenolic compounds from dry olive mill residue. Chemosphere 89 (6), 708–716.

Gong, J.L., Wang, B., Zeng, G.M., Yang, C.P., Niu, C.G., Niu, Q.Y., et al., 2009. Removal of cationic dyes from aqueous solution using magnetic multi-wall carbon nanotube nanocomposite as adsorbent. J. Hazard. Mater. 164 (2–3), 1517–1522.

Gottschalk, F., Sun, T., Nowack, B., 2013. Environmental concentrations of engineered nanomaterials: review of modeling and analytical studies. Environ. Pollut. 181 (6), 287–300.

Grossklaus, D., Bailao, A.M., Rezende, T.C.V., Borges, C.L., Oliveira, M.A.P., Parente, J.A., et al., 2013. Response to oxidative stress in *Paracoccidioides* yeast cells as determined by proteomic analysis. Microbes Infect. 15, 347–364.

Guo, Z., Chen, G., Zeng, G., Li, Z., Chen, A., Yan, M., et al., 2014. Ultrasensitive detection and co-stability of mercury (II) ions based on amalgam formation with Tween 20-stabilized silver nanoparticles. RSC Adv. 4 (103), 59275–59283.

Guo, Z., Chen, G., Zeng, G., Liang, J., Huang, B., Xiao, Z., et al., 2016. Determination of inequable fate and toxicity of Ag nanoparticles in a *Phanerochaete chrysosporium* biofilm system through different sulfide sources. Environ. Sci. Nano 3 (5), 1027–1035.

Handy, R.D., Al-Bairuty, G., Al-Jubory, A., Ramsden, C.S., Boyle, D., Shaw, B.J., et al., 2011. Effects of manufactured nanomaterials on fishes: A target organ and body systems physiology approach. J. Fish Biol. 79 (4), 821–853.

He, D., Bligh, M.W., Waite, T.D., 2013. Effects of aggregate structure on the dissolution kinetics of citrate-stabilized silver nanoparticles. Environ. Sci. Technol. 47 (16), 9148–9156.

Hu, X., Wang, J., Liu, Y., Li, X., Zeng, G., Bao, Z., et al., 2011. Adsorption of chromium (VI) by ethylenediamine-modified cross-linked magnetic chitosan resin: isotherms, kinetics and thermodynamics. J. Hazard. Mater. 185 (1), 306–314.

Hu, L., Zhang, C., Zeng, G.M., Chen, G.Q., Wan, J., Guo, Z., et al., 2016. Metal-based quantum dots: synthesis, surface modification, transport and fate in aquatic environments and toxicity to microorganisms. RSC Adv. 6, 78595–78610.

Hu, L., Wan, J., Zeng, G.M., Chen, A.W., Chen, G.Q., Huang, Z.Z., et al., 2017. Comprehensive evaluation of the cytotoxicity of CdSe/ZnS quantum dots in Phanerochaete chrysosporium by cellular uptake and oxidative stress. Environ. Sci. Nano https://doi.org/10.1039/C7EN00517B.

Huang, D.L., Zeng, G.M., Feng, C.L., Hu, S., Jiang, X.Y., Tang, L., et al., 2008. Degradation of lead-contaminated lignocellulosic waste by *Phanerochaete chrysosporium* and the reduction of lead toxicity. Environ. Sci. Technol. 42 (13), 4946–4951.

Huang, Z., Chen, G., Zeng, G., Guo, Z., He, K., Hu, L., et al., 2017. Toxicity mechanisms and synergies of silver nanoparticles in 2,4-dichlorophenol degradation by *Phanerochaete chrysosporium*. J. Hazard. Mater. 321, 37–46.

Huang, Z., Xu, P., Chen, G., Zeng, G., Chen, A., Song, Z., et al., 2018. Silver ion-enhanced particle-specific cytotoxicity of silver nanoparticles and effect on the production of extracellular secretions of *Phanerochaete chrysosporium*. Chemosphere 196, 525–584.

Kaegi, R., Voegelin, A., Ort, C., Sinnet, B., Thalmann, B., Krismer, J., et al., 2013. Fate and transformation of silver nanoparticles in urban wastewater systems. Water Res. 47 (12), 3866–3877.

Kahru, A., Dubourguier, H.C., Blinova, I., Ivask, A., Kasemets, K., 2008. Biotests and biosensors for ecotoxicology of metal oxide nanoparticles: a minireview. Sensors 8, 5153–5170.

Keller, A.A., McFerran, S., Lazareva, A., Suh, S., 2013. Global life cycle releases of engineered nanomaterials. J. Nanopart. Res. 15 (6), 1–17.

Kennedy, A.J., Chappell, M.A., Bednar, A.J., Ryan, A.C., Laird, J.G., Stanley, J.K., et al., 2012. Impact of organic carbon on the stability and toxicity of fresh and stored silver nanoparticles. Environ. Sci. Technol. 46 (19), 10772–10780.

Laban, G., Nies, L., Turco, R., Bickham, J., Sepulveda, M., 2010. The effects of silver nanoparticles on fathead minnow (*Pimephales promelas*) embryos. Ecotoxicology 19 (1), 185–195.

Levard, C., Reinsch, B.C., Michel, F.M., Oumahi, C., Lowry Jr., G.V., Brown, G.E., 2011. Sulfidation processes of PVP-coated silver nanoparticles in aqueous solution: impact on dissolution rate. Environ. Sci. Technol. 45 (12), 5260–5266.

Lowry, G.V., Espinasse, B.P., Badireddy, A.R., Richardson, C.J., Reinsch, B.C., Bryant, L.D., et al., 2012. Long-term transformation and fate of manufactured ag nanoparticles in a simulated large scale freshwater emergent wetland. Environ. Sci. Technol. 46 (13), 7027–7036.

Massarsky, A., Dupuis, L., Taylor, J., Eisa-Beygi, S., Strek, L., Trudeau, V.L., et al., 2013. Assessment of nanosilver toxicity during zebrafish (*Danio rerio*) development. Chemosphere 92, 59–66.

Norie, T., Kazutaka, N.A., Ishimaru, K., 2003. Antioxidative capacity of extracts and constituents in *Cornus capitata* adventitious roots. J. Agric. Food Chem. 51 (20), 5906–5910.

Pietrobelli, J.M.T.deA., Modenes, A.N., Fagundes-Klen, M.R., Espinoza-Quiñones, F.R., 2009. Cadmium, copper and zinc biosorption study by non-living *E. densa* biomass. Water Air Soil Pollut. 202 (1–4), 385–392.

Qiu, R.L., Zhao, X.Y., Tang, T., Yu, F.M., Hu, P.J., 2008. Antioxidative response to Cd in a newly discovered cadmium hyperaccumulator. *Arabis paniculata* F. Chemosphere 74 (1), 6–12.

Quadros, M.E., Marr, L.C., 2011. Silver nanoparticles and total aerosols emitted by nanotechnology-related consumer spray products. Environ. Sci. Technol. 45 (24), 10713–10719.

Ramskov, T., Forbes, V.E., Gilliland, D., Selck, H., 2015. Accumulation and effects of sediment-associated silver nanoparticles to sediment-dwelling invertebrates. Aquat. Toxicol. 166, 96–105.

Vance, M.E., Kuiken, T., Vejerano, E.P., McGinnis, S.P., Hochella, M. F., Rejeski, D., et al., 2015. Nanotechnology in the real world: redeveloping the nanomaterial consumer products inventory. Beilstein J. Nanotechnol. 6, 1769–1780.

Wang, J., Wang, W., 2014. Low bioavailability of silver nanoparticles presents trophic toxicity to marine Medaka (*Oryzias melastigma*). Environ. Sci. Technol. 48 (14), 8152–8161.

Xu, P., Zeng, G.M., Huang, D.L., Feng, C.L., Hu, S., Zhao, M.H., et al., 2012. Use of iron oxide nanomaterials in wastewater treatment: a review. Sci. Total Environ. 424 (4), 1–10.

Yang, C.P., Chen, H., Zeng, G.M., Yu, G.L., Luo, S.L., 2010. Biomass accumulation and control strategies in gas biofiltration. Biotechnol. Adv. 28 (4), 531–540.

Zeng, G., Chen, A., Chen, G., Hu, X., Guan, S., Shang, C., et al., 2012. Responses of *Phanerochaete chrysosporium* to toxic pollutants: physiological flux, oxidative stress, and detoxification. Environ. Sci. Technol. 46 (14), 7818–7825.

Zeng, G.M., Chen, M., Zeng, Z., 2013. Shale gas: surface water also at risk. Nature 499 (7457), 154.

Zeng, G.M., Wan, J., Huang, D.L., Hu, L., Huang, C., Cheng, M., et al., 2017. Precipitation, adsorption and rhizosphere effect: the mechanisms for phosphate-induced Pb immobilization in soils — a review. J. Hazard. Mater. 339, 354–367.

Zhang, Y., Zeng, G.M., Tang, L., Huang, D.L., Jiang, X.Y., Chen, Y.N., 2007. A hydroquinone biosensor based on immobilizing laccase to modified core-shell magnetic nanoparticles supported on carbon paste electrode. Biosens. Bioelectron. 22 (9), 2121–2126.

Zuo, Y.N., Chen, G.Q., Zeng, G.M., Li, Z.W., Yan, M., Chen, A.W., et al., 2015. Transport, fate, and stimulating impact of silver nanoparticles on the removal of Cd(II) by *Phanerochaete chrysosporium* in aqueous solutions. J. Hazard. Mater. 285, 236–244.