Toxicity effects of silver nanoparticles on the freshwater bivalve *Corbicula fluminea*

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Abstract

Toxicity effects of silver nanoparticles (AgNPs) on the freshwater bivalve *Corbicula fluminea* (*C. fluminea*) were investigated through experiments. In this study, *C. fluminea* promoted the sedimentation of AgNPs and affected the fate and transformation of AgNPs. A series of biomarkers of *C. fluminea in vivo* were evaluated after 14 days exposure to various doses (0-2 $mg \cdot L^{-1}$) of polyvinyl pyrrolidone (PVP) coated AgNPs. The levels of antioxidants increased obviously in 2 $mg \cdot L^{-1}$ AgNPs treatments to protect *C. fluminea* from oxidative damage. Glutathione peroxidase (GPx) and glutathione (GSH) played important roles in tissues detoxification in 0.1 and 0.5 $mg \cdot L^{-1}$ exposure, respectively. The biological behaviors (feeding rate, ammonia excretion rate) were inhibited at 0.1 $mg \cdot L^{-1}$, inducted at 0.5 $mg \cdot L^{-1}$, and inhibited again at 2 $mg \cdot L^{-1}$ AgNPs, which indicated that AgNPs influenced the physiological metabolism of *C. fluminea*. Ag contents in tissue and shell of *C. fluminea* were much higher than blank groups. In addition, *in vivo* tissues were more sensitive to low AgNPs concentration compared with shells, indicating that *C. fluminea* could be used as a good indicator for AgNPs freshwater pollution. No Ag was detected in feces probably implying that nanoparticles had long gut retention time in *C. fluminea*. Overall, this study reveals the interactions between AgNPs and *C. fluminea* and provides important implications about the fate and toxicity of AgNPs in natural aquatic environment.

Keywords:

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Silver nanoparticles; *Corbicula fluminea*; Fate; Antioxidants; Behaviors

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Highlights

- *C. fluminea* promoted the sedimentation of AgNPs.
- PVP-coated AgNPs induced oxidative stress in *C. fluminea*.
- *C. fluminea* could be a good indicator for AgNPs freshwater pollution.
- AgNPs had long gut retention time in *C. fluminea*.

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1. Introduction

There are many environmental stressors such as heavy metal [1], pesticides [2,3], drugs [4], atmospheric pollutants [5], fossil energy [6], nanomaterials [7,8], etc. Among these, engineered nanomaterials bring various benefits for our society due to their excellent properties. However, the increasing applications of nanomaterials can cause potential risk to human health and ecosystems [9,10,11]. Therefore, safety assessments of engineered nanoparticles in the environment has already become a main issue globally. Silver nanoparticles (AgNPs), one of the most widely used engineered nanomaterials, have been applied to manufacturing industries including textiles, chemicals, medical service products, and others, due to their beneficial properties [12]. Nevertheless, it has been reported that AgNPs could cause negative effects on environmental biota, such as fungi [13], algae [14], and nematode [15], etc. The benthic species play a vital role in the structure and function of estuarine and marine ecosystems [16]. Once AgNPs are released from commodities into aquatic environment, the aquatic species, especially the benthic organisms, could be at risk [17]. However, there are many important issues about the toxicity of AgNPs on benthic organisms that are not well understood. As vital benthic organisms, filter-feeding bivalves not only play a vital role in the structure and function of marine and estuarine ecosystems [16], but also can be used as a typical sentinel for environmental pollutants [18,19]. Some studies focused on the toxicity of AgNPs on bivalves have been reported. A marine mesocosm study showed that AgNPs induced

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DNA damage and oxidative damage in *Scrobicularia plana* [20]. AgNPs were also reported to cause hepatopancreas pathology and the occurrence of early apoptosis of *Mytilus galloprovincialis* [21]. However, most studies in China and abroad have focused on the effects of AgNPs on marine bivalves, and there wasn't any study about the influence of AgNPs on freshwater bivalves. Given the disparity among studies using marine and freshwater species, it is necessary to obtain more information.

Corbicula fluminea (*C. fluminea*) was chosen as a typical freshwater bivalve for further understanding the toxicity effect of AgNPs on freshwater bivalves. *C. fluminea* is widely distributed in Southern and East Asia. Due to the highly efficient filter-feeding ability [22], *C. fluminea* is commonly used in field and laboratory studies to evaluate effects of toxicity and bioreactivity [23]. Studies have shown that antioxidant responses [4,24] and biological behaviors [25] in *C. fluminea* could be used as biomarkers to evaluate the toxicity of environmental pollutants.

The study aimed at (1) estimating the fate of PVP-coated AgNPs in experimental systems, (2) investigating the bioaccumulation and toxicity effects of PVP-coated AgNPs on *C. fluminea* (physiological behaviors, oxidative stress and antioxidation mechanism). One pulse treatment of AgNPs was selected, and AgNPs were added into upper water in order to emulate the scenario of AgNPs water pollution. Bivalves were treated with PVP-coated AgNPs of different initial concentrations (0, 0.1, 0.5, and 2 $mg \cdot L^{-1}$ AgNPs). Total Ag concentrations in water were detected throughout the whole experimental period. Ag accumulation in sediment, Ag bioaccumulation,

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feeding rate, ammonia excretion rate, oxidative damage (LPO levels) and antioxidants (catalase, glutathione S-transferase, glutathione, superoxide dismutase, and glutathione peroxidase) were determined after exposure. To our knowledge, this work is the first report on the toxicity effects of AgNPs on freshwater bivalves *C. fluminea.*

2. Materials and methods

2.1. Animal collection and culturing

Bivalves were obtained from Heishui River (22°57′56″N, 106°42′19″E) in Jingxi County, Guangxi Zhuang Autonomous Region (Fig. 1). All specimens with similar sizes of shell length (22 ± 3 mm) and shell height (12 ± 2 mm) were selected in this study. After transferring to the laboratory, all selected specimens were acclimated in a 60 L tank containing about 2 cm quartz sand and reverse osmosis (RO) water, for 14 days. Bivalves were fed with about 50 mg fish food every 2-3 days and feeding was stopped in 2 days before the exposure experiment began.

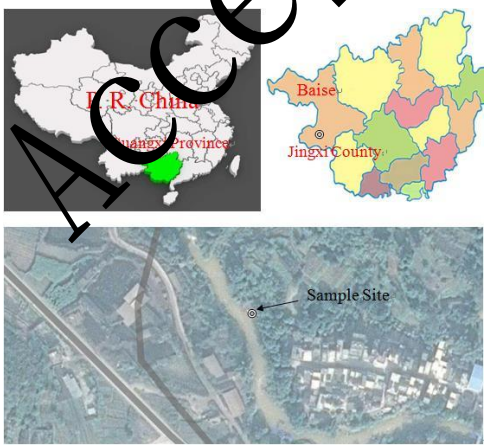

Fig. 1. Location of the sampling site.

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2.2. Preparation and characterization of AgNPs

PVP-coated AgNPs solution was prepared following the method [26] with slight changes. 8 mL of 0.3% PVP and 24 mL of 150 mM sodium borohydride were added into an ice bath with vigorous stirring, then 24 mL of 20 mM silver nitrate was added into the mixture. Three minutes later, the mixed solution continued to stir in ambient temperature for another 2 h. Dialysis of all particle suspension was conducted using a 1 kDa regenerated cellulose membrane to remove impurities, including excess PVP and silver ions. After being digested by concentrated nitric acid, the Ag concentration of PVP-coated AgNPs stock solution was measured by flame atomic absorption spectrometry (FAAS) (AAS700, PerkinElmer, USA). The hydrodynamic diameter and zeta potential of AgNPs were determined using a Zetasizer Nanoseries (Malvern Instruments). The morphology of AgNPs was also visually assessed by a transmission electron microscopy (TEM) operating at 100 kV.

2.3. Sediment preparation

All natural sediments used in the experiment were collected from Chongming island. Large debris were removed from sediments. Total organic carbon was measured by loss-on-ignition method [27]. Briefly, 1.0 g sediment sample was dried at 105 ℃ for 10 h, then weighed and transferred to muffle furnace in order to heat for 8 h to constant weight. Water extracts of carbon (WEOC) was measured with a TOC analyzer (TOC-VCPH, Shimadzu, Japan), after shaking the sediment-water mixture for 3 h, followed by centrifugation and filtration.

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2.4. Experimental setup

Whole experimental plastic beakers were acid washed and rinsed repeatedly with RO water. Natural sediment (200 g wet weight, ww) and 800 mL RO water were carefully added to each beaker (1000 mL), and then settled for 2 hours. AgNPs stock solutions were one-off injected into overlying water in order to achieve a series of Ag nominal concentrations. Two kinds of test groups were set in this experiment: (i) experimental groups: AgNPs with bivalves: 0, 0.1, 0.5, 2 mg Ag L^{-1}, (ii) control groups: AgNPs without bivalves: 0.1, 0.5, 2 mg Ag L^{-1}. Three replicates were used for each exposure treatment with 6 bivalves per replicate. The chosen concentrations of AgNPs were related to those used in studying the toxicity of AgNPs on *Chironomus riparius*, which clearly showed the toxicity of AgNPs [28]. There was no feeding during the 14 days experimental period, because natural sediment could provide enough food for bivalves. All experimental systems were static without water aeration. The mortality of *C. fluminea* were checked during the experimental period.

2.5. Quantitative analysis of Ag

2.5.1. Quantification of total silver in the experimental systems

During the 14 days exposure, aliquot water samples near to the water-sediment interface were taken at specific time intervals (0, 3, 7, 11, and 14 day), and then digested with nitric acid for FAAS analysis. At the above time intervals, the zeta potential of upper layer water was determined by dynamic light scattering using a nanoseries zetasizer (Malvern Instruments).

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Sediment samples were taken at the 14th day and air-dried, sieved (≤ 150 μm), and then digested with three kinds of acid (nitric acid, hydrofluoric acid and perchloric acid, 5: 5: 3 v/v/v) through a graphite digester. The digestion solutions were diluted to constant volume. The Ag concentrations in sediment samples were also measured by FAAS.

2.5.2. Quantification of total silver in biota

At the 14th day, the whole soft tissues and shells of bivalves were dissected and frozen at -80 ℃ until used. Each soft tissue of bivalves was thawed and charred in a crucible with hot plate first. Afterwards, crucibles were moved to muffle furnace at 550 ℃ for 6 h, cooled at room temperature, and then the ashed samples were digested with acid solution (nitric acid and hydrochloric acid, 1: 1 v/v). The resulting solutions were analyzed by FAAS as described above.

The thawed shells were rinsed and then dried at 60 ℃ to constant weight. After that, shells were ground into powder using a mortar and pestle. Then the powder was acid digested using graphite digester and the obtained digestion solutions were analyzed by FAAS.

Emptying guts tests were conducted at end of exposure. Tested bivalves of each group were placed in 100 mL glass beakers containing 100 mL RO water, and then excreta in the beakers were gathered through low speed centrifugation. The collected fecal sample of each group was divided into two parts. One of them was used to determine Ag concentration in feces by FAAS. The other sample was treated with

freeze-drying and then spray-gold. Fecal morphology and composition were studied by SEM and EDX analysis. Feces were investigated in a SEM (FEI Quanta 200), which was operated at an accelerating voltage of 30 kV. The microscope was operated in scanning mode. Feces were localized and analyzed for their elemental composition, using an EDX analysis system (EDAX Inc, Mahwah, NJ, USA).

2.6. Behavior test

2.6.1. Filter-feeding test

To understand the effects of PVP-coated AgNPs on *C. fluminea*, filter-feeding rates, algal clearing rates assays of bivalves were measured after 14 days exposure. The blue algae (*Microcystis aeruginosa*) was cultivated under controlled conditions (25 ℃, light: dark cycle of 12: 12 h) in BG11 medium, and the chlorophyll *a* contents of *Microcystis aeruginosa* used for this tests was 0.615 ± 0.027 mg·L^{-1}. Each 100 mL beaker was filled with 25 mL RO water and 25 mL algae solution, and one bivalve was placed into it. The initial chlorophyll *a* content was determined using the method described by Hua et al. [29] at the beginning of tests. After 2 h, bivalves were removed from the beakers, and the contents of chlorophyll *a* were determined again. According to the difference in chlorophyll *a* concentrations, filter-feeding rates were calculated.

2.6.2. Ammonia excretion test

The ammonia excretion tests were conducted through measurement of ammonia content after 14 days of exposure. Briefly, each bivalve selected randomly from

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exposure beakers was put into a 100 mL beaker containing 100 mL RO water. After 2 h, the ammonia content of RO water was determined by Nesster's reagent colorimetry [30].

2.7. Biochemical markers

2.7.1. Tissue preparation

Bivalves collected at end of experiment were dissected, and then the tissues were kept at -80 ℃ for subsequent analysis. Once thawed, tissues were homogenized in 5 mL of phosphate buffered solution (PBS) (0.1 M, pH = 7.4) and then centrifuged at 10,000 r/min (30 min, 4 ℃). The supernatant fractions were used to determine Glutathione-S-transferase (GST), Catalase (CAT), Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) level [31].

Two selected thawed tissues were treated in different ways to measure LPO and GSH. Briefly, one of them was homogenized in 5 mL 10% trichloroacetic acid, followed by centrifugation at 4000 r/min (10 min), and this supernatant was analyzed for LPO. The other one was homogenized in 5 mL precooled 50 g/L trichloroacetic acid and then centrifuged at 12,000 g (20 min, 4 ℃), the collected supernatant was used to measure GSH.

2.7.2. Glutathione-S-transferase

The method determining GST was described in previous study [32]. GST can catalyze the conjugation of GSH and l-chloro-2, 4-dinitrobenzene (CDNB). The activity of GST was measured at 340 nm, using 1.25 mM 1-chloro-2,

4-dinitrobenzene (CDNB, Sigma), 10 mM reduced GSH, and PBS (0.1 M, pH 6.5). The increase of absorbance in CDNB conjugate was monitored for 5 min spectrophotometrically, and the unit of GST was defined as 1 nmol CDNB conjugate per min per mg protein, expressed as U/mg.

2.7.3. Catalase

CAT activity was quantified [33] by measuring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm for 1 min in 0.2 mL supernatant, and the definition of one CAT unit was the enzyme content capable of degrading 1 μmol of H_2O_2 per min per mg of protein, expressed as CAT K mg^{-1} protein.

2.7.4. Superoxide dismutase

SOD activity was evaluated according to nitro-blue tetrazolium (NBT) colorimetric method [33], which measured the inhibitory degree of NBT reduction by superoxide anion radicals generated by oxidation of reduced riboflavin in aerobic conditions. Results were expressed as SOD Unit per mg protein (SOD Unit mg^{-1} protein). One SOD activity unit was defined as the amount of enzyme producing 50% inhibition of NBT per mg protein.

2.7.5. Glutathione peroxidase

The activity of GPx was determined according to the method described by Martínez et al. [34]. GPx catalyze oxidation of GSH followed by reduction reaction with peroxide, so xanthous 5-glucosinolates, 2-nitro benzoic acid anions, as the production of reaction between GSH and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB),

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could be measured spectrophotometrically at 340 nm. According to the calculation of GSH reduction, the GPx activity was obtained. One GPx activity unit was expressed as the amount of enzyme inducing 1 μmol decrease of GSH per mg protein.

2.7.6. Lipid peroxidation

LPO was assessed by determining absorbance at 532 nm of resultant (trimethyl compounds) produced in the reaction between malondialdehyde (MDA) and thiobarbituric acid in the condition of acidity and high-temperature [34]. Based on the absorbance, MDA content was calculated. Results were expressed in MDA nmol·g^{-1} wet weight (ww).

2.7.7. Glutathione

Prepared supernatant was processed with potassium phosphate buffer (0.1 M, pH = 7.7). During the oxidation of glutathione by DTNB, 2-nitro-5 thiobenzoic acid is formed. Absorption can be spectrophotometrically read at 412 nm. The reduced glutathione concentration was obtained from a reduced glutathione standard curve. Results were indicated as GSH μmol·g^{-1} wet weight (ww) [34].

2.7.8. Total protein

The method measuring protein content was referred to that described by Bradford [35]. On the basis of protein reacting with Coomassie Brilliant Blue G-250 dye, absorbance was read at a wavelength of 595 nm in a spectrophotometer, using bovine serum albumin as the standard.

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2.8. Statistical analysis

Each of the assays was processed with statistical analysis. All data is expressed as the mean ± standard deviation (SD). Significant differences between all tests were analyzed by using one-way analysis of the variance (ANOVA), and statistical evaluation of the results was determined by Tukey post hoc test. All the statistical analyses were performed using the IBM SPSS statistics 19. The results shown in the figures represent the average of three independent replicate treatments. The significant level was established at $p < 0.05$. The different letters (a, b, ab) in Fig. 6 and Fig. 7 represent that there are significant differences between groups.

3. Results and discussion

3.1. Sediment properties

Carbon content of natural sediment in the water extracts was 193.03 ± 8.01 mg·kg^{-1} and the TOC of sediment was 5.60 ± 0.46%. There was no detection of Ag in the sediment through FAAS analysis.

3.2. Characteristics of AgNPs

The zeta potential and mean hydrodynamic diameter of PVP-coated AgNPs suspension were measured by dynamic light scattering analysis. Zeta potential of stock solution was -30.5 ± 3.5 mV. The DLS analysis indicated that the particles in the moderate dispersed solution had an average diameter of 27.66 ± 0.80 nm. The particle shape of nanoparticles was characterized by TEM (Fig. 2), and the results turned out that the nanoparticles were spherical without intense aggregation.

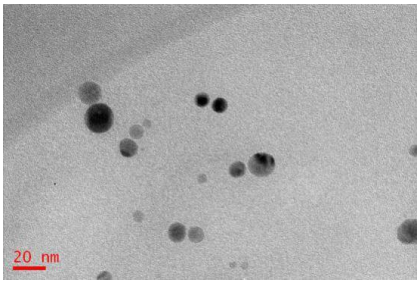

Fig. 2. TEM image of the AgNP solution. Scale bar is 20 nm.

3.3. Ag fate in sediment-water systems

During the experimental period, Ag concentration (Fig. 3) and the absolute value of zeta potential (Fig. 4) in the overlying water presented a decreasing trend. In experimental groups, it was clear that Ag concentration appeared to plummet two times during the exposure and the absolute value of zeta potential dropped to the lowest value on the 3rd day, then increased gradually. While, in control groups, Ag content no longer decreased and reached equilibrium since the 11th day, and Ag concentrations were much higher than that in experimental groups. The absolute value of zeta potential decreased on the 3rd day, increased a bit on the 7th day, and then continued to decline until the end of experiment.

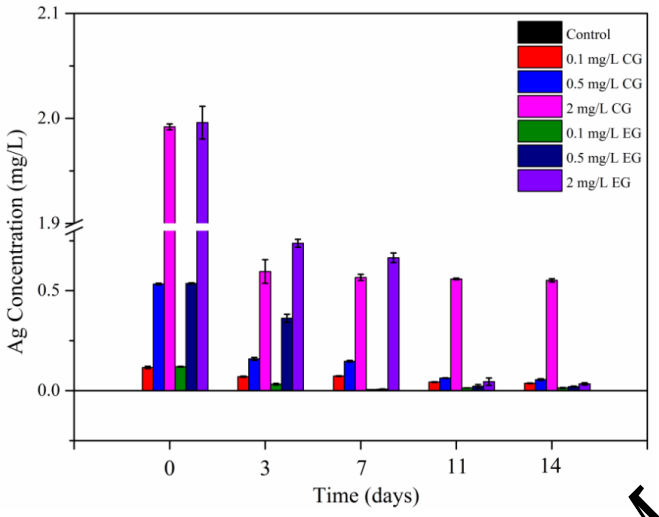

Fig. 3. Concentration of Ag in the upper water after exposure to the four experimental conditions (0, 0.1, 0.5, and 2 mg·L^{-1} AgNPs). CG, EG represent control groups, experimental groups respectively.

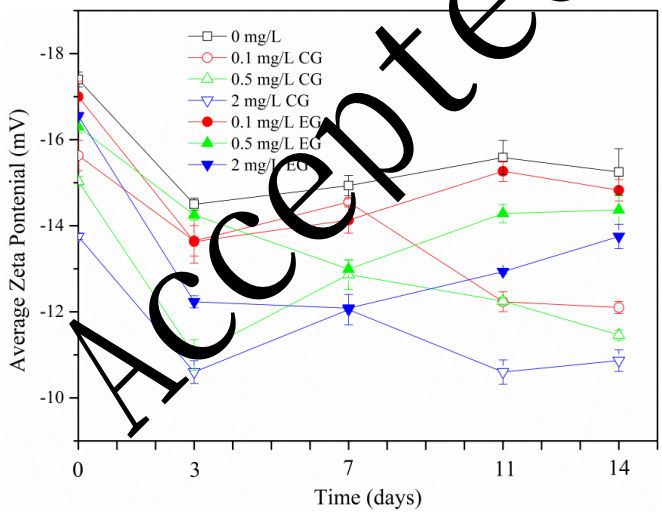

Fig. 4. Zeta potential of overlying water after exposure to the seven experimental conditions (0, 0.1, 0.5, and 2 mg·L^{-1} AgNPs). CG, EG represent control groups, experimental groups respectively.

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Generally speaking, the fate and transformation of AgNPs in environmental conditions were influenced by their intrinsic properties, environmental factors (pH, dissolved oxygen, natural organic matter, and sulfide) etc [13]. A number of recent studies showed that AgNPs probably underwent oxidative dissolution to release Ag^+, adsorption of NOM, reactions with sulfur species or chloride, or aggregation [36].

In this study, a similar sharp drop in Ag content and absolute value of zeta potential happened at the 3rd day in both control and experimental groups, indicating that AgNPs probably settled into sediment. An artificial ecosystem experiment reported that AgNPs underwent rapid oxidative dissolution at initial 12 h, and then dissolved silver contents reached a plateau [37]. Thus it could be inferred that AgNPs probably occurred to aggregation in the form of Ag^+ complexes or AgNPs aggregates [38]. The second sharp fall of Ag concentrations and the increase in absolute value of zeta potential in experimental groups suggest that AgNPs might be settle into sediment again. The absolute value of zeta potential gradually decreased and Ag contents no longer decreased in control groups, which demonstrated that the main transformation form of AgNPs could be oxidative dissolution, thus amounts of released Ag^+ existed in the water phase with an increase of cations. Contrasting the two above-mentioned phenomena, it could be concluded that *C. fluminea* affected the fate and transformation of AgNPs and promoted the sedimentation of AgNPs. After bivalves overcoming the adaptive phase, behaviors of bivalves probably promoted the movement of AgNPs to sediment.

The Ag accumulation in the sediment for each treatment in the 14th day was presented in Fig. 5. In microcosms, various extents of Ag accumulation were observed: 0.383 ± 0.058, 1.648 ± 0.058, and 6.438 ± 0.106 $\mu g \cdot g^{-1}$ dw sediment for microcosms contaminated by 0.1, 0.5, and 2 $mg \cdot L^{-1}$ AgNPs, respectively, indicating that Ag accumulation increased with the increasing concentrations of AgNPs. Majority of Ag were deposited in sediment, which was consistent with Lowry's report: AgNPs injected to mesocosm experiment were mainly gathered in the surface layer of sediment [38].

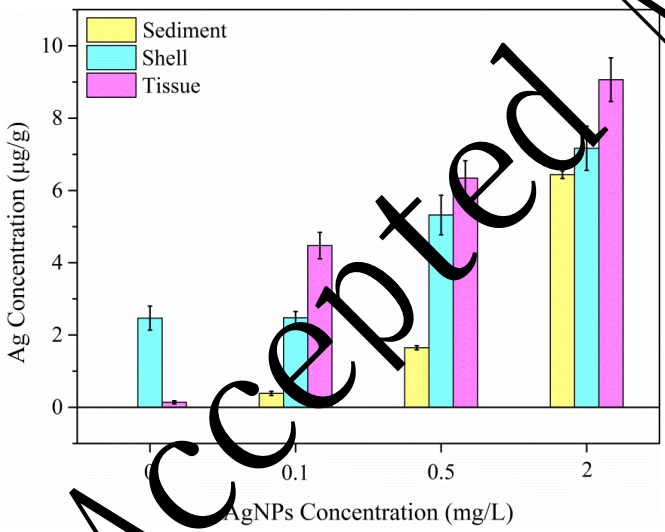

Fig. 5. Ag concentrations in sediment, shell, and tissue after 14 days exposed to the four experimental conditions (0, 0.1, 0.5, and 2 $mg \cdot L^{-1}$)

3.4 Ag bioaccumulation

As is well known, metals can pose as a serious risk to organisms due to their bioaccumulation and toxicity [39]. However, bioaccumulation has drawn more attention for scavenging heavy metal ions [40]. The bioaccumulated Ag content

possibly depends on exposure concentrations and media conditions, as well as the difference among species [15,20,41]. Ag concentration in whole soft tissues and shells were illustrated in Fig. 5. Ag concentration in body burden increased significantly from 4.475 to 9.064 $\mu g \cdot g^{-1}$ ww in accordance with exposure concentration. According to experimental phenomenon, there probably were two ways in which to accumulate Ag in *C. fluminea*: (i) siphon uptaking from overlying water after the adaptation period (ii) siphon uptaking from pore water in sediment after moving into sediment.

Some studies have reported that shells of bivalves could be a sentinel to monitor environmental pollution, particularly heavy metal pollution [42]. Ag concentration in shells increased by 3 times in 2 $mg \cdot L^{-1}$ than in 0.1 $mg \cdot L^{-1}$ AgNPs treatments. Nevertheless, *C. fluminea* exposed to 0.1 $mg \cdot L^{-1}$ AgNPs had an analogous low Ag shell burden levels as the blank group. The background Ag concentration in blank groups were similar with that in *Mytilus edulis trossulus* [43].

The partitioning factor (PF, defined as the ratio between the mean metal concentrations in soft tissues and shells) [44] of AgNPs varied from 1.8 in 0.1 $mg \cdot L^{-1}$ to 1.2 in 0.5 $mg \cdot L^{-1}$. Therefore, the similar concentrations in the soft tissues and shells indicated that both the shells and soft tissues of *C. fluminea* could be good indicators for freshwater AgNPs contamination. There also were differences in Ag content between *in vivo* and shells: Ag content of tissue in 0.1 mg/L was almost 3 times more than that in 0.5 mg/L, while, Ag content of shells in 0.1 mg/L was much less than that in 0.5 mg/L. Therefore, Ag tend to accumulate *in vivo* in lower concentrations. On the

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contrary, Ag is more likely to adsorb on shells than store up in tissues with the increase of Ag initial concentration. The self-protection mechanism of *C. fluminea* with closed shell may result in this difference. Hence, *in vivo* tissue can be a preferable choice for low concentration contamination due to its high sensitivity, while, shell is a better indicator for high concentrated AgNPs pollution compared with tissues.

3.5. Biomarkers responses

In the present research, no mortality was observed at different AgNPs concentrations after 14 days exposure, indicating that *C. fluminea* revealed strong resistance to AgNPs. Based on the data of oxidative stress, it was obvious that AgNPs caused different damage to bivalves and triggered the responses of antioxidant mechanism.

Analysis of antioxidants levels and LPO levels in *C. fluminea* are presented in Fig. 6 and Fig. 7, respectively. Our study demonstrated that Ag bioaccumulation in *C. fluminea* varied among treatments (Fig.5), thus antioxidant mechanism responded differently. SOD is reported to be the first line of enzymatic defense mechanism, which can catalyze the chemical transformation of superoxide (O^{2-}) into oxygen and hydrogen peroxide. Nonetheless, the detrimental by-product hydrogen peroxide needs to be eliminated. CAT plays an important role in the process of the inactivation of hydrogen peroxide. The obtained results showed the increased activities of SOD and CAT in 2 mg·L^{-1} groups. These results were in agreement with those found by Buffet

et al. [45], where oxidative stress was observed in *Scrobicularia plana* exposed to copper oxide nanoparticles and there also was an increase in SOD and CAT levels. In comparison with other experimental groups, the prominent SOD and CAT levels suggest that the oxidative stress induce overproduction of both radical superoxide and hydrogen peroxide under higher AgNPs concentration. MDA can be considered as an indicator of membrane damage from ROS (reactive oxygen species) [46]. Although a significant Ag bioaccumulation were observed in *C. fluminea*, there was no lipid peroxidation observed in 0.1 and 0.5 $mg \cdot L^{-1}$ AgNPs exposure, because lipid peroxidation possibly happened when the content of reactive oxidants exceeded the scavenging capacity required by the antioxidant defense [47].

The metabolism of GSH is crucial to maintain cellular homeostasis and resist toxicity from hazardous substances and oxidative stress, which can convert into its oxidized form (GSSG) through oxidation. An increased GSH level is normal reaction of cell in response to a stressor and the compensation in antioxidant system. GSH reduction reflect imbalance between antioxidants and oxygen radicals and consequently induce oxidative stress [48]. The up-regulation of GSH level was observed for 0.5 $mg \cdot L^{-1}$ treatments, however, no increase in lipid peroxidation, SOD, CAT, and GST activities were found in 0.5 $mg \cdot L^{-1}$ treatment, probably because of the occurrence of an adaptive and transient antioxidant response at low bioaccumulation of AgNPs [47]. GST is involved in Phase II metabolic processes, which can catalyze the conjugation of the reduced form of GSH to xenobiotic substrates. Furthermore,

GST plays a protective role in oxidative stress due to peroxidase and isomerase activities. A distinct increase was observed in GST levels for the bivalves exposed to the highest concentrations of AgNPs compared to blank groups in the present study, indicating its protective effect to stress. The biological function of GPx is to protect organisms from oxidative stress via reducing lipid hydroperoxides to their corresponding alcohols and reducing free hydrogen peroxide to water. The increase of GPx activity in 2 $mg \cdot L^{-1}$ AgNPs exposure probably resulted from the reaction to oxidative damage. However, down-regulated GPx levels were observed in 0.5 $mg \cdot L^{-1}$ treatment. This was likely to associate with increased GSH level, because GPx catalyze the transformation of GSH into GSSH accompanied by the reduction of harmful hyperoxides to nontoxic hydroxyl compounds. In 0.1 $mg \cdot L^{-1}$ AgNPs treatments, a decrease of GPx level was found. The change trend of GPx activities was similar to the results of GPx activities in neretrix after 15 days exposure to STPs effluent [49], which confirmed the "Inhibition-Induction" pattern [50]. Ag *in vivo* induced the generation of free radicals at low Ag concentration, resulting in the damage of physiological balance *in vivo* and significant inhibition of GPx levels. As Ag concentrations increased, the stress-response system and antioxidant defense system *in vivo* were fully activated; so GPx activity increased gradually to remove excess free radicals and protected the body from oxidative damage. In conclusion, *C. fluminea* probably processed specific detoxification mechanisms associated with Ag or Ag complexes in tissues. GPx and GSH played important roles in tissues

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detoxification in 0.1 and 0.5 mg·L^{-1} AgNPs exposure, respectively. In 2 mg·L^{-1} treatments, the activities of antioxidant enzymes and GSH increased significantly to relieve toxicity.

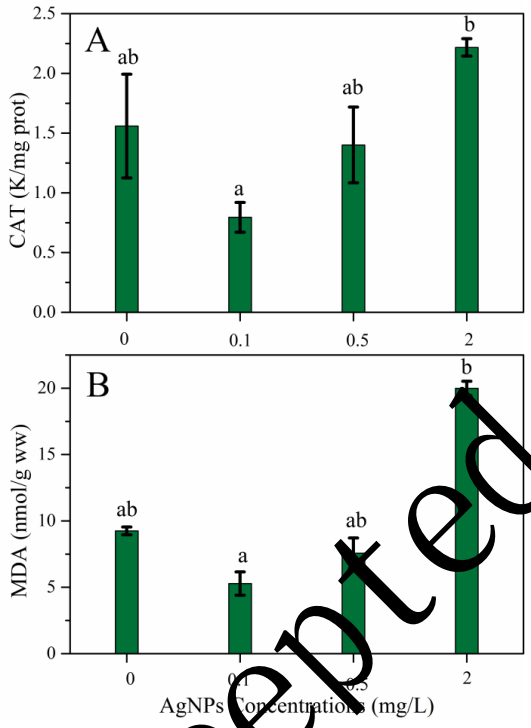

Fig. 6. Effects of PVP-coated AgNPs on CAT (A) and MDA (B) of *C. fluminea in vivo* for 14 days. Data is mean and standard deviation based on wet tissue weight.

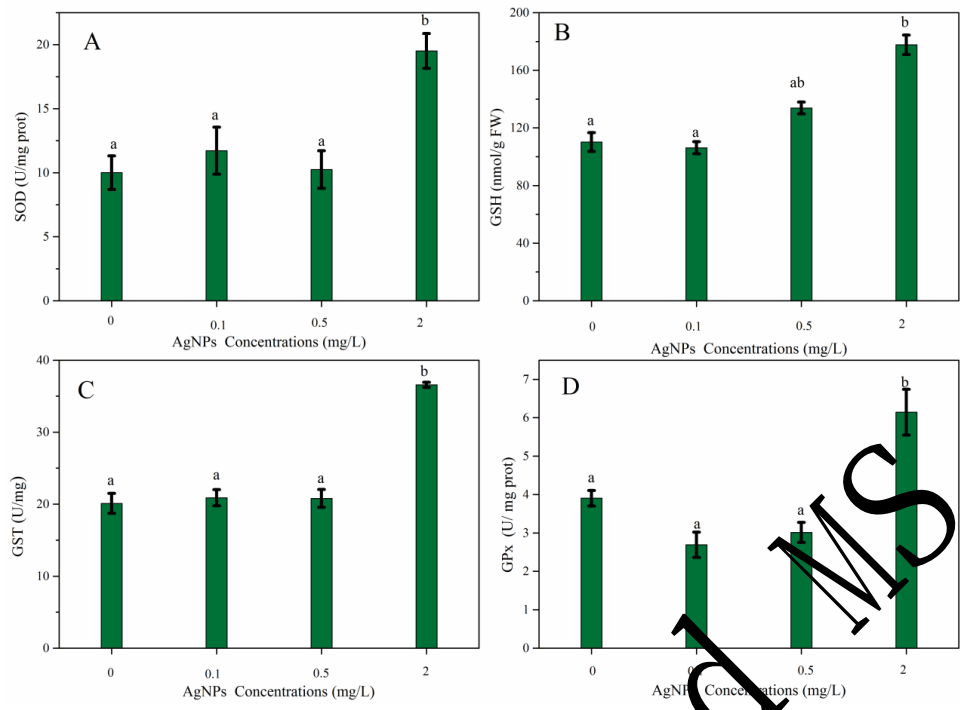

Fig. 7. Effects of PVP-coated AgNPs on SOD (A), GSH (B), GST (C), and GPx (D) of *C. fluminea in vivo* for 14 days. Data is mean and standard deviation based on wet tissue weight.

3.4. Physiological effects

Ammonia excretion and filter-feeding rates have been reported to be sensitive indicators reflecting the toxicity of heavy metals [24,51,52,53]. As shown in Fig. 8, no significant difference in ammonia excretion rate was found between the blank and 0.1 mg·L^{-1} experimental groups. Moreover, the ammonia excretion rates of *C. fluminea* in 0.1 mg·L^{-1} AgNPs were lower than that in 0.5 and 2 mg·L^{-1} AgNPs. There was no significant difference among the four experimental groups in feeding rates of *C. fluminea* (Fig. 8). Ammonia excretion and feeding rates showed similar tendency,

followed the “Inhibition-Induction-Inhibition” pattern, which was also similar with the result analyzed by Zeng et al. [54]. When the concentration of AgNPs was below 0.1 mg·L^{-1}, the rates of ammonia excretion and feeding decreased slightly. The rates both increased to maximum, whereas the rates were inhibited again at 2 mg·L^{-1}. The ammonia excretion rates showed a good positive linear correlation with the filter-feeding rates (Fig.9). This tendency indicated that lower concentration exposures induced the accumulation of ROS in the body, which resulted in a certain degree of oxidative damage in cell, while the body antioxidant enzyme defense system had not yet been activated. This point of view was also proved by the decrease of GPx level. Thus ROS damaged the structure and function of membrane and led to a series of physiological and biochemical metabolic disorders. The physiological metabolism of *C. fluminea* decreased, which was presented as the slight decrease of feeding rates and ammonia excretion rates. When the concentration of AgNPs increased, the antioxidant enzyme defense system produced a large number of enzymes to eliminate ROS, then protecting the body from oxidative damage and increasing feeding and excretion capacity. Higher LPO levels at high AgNPs concentration indicated that cells suffered from more serious damage and the free radical content exceeded the scavenging capacity of the enzyme system, leading to the decline in physiological and metabolic functions.

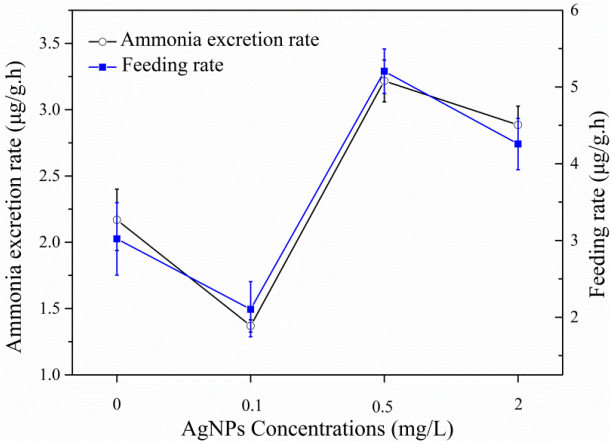

Fig. 8. Effects of PVP-coated AgNPs on ammonia excretion rates and feeding rates of *C. fluminea in vivo* for 14 days. Data is mean and standard deviation based on wet tissue weight.

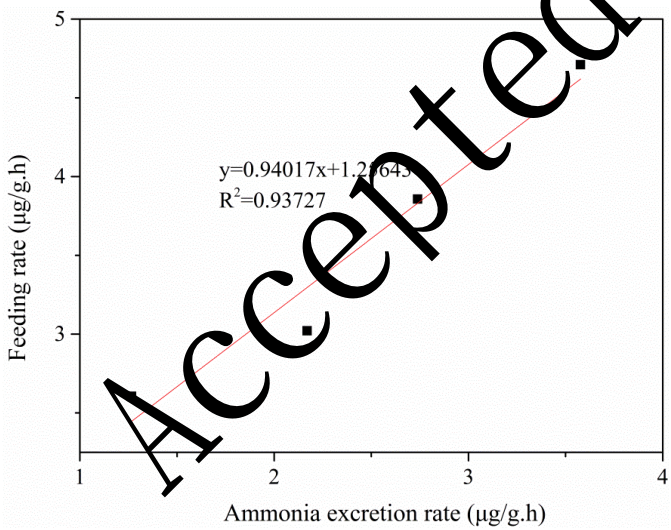

Fig. 9. Liner correlation of Ammonia excretion rates and feeding rates in *C. fluminea.*

According to results of FAAS and SEM (Fig. 10), no Ag was found in feces of *C. fluminea*, possibly due to the fact that nanoparticles had long gut retention time [55]. The compositions of feces in different concentrations were obtained by SEM-EDAX.

These results revealed that the constitution of feces in higher AgNPs concentration presented more littery than that in 0.1 mg·L^{-1} AgNPs. Calcium was detected in the feces in 2 mg·L^{-1} AgNPs treatment, while no detection was found in 0.1 mg·L^{-1} AgNP treatment, which probably indicated AgNPs affected the normal calcium metabolism *in vivo*, thus calcium was excreted from the body. Generally, the developed calcium storage mechanism *in vivo* is essential to freshwater mollusk, and calcospherite acts as calcium storehouse and plays an important role in the transport and the dynamic balance of calcium [56]. However, glycoprotein, a component of calcospherite, tended to react with metal cations [57]. Glycoprotein probably combined with Ag leading to the disintegration of calcospherite, and finally, calcium excreted with excretion.

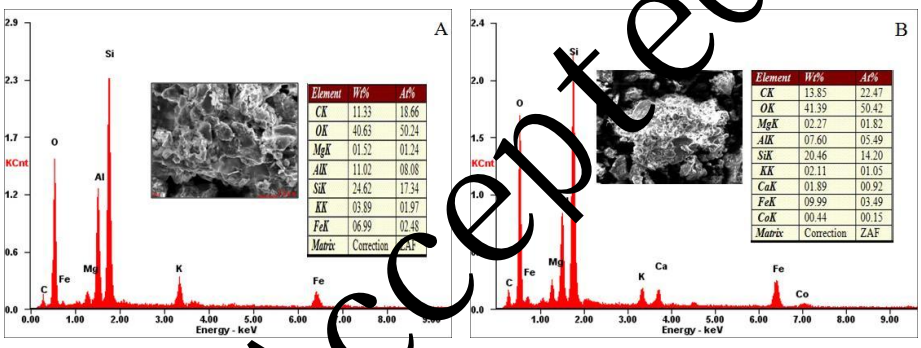

Fig. 10. SEM-EDX micrographs of feces of *C. fluminea* following exposure to PVP-coated AgNPs: (A) 0.1 mg·L^{-1}, (B) 2 mg·L^{-1}.

5. Conclusions

This study was conducted with freshwater bivalve exposed to PVP-AgNPs. In this study, *C. fluminea* facilitated the movement of AgNPs from water to sediment and affected the fate and transformation of AgNPs. Hence, a large amount of Ag

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accumulated in sediment and posed a threat to benthic organisms. The results revealed that the bioaccumulation of Ag resulted in oxidative damage and the prohibition of physiological metabolism in *C. fluminea*. Different detoxification mechanism was found in *C. fluminea* at varied doses of AgNPs. According to AgNPs bioaccumulation in body and shells, shells are better choice for high AgNPs concentration indicators, whereas tissues are more sensitive to lower concentration. No Ag was found in feces of *C. fluminea*, possibly because AgNPs had longer gut retention time compared to other substances. Furthermore, high doses of AgNPs probably induced the disintegration of calcospherite and the loss of calcium. This study furthers the understanding of the interaction between AgNPs and *C. fluminea*, providing meaningful information on the fate and toxicity of AgNPs occurring in natural aquatic environment.

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References

[1] Hu, X.J., Wang, J.S., Liu, Y.G., Li, X., Zeng, G.M., Bao, Z.L., Zeng, X.X., Chen, A.W., Long, F., 2011. Adsorption of chromium (VI) by ethylenediamine-modified

cross-linked magnetic chitosan resin: Isotherms, kinetics and thermodynamics. J. Hazard. Mater. 185, 306-314. http://dx.doi.org/10.1016/j.jhazmat.2010.09.034.

[2] Tang, L., Zeng, G.M., Shen, G.L., Li, Y.P., Zhang, Y., Huang, D.L., 2008. Rapid detection of picloram in agricultural field samples using a disposable immunomembrane-based electrochemical sensor. Environ. Sci. Technol. 42, 1207-1212. http://dx.doi.org/10.1021/es7024593.

[3] Zeng, G.M., Chen M., Zeng, Z.T., 2013. Risks of Neonicotinoid Pesticides. Science 340, 1403. http://dx.doi.org/10.1126/science.340.6139.1403-a.

[4] Chen, H.H., Zha, J.M., Yuan, L.L., Wang, Z.J., 2015. Effects of fluoxetine on behavior, antioxidant enzyme systems, and multixenobiotic resistance in the Asian clam *Corbicula fluminea*. Chemosphere 119, 856-862. http://dx.doi.org/10.1016/j.chemosphere.2014.08.062.

[5] Cheng, Y., He, H.J., Yang, C.P., Zeng, G.M., Li, X., Chen, H., Yu, G.L., 2016. Challenges and solutions for biofiltration of hydrophobic volatile organic compounds. Biotechnol. Adv. 34, 1091-1102. http://dx.doi.org/10.1016/j.biotechadv.2016.06.007.

[6] Zeng, G.M., Chen, M., Zeng, Z.T., 2013. Shale gas: surface water also at risk. Nature 499, 154. http://dx.doi.org/10.1038/499154c.

[7] Gong, J.L., Wang, B., Zeng, G.M., Yang, C.P., Niu, C.G., Niu, Q.Y., Zhou, W.J., Liang, Y., 2009. Removal of cationic dyes from aqueous solution using magnetic multi-wall carbon nanotube nanocomposite as adsorbent. J. Hazard. Mater. 164, 1517-1522. http://dx.doi.org/10.1016/j.jhazmat.2008.09.072.

[8] Liang, J., Liu, J.F., Yuan, X.Y., Dong, H. R., Zeng, G.M., Wu, H.P., Wang, H., Liu, J.Y., Hua, S.S., Zhang, S. Q., Yu, Z.G., He, X. X., He, Y., 2015. Facile synthesis of alumina-decorated multi-walled carbon nanotubes for simultaneous adsorption of cadmium ion and trichloroethylene. Chem. Eng. J. 273, 101-110. http://dx.doi.org/10.1016/j.cej.2015.03.069.

[9] Hu, L., Zhang, C., Zeng, G.M., Chen, G.Q., Wan, J., Guo, Z., Wu, H.P., Yu, Z.G., Zhou, Y.Y., Liu, J.F., 2016. Metal-based quantum dots: synthesis, surface modification, transport and fate in aquatic environments and toxicity to microorganisms. RSC Adv. 6, 78595-78610. DOI: 10.1039/C6RA13016J.

[10] 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. http://dx.doi.org/10.1016/j.aquatox.2015.07.002.

[11] Zeng, G.M., Wan, J., Huang, D.L., Hu, L., Huang, C., Cheng, M., Xue, W.J., Gong, X.M., Wang, R.Z., Jiang, D.N., 2017. Precipitation, adsorption and rhizosphere effect: the mechanisms for Phosphate-induced Pb immobilization in soils-A review. J. Hazard. Mater. 339, 354-367. https://doi.org/10.1016/j.jhazmat.2017.05.038.

[12] Yi, F., Chen, G.Q., Zeng, G.M., Guo, Z., Liu, W.W., Huang, Z.Z., He, K., Hu, L., 2016. Influence of cysteine and bovine serum albumin on silver nanoparticle stability, dissolution, and toxicity to *Phanerochaete chrysosporium*. RSC Adv. 6, 106177-106185. http://dx.doi.org/10.1039/c6ra23675h.

[13] Huang, Z.Z., Chen, G.Q., Zeng, G.M., Guo, Z., He, K., Hu, L., Wu, J., Zhang L.H., Zhu, Y., Song, Z.X., 2017. Toxicity mechanisms and synergies of silver nanoparticles in 2, 4-dichlorophenol degradation by *Phanerochaete chrysosporium*. J. Hazard. Mater. 321, 37-46. http://dx.doi.org/10.1016/j.jhazmat.2016.08.075.

[14] Navarro, E., Wagner, B., Odzak, N., Sigg, L., Behra, R., 2015. Effects of differently coated silver nanoparticles on the photosynthesis of *Chlamydomonas reinhardtii*. Environ. Sci. Technol. 49, 8041-8047. http://dx.doi.org/10.1021/acs.est.5b01089.

[15] Yang, X.Y., Jiang, C.J., Kim, H.L.H., Badireddy, A.R., Dykstra, M., Wiesner, M., Hinton, D.E., Meyer, J.N., 2014. Silver nanoparticle behavior, uptake, and toxicity in *Caenorhabditis elegans*: Effects of natural organic matter. Environ. Sci. Technol. 48, 3486-3495. http://dx.doi.org/10.1021/es404444n.

[16] Meysman, F.J.R., Middelburg, J.J., Heip, C.H.R., 2006. Bioturbation: a fresh look atDarwin's last idea. Trends Eco. Evol. 21, 688-695. https://dx.doi.org/10.1016/j.tree.2006.08.002.

[17] Cong, Y., Bantaa, G.A., 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, 403-411. http://dx.doi.org/10.1016/j.aquatox.2011.07.014.

[18] Arini, A., Daffe, G., Gonzalez, P., Feurtet, M.A., Baudrimont, M., 2014. Detoxification and recovery capacities of *Corbicula fluminea* after an industrial metal

553 contamination (Cd and Zn): A one-year depuration experiment. Environ. Pollut. 192,
554 74-82. http://dx.doi.org/10.1016/j.envpol.2014.04.012.
555 [19] Hull, M.S., Chaurand, P., Rose, J., Auffan, M., Bottero, J.Y., Jones, J.C., Schultz,
556 I.R., Vikesland, P.J., 2011. Filter-feeding bivalves store and biodeposit colloidally
557 stable gold nanoparticles. Environ. Sci. Technol. 45, 6592-6599.
558 http://dx.doi.org/10.1021/es200809c.
559 [20] Buffet, P.E., Vergnoux, A.Z., Châtel, A., Berthet, B., Métais, I., Ettajani, H.P.,
560 Poirier, L., Acosta, A.L., Guyon, H.T., Faverney, C.R., Guibbolini, M., Gilliland, D.,
561 Jones, E.V., Mouneyrac, C., 2014. A marine mesocosm study on the environmental
562 fate of silvernanoparticles and toxicity effects on two endobenthic species: The
563 ragworm *Hediste diversicolor* and the bivalve mollusc *Scrobicularia plana*. Sci. Total
564 Environ. 470-471, 1151-1159. http://dx.doi.org/10.1016/j.scitotenv.2013.10.114.
565 [21] You, L.P., 2014. Studies on the toxicological effects of silver nanoparticles on
566 *Mytilus galloprovincialis* (Doctor thesis). University of Chinese Academy of Sciences,
567 Beijing, China.
568 [22] Geng, N., Wang, P.F., Wang, C., Hou, J., Qian, J., Miao, L.Z., 2016.
569 Mechanisms of cadmium accumulation (adsorption and absorption) by the freshwater
570 bivalve *Corbicula fluminea* under hydrodynamic conditions. Environ. Pollut. 212,
571 550-558. http://dx.doi.org/10.1016/j.envpol.2016.01.091.

572 [23] Frenzel, M., Harrison, R.J., Harper, E.M., 2012. Nanostructure and
573 crystallography of aberrant columnar vaterite in *Corbicula fluminea* (Mollusca), J.
574 Struct. Biol. 178, 8-18. http://dx.doi.org/10.1016/j.jsb.2012.02.005.
575 [24] Cid, A., Picado, A., Correia, J.B., Chaves, R., Silva, H., Caldeira, J., Matos,
576 A.P.A., Diniz, M.S., 2015. Oxidative stress and histological changes following
577 exposure to diamond nanoparticles in the freshwater Asian clam *Corbicula fluminea*
578 (Müller, 1774), J. Hazard. Mater. 284, 27-34.
579 http://dx.doi.org/10.1016/j.jhazmat.2014.10.055.
580 [25] Zeng, L.X., Chen, G.Z., Wu, H.H., 2007. Toxicity Effects of Cd and Cu on the
581 respiration and excretion metabolism of Asian clam. J. Agro-Environ. Sci. 26,
582 175-178. http://dx.doi.org/10.3321/j.issn:1672-2043.2007.01.034.
583 [26] Guo, Z., Chen, G.Q., Zeng, G. M., Liang, J., Huang, B.B., Xiao, Z.H., Yi, F.,
584 Huang, Z.Z., He, K., 2016. Determination of inequable fate and toxicity of Ag
585 nanoparticles in *Phanerochaete chrysosporium* biofilm system through different
586 sulfide sources. Environ. Sci-Nano 3, 1027-1035.
587 http://dx.doi.org/10.1039/c6en00156d.
588 [27] Liang, J., Yang, Z.X., Tang, L., Zeng, G.M., Yu, M., Li, X.D., Wu, H.P., Qian,
589 Y.Y., Li, X.M., Luo, Y., 2017. Changes in heavy metal mobility and availability from
590 contaminated wetland soil remediated with combined biochar-compost. Chemosphere.
591 181, 281-288.

592 [28] Lee, S.W., Park, S.Y., Kim, Y.H., Im, H., Choi, J.H., 2016. Effect of sulfidation
593 and dissolved organic matters on toxicity of silver nanoparticles in sediment dwelling
594 organism, *Chironomus riparius*. Sci. Total Environ. 553, 565-573.
595 http://dx.doi.org/10.1016/j.scitotenv.2016.02.064.
596 [29] Hua, Q, Liu, Y.G., Yan, Z.L., Zeng, G.M., Liu, S.B., Wang, W.J., Tan, X.F.,
597 Deng J.Q., Tang, X., Wang Q.P., 2018. Allelopathic effect of the rice straw aqueous
598 extract on the growth of *Microcystis aeruginosa*. Ecotoxicol. Environ. Safe.
599 148,953-959. https://doi.org/10.1016/j.ecoenv.2017.11.049.
600 [30] Wang, W.L., 2009. Dicussion about Factors Influencing in Determination of
601 Ammonia·Nitrogen by Nessler's Reagent Colorimetry Method. Environmental
602 Monitoring in China 25, 29-32.
603 [31] Jin, X.W., Zha, J.M., Xu, Y.P., Wang, Z.J., 2009. Toxicity and oxidative stress of
604 three chlorophenols to freshwater clam *Corbicula fluminea*. Asian J. Ecotoxicol. 4,
605 816-822.
606 [32] Martínez, G.V.A., DelValls, A.T., Díaz, M.L.M., 2015. Yes, caffeine, ibuprofen,
607 carbamazepine, novobiocin and tamoxifen have an effect on *Corbicula fluminea*
608 (Müller, 1774). Ecotoxicol. Environ. Safe. 120, 142-154.
609 http://dx.doi.org/10.1016/j.ecoenv.2015.05.036.
610 [33] Qiu, R.L., Zhao, X.Y., Tang, T., Yu, F. M., Hu, P.J., 2008. Antioxidative
611 response to Cd in a newly discovered cadmium hyperaccumulator. Arabis paniculata
612 F. Chemosphere 74 (1), 6-12. http://dx.doi.org/10.1016/j.chemosphere.2008.09.069.

[34] McCarthy, M.P., Carroll, D.L., Ringwood, A.H., 2013. Tissue specific responses of oysters, *Crassostrea virginica*, to silver nanoparticles, Aquat. Toxicol. 138-139, 123-128. http://dx.doi.org/10.1016/j.aquatox.2013.04.015.

[35] Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantifies of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72, 248-254. http://dx.doi.org/10.1016/0003-2697(76)90527-3.

[36] Liu, J.Y., Wang, Z.Y., Liu, F.D., Kane, A.B., Hurt, R.H., 2012. Chemical Transformations of Nanosilver in Biological Environments. ACS nano 6 (11), 9887-9899.

[37] Zeng, J.W., Xu P., Chen, G.Q., Zeng, G.M., Chen, A.W., Liu, L., Huang, Z.Z., He, K., Guo, Z., Liu, W.W., Wu, J., Shi, J.B., 2018, Effects of silver nanoparticles with different dosing regimens and exposure media on artificial ecosystem. J. Environ. Sci. (In press).

[38] Lowry, G.V., Espinasse, B.P., Badireddy, A.R., Richardson, C.J., Reinsch, B.C., Bryant, L.D., Bone, A.J., Deonarine, A., S. Chae, Therezien, M., Colman, B.P., Kim, H.H., Bernhardt, E.S., Matson, C.W., Wiesner M.R., 2012. Long-term transformation and fate of manufactured Ag nanoparticles in a simulated large scale freshwater emergent wetland. Environ. Sci. Technol. 46, 7027-7036. http://dx.doi.org/10.1021/es204608d.

[39] Feng, Y., Gong, J.L., Zeng, G.M., Niu, Q.Y, Zhang, H.Y., Niu, C.G., Deng, J.H., Yan, M., 2010. Adsorption of Cd (II) and Zn (II) from aqueous solutions using

magnetic hydroxyapatite nanoparticles as adsorbents. Chem. Eng. J. 162, 487-494. http://dx.doi.org/10.1016/j.cej.2010.05.049.

[40] Fan, T., Liu, Y.G., Feng, B.Y., Zeng, G.M., Yang, C.P., Zhou, M., Zhou, H.Z., Tan, Z.F., Wang, X., 2008. Biosorption of cadmium(II), zinc(II) and lead(II) by *Penicillium simplicissimum*: Isotherms, kinetics and thermodynamics. J. Hazard. Mater. 160, 655-661. http://dx.doi.org/10.1016/j.jhazmat.2008.03.038.

[41] Cleveland, D., Long. S. E., Pennington, P. L., Cooper E., Fulton, M. H., Scott, G. I., Brewer, T., Davis, J., Petersen, E. J., Wood, L., 2012. Pilot estuarine mesocosm study on the environmental fate of Silver nanomaterials leached from consumer products. Sci. Total Environ. 421-422,267-272. https://doi.org/10.1016/j.scitotenv.2012.01.025.

[42] Koide, M., Dong, S.L., Goldbergm, E.D., 1982. Metal and transuranic records in mussel shells, byssal threads and tissues. Estuar. Coast. Shelf S. 15, 679-695. http://dx.doi.org/10.1016/0272-7714(82)90079-8.

[43] Szefer, P., Freleka, K., Szefer, K., Lee, C.B., Kim, B.S., Warzocha, J., Zdrojewska, I., Ciesielski, T., 2002. Distribution and relationships of trace metals in soft tissue, byssus and shells of *Mytilus edulis trossulus* from the southern Baltic. Environ. Pollut. 120, 423-444. https://doi.org/10.1016/S0269-7491(02)00111-2.

[44] Cravoa, A., Bebiannoa, M.J., Fosterb, P., 2004. Partitioning of trace metals between soft tissues and shells of Patella aspera. Environ. Int. 30, 87-98. http://dx.doi.org/10.1016/S0160-4120(03)00154-5.

655 [45] Buffet, P.E., Tankoua, O.F., Pan, J.F., Berhanu, D., Herrenknecht, C., Poirier, L.,
656 Triquet, C.A., Amiard, J.C., Bérard, J.B., Risso, C., Guibbolini, M., Roméo, M., Reip,
657 P., Jones, E.V., Mouneyrac, C., 2011. Behavioural and biochemical responses of two
658 marine invertebrates *Scrobicularia plana* and *Hediste diversicolor* to copper oxide
659 nanoparticles. Chemosphere 84, 166-174.
660 http://dx.doi.org/10.1016/j.chemosphere.2011.02.003.
661 [46] Ringwood, A.H., Conners, D.E., Keppler, C.J., Dinovo, A.A., 1999. Biomarker
662 studies with juvenile oysters (*Crassostrea virginica*) deployed in situ. Biomarkers
663 Biochemical Indicators of Exposure Response and Susceptibility to Chemicals, 1999,
664 4(6): 400-415. http://dx.doi.org/10.1080/135475099230570.
665 [47] Correia, B., Freitas, R., Figueira, E., Soares, A.M.V.M., Nunes, B., 2016.
666 Oxidative effects of the pharmaceutical drug paracetamol on the edible clam
667 *Ruditapes philippinarum* under different salinities. Comp. Biochem. Phys. C. 179,
668 116-124. http://dx.doi.org/10.1016/j.cbpc.2015.09.006.
669 [48] Halliwell, B., Gutteridge, J.M.C., 1990. Free radicals in biology and medicine,
670 Oxford University Press, Oxford.
671 [49] Su, E.P., Meng, F.P., Sun, T., Fu, W.C., Wang, Q., Li, Y.F., Hu, H., 2012.
672 Responses of the antioxidant enzymes in Meretrix meretrix exposed to effluent from
673 municiplal sewage treatment plants for short term. J. Environ. Sci. China 34, 511-517.

[50] Wang, F., Zhao, Y.F., Lu, J.C., Liu, C.F., 2008. Effect of Cu^{2+} on the antioxidant enzyme of scallop chlamys farreri's viscera. Fisheries Sci. 27, 622-624. http://dx.doi.org/10.3969/j.issn.1003-1111.2008.12.004.

[51] Cheung, S.G., Cheung, R.Y.H., 1995. Effects of heavy metals on oxygen consumption and ammonia excretion in creen-lipped mussels (*Perna viridis*). Mar. Pollut. Bull. 31, 4-12. http://dx.doi.org/10.1016/0025-326X(95)00137-C.

[52] Galvez, F., Hogstrand, C., Wood, C.M., 1998. Physiological responses of juvenile rainbow trout to chronic low level exposures of waterborne silver. Comp. Biochem. Physiol. 119C, 131-137. http://dx.doi.org/10.1016/S0742-8413(97)00199-0.

[53] Soucek, D.J., 2007. Sodium sulfate impacts feeding, specific dynamic action, and growth rate in the freshwater bivalve *Corbicula fluminea*. Aquat. Toxicol. 83, 315-322. http://dx.doi.org/10.1016/j.aquatox.2007.05.006.

[54] Zeng, L.X., Zhang, Y.J., Kang, Y., Zhang, Q.Y., 2014. Effects of BPA and NP on respiratory metabolism and antioxidant enzymes in Asian calm. Ecol. Environ. Sci. 23, 122-128. http://dx.doi.org/10.3969/j.issn.1674-5906.2014.01.018.

[55] Ward, J.E., Kach[illegible]L., 2009. Marine aggregates facilitate ingestion of nanoparticles by suspension-feeding bivalves. Mar. Environ. Res. 68, 137-142. http://dx.doi.org/10.1016/j.marenvres.2009.05.002.

[56] Davis, W.L., Jones, R.G., Hagler, H.K., 1979. Calcium containing lysosomes in the normal chick duodenum: A histochemical and analytical electron microscopic study. Tissue. Cell 11, 127-138. http://dx.doi.org/10.1016/0040-8166(79)90013-2.

[57] Huang, Z.Z., Chen, G.Q., Zeng, G.M., Chen, A.W., Zuo, Y.N., Guo, Z., Tan, Q., Song, Z.X., Niu, Q., 2015. Polyvinyl alcohol-immobilized *Phanerochaete chrysosporium* and its application in the bioremediation of composite-polluted wastewater. J. Hazard. Mater. 289, 174-183. http://dx.doi.org/10.1016/j.jhazmat.2015.02.043.