Humic Acid Alleviates the Toxic Effect of AgNPs on *Tigriopus Japonicus*

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Abstract: Silver nanoparticles (AgNPs) with excellent physical and chemical properties are one of the most widely used nanoparticles (NPs). However, with the increase of the production of AgNPs, there will be certain risks to aquatic organisms when they are discharged into the water environment. Here, we systematically studied the ecotoxic effect of AgNPs on *Tigriopus* under the influence of humic acid (HA) in natural organic matter (NOM). From the perspective of individual level, the effects of AgNPs on feeding behavior of *Tigriopus* under the action of HA were explored. As a link between primary and higher nutrient levels in the ocean, it is of great significance to study the feeding behavior of copepods. Compared with acute and chronic toxicity indexes, behavioral indexes are more sensitive to evaluate the toxic effects of pollutants. With the increase of AgNPs concentration, the feeding rate and water filtration rate of *Tigriopus* decreased, indicating that the presence of nanoparticles (NPs) could significantly inhibit the feeding behavior of the tested organisms. The presence of humic acid (HA) alleviated the inhibition of AgNPs on feeding rate and water filtration rate of *Tigriopus*. In addition, this indicated that there was a certain interaction between NPs and HA, which would alleviate the toxic effects of nanoparticles on copepods, and ultimately affected the individual level and population dynamics of copepods.

1 INTRODUCTION

Nanoparticles are defined as ultra-fine particles with a particle size of 1-100 nm. Due to their chemical composition, shape, size, density, aggregation, surface properties and unique physical and chemical properties (such as magnetic, optical and electrochemical properties) (Shevlin, 2018). In the process of synthesis, production and use, nanomaterials will inevitably be discharged into water through a variety of ways. Their unique physical and chemical properties can be transmitted and transferred through the food chain of aquatic organisms, enrich in the body of organisms with higher trophic levels, and have toxic effects on biological cells and individuals (Vance, 2015). Existing nanomaterials have confirmed that silver nanoparticles (AgNPs) are toxic to aquatic organisms, playing a toxic role by causing membrane damage, production of reactive oxygen species, protein degeneration, mitochondrial dysfunction, DNA damage, and inhibition of cell proliferation Silver nanoparticles: Toxicity in model organisms as an overview of its hazard for human health and the environment (Tortella, 2020). Their effects on aquatic organisms should be fully studied (Williams, 2019). Their position in marine food chains is very prominent, especially with regard to the transfer of energy (Jeong, 2020). From the study's endpoints, *Tigriopus* is not only suitable for short-term acute exposure but also for multigenerational subchronic or chronic toxicity studies. From the perspective of research methods, there are individual level of apparent research, biochemical level of micro research and individual level of research indicators, such as mortality, growth and development, sex ratio, spawning capacity, reproduction and feeding.

Natural organic matter (NOM), which is a complex mixture of a wide range of molecular weights, is presents in the environment at concentrations ranging from 1 to 10 $mg \cdot L^{-1}$ (Li, 2018). The results showed that Suwannee River humic acid (SRHA) could decompose the surface layer of AgNPs at the concentration of 10 $mg \cdot L^{-1}$, and the release amount of Ag^{+} decreased with the increase of SRHA concentration (Gunsolus, 2015). At the same time, there may be a reversible process in the light environment where NOM (e.g.HA) reduces Ag^{+}

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to AgNPs, depending on the concentrations of Ag^+ and HA (Zhou, 2016).

In this study, *Tigriopus* was selected as the research object, and the research method combined with laboratory culture, determination and analysis was planned to conduct the following explorations. In terms of individual level, this paper explored the effects of different concentrations of AgNPs on feeding behavior of *Tigriopus*, and the effects of AgNPs on feeding behavior of *Tigriopus* in the presence of humic acid (HA). This study provides data support and scientific basis for evaluating the potential effects of silver nanoparticles (AgNPs) on marine copepods under the influence of humic acid (HA) in natural organic matter (NOM).

2 MATERIALS AND METHODS

2.1 Preparation of Experimental Materials

(1) Preparation of AgNPs reserve solution

AgNPs (<100 nm) powder purchased from Sigma-Aldrich. When preparing the reserve solution, 0.015g AgNPs powder was placed in a 100 mL beaker, and the volume was fixed in a 250 mL volumetric flask. The powder was prepared into 60 mg/L reserve solution and stored in a refrigerator at 4℃. Before each experiment, the reserve solution was diluted into solutions of different concentrations, and ultrasound was required for 30 min in advance.

(2) Preparation of HA and AgNPs mixed solution

In the preparation of mixed solutions of HA and AgNPs, quantitative AgNPs and HA should be added into the test tube with sterilized seawater and oscillated continuously for 24 h in an oscillating chamber (37℃, rotation speed 180 r/min). The process of sterilizing seawater was as follows: the purchased natural seawater was aerated with an air pump, filtered by 0.45 μm cellulose nitrate membrane before the experiment, and then sterilized with an autoclave. The sterilization condition was 121℃ for 20 min.

(3) Culture of Tigriopus

The *Tigriopus* used in this experiment was provided by Ecotoxicology Laboratory of Ocean University of China. *Isochrysis galbana* and *Phaeodactylum tricornutum* were used as mixed bait and fed once a day. The density of microalgae was 1×10^6 cell·mL^{-1}. Incubate in a constant temperature incubator with light to dark ratio of 12 h: 12 h, light intensity of 2100 lux and light temperature of 24℃. The seawater was changed once a week. The seawater was extracted and filtered by 0.45 μm cellulose nitrate lipid membrane. The seawater was sterilized in an autoclave at 121℃ for 20 min and cooled to room temperature for later use. The pH of the sterilized seawater is 8.2 and the salinity is 31‰.

(4) Culture of microalgae

Isochrysis galbana and *Phaeodactylum tricornutum* used in this experiment were provided by Ecotoxicology Laboratory of Ocean University of China. Shake well once every morning and evening to prevent microalgae from settling or sticking to the wall.

2.2 Experimental Method

In order to study whether the presence of humic acid (HA) would affect the feeding behavior of AgNPs on *Tigriopus*, a feeding experiment was designed. There were 5 adult in each culture plate. The volume of solution in each well of culture plate was 5 mL. The experiment was carried out under 24 light protection conditions. The vibration was carried out every half hour in the vibration chamber. After exposure for 4 h, the number of algal cells was counted under the microscope with a blood cell counting plate. The algal cells used in the experiment were *Isochrysis galbana*.

3 RESULTS AND DISCUSSION

3.1 Feeding, Filtering Rates of AgNPs to *Tigriopus* in the Presence of HA

As shown in Fig.1, compared with the control group, even in the experimental group with the smallest concentration of AgNPs, the feeding rate and water filtration rate were reduced by 41.14% and 50.64%, respectively. The experimental group with the largest concentration of AgNPs had even lower feeding rate and water filtration rate 67.28%, 74.55%. It can be seen that the presence of AgNPs inhibited the feeding and filtering behavior of the *Tigriopus*. However, after adding different concentrations of HA, the feeding rate and water filtration rate changed. For the low concentration AgNPs experimental group, the feeding rate of the experimental group with 5 mg·L^{-1} HA increased by 18.26% compared with the AgNPs experimental group, and the water filtration rate also showed an upward trend, increasing by 23.08%. Under the influence of HA, the change trend of feeding rate and water filtration rate in the high concentration AgNPs group was consistent with that in the low concentration group.

Behavioural effects of organisms are of great significance in the study of the toxic effects of pollutants such as nanoparticles. Compared with mortality, behavioral indicators are more sensitive to the evaluation of the toxic effects of pollutants. Therefore, the influence of AgNPs on the feeding behavior of *Tigriopus* was investigated in the presence of different concentrations of HA. As can be seen from Fig.1, the feeding rate of *Tigriopus* decreased with the increase of AgNPs concentration, which may be because *Tigriopus* transferred the energy used for feeding behavior to resist the external environmental stress, resulting in a decrease in feeding rate. Studies have shown that the ability of *Tigriopus* to feed may be related to metabolism, as hard-to digest food will stay in the intestines for longer, resulting in a sense of satiety, resulting in a decreased ability to feed (Yu, 2020). The exposure of AgNPs in *Tigriopus* produced oxidative stress effect. To resist oxidative stress effect, the organism must consume energy, and the energy consumption accumulated to a certain extent can further affect the normal physiological activities of the organism and lead to toxicity effects at the individual level (such as changes in feeding behavior). According to the study of Mattsson et al. (Mattsson, 2016), 24 h after daphnia was exposed to silver nanowires (Ag NWs), Ag NWs would accumulate in the intestine of daphnia and transfer to the body cavity through the intestinal epithelium. Due to the damage of digestive organs, nanoparticles would destroy the nutrition and digestive function of daphnia. Keller et al. (Keller, 2012) pointed out that after the organism ingents nanoparticles, nanoparticles adsorb on the surface of the cell membrane through coordination and electrostatic action, interfering with signal transmission on the cell membrane, leading to abnormal cell membrane function and even changing the permeability of the cell membrane, resulting in cell damage. The feeding rate of *Tigriopus* in the presence of HA was higher than that in the presence of AgNPs alone. This may be the Zeta potential change caused by the adsorption of HA on the surface of AgNPs. Meanwhile, the surface charge of NPs was increased to form a higher double electric layer repulsed energy, which lead to the uniform dispersion of aggregates in the medium and inhibits the uptake of NPs by *Tigriopus* (Mohd, 2014).

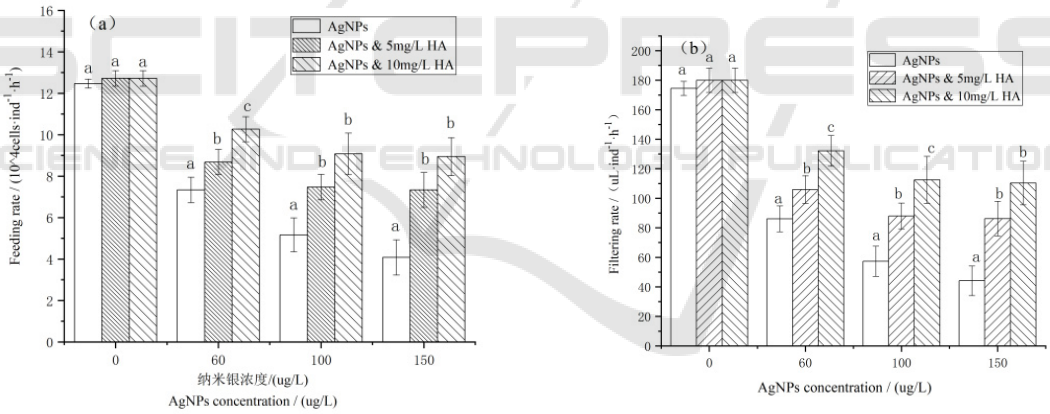

Figure 1: Effects of AgNPs on feeding rate (a) and filtering rate (b) of *Tigriopus* in the presence of different concentrations of HA.

4 CONCLUSIONS

With the increase of AgNPs concentration, the feeding rate and filtrating rate of *Tigriopus* decreased, indicating that the presence of nanoparticles (NPs) could significantly inhibit the feeding behavior of the tested organisms. This may be that nanoparticles (NPs) were ingested by *Tigriopus* and entered the intestinal mucosa, which caused mechanical damage to the intestinal mucosa. The nanoparticles (NPs) accumulated in the intestinal mucosa were difficult to digest and affected the feeding behavior of *Tigriopus*. The presence of humic acid (HA) alleviated the inhibition effect of Ag NPs on the feeding rate and water filtration rate of *Tigriopus*. This may be because the adsorption of HA on the surface of AgNPs causes a change in Zeta potential, resulting in agglomerates being evenly dispersed in the medium, reducing the intake of AgNPs by *Tigriopus*. In addition, due to the adsorption of HA on the surface of AgNPs, the total amount of Ag^{+} released was reduced, which weakened the degree of internalization of AgNPs by organisms and alleviated the toxic effect of AgNPs on *Tigriopus.*

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