参赛队员姓名：韩嘉

中学：北京师范大学附属实验中学

省份：北京

国家/地区：中国

指导教师姓名：范文宏；梁丁元

指导教师单位：北京航空航天大学

论文题目：不同粒径和表面包覆的纳米银沿食物链传递的影响

The influence of AgNPs of different sizes and surface coatings along the food chain

Abstract

The cytotoxicity risk of nanoparticles along the food chain is understudied, with the few available studies mainly focused on the toxic effects of nanoparticles under direct exposure. The characteristics of silver nanoparticles (AgNPs) influence cytotoxicity risks; however, such risks have not been investigated along a food chain. Therefore, this study assessed how distinct characteristics (different sizes and surface coatings) of AgNP affect its accumulation along a minor food chain comprising *Escherichia coli* (*E. coli*) and *Tetrahymena thermophila* (*T. tetrahymena*). Ag accumulation was measured after the initial intake in *E. coli* and then intake in *T. tetrahymena* through water phase exposure, food phase exposure, as well as food and water phase exposure.

The research found that for different types of aquatic organisms, the impact of sizes and surface coatings vary greatly. The high intake ratio and current researches on food chain both indicates that nanoparticles can accumulate along the food chain and may amplify as trophic level increases. In the food and water phase exposure which is the closest to the natural environment, Ag accumulation was reduced in water and food exposure group compared to water/food exposure separately due to the exocytosis of nanoparticles. However, further research is required to explain these results more thoroughly. The results highlighted the environmental risk AgNPs could have on the environment and humans. Therefore, AgNPs used daily should be carefully designed to avoid possible environmental risks, and such possible risks should be evaluated during the design.

KEYWORDS: AgNPs, Ag accumulation, food chain, size, surface coating

CONTENTS

Introduction

1.1 Overview of silver nanoparticles

The special physical characteristics of silver nanoparticles (AgNPs) have made AgNPs widely utilized in the process of biomedicine, catalysis, sterilization, and kitchenware design, amongst others (Peters et al., 2018; Calderoń-Jimeńez, 2017; Konop, 2016; Wei et al., 2015; Chernousova and Epple, 2013). However, with their production, usage, and disposal, nanoparticles can be inevitably released into the environment and pose potential risks. Such potential risks include elevated (order of ng/L to μg/L) Ag accumulation in the aquatic environment (Xiao, 2019), threatening the organisms within (Mitrano et al., 2016; Kittler et al., 2010) and even human health. Therefore, investigating the possible environmental and health risks of AgNPs is of interest to environmental scientists.

1.2 The cytotoxicity risk of AgNPs in the aquatic environment

Researches focusing on the cytotoxicity risk of AgNPs to single aquatic organisms such as *Escherichia coli* and *Daphnia magna* are ample in the field (Kim et al., 2016; Sondi and Salopek-Sondi, 2004), accounting for the cytotoxicity mechanism of AgNPs (Soenen et al., 2015; Le Ouay and Stellacci, 2015). However, these studies only explore the biological effects under direct exposure, ignoring the possibilities of other exposure pathways such as along the food chain. Under the exposure through the food chain, AgNPs can be captured by aquatic organisms, affecting the evaluation of biological effects. Due to the flow of energy in the food chain, Ag accumulated along the food chain can be transferred, which enlarges the harm to species at higher trophic levels (e.g., humans), potentially causing damage to such species. Due to the large complexity of the food chain (which involves different interacting factors and species), only a few in-depth studies on Ag accumulation of AgNP exist (Matos et al., 2020; Wang et al., 2016). Therefore, more studies on the accumulation of AgNPs along the food chain are presently required.

1.3 Factors affecting the toxicity of nanoparticles

Previous studies have elucidated two main categories of factors that influence the toxicity of nanoparticles, namely: (1) the characteristics of nanomaterial, including the solubility, particle size, particle shape, and surface properties of nanoparticles (Xu et al., 2018; Xu et al., 2013), and (2) environmental factors such as pH level, light intensity and organic matter (Sharma, 2014).

1.4 Research Significance

The wide application of nanomaterials in the daily use of antibacterial products has drawn attention to the accumulation of Ag particles in the water environment. Because nanomaterials are passed into the ecosystem through water and the food chain, the exposure of the latter can be passed step by step to expand their reach and even endanger human health at the end. Hence, experiments on Ag accumulation of AgNPs across the food chain should be emphasized. However, due to the complexity of such experiments, few studies have looked at the accumulation of nanosilver along the food chain. Besides the importance of experiments through the food chain, studies have shown that Ag accumulation is influenced by the properties of AgNPs and environmental factors. Therefore, by studying how nanoparticles of different properties accumulate along the food chain, a deeper understanding of the actual ecosystem can be obtained. Such an understanding is of great significance for the comprehensive evaluation of the accumulation of AgNPs and for designing better nanoparticles.

In most experiments studying Ag accumulation along the food chain, a single aquatic organism is generally considered; however, only a few studies have considered food phase and food and water phase exposures. Therefore, compared with previous experiments, this experiment had two bright spots, one was to study the food chain exposure pathway, and the other was to change the natural properties of nanosilver.

2. Materials and methods

2.1 Materials and property characterization

AgNPs of 5 nm were purchased from *Beijing Biotech Biotechnology Co., Ltd,* while 20 nm particles were purchased from *Shanghai Husheng Technology Co., Ltd*. The hydrodynamic size and zeta potential were observed by dynamic light scattering (DLS; Setasizer nano, Malvern, UK). Ultraviolet-visible (UV-Vis) spectrophotometer (U3900, Hitachi, Japan) was used to determine the surface plasmon resonance.

2.2 Bacterial cultivation

The bacterial strain *E. coli* K-12 (KCTC1116) was grown in a Luria-Bertani (LB) broth (5% tryptone, 5% yeast extract, and 10% NaCl). The ciliate *T. tetrahymena* SB210 was obtained from *the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan,* and grown on a nutritional growth medium (2% proteose peptone, 0.1% yeast extract, 0.003% Fe-EDTA, 100 units mL penicillin G, 100 mg/L streptomycin sulfate, and 0.025 mg/L amphotericin B).

2.3 Experiment

2.3.1 Experiment process

Precisely 10 mL of *E. coli* suspension (OD_{600} of 0.77) was first mixed 10 mL EPA (US Environmental Protection Agency) freshwater containing AgNPs with three different types (20 nm-PVP-Ag; 5 nm-PVP-Ag, and 5 nm- Citrate-Ag). These three types differed in surface coatings and sizes. The solution was divided into three parts. For water and food phase exposure, 10 mL of the exposed solution was directly mixed with 1 mL of *T. tetrahymena* suspension (to a final concentration of 30000 cells/mL). The rest of the solution was washed through centrifugation (2000G, 5min) twice. After that, food phase exposure was performed by mixing 8 mL of the exposed solution with 0.8 mL of *T. tetrahymena* suspension as previously described, while the remaining 2 mL was not used for further exposure. Water exposure was done by using 5 mL of

EPA freshwater with AgNPs and 0.5 mL *T. tetrahymena* suspension (30000 cells/mL). Each experimental group was replicated three times, and exposures were done for 24 hours in an environment of 28°C and 110 Revs/min. After the exposure, samples undergoing water phase exposure were washed twice through centrifugation (600 G, 5min). Samples were filtered on 5.0 μm freshwater to separate *E. coli* and *T. tetrahymena*. To be more specific, two superimposed filters were employed to calibrate the adsorptive loss of Ag. Filters supporting the *T. trtrahymena* were rinsed with EPA freshwater to remove AgNPs/Ag+ adsorbed on the outer cell wall (Yan et al.,2021). Finally, all samples were subjected to acid digestion (80°C, 5 hours), and Ag accumulation was measured using Inductively Coupled Plasma-Mass-Spectrometry (ICP-MS).

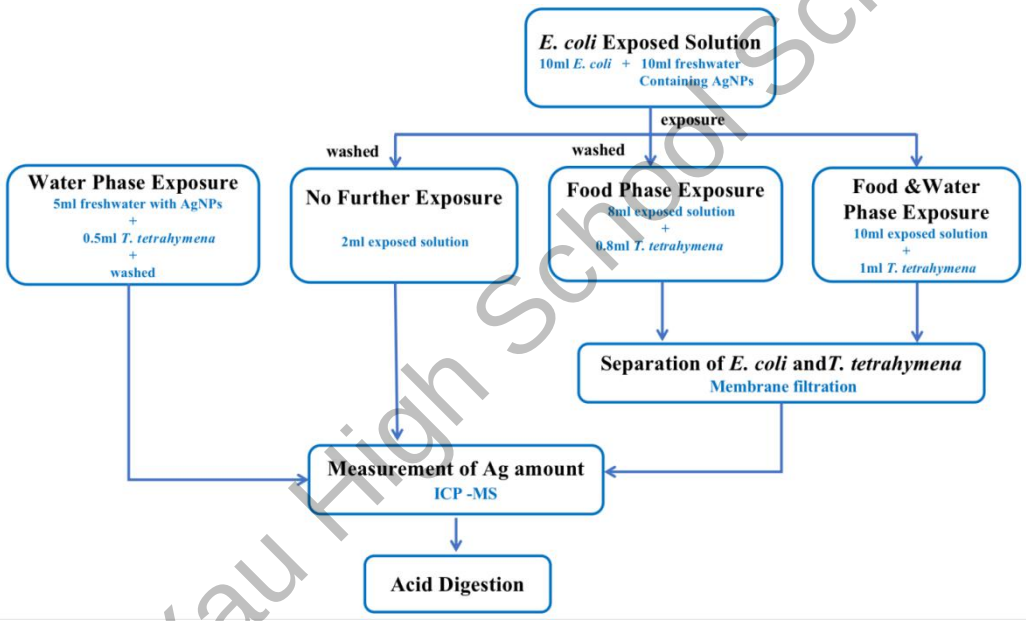


Fig.1 flow chart of the experiment process

2.3.2 TTF ratio calculation and statistical analysis

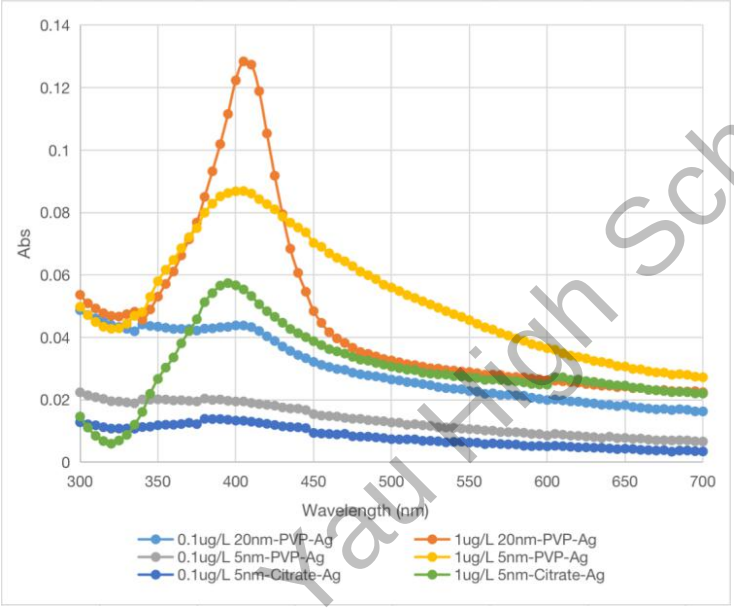
The trophic transfer factor (TTF) was calculated by dividing the Ag amount at a higher trophic level with the Ag amount at a lower trophic level as follows:

One-way analysis of variance was performed in Statistical Product and Service Solutions (SPSS 28.0.0.0) to determine the significance between all the treatment groups. Statistical significance was set at $p < 0.05$.

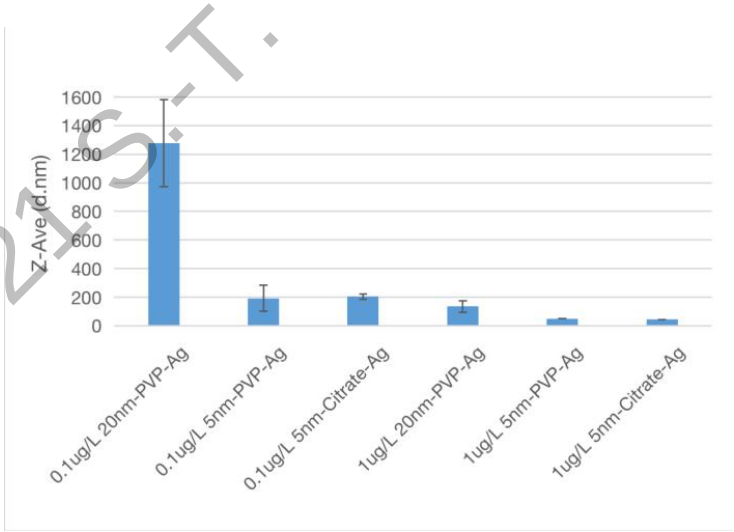
3. Result and discussion

3.1 The characteristics of Ag particles

The 20 nm-PVP-Ag and 5 nm-PVP-Ag peaked at 400 nm and 380 nm in the UV spectrum, respectively (Fig. 2a), confirming the size of the nanoparticles purchased. Based on DLS, the hydrodynamic diameter was 135.5 nm, 50.2 nm, and 44.5 nm for 1 μg/L 20 nm-PVP-Ag, 1 μg/L 5 nm-PVP-Ag, and 1 μg/L 5 nm-Citrate-Ag, respectively (Fig. 2b). The hydrodynamic diameter of 20 nm nanoparticles was more significant ($p < 0.05$) than those of 5 nm nanoparticles (Fig. 2b). The zeta potential was -7.8 mV, -9.0 mV and -13.0 mV for 20 nm-PVP-Ag, 5 nm-PVP-Ag, and 5 nm-Citrate-Ag, respectively (Fig. 2c). Both the hydronamic sizes and zeta potential further confirmed the information of nanoparticles.



a



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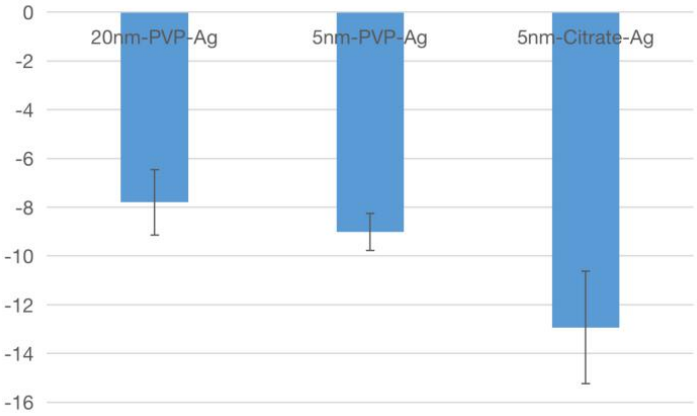

c

Fig.2 Characteristic of Ag colloids. a) absorbance peaks of Ag colloids; b) hydrodynamic size of Ag colloids; c) zeta potential of Ag colloids

3.2 The accumulation of Ag in *E. coli*

Within the period of AgNPs exposure, 5 nm-Citrate-Ag had much higher ($p < 0.05$) Ag accumulation in *E. coli* than both 5 nm-PVP-Ag and 20 nm-PVP-Ag (Fig. 3). However, the Ag accumulation in *E. coli* after exposure to 20 nm-PVP-Ag was approximately the same as that of 5 nm-PVP-Ag ($p > 0.05$). Therefore, it can be estimated that surface coatings of AgNPs have a more significant effect on Ag accumulation in *E. coli* than AgNPs sizes.

The possible reason for this observation is the effect of steric hindrance. PVP coating is a large-sized, non-toxic and non-ionic polymer (Koczkur et al., 2015). On the other hand, the citrate covering was thinner. With a larger volume, PVP prevents the aggregation of AgNPs by the repulsive forces that arise from its hydrophobic carbon chains, which extend into solvents, thereby forming great steric hindrance (Koczkur et al., 2015). Therefore, the steric hindrance of PVP coating reduced the amount of AgNPs that could attach to the surface of *E. coli*. On the contrary, because the layer of citrate is thin, it is much easier for AgNPs to attach to the surface of *E. coli.* Another factor that may contribute to the result is the zeta potential of the particles. Because particles coating in citrate has the lowest zeta potential (highest absolute value), steric hindrance has a more significant effect on Ag accumulation in *E. coli*.

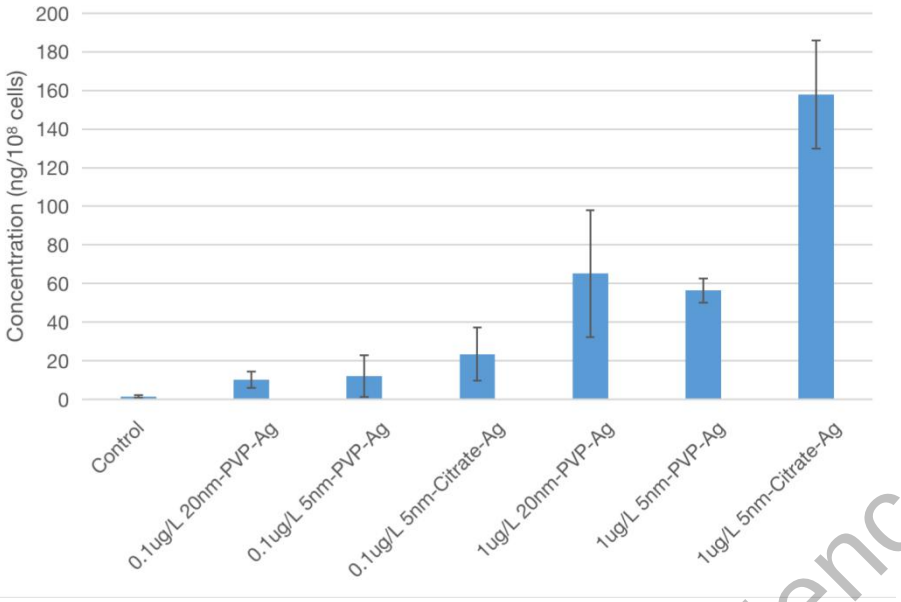

Fig.3 AgNPs Concentration in E. coli after exposure

3.3 The accumulation of Ag in *T. tetrahymena* through water phase exposure

For the water phase exposure, 20 nm-PVP-Ag had significantly ($p < 0.05$) lower Ag accumulation in *T. tetrahymena* than 5 nm-Citrate-Ag and 5 nm-PVP-Ag at both concentrations (Fig. 3), while the Ag accumulation in either coating of 5 nm nanoparticles is approximately the same ($p > 0.05$). Because the Ag accumulation was high in 5 nm nanoparticles, what affects the Ag accumulation in *T. tetrahymena* most significantly is the size of the particles rather than the coatings of the nanoparticles (mainly affecting the accumulation of nanomaterial in *E. coli* concluded from the water phase exposure).

Reasons for the larger amount of Ag discovered in 5 nm particles compared to the 20 nm particles can be explained by different pathways through which nanoparticles approach the cell. Researches have already shown that the pathways that nanoparticles choose are strongly correlated with the hydrodynamic size of the particles (Huang, 2012; Ma, 2011). For smaller sizes (smaller than 10 nm), nanoparticles would pass through membrane pores and directly enter the cytoplasm. In addition, particles entering the cytoplasm are difficult to remove due to the limited number of discharging channels. Therefore, 5 nm nanoparticles would stay in the cytoplasm and accumulate. On the other hand, particles with a larger size (larger than 10nm) will go through receptor-mediated

endocytosis, in which particles pass through the cell membrane through channel proteins. Ag particles will first be adhered to the cell membrane and then they will be further transported in the form of vesicles. When *T. tetrahymena* absorbs enough nanoparticles, they will emit the waste outside the body.

The likely reason that can explain the different Ag accumulation amounts in *E. coli* and *T. tetrahymena* is the different biological structures that *E. coli* and *T. tetrahymena* possess. *E. coli* contains a cell wall consisting of an outer membrane and an inner cytoplasmic membrane (Wang et al., 2021; *Escherichia coli*, n.d.), making nanoparticles difficult to pass through (Rajeshwari et al., 2011), especially when the concentration of nanoparticles is low. In contrast, *T. tetrahymena* has a strong ability to uptake nanoparticles. Therefore, it is easier for Ag particles to enter the cells of *T. tetrahymena* compared with *E. coli.* The appearance of Ag in the experiment is solely because some particles are attached to the surface of *E. coli* (Dong, 2017).

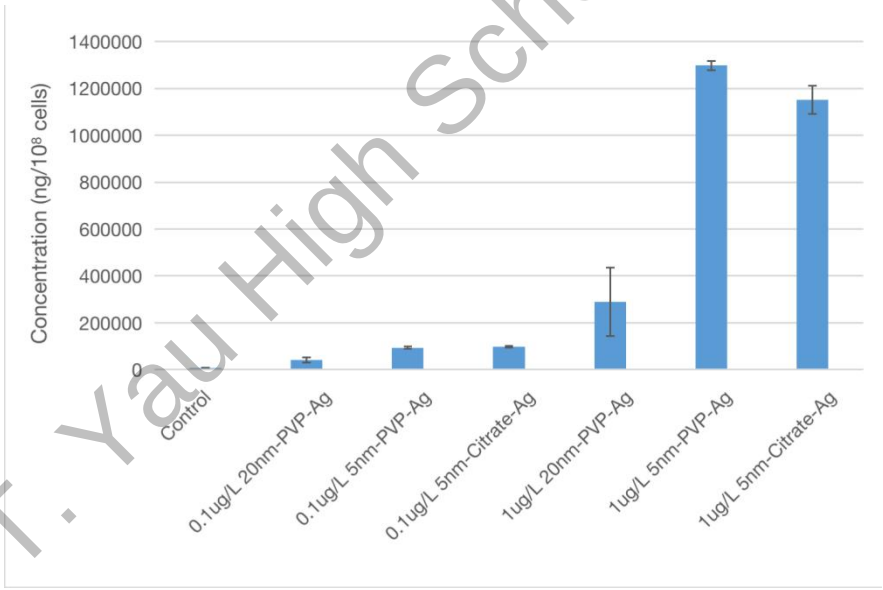

Fig. 4 AgNPs Concentration of water exposure

3.4 The accumulation of Ag through food phase exposure and food and water phase exposure

The intake ratio was approximately equal to 1 (Fig. 6), suggesting that *T. tetrahymena* took up all the *E. coli* and did not discharge any Ag (i.e., all the Ag particles were retained inside the body). Evidence shows that *T. tetrahymena* itself has high tolerance towards toxic particles (Wei,

2016; Wei et al., 2016). However, the amount of Ag accumulated inside *T. tetrahymena* is high, indicating the high environmental risk of AgNPs. According to the accumulation theory, species such as humans at higher trophic levels of the food chain will have larger risks during exposures.

The observed TTF ratio of less than 1 (Fig. 7) indicates that the Ag amount along the food chain does not show the phenomena of bioamplification. This observation may be because the conditions for the bioamplification are not met for the total cell concentration of *T. tetrahymena* (approx.30,000 cells/mL) used in the experiment. However, in the experiment, *T. tetrahymena* already intakes nearly all the *E. coli* and discharged only small amounts of particles, indicating that the accumulation is already strong enough. In addition, previous studies have already shown that bioamplification could happen in this trophic level (Werlin, 2010) and that bioamplification is also possible in the environment since *T. tetrahymena* cannot reach the experimental concentration of 30,000 cells/ml in nature.

The accumulation through water and food exposure as a whole (Fig. 8) is approximately similar (or even lower) to that of through water and food exposure, respectively (Fig. 4 and Fig. 5). Therefore, it is likely that under food and water exposure as a whole, both effects have been restrained due to the discharge of nanoparticles. However, though this exposure pathway is the closest to natural environment, this phenomena have not been observed and explained before according to current researches. Due to the complexity of the environment and multiple factors affecting the Ag accumulation, further research is needed to elucidate the cause of this phenomenon.

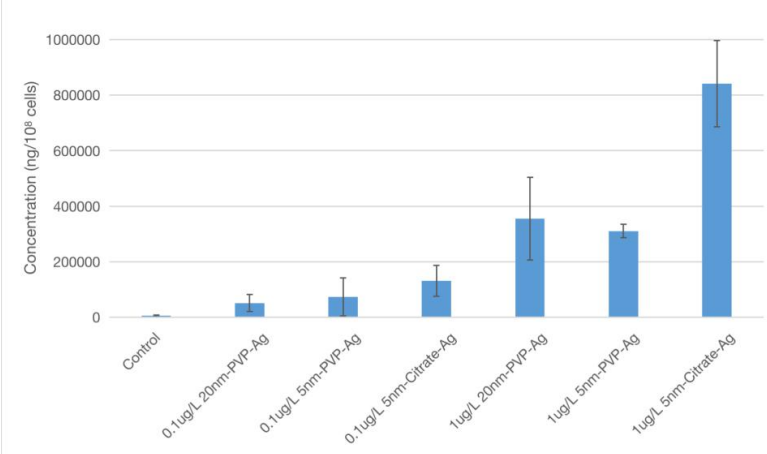

Fig.5 AgNPs Concentration through food exposure

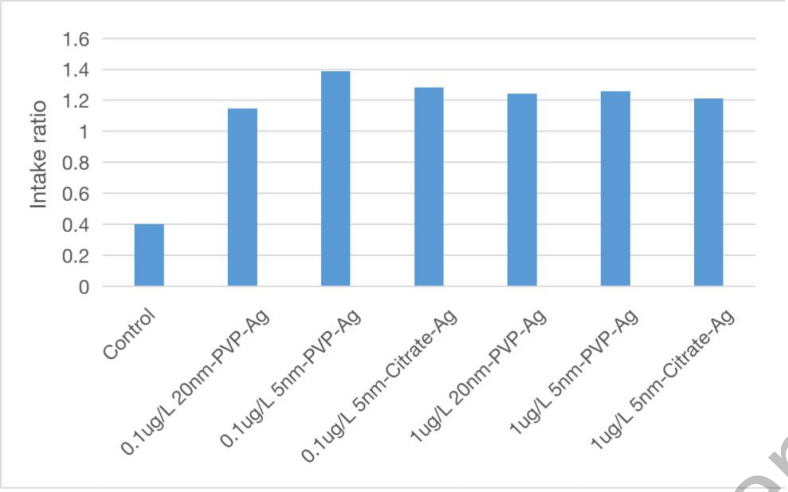

Fig.6 Intake ratio

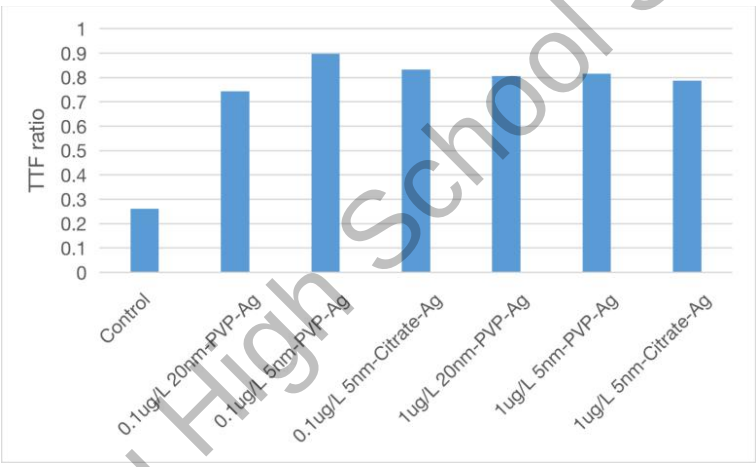

Fig.7 TTF ratio (Ag accumulation in *T. tetrahymena* / Ag accumulation in *E. coli*)

4. Conclusion

The experiment established a minor food chain from *E. coli* to *T. tetrahymena*. Nanoparticles with different characteristics were used to examine effects on Ag accumulation. It was found that sizes and coatings are significant factors for Ag accumulation. In addition, for different types of aquatic organisms, the impact of sizes and surface coatings vary greatly. The high intake ratio observed in this study and current researches on the food chain both indicate that nanoparticles can accumulate along the food chain and may amplify as the trophic level increases. Therefore, the bioamplification effect can happen to the food chain at large possibilities, which further reminds us that we should pay even more attention to nanoparticles' environmental and health risks to ensure safety for aquatic organisms and humans.

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