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Inhibitory effects of silver nanoparticles in two green algae, *Chlorella vulgaris* and *Dunaliella tertiolecta*

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ABSTRACT

Freshwater microalga *Chlorella vulgaris* and marine microalga *Dunaliella tertiolecta* were used to investigate toxic effects induced by 50 nm silver nanoparticles (AgNPs). To induce AgNPs effect, we exposed *Chlorella vulgaris* and *Dunaliella tertiolecta* for 24 h to 0–10 mg/L. We showed that growth media had different effects in AgNPs agglomerates' formation. Cellular viability, reactive oxygen species (ROS) formation and lipids peroxidation were employed to assess the toxic effects of AgNPs. AgNPs were able to interact directly with the *Chlorella vulgaris* cells surface and large aggregates were observed. AgNPs have a negative effect on *Chlorella vulgaris* and *Dunaliella tertiolecta*, as manifested by a strong decrease in chlorophyll content, viable algal cells, increased ROS formation and lipids peroxidation. The variability in sensitivity of both algae towards AgNPs was observed. We conclude that AgNPs have a negative effect on aquatic algae and these alterations might have serious consequences on structure and function of aquatic plant communities.

1. Introduction

Silver nanoparticles (AgNPs) are one of the most widely used nanomaterials in consumer products (www.nanotechproject.org). AgNPs are used in industrial products, for medical needs due to their antibacterial and antifungal activities and they are added as active compounds in detergent (Sambhy et al., 2006; Pal et al., 2007; Rai et al., 2009). AgNPs in water may have a high mobility and they can be easily transported to large aquatic environment (Blaser et al., 2008). However, their environmental impact on aquatic ecosystems is still unknown.

Algae species vary widely in their response to different toxic chemicals (Boyle, 1984). Park et al. (2010) reported that AgNPs have selective inhibitory effects on the harmful cyanobacterium *Microcystis aeruginosa* and this alga was more sensitive to AgNPs than green algae. Klaine et al. (2008) reported that major differences exist in the chemical behavior of nanoparticles in seawater compared to freshwater that will impact on the behavior of nanoparticles and therefore the habitats or organisms being exposed. Nanoparticles' toxicity could be due to algae cell-wall. Indeed, Perreault et al. (2011) compared aggregates' formation in the wild type *Chlamydomonas reinhardtii* to its cell wall-deficient mutant exposed 48 h to glycodendrimer-coated gold NPs. They observed that the wild type *Chlamydomonas reinhardtii* formed large aggregates while no aggregates were observed when the cell-wall was lacking. Furthermore, such aggregates' formation has been reported for other nanoparticles. It was reported that SiO_2 and TiO_2 NPs were able to interact directly with the algal cells' surface through adsorption to the cell walls (Van Hoecke et al., 2008; Sadiq et al., 2011). Aggregates' formation might reduce the light available to algal cells and thus inhibit their growth (Navarro et al., 2008; Perreault et al., 2011), or alter the cellular acquisition of essential nutrients by clogging to the walls (Wei et al., 2010).

Large surface area to mass ratio of small size AgNPs provides strong reactive interactions with cellular and intercellular compartments (Pal et al., 2007; Carlson et al., 2008). It is known for AgNPs to generate free radicals in microorganisms and deteriorate cellular functions (Kim et al., 2007). Oxidative stress is an important factor in nanoparticles-induced toxicity (Nel et al., 2006). Induction of oxidative stress by AgNPs was observed in different organisms (Kim et al., 2009; Ahamed et al., 2010; Choi et al., 2010) and the toxicity of AgNPs to the photosystem II quantum yield of a freshwater alga (*Chlamydomonas reinhardtii*) was demonstrated (Navarro et al., 2008). The latter authors suggested that toxicity of AgNPs to *Chlamydomonas reinhardtii* presents indirect evidence that toxicity of AgNPs is mediated by Ag^+. Therefore, better knowledge of AgNPs toxicity mechanism is required in order to evaluate the environmental risk of their toxicity and the possible change of ecosystem equilibrium.

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Toxicological studies have shown that nanoparticle size and aggregation play an important role in determining toxicity (Sager et al., 2007; Panessa-Warren et al., 2009). Aggregation of nanoparticles depends on particle concentration, pH, ionic strength, ionic composition, concentration and composition of natural organic matter, and other characteristics of the aqueous media (Keller et al., 2010). In the present study, a freshwater microalga *Chlorella vulgaris* and a marine microalga *Dunaliella tertiolecta* were used to investigate toxic effects induced by 50 nm AgNPs. Cellular viability, reactive oxygen species (ROS) formation and lipids peroxidation were employed to assess the toxic effects of AgNPs. The results from this study could facilitate a better understanding of the potential toxicity risks of AgNPs in aquatic environments and compare differential sensitivity among two green algae.

2. Materials and methods

2.1. Algal culture

The freshwater microalga *Chlorella vulgaris* and the marine green alga *Dunaliella tertiolecta* (CPCC-420) were obtained from the Canadian Phycological Culture Center (CPCC, University of Waterloo, Canada). The microalga *Chlorella vulgaris* was grown in sterile BG-11 liquid medium (Rippka et al., 1979) and *Dunaliella tertiolecta* (CPCC-420) was cultivated in seawater growth medium according to McLachlan (1960). The cells were grown under continuous and constant light intensity (100 μmol m^{-2} s^{-1}, SYLVANIA GRO-LUX Wide Spectrum light F40/GRQ/AQ/WS) at 24 °C. The stock culture was aerated with bubbling air. Aliquot of algal samples was used when cellular cultures were in their exponential growth phase. We note here that *Dunaliella tertiolecta* is a cell-wall lacking alga (Oliveira et al., 1980).

2.2. AgNPs characterization

Spherical silver nanopowder was purchased from MTI Corporation (Richmond, CA, USA). According to the manufacturer the diameter of AgNPs was 50 nm, purity was 99.9% and the specific surface area was 5–10 m^2/g. In this study, AgNPs size was evaluated by transmission electronic microscopy (TEM) and a suspension of 1 mg/L was prepared in: nanopure water (pH=6.8), BG-11 (pH=7) and McLachlan (1960) (pH=6) media. AgNPs particle distribution was determined by dynamic light scattering (DLS) with a ZetaPlus particle sizer (Brookhaven Instruments Corporation, USA) using 90Plus Particles Sizing Software Ver. 4.20. Three suspensions were prepared in: nanopure water, BG-11 and McLachlan (1960) media. A stock suspension of 100 mg/L was prepared and sonicated before use for 2 min with a sonicator. Zeta potential in the culture media was determined by the electrophoretic mobility method with the ZetaPlus system.To determine the solubility of the AgNPs, suspensions of 0–10 mg/L were prepared for 24 h and incubated in the condition as described above for algal culture. The suspensions were filtered on a 0.45 μM filter. 10% nitric acid was added for analysis by atomic emission spectroscopy using a Varian SpectrAA 220 FS system.

2.3. Algal exposure to AgNPs

Aliquots of 100 mL of the algal culture (1×10^6 cells/mL) in growing media (BG-11 and McLachlan (1960)) were exposed to 0, 0.01, 0.1, 1 and 10 mg/L of AgNPs and incubated in the condition as described above for 24 h.

2.4. Algal optical microphotographs

Morphological changes in algal culture exposed to 0, 0.1 and 10 mg/L of AgNPs for 24 h was determined using a Nikon Eclipse TS100 microscope and pictures were recorded with a Pixelink camera.

2.5. Determination of total chlorophyll

Total chlorophyll extraction was done in 100% of methanol at 65 °C and quantitative determination was done according to Lichtenthaler (1987).

2.6. Determination of viable cells

Fluorescein diacetate (FDA) is a non-polar ester that passes through cell membranes. Once inside the cell, FDA is hydrolyzed by esterase (an enzyme present in viable cells) to produce fluorescein, which accumulates inside viable cell walls and fluoresces under UV light (Regel et al., 2002). Viability of algal cells was estimated using the FDA method (Mayer et al., 1997). Each AgNPs' treatment and control was treated with 5 μM of FDA in 1 mL of solution. The fluorescence was measured using an excitation wavelength of 485 nm and an emission wavelength of 530 nm. All the fluorescence data were collected using a fluorescence plate reader.

2.7. Determination of reactive oxygen species (ROS) formation

ROS formation was measured using the cell permeable indicator 2′,7′-dichlorodihydro fluorescein diacetate (H_2DCFDA) (Gerber and Dubery, 2003). Cellular esterases hydrolyze the probe to the non-fluorescent 2′,7′-dichlorodihydrofluorescein (H_2DCF), which is better retained in the cells. In the presence of ROS and cellular peroxidases, H_2DCF is transformed to the highly fluorescent 2′,7′-dichlorofluorescein (DCF). Each AgNPs' treatment and control was treated with 5 μM of H_2DCFDA in 1 mL of solution. The DCF fluorescence was measured using an excitation wavelength of 485 nm and an emission wavelength of 530 nm. All the fluorescence data were collected using a fluorescence plate reader.

2.8. Determination of lipids peroxidation

Lipids peroxidation was measured using the cell permeable indicator C11-BODIPY$^{581/591}$. Each AgNPs' treatment and control was treated with 5 μM of C11-BODIPY$^{581/591}$ (Pap et al., 1999) in 1 mL of solution. The fluorescence was measured using an excitation wavelength of 485 nm and an emission wavelength of 530 nm. All the fluorescence data were collected using a fluorescence plate reader.

2.9. Data analysis and statistics

All treatments were done in triplicates. Means and standard deviations were calculated for each treatment. Significant differences between control samples and AgNPs exposed algal samples were determined by analysis of variance (ANOVA) and Tukey Honestly Significant Differences (HSD) test where p value less than 0.05 was considered to be significant.

3. Results

3.1. Characterisation of AgNPs

Transmission electronic microscopy (TEM) images confirm that AgNPs have a spherical size of 50 nm (Fig. 1). However, AgNPs agglomerates' formation was observed in all tested media.

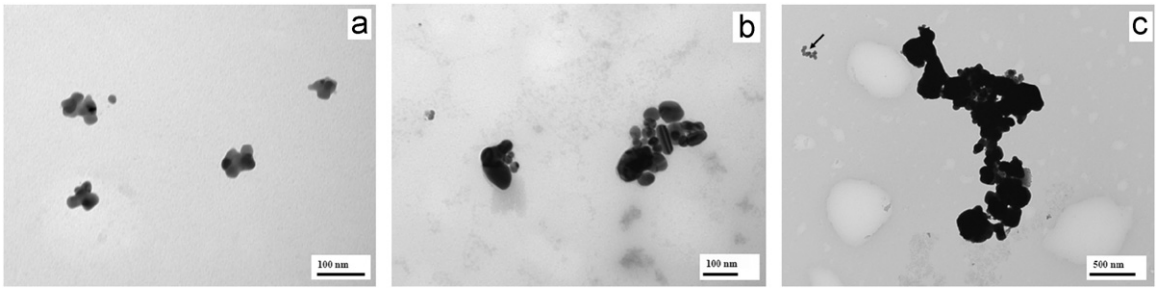

Fig. 1. Transmission electron microscopy (TEM) image of silver nanoparticles (1 mg/mL) in three suspensions media: nanopure water (a), BG-11 (b) and McLachlan (1960) (c). The arrow in (c) shows AgNPs of 50 nm.