Phytotoxicity of Silver Nitrate and Silver Nanoparticles on *Elodea canadensis*

Leonard Bernas

Faculty Advisors: Dr. Kurt Winkelmann, Department of Chemistry; Dr. Andrew Palmer, Department of Biological Sciences, Florida Institute of Technology

Abstract

This project demonstrates that *Elodea canadensis*, a common aquatic plant, can absorb silver cations and silver nanoparticles from its environment. *E. canadensis* was chosen for study because it is a model aquatic plant for measuring toxicity. Exposures of *E. canadensis* to concentrations of silver nitrate ≤ 100 mg/L and silver nanoparticles ≤ 30 mg/L were monitored for one week. Phytotoxicity was determined based on changes in photosynthetic pigment levels and silver uptake by the plant. Silver nanoparticles are toxic at concentrations greater than 20 mg/L while silver cations are toxic at concentrations of 30 mg/L. These results are significant in two ways. (1) *E. canadensis* can absorb silver cations and nanoparticles, suggesting their use for heavy metal decontamination of aquatic environments. (2) The sensitivity of *E. canadensis* to nanoparticles raises concerns about the safe and ethical use of nanoparticles.

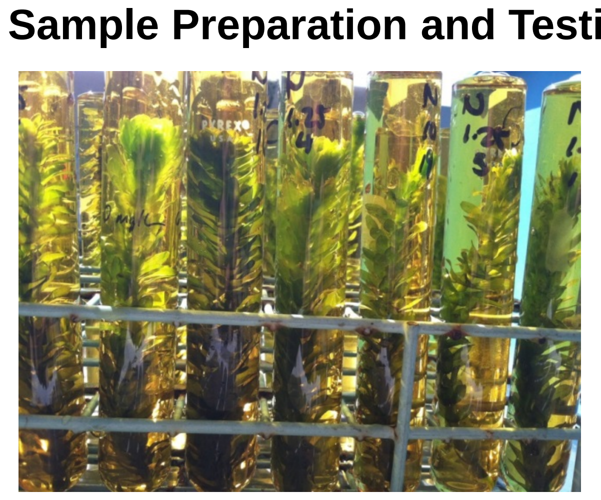
Introduction

Releasing silver into the environment threatens aquatic ecosystems. Silver nanoparticles are increasingly common in industrial processes and consumer products. Silver can enter waterways through product breakdown and waste disposal.

Silver cations enter a biological cell through sodium and copper channels and can accumulate in organisms through membrane ion transporters.[1] Silver nanoparticles can severely impact aquatic plants because they can enter the cell via endocytosis, causing invagination of the plasma membrane and forming vesicles that transport particles throughout the cell. Silver nanoparticles can release silver cations into the cell, causing additional damage. Silver causes oxidative stress on proteins and enzymes.[2]

Elodea canadensis is a model aquatic organism and has uses in bioremediation for copper. If introduced to areas with critical levels of heavy metals, aquatic plants can be used for detoxification.[3] Furthermore, naturally occurring humic acids can reduce silver cations and form a stabilizing coating around silver nanoparticles in aquatic systems.[4] Understanding toxicity of these particles on plants can provide insight into the environmental consequences of silver bioaccumulation.

Sample Preparation and Testing



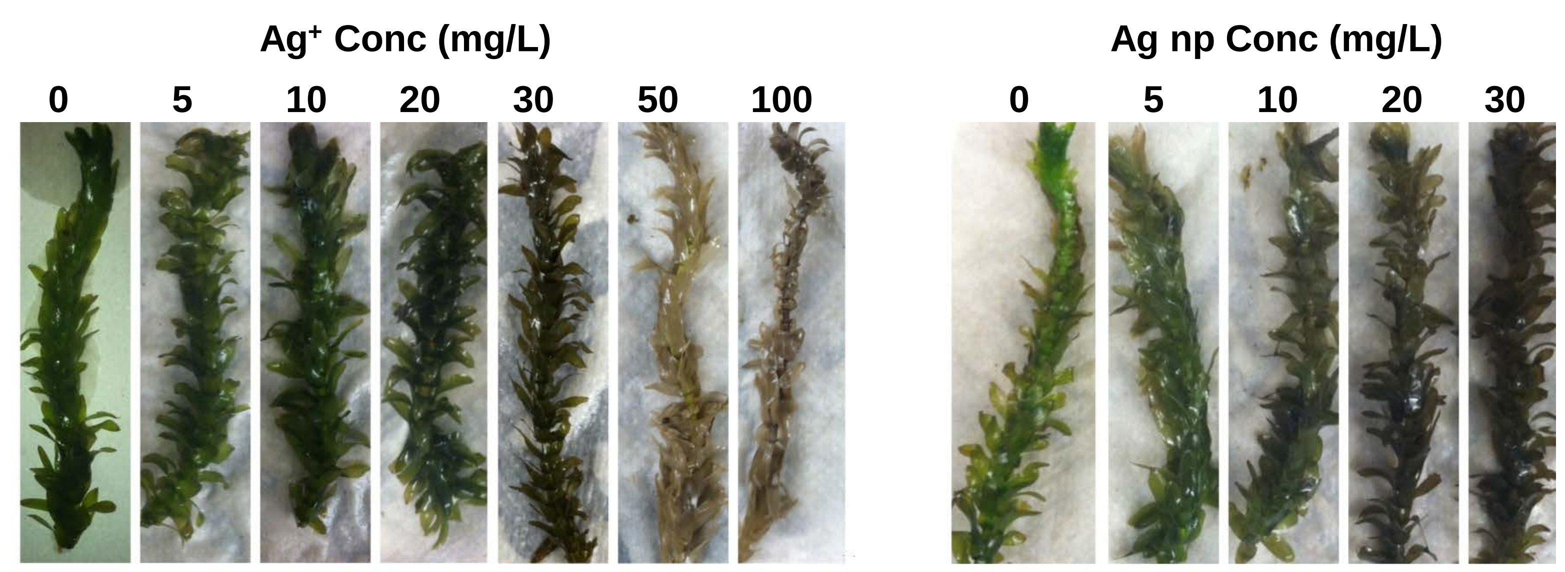
- React $AgNO_3$ with Sewanee River Humic Acid Standard II at 90 °C for 1 hr to form Ag nanoparticles.[4]
- Acclimate *E. canadensis* in R/O water.
- Place 12 cm stalks in 50 mL test tubes.
- Add moderately hard synthetic freshwater containing Ag^+ or Ag nanoparticles.
- Prepare blank and control samples.
- Maintain samples in the Florida Tech greenhouse for 1 week.[5]

Phytotoxicity Testing

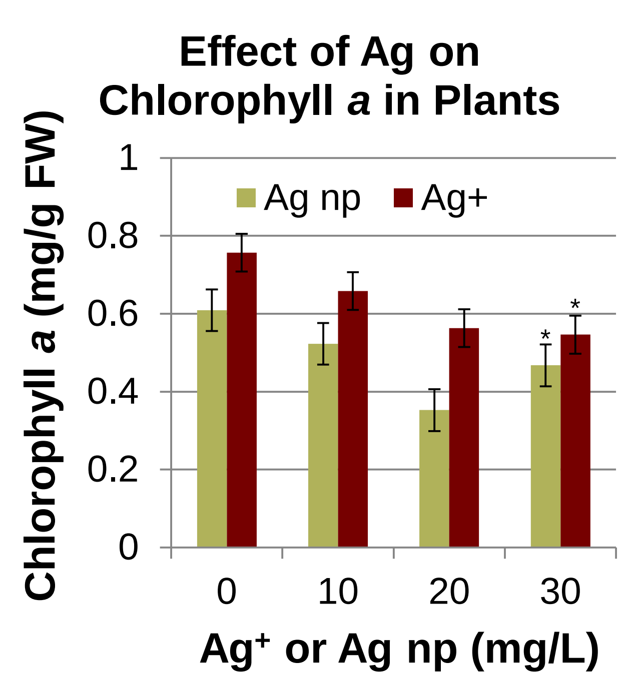
- Visually assess the condition of plants during and after the plants' exposure to silver solutions.
- Determine concentrations of carotenoid, chlorophyll *a*, and chlorophyll *b* by submerged leaves in 80% acetone for 24 h.[6]
- Measure chlorophyll and carotenoid pigment absorbances by UV-Vis spectroscopy.
- Determine pigment concentrations using Lichtenthaler coefficients.[7]
- Measure silver bioaccumulation with flame atomic absorption. AA samples were first washed then dissolved in nitric acid.

Results and Discussion

Visual analysis shows a significant effect on the plants after one week of exposure to the silver solutions. Higher concentrations of $AgNO_3$ had a more pronounced effect on the plants for all measurements. Nanoparticle samples were limited to 30 mg/L.



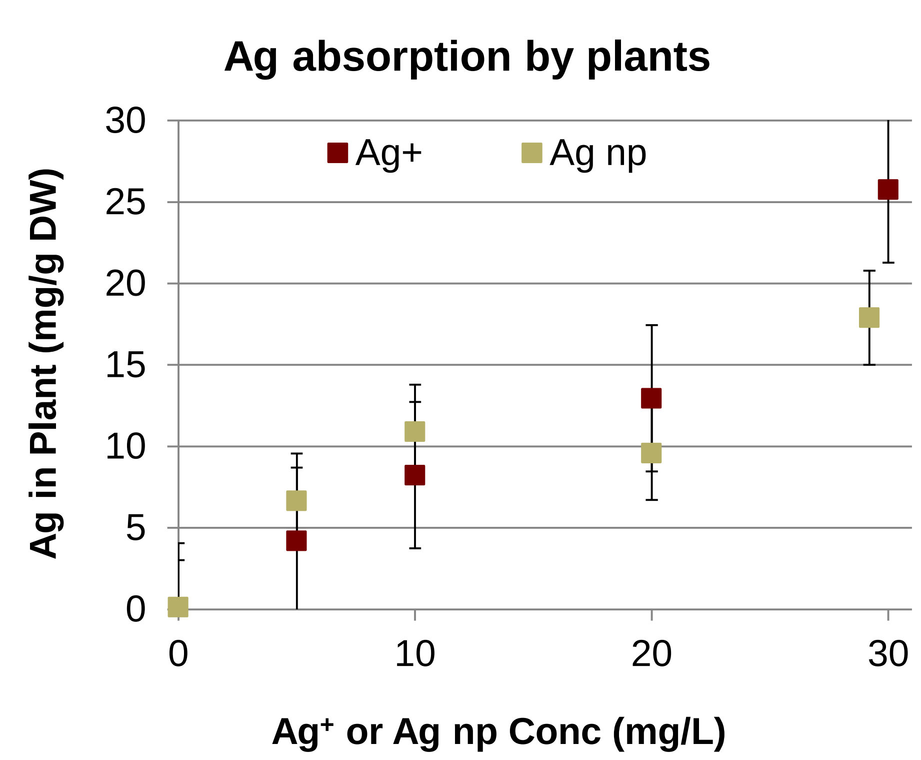
Higher concentrations of silver nanoparticles and $AgNO_3$ caused a small to moderate decline in most pigment concentrations in plants.



Decline in Pigment Conc Compared to Controls ($p<0.05$)

Conc (mg/L)	Chl a	Chl b	Ttl Chl	Car	Chl a/b ratio
5					Ag^+, np
10					Ag^+, np
20	Ag^+, np		Ag^+, np	np	np
30	Ag^+, np			Ag^+	Ag^+, np

Although nanoparticles had a greater effect on pigment loss, this is to be expected given that each nanoparticle can release many cations when it is oxidized in the cell. With this in mind, the effect of the nanoparticles is surprisingly low, perhaps due to the stability provided by their humic acid coating.



Plants absorbed amounts of silver from both the nanoparticle and $AgNO_3$ solutions. Despite the large number of silver atoms present in a nanoparticle, the amount of silver absorbed by plants from both types of solution is similar.

The trend of higher silver content in plants was also observed for $AgNO_3$ concentrations of 50 and 100 mg/L. Similar tests were not performed for nanoparticles because they are unstable at those concentrations.

Summary and Conclusions

Silver cations and silver nanoparticles have an obvious impact on the photosynthetic processes of *E. canadensis*. Visual analysis showed that the plant turns brown and wilted after a week of exposure to silver nanoparticles at concentrations ≥ 10 mg/L and at concentrations of ≥ 20 mg/L for silver cations.

Analysis of the pigment concentrations in plant leaves showed statistically significant ($p < 0.05$) decreases in the amount of chlorophyll *a* in plants exposed to higher concentrations of silver nanoparticles and $AgNO_3$ Changes in chlorophyll *b* and carotenoid concentrations showed smaller or inconsistent changes. Although changes in chlorophyll *a* and *b* concentrations were not always significant, the ratio of the two absorbances did change for plants at all silver nanoparticle and $AgNO_3$ concentrations. This ratio is commonly reported for toxicological studies of plants.

Pigment levels were consistently lower for plants exposed to nanoparticles compared to plants exposed to $AgNO_3$. Since each nanoparticle contains thousands of silver atoms, it is surprising that the nanoparticles did not have a greater effect on the plants. This suggests that the humic acid formed a protective coating around the nanoparticles which prevented them from reacting with the cells.

From the literature, it is known that silver nanoparticles are more toxic to plants than animals, although the destruction of plants can then impact the animals' food sources. Silver ions are more toxic to animals.[1] The lower-than expected impact of silver nanoparticles to cations found here is consistent with the idea that these silver species undergo different mechanisms of absorption and cellular reactions. Finally, these results show that *E. canadensis* absorbs silver and suggests that it can be used for bioremediation of contaminated areas with significant damage to the plant only at higher concentrations of silver.[3]

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Acknowledgements

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