I, Nicole A Hanks, hereby submit this original work as part of the requirements for the degree of Doctor of Philosophy in Chemistry.

It is entitled:
Silver Nanoparticle and Silver Ion Water Contamination: Assessment of phytoremediation and point-of-use filtration media

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Silver Nanoparticle and Silver Ion Water Contamination: Assessment of phytoremediation and point-of-use filtration media

A dissertation submitted to the graduate school of the University of Cincinnati in partial fulfillment of the requirements for the degree of Doctor of Philosophy

In the Department of Chemistry of the College of Arts and Sciences

By

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Abstract

Silver ion and silver nanoparticle water contamination has become a growing threat to fresh water sources. In the past, silver was not considered a hazard as it was naturally removed from the environmental waters through precipitation. Therefore, silver did not remain suspended in the water and did not pose a threat to human consumption. Now, the silver concentration is increasing the presence of silver in environmental waters as the use of silver in consumer products continues to climb. This growing concern to find effective removal techniques for silver contaminates in drinking water has promoted this research to analyze the effectiveness of phytoremediation and point-of-use water filtration media.

Phytoremediation, which utilizes plants to remove contaminants from a water source, has gained popularity in regions of the world where drinking water facilities are not available to provide safe drinking water to the community. *Pistia stratiotes* is a common floating water plant used for phytoremediation of drinking water and is currently implemented around the world. In order to study the phytoremediation abilities of silver ions and silver nanoparticles with *P. stratiotes*, each silver form was tested at varying concentrations within the water of the plant. The plant’s water and biomass were analyzed to identify removal capabilities of silver forms and the location in which the plant isolates silver in the biomass.

In addition to comparing *P. stratiotes* for silver removal capabilities, plants were also analyzed when silver nanoparticles were in the presence of environmental cations, concentrating on strontium. The strontium ion’s divalent cationic charge interacts with the electrons around the nanoparticle surface, inducing agglomeration. These agglomerates were introduced to *P. stratiotes* without reduction of accumulation potential by the plants. Furthermore, strontium and calcium were found to quench silver nanoparticle degradation of hydrogen peroxide and reduced
silver nanoparticle mediated production of reactive oxygen species. However, silver nanoparticles were found to exacerbate sodium-mediated production of reactive oxygen species within *P. stratiotes* leaves.

Finally, silver forms were tested on point-of-use filtration media. In the United States, the last defense from water contaminants before direct ingestion by an individual relies on in-line filtration units and after faucet filtration units. By using activated carbon, silica, ceramic spheres and KDF-55, the removal of silver ion and silver nanoparticle contamination in water was monitored and then rinsed with doubly deionized water to observe continual elution of silver from the filtration bed. The removal capabilities of each media were reliant on chemical reactivity and filtration bed size. The mechanical removal of silver forms was not effective in removing higher concentrations of the silver contaminants.
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Chapter One | Silver forms and phytoremediation of contaminated waters

1.1 Silver

Silver has been used since ancient times for its antibacterial capabilities. The Romans would line the bottom of their wells with silver to reduce bacterial and microbial growth within their water (Emsley, 2011). Silver is one of the most toxic metals for smaller organisms and when exposed to microorganisms, such as bacteria and viruses, the silver attaches to the sulfur bonds on their enzymes causing the essential components of cellular activity to denature, leading to death of the microorganism. For larger organisms like humans, this metal has not been considered a major threat. The hydrochloric acid in the stomach stops about 90% of the ingested silver from being absorbed by the body (Emsley, 2011). It precipitates out the silver into an insoluble form, thereby decreasing harm to the body.

1.1.1 Nanoparticles

With nanotechnologies becoming more prevalent in modern society, silver toxicity has become more of a concern. 50% of all produced nanoparticle containing consumer products use silver as the major nanoparticle composite (The Project on Emerging Nanotechnologies, 2013). Nanoparticles are the transitional region between individual molecules or atoms and its bulk material counterpart. These particles range from 1 to 100 nm in size allowing for variation of their physical and chemical properties. When materials approach the nanoscale, the percentage of atoms/molecules on the particle’s surface is greater per weight than of larger particles. This causes the nanoparticles to be more reactive than their bulk or ion counter parts. The high surface area is crucial for better catalytic and electrolytic uses, thus improving the material’s performance in technology as batteries and sensors. It can also lead to increased chemical
strength, optical properties, and intensified chemical or heat resistance.

1.1.2 Nanoparticle Applications and Optical Properties

Nanoparticles can be made from a variety of materials (i.e. metals, inorganic and organic compounds, and proteins). However, the most widely applied are silver nanoparticles (AgNPs). AgNPs are known for their antibacterial and antimicrobial properties, these nanoparticles are utilized in consumer products and medical applications to take advantage of these properties. Nanoparticles are in fabrics, bandages, medications and as coatings for appliances to reduce potential infection by providing protection against bacteria (Panacek, et al., 2006). In addition to their sanitizing abilities, AgNPs have unique optical, electrical, and thermal properties. AgNPs are used in conductive inks and paints due to their high electrical conductivity and stability. By understanding how changing the nanoparticle size, shape, and surface chemistry, these particles can be optimized for performance and application capabilities.

As mentioned prior, nanoparticles have a useful optical property that allows them to be used as sensors. AgNPs have an absorbance peak around 400 nm, which is not observed in their ion counterpart. This peak occurs due to surface plasmon resonance. When nanoparticles are exposed to radiation (i.e. light), the collective oscillation of conductive electrons around the particle, known as the surface plasmon, absorb specific wavelengths of light (Figure 1.1). This is known as the surface plasmon band. The absorption of light is what gives a colloidal solution color. This phenomenon shows that only the electrons on the surface are affected, and the size and shape of the nanoparticle altered the color and intensity of the absorbance. A smaller particle requires a large amount of energy to excite the surface plasmon electrons. The use of plasmon resonance for AgNP solutions is further explored in chapters 3-5.
1.1.3 Nanoparticle coatings and alterations

Nanoparticles may undergo size and shape changes due to their environmental interactions. By destabilizing the ions between particles, these nanoparticles may form larger particles or completely destabilize the nanoparticle to the ion form. Agglomerations occur when nanoparticles gather to form clusters of the primary particles while retaining their crystalline structures and shapes. Aggregates are larger mass particles formed by merging without retaining the initial particle shape or structure.

It is common practice to add protective agents to prevent nanoparticle agglomeration. These coatings, known as capping agents, can be made of inorganics (i.e. metal oxide, alloys), organic molecules (i.e. hydrocarbonate, starch, citrate), polymers (i.e. polyvinylpyrrolidone, polyvinyl alcohol), and even biological compounds (i.e. proteins, polysaccharides). These coatings may change the nanoparticle's physically (i.e. charges, magnetism, hydrophobicity), optically (i.e. fluorescence, absorbance), and even how they interact with their environment (i.e. biocompatibility, reactivity, stability). This research focuses on uncoated AgNPs. The capping
of nonionic polymers allow for the repulsive electrostatic forces between the polymer chains to prevent nanoparticles aggregation. Both the polymer and surfactant also give some resistance to oxidation and aggregation of the nanoparticle by their environment. Therefore, these nanoparticles remain soluble and stabilized in conditions that would cause their ion counterparts to experience precipitation causing more interactions between the particles and organisms possible.

1.2 Current water treatment processes

Nanoparticles are now making their appearance in waterways, threatening the purity of drinking water sources and increasing the potential toxic exposure to humans. The need for clean drinking water has spurred treatment and regulation of public water around the world. Organizations such as the World Health Organization (WHO) and the United States Environmental Protection Agency (EPA) have placed maximum contamination limits (MCL) on various heavy metals present in public drinking water to help reduce human exposure to large quantities of toxic metals (Abbott Chalew, et al., 2013). Initialization, consumer products, and public waste increase the heavy metals now found in the environment and fresh water sources (Eastman Kodak Company, 2003). These metal contaminants have propelled studies to predict and counter their environmental transport, as well as their ultimate impact to any ecosystem, out of concern for the quality of the available water supply.

To reduce contamination of our water sources, water treatment facilities work to prevent high concentrations of heavy metals and nanoparticles from entering the environment. A typical water treatment facility utilizes coagulation, sedimentation, filtration, and disinfection to treat drinking water for distribution (Figure 1.2). When the water enters the facility, it is introduced to a process called coagulation where chemicals, such as alum, are added to the water in order to
destabilize dissolved particles (Henry, et al., 2009). The destabilized molecules aggregate and agglomerate into larger particles, which are removed by gravity in the sedimentation chambers. After the larger particles are removed from the water, smaller particles may remain. These smaller particles, such as nanoparticles, pass through to the filtration chamber where low-pressure membrane filtration relies on sieving to physically remove the particulate contaminants (Abbott Chalew, et al., 2013).

Figure 1.2. An illustration of the typical process of water treatment for drinking water distribution in the United States.

In today's conventional treatment of drinking water in the United States, there are large quantities of nanoparticles still present after treatment. For more stable nanoparticles such as zinc oxide, 45-99% of nanoparticles still make it past the chemical treatments (Li, et al., 2013). For less stable nanoparticles like silver, 2-20% of the AgNPs breakthrough; their breakthrough increases to 1-45% when only microfiltration is employed, which is common in smaller treatment plants (Li, et al., 2013) (Panacek, et al., 2006). The use of ultrafiltration and nanofiltration are able to keep out the smaller nanoparticles, of sizes 0.1 to 100nm, but these
filters are expensive causing them often not to be utilized in the drinking water filtration process (Li, et al., 2013).

For many countries around the world, these large-scale filtration systems are too costly and are not employed. If developing countries employ filtration systems, they are portable or personal filtration systems like activated carbon columns that require suction or gravity to filter. However, many times even these portable systems are too costly to employ and replace, causing most people to drink polluted water. Due to the high cost to develop and employ, many communities and corporations are utilizing plants as a cheap and natural way to clean their water by phytoremediation.

1.2.1 Point-of-use filtration media

Point-of-use filtration methods are located at the tap where water is dispensed. These systems utilize various filtration media to remove organic and inorganic contaminants from the faucet or tap. Most point-of-use filtration methods utilize chemically active media to remove unwanted organic and inorganic contaminants, which affect the taste or smell of the water. Chemically active filtration media are activated carbon, kinetic degradation fluxion (KDF), and ionic resins to promote reduction-oxidation reactions with water contaminants (Sutherland, 2008). Mechanically driven water filters such as ceramic balls, silica gel, and granular sand or rock allow small particles and water to pass through the media while large particles are trapped or blocked from continuing through the filter. Some of these chemical and mechanical filtration media allow adsorption to take place on the surface of the media. Adsorption is the process by which a molecule or atom binds to the surface of a solid. This varies from absorption, which permeates the solid by holding both the molecules and the water within its mass.
1.2.2 Phytoremediation

Phytoremediation is an in situ process utilizing plants to remove contaminants from a waste site through a variety of processes. Using plants for waste treatment is regarded as an economically favorable and environmentally friendly method in comparison to current forms of water purification and remediation. While phytoremediation for treatment of soil is a time consuming process in comparison to some ex situ processes, using plants for water remediation is a quick and effective method in extracting a variety of contaminants in a short amount of time called a hydroponic system. Previous studies have focused on the natural ability of top-floating aquatic plants, which accumulate an abundance of contaminants in their large root systems, while maintaining normal growth, for effective wastewater cleanup called hydroponic system treatment (Odjegba, et al., 2004). Included in the cited references are some reviews on the incorporation of plants to remove heavy metals from contaminated sources (Odjegba, et al., 2004) (McCutcheon, et al., 2003) (Kadukova, et al., 2010).

By understanding the process by which plants extract, accumulate, and modify contaminants will help to successfully apply plants for effective phytoremediation. Figure 1.3 illustrates the basic potential metabolic pathways within a plant during remediation of contaminated waters. When a plant participates in phytoremediation the plant can cleanup pollutants by extracting contaminants with their roots from the environment. The roots can either convert the pollutants to a less harmful form or translocate them to the leaves for conversion. Once in the leaves, the plant is able to convert select types of contaminants (i.e. organics) into vapors, which are released into the air. Another process that the plant uses to decrease contamination, but still remediate the water source is rhizosphere degradation. Rhizosphere degradation takes place when contaminants stick to the roots of the organism and microbes or
bacteria breakdown the adsorbed contaminants to a less harmful chemical form.

**Figure 1.3.** Diagram of a plant’s metabolic pathways of phytoremediation.

Currently, research shows phytoremediation breaks down organic contaminants in the soil and water, while inorganics are unable to be degraded. For the most part, inorganic contaminants occur naturally from erosion of the earth; these pollutants increase in the environment due to mining, industrial and day-to-day activities of the human population (Koontz, et al., 1980) (McCutcheon, et al., 2003) (Kadukova, et al., 2010). Despite their inability to degrade, inorganics are stabilized or sequestered in the plant tissue to reduce internal harm. Inorganic materials are broken down for macronutrients, such as nitrates and phosphates (Kadukova, et al., 2010), and trace elements required for cellular activity, such as Cu, Fe, Mo and Zn (Prasad, 2004). For those nonessential elements such as radioactive contaminants like $^{90}$Sr or precious metals like Ag and Au, they are still removed from the environment by the plant. However, the plant works to immobilize these toxic metals within its biomass to decrease further cellular damage promoted by circulation within the plant.
While plants provide promising remediation abilities for soil and water, there are still major limitations to the effectiveness of this process. Currently, there is not an established understanding on whether contaminants that are collected in the roots or leaves will reenter the environment when the plant waste is combusted or composted. In addition, phytoremediation is dependent on the plant’s health and the contamination concentration. Higher concentrations of contaminants interrupt the plant’s ability to maintain metabolic processes and may ultimately lead to the plant’s death.

1.2.3 Uptake of Heavy Metals in Plants

When metals are near plants, they either attach to the surface of the roots or are absorbed into the plant through natural nano- or micro-meter scale uptake pathways within the epidermis. Upon uptake into the plant's roots, these nanoparticles and ions can pass through three pathways: transmembrane, symplast, and apoplast (Figure 1.4). The transmembrane and symplastic pathways are known as cell-to-cell selective uptake mechanisms that move nutrients either through the cell walls via ion specific uptake channels or around the cellular membranes and through the plasmodesmata channels between cells, respectively (Meyer, et al., 2004). Passing through a plant using apoplastic pathways is non-selective, unlike the previous two uptake pathways. By using apoplastic pathways, nutrients and other materials pass between cells in a water-filled channel to the xylem for further transport through the plant. These water filled channels vary in diameter from 5 to 30 nanometers (Meyer, et al., 2004).
Once the metal passes through the epidermis, it proceeds to the cortex cell layer of the roots. This layer becomes more selective in allowing metals to pass through to the core of the plant. While the apoplastic pathways are still available for transport, these paths between the cells are less than 10 nanometers in size and can easily clog. Those metal particles or compounds, which are too big to pass through the smaller channels, will not proceed further through the plant.

The metals that make their way through the cortex are reliant only on selective means of transport through the endodermal layer of cells. These cells do not allow for the apoplastic flow of water between the cells, as the casparian bands block the pathway. Casparian bands are thickened areas of the cell wall that prevent larger particles to be pulled directly to the center of the plant. Therefore, any metal or compound that is able to proceed through the endodermal layer must interact with the cells to enter the xylem for circulation through the plant.

Within the plant, inorganic compounds are removed from the ion uptake pathways by extraction via membrane transporter proteins. The transport of inorganics can be a normal
process for nutrients such as Cu, Mg, and Zn. These pathways can also transport contaminants, which are chemically similar to their nutrients, allowing them to be taken into the plant accidentally as strontium by calcium transporters (Comar, et al., 1957) and silver by sulfur containing transporters (Koontz, et al., 1980). Inorganics usually exist in the water as ions and are unable to pass through the cellular membranes without the aid of membrane transporter proteins. As there are specific protein transporters to each metal, the uptake of inorganics depend on a specific number of membrane proteins in a controlled manner following Michaelis Menten kinetics (Prasad, 2004). When the cells accumulate inorganic contaminants, they often cause toxicity through damage to the cell structure by oxidative stress due to redox activity as well as replacing other essential nutrients within the cells (Prasad, 2004).

1.2.4 Silver ion and silver nanoparticle interactions within plants

Silver’s presence in a plant can be significant. Silver has a high affinity for amino, imino and sulfhydryl groups within the plant causing binding or complexing of silver with cellular enzymes (Koontz, et al., 1980). This inhibition of enzymes alters the cellular permeability. Ionic silver has a strong electronegativity that allows it to displace other cations from sites on the cell wall, membrane, and even alter DNA molecules. However, plants work to reduce these toxic interactions by sequestering silver into localized areas within the plant’s biomass (Koontz, et al., 1980). At lower concentrations, this approach can help the plant maintain normal biological functions. At higher concentrations, the silver tends to inhibit root uptake of nutrients as well as encroach on metabolic processes (Odjegba, et al., 2004). Even when silver is no longer prevalent for uptake, it is still able to translocate through the plant as a small percentage if the silver is still mobile within the plant (Koontz, et al., 1980).

To use plants for remediation of water sources, there are other interactions that take place
to change the overall nanoparticle formation. Nanoparticle toxicity within plants depends on its size, catalytic activities with the plant surface and affinity-based interactions (Dietz, et al., 2007). Nanoparticles travel through natural openings within the plant during uptake. However, once inside the plant, the larger particles have more difficulty moving through the cell walls undamaged. These particles can clog cellular and apoplastic uptake pathways. Particles less than five nanometers are better able to translocation through the plant's cellular pathways and penetrate further into the plant's biomass (Dietz, et al., 2007).

By interacting with the plant’s cellular processes, the nanoparticle often causes redox changes within the plant due to metal-exposure and oxidative stress (Dietz, et al., 2007). Many metal nanoparticles such as silver, gold and platinum catalyze redox reactions within the plant. However, these effects are dependent on the concentrations introduced into the cells of the plant. AgNPs tend to dissolve slowly and attach to the plant's surface. Thus, major toxicity takes place when large concentrations of these nanoparticles or their ion counterparts are present in a localized area (Dietz, et al., 2007). This means that for toxicity to occur it is not always essential for internalization of nanoparticles, but can also take place due to interactions on the surface of the cells. The degradation or dissolving of nanoparticles may occur under oxidative conditions that are further affected by pH, surface coatings, and temperature (Cong, et al., 2011).

1.2.5 *Pistia stratiotes*

As biological processes in plants are solar-driven, phytoremediation is tenfold cheaper than engineering-based methods such as pump-and-treat systems (McCutcheon, et al., 2003). While economically more ideal, an efficient plant for phytoremediation is required to have favorable properties for successful results. These ideal plants must have fast growth and reproduction capabilities, high biomass, and must be competitive, hardy and tolerant of organic
and inorganic pollutants. More specifically for water treatment, these plants need to have large, dense root systems for increased depth into the water system and to promote microbial growth on the root surface (Prasad, 2004). Current plants used for phytoremediation are *Eichhornia crassipes* (water hyacinth), *Lemnoideae* (duckweed), and *Pistia stratiotes* (water lettuce) (Gupta, *et al*., 2012). For the research in this dissertation, *P. stratiotes* was chosen and used in experiments in chapters 3 and 4.

*P. stratiotes* is a floating freshwater plant belonging to the Araceae family (Figure 1.5). These plants float on the surface of the water with roots fully submerged under the floating leaves. The leaves are able to grow up to 14 cm long and are covered in short hairs which allow the plant to trap air bubbles to increase the plant’s buoyancy (Odjegba, *et al*., 2004). This plant utilizes sexual reproduction via pollination of its dioecious flowers or vegetatively (Muniappan, *et al*., 2009). *P. stratiotes* is not a winter-hardy plant and has a minimum growth temperature at 15°C, which limits its invasive spread to regions that experience winter (Muniappan, *et al*., 2009). For warmer regions, this plant spreads rapidly. Documentation shows *P. stratiotes* as a medicine and fodder for cattle in Africa since 77 A.D. and found its way to Australia and New Zealand in the 1970s (Muniappan, *et al*., 2009).
These plants were chosen for this research for their fast growth rate, metal accumulation, and presence across the globe. *P. stratiotes* requires solar radiation to thrive allowing it to be an ideal plant for use in tropical or subtropical areas, where it is currently used in phytoremediation water systems (Odjegba, *et al.*, 2004). Compared to native plants in these regions, this invasive plant has a higher nutrient uptake capacity and high nutrient removal efficiency. *P. stratiotes* has a fast growth rate that allows it to double in biomass in just five days (Odjegba, *et al.*, 2004). A phytoremediation pond requires careful management with periodic harvesting to maintain a healthy biomass and to ensure contaminants are not reintroduced into the pond (Odjegba, *et al.*, 2004). Further study using *P. stratiotes* for phytoremediation may be found in chapters 3 and 4.

1.3 Research Objectives

Due to their vast use in consumer products, nanoparticles and their ion counterparts are continually increasing in concentration in environmental and public water sources. The research for water treatment facilities’ abilities to remove these contaminants is gaining momentum; however, phytoremediation techniques do not have the same support. It is pertinent to research the capabilities of these nanoparticles and ions to identify if there are any variations in their
capabilities in removing both forms from their water sources.

While low concentrations are naturally found in the environment (5 ng L$^{-1}$ in fresh water), larger concentrations are becoming more prevalent. Silver contamination in the air, soil, and water are becoming more prominent due to the use and disposal of silver containing industrial and consumer products. Although silver is one of the most toxic heavy metals, it is not normally considered a major hazard due to solubility issues. However, with the mass production of AgNPs, soluble forms of silver are becoming more widespread and are now a liability.

*P. stratiotes* was assessed for its phytoremediation abilities for silver contaminates as it is widely utilized for phytoremediation and is not well researched for such interactions. This dissertation concentrates on the interactions between AgNP and ion forms interacting with *P. stratiotes* to identify if the form, concentration and antibacterial properties of these compounds vary the plant’s water remediation abilities. The specific objectives of this dissertation are stated below:

- Evaluate the effectiveness of *P. stratiotes* in removing AgNPs and silver ions from a contaminated water source.
- Assess the accumulation capabilities of *P. stratiotes* and its isolation of silver within the plant’s biomass.
- Identify how natural strontium ions interact with AgNPs.
- Analyze variation in the production of reactive oxygen species by the presence of AgNPs and silver ions with environmental ions such as strontium, calcium and sodium.
- Assess the removal capabilities of silver forms on various types of point-of-use filtration media and retention of contaminant forms.
1.3 References


Chapter Two | Instrumentation of phytoremediation and nanoparticle analysis

2.1 Mass spectrometric techniques

For many real world samples, the determination of trace analytes can be challenging and is often a problem faced by analytical chemists. To solve this problem, chemists use mass spectrometric instruments for quantification of a sample’s contents. These instruments are used to measure select analytes by ionization of molecules or ions within the sample. This allows for determining the sample’s elemental or molecular composition. This research focuses on the use of elemental mass spectrometry for quantitation of total present metals within biological samples.

2.1.1 Inductively coupled plasma mass spectrometry

The inductively coupled plasma mass spectrometry (ICP-MS), an elemental-specific detector, utilizes quadrupole technology to quantitatively monitor multiple elements in the periodic table simultaneously. As a relatively new technique in elemental analysis, the first journal article was published in 1980 and within three years, the technique matured rapidly for commercial instruments due to its quick development in both the industrial and academic fields (Houk, et al., 1980). The ICP-MS is capable of detection limits of the sub to low parts per trillion levels with a power range up to nine orders of magnitude. More information using ICP-MS for experimentation is found in chapters 3, 4, and 5.

Currently, ICP-MS is used in many applications and fields of study. Some of these applications include the environmental, medical and forensic fields, specifically for toxicology purposes. Clinical laboratories employ ICP-MS for quantification of heavy metals in blood, urine, serum, as well as plasma to identify any metabolic concerns or heavy metal poisoning. Most of the research in this dissertation uses ICP-MS in monitoring a variety of elements in
varying environmental matrices for the remediation of contaminated water sources. More information on the applications of ICP-MS may be found in the cited references (Houk, et al., 1980) (2004) (2010).

2.1.1.1 Instrument setup and theory

ICP-MS is an effective technique for elemental quantification. A diagram of the instrumentation for this analysis is presented in Figure 2.1. A liquid sample is passed through a nebulizer by a peristaltic pump or an additional interface such as liquid chromatography. The nebulizer is comprised of an internal capillary allowing for liquid flow and surrounded by a high-velocity stream of argon gas. The argon gas stream forces the solvent out of the nebulizer into aerosol droplets, which pass into a cooled spray chamber and are separated by size. The larger droplets of solvent are condensed and discarded as waste. The smaller droplets, between 5 and 10 microns, are desolvated to particles (in 1.0 to 1.5 milliseconds), vaporized (less than 150 microseconds), followed by atomization and ionization of elements to form positively charged ions (on the order of microseconds) in the argon plasma. The argon plasma temperature ranges from 6000K to 10000K depending on the monitored plasma region.

Figure 2.1. An illustration depicting the general schematic of an ICP-MS instrument.
After the plasma, the formed ions pass through the sampling and skimmer cones. This interface is the pressure transition from atmospheric to vacuum by a turbomolecular pump. This region uses cones to force the ion flow to the cone diameter of 0.4 to 1.0 millimeters by influencing electrodynamic forces between ions, known as the Debye length, normally $10^{-4}$ to $10^{-3}$ millimeters. The ions are then focused by the extraction lenses into the octopole ion guide. The octopole is a collision/reaction cell used for interference removal; further description is in section 2.1.1.2.

The ions possessing sufficient kinetic energy to pass through the octopole move to the quadrupole for selective mass filtering. This is accomplished by utilizing a direct current on one pair of rods and a radio frequency on the opposite pair of rods. This results in an electrical field that allows only the selected ions of interest that possess a stable trajectory to reach the detector. A mass range of 2 to 260 Daltons are allowed under general parameters of scanning with sampling times for a selected mass to be set at 0.1 seconds. The selected ions, filtered through this process, are detected by the electron multiplier.

2.1.1.2 **Kinetic energy discrimination of interferences**

What makes ICP-MS a competitive instrument in the analytical field is its low detection limits and discrimination of polyatomic ions from a singular ion. The quadrupole and octopole mass filters are what cause this instrument to be effective and versatile. A general comparison of the triple quadrupole mass filter versus the commonly used octopole and quadrupole mass filter setup is shown in Figure 2.2. After the selected mass parent ions pass through the first quadrupole, of the triple quadrupole mass filter setup, they then travel into the octopole where an inert or reactive gas causes the dissociation of the parent to form the daughter ions or cause a reaction to form daughter ions. This quadrupole-octopole-quadrupole setup provides additional
separation abilities to discriminate between the isobaric and polyatomic interferences.

**Figure 2.2.** Illustration of the triple quadrupole and common mass spectrometry setup for ICP-MS.

It is important during method development to select an appropriate cell gas as there are a number of factors that are dependent upon the choice. These factors include sample matrix, elution solvent, isotopes of interest, and formation of isobaric adducts. Common interferences caused by adduct formations within the desired analytes includes oxides, hydrides, chlorides, and bromides. **Table 2.1** holds a list of commonly used gases for ICP-MS for a wide range of samples and applications.
Table 2.1. Commonly used gases for ICP-MS analysis adapted from Koppenaal et al. (Koppenaal, et al., 2004)

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Gases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collision</td>
<td>He, Ar, Ne, Xe</td>
</tr>
<tr>
<td>Charge exchange</td>
<td>H₂, NH₃, Xe, CH₄, N₂</td>
</tr>
<tr>
<td>Oxidation reagent</td>
<td>O₂, N₂O, NO, CO₂</td>
</tr>
<tr>
<td>Reduction reagent</td>
<td>H₂, CO</td>
</tr>
<tr>
<td>Other reactions (adduction)</td>
<td>CH₄, C₂H₆, C₂H₄, CH₃F, SF₆, CH₃OH</td>
</tr>
</tbody>
</table>

By utilizing various mechanisms, it is possible to remove analyte interferences. In chapters 3-5, helium is used as an inert collision gas to remove polyatomic interferences. Figure 2.3 illustrates the use of helium to discriminate voltages caused by the difference in spatial size of the analyte and polyatomic interference. Compared to the analyte of interest, a polyatomic is larger, allowing it to statistically encounter more collisions than with the chosen cell gas. These collisions cause an overall reduction in the polyatomic kinetic energy. By tuning the instrument to be selective for the interested analytes, an energy barrier is created by a difference in quadrupole and octopole bias voltages. The analyte of interest possesses the needed kinetic energy to pass through the energy barrier. The polyatomic ion has a lower kinetic energy and cannot cross the energy barrier due to the collisions with the cell gas reducing the polyatomic overall energy.
Figure 2.3. Illustration of kinetic energy discrimination of interferences within the collision/reaction cells.

In addition to the kinetic energy difference between the analyte and polyatomic, it is possible to have discrimination due to charge transfer from the interfering atoms’ collisions with the neutral cell gas atom. These particles are then destabilized and removed from the ion stream. Similar results are achieved with collision-induced dissociation allowing for bond cleavage and therefore removing the interference. Reaction gases can achieve similar results by applying a reactive cell gas for elemental detection of high interference prone elements. Further information may be found in the cited references (Houk, et al., 1980) (Koppenaal, et al., 2004).

2.2 Spectroscopic techniques

Spectroscopic techniques utilize the absorption or emittance of energy by a chemical under specific conditions. These techniques operate over different, limited frequencies. These variations of optical properties are dependent on the direction of electromagnetic radiation
propagation because of scattering, dispersion, diffraction or reflection of the sample. Spectroscopic techniques use similar instrumental components, including an energy source, a means for isolation of narrow range wavelengths, and a detector for measuring the signal from the sample. For the purpose of the research in this dissertation, the optical properties monitored are within the ultraviolet-visible region of the electromagnetic spectrum.

2.2.1 Ultraviolet visible spectroscopy

While ICP-MS and CPE are useful in conjunction for identifying metal species, ultraviolet-visible (UV-Vis) spectroscopy can also provide more information as to the location and formation of these metal species in a sample. A UV-Vis detector can help to identify biological microanatomy (i.e. proteins, lipids, cellular membranes), organic molecules (i.e. chlorophyll), as well as metal nanoparticles that vary in optical properties than their ion counterparts. For more information on this technique, see chapters 3-5.

2.2.1.1 Instrumental setup and theory

UV-Vis spectroscopy is able to determine the absorption or transmission of light in the ultraviolet and visible spectral region when the sample is exposed to a light source. Figure 2.4 is an illustration of the internal components of a UV-Vis spectrometer. Further information involving UV-Vis spectroscopy in current research may be found in the cited references (Antonov, et al., 2000) (Ojeda, et al., 2012).
While samples are required to be transparent and aqueous, they can be placed into a cuvette made of glass, plastic, or fused silica (for ultraviolet light) depending on the range of spectral interest. The cuvette is placed in the sample cell where light will pass through the sample. The light source is comprised of two lamps: tungsten (190-380 nm) and deuterium (380-900 nm). The light from the lamps shine onto a monochromator that isolates specific wavelengths from the source light and directs the desired wavelength to pass through the sample and reference cells. These selected wavelengths may be scattered or absorbed by the sample. Any light that passes through the sample consistent with its initial trajectory will continue into the photodiode detector. The photodiode detector converts the light into a current that is communicated to a computer readout of the intensities of light passing through the sample. This instrument is able to give the intensity of how the sample absorbs or transfers light for a select wavelength or over a scanning spectrum of multiple wavelengths. For this dissertation, UV-Vis is utilized for the identification for the presence of nanoparticles from its metal ion counterpart as well as to identify the presence of biological components and organic compounds within plant
samples in chapters 3-5.

**2.2.2 Dynamic light scattering**

Dynamic light scattering (DLS), also known as photon correlation spectroscopy or quasi-elastic light scattering, is a technique that utilizes the way light scatters off particles in a suspension to calculate a relative particle size distribution and stability within a suspension. While this technique is utilized within the medical field for detection of bacteria or proteins present in biological samples, the most common use of DLS is to analyze nanoparticles. In general terminology, this technique is used to identify the measurement of particle sizes that vary on the micron to nanometer scale and distinguish between a molecule (i.e. protein) and a particle (i.e. nanosilver). Chapters 3-5 provide more information on using the DLS for experimentation. Additional information on DLS can be found in the cited references (Webb, 2000) (2014).

**2.2.2.1 Instrument setup and theory**

A typical setup for a DLS instrument is illustrated in Figure 2.5. DLS instruments utilize a He-Ne laser with a fixed wavelength at 633 nm. This monochromatic light source is scattered by the particles in a colloidal solution. A photodiode detector collects the light scattered at 90 degrees. The fluctuations of light intensity are converted into electrical pulses that are generated into a representative plot of the particle size distribution of the solution.
2.2.2.2 Zeta potential theory of stability

Dynamic light scattering utilizes the zeta potential of a colloidal solution to identify the electrokinetic potential between the surface of a colloid and any point in the mass of the suspending liquid, better known as the surface potential. Visualization of the charged surroundings of an ionic colloid is known as the double layer (Figure 2.6). The positive ions, called counter-ions, when in a solution around the colloid will initially experience attraction from the negative colloid. These counter-ions attach firmly to the surface of the colloid in a layer...
called the Stern layer. With the Stern layer in place, positive ions are still attracted to the negative colloid but are repulsed by the Stern layer and the other approaching positive ions outside of the Stern layer. These additional counter-ions create a charged atmosphere called the diffuse layer. The diffuse layer has a higher concentration of ions near the colloidal surface and gradually decreases as the distance increases from the surface until an equilibrium is reached with all ions in solution. Overall, the charge density is greatest around the colloid surface. The charge density differs at any distance from the colloid due to the variance of the concentration of positive and negative ions at a specific point. Gradually the charge density will decrease to zero as the positive and negative ions merge.

Figure 2.6. Illustration of the double layer of a colloidal particle and its potential curve for
identifying the zeta potential based on the electrical strength between the colloid and the overall solution.

The double layer consists of the Stern and diffuse layers. Their layer of thickness depends on the solution’s ion concentration and the types of ions present. The double layer forms in order to neutralize the charged colloid and in turn creates an electrokinetic potential between the solution at any location and the surface of the colloid. This variance in voltage is in the millivolt range and is known as surface potential. The further from the Stern layer, the faster the potential drops to approach zero. The potential curve from Figure 2.6 shows the electrical strength between the colloid and the overall solution.

The charged colloid moves into a voltage field at a fixed velocity due to electrophoresis. These colloids are related to the dielectric constant, the viscosity of the solution, and the electrical potential at the boundary between the Stern and the diffuse layer, commonly known as the slipping plane. The Stern layer is a ridged layer attached to the colloid, and the diffuse layer is not attached. The electrical potential at this junction relates to the mobility of the overall particle called the zeta potential. The zeta potential is quantified by tracking the colloidal particles as they move in a voltage field. Larger positive or negative zeta potential values indicate high stability of the colloidal solution. Lower zeta potential values indicate that the particles weakly repel other particles and result in nanoparticle aggregation and agglomeration.

2.2.3 Fluorescence spectroscopy

Fluorescence spectroscopy is a technique that measures the intensity of fluorescence emitted from a sample or the fluorescence probe molecule that absorbs photons. Due to its high sensitivity, fluorescence is an important tool in real-time structure and biological samples.
2.2.3.1 Instrumental setup and theory

Fluorescence takes place when a fluorophore, a material capable of fluorescence, is excited to a higher electronic state. This occurs when the fluorophore absorbs an incident photon and is unable to return to the ground state level unless it emits a photon. In order to monitor fluorescence, instruments like microplate readers are employed (Figure 2.7). All fluorescent detection instruments use an excitation light source such as a xenon arc lamp. A monochromator filters to the specific wavelength to excite the fluorophore within the sample before isolating the light produced from the lamp. The fluorescence intensity is then filtered again by another monochromator before detection by the photomultiplier tube. The microplate reader reports the mean fluorescent intensity of a sample.

![Figure 2.7. A block diagram of a fluorescence microplate reader.](image)

2.2.3.2 Dyes and detection

Often, dyes are required in order to monitor biological functions or chemical reactions by fluorescence. Many reactions are quick processes that make normal methods of detection extremely difficult to monitor. Therefore, fluorescent dyes are employed to help monitor these
short-lived reactions (Kreslaviski, et al., 2012) (Cash, et al., 2007). Each fluorescent dye is unique and is chosen for its specificity for a particular reaction. For example, luminol (3-aminophthalhydrazide) is used to identify reactive oxygen species produced in cells by metal ion interactions. When luminol encounters hydrogen peroxide (H₂O₂), hydroxide (OH⁻) or peroxide radical (O₂⁻), it is oxidized to form 3-aminophthalate, which fluoresces at 425 nm when excited by 305 nm wavelength (Figure 2.8).

Figure 2.8. Reaction of luminol with hydrogen peroxide to create fluorescent 3-aminophthalate.

Sodium terephthalate is also used as a fluorescence probe. When a hydroxide radical
interacts with sodium terephthalate, it forms 2-hydroxyterephthalate ($\lambda_{\text{excitation}}=310$ nm, $\lambda_{\text{emission}}=430$ nm). A depiction of this reaction is shown in Figure 2.9.

![Reaction of sodium terephthalate with HO● to produce 2-hydroxyterephthalate](image)

**Figure 2.9.** Reaction of sodium terephthalate with HO● to produce 2-hydroxyterephthalate.

### 2.3 Microscopic techniques

Microscopic instruments amplify a specimen not visible to the naked eye by using lenses and filters to produce a magnified image. Microscopy is divided into three factions: optical, electron, and scanning probe. This research focuses on electron microscopy. As its name reflects, electron microscopy utilizes an electron beam for a higher resolution of the specimen, instead of the conventional use of light in optical microscopy, which limits resolution to about 0.2 micrometers. The electron beam impacts the sample and the detector to create an amplified image of the collected, scattered, and transmitted electrons.

#### 2.3.1 Scanning electron microscope

A scanning electron microscope (SEM) generates an image of the surface of a sample utilizing a focused beam of high-energy electrons instead of light, which is used in most microscopes. The signals from the electrons’ interactions with the sample reveal information about the sample's chemical composition, external texture, and even the crystalline structure and orientation of the surface of the sample. The resulting two-dimensional image allows the sample
to be observed at a resolution of one centimeter to five microns in width. Chapter 3 and 5 provides additional information for using SEM for experimentation. More information is available in the cited references for SEM applications and background (Goldstein, et al., 2003) (Hafner, 2007).

2.3.1.1 Instrument setup and theory

SEM is able to determine the texture of a sample's surface, but it utilizes high-energy electrons instead of the use of light as in most common microscopes. Figure 2.10 is a schematic representation of an SEM with both electron and X-ray detection. The electron gun utilizes a tungsten filament source to emit electrons accelerated at energies between 1 and 30 kV. The magnetic condenser lenses direct the electron beam to the objective lens that determine the size of the beam to interact with the sample’s surface. Overall, the magnetic condensers and objective lens reduce the diameter of the electron beam to 2-10 nm when it finally reaches the sample.
Figure 2.10. A block diagram of an SEM with X-ray and electron detectors.

The electromagnetic coils located within the magnetic objective lenses allow for scanning. While one pair deflects the beam in the x-direction across the sample, the other coil and lens pair deflects the beam in the y-direction. By correlating the electrical signal to these coils as a function of time, the electron beam moves in a straight line across the sample before moving back to its beginning location. This process is repeated at various y-positions. The rapid movement of the beam over the sample surface cases the surface electrons to be irradiated allowing a digital representation of the scanned surface to be reproduced.

The sample chamber requires a large-capacity vacuum pump to hold an ambient pressure of $10^{-6}$ torr. The sample chamber also contains the sample holder allowing multiple samples to
be scanned in succession. The sample holder has the ability to move in the x, y, and z directions, allowing most samples to be viewed from almost any perspective. Conductive samples decrease the possible thermal degradation of the sample. Biological and environmental samples generally require a thin metallic coating, as they are non-conductive samples. A metallic coating of about 10 nm is usually produced by sputtering or by vacuum evaporation.

2.3.1.2 Electron beam and sample surface interactions

A SEM is very useful for the study of solids due to a wide variety of signals, which are generated when the electron beam interacts with the solid sample. **Figure 2.1** is a diagram of the signals that result from the electron beam and sample interactions. Two types of interactions are generated by the collision of the incident beam with the solid specimen: elastic and inelastic.

Elastic interactions are the changes in trajectory of the electrons when they meet the solid without significantly altering their energies. These electrons are called backscattered electrons. The backscattered electrons maintain the same velocity and essentially a constant kinetic energy while its direction changes. The angles of the deflected electrons are random and may vary from 0 to 180 degrees, causing the diameter of the backscattered electrons to be much larger than the incident beam.
The second type of interaction is inelastic. Inelastic interactions are a result in the transfer of all or part of the energy from the electron to the sample. X-rays, larger wavelength photons or secondary electrons are then emitted from the sample’s surface. Secondary electrons are generally less than half of the number of electrons that backscatter. The secondary electrons are produced by the collision of the incident beam electrons with the weakly bound electrons on the sample’s surface. This leads to an ejection of the electrons from the surface. Prevention of secondary electrons reaching the detector is possible with the application of a small negative bias to the transducer housing. X-ray photons are emitted as a third possible outcome of electron bombardment of a sample. This radiation allows for X-ray fluorescence analysis of SEM images.

### 2.3.2 Transmission electron microscope

A transmission electron microscope (TEM) is similar to a compound optical microscope. The TEM utilizes an electron beam instead of light to pass through the specimen, while interacting with the specimen as it transmits through. This process of magnification allows for a two-dimensional image of the specimen’s crystallographic and morphologic structure and form.
with the maximum magnification being one nanometer. Use of a TEM for nanoparticle structural information is in chapter 4.

2.3.2.1 Instrument setup and theory

An illustration of the instrumental setup of a TEM is shown in Figure 2.12. A TEM utilizes a cathode to produce an electron beam (100-300 kV). This beam uses electromagnetic lenses to focus the electrons into a tight beam by controlling the intensity and angular aperture. The first lens produces a reduced image of the beam that is used to control the size of the beam produced of the specimen. The second lens is used to place the controlled beam onto the specimen. The use of a smaller beam is ideal to help minimize any potential destruction of the specimen due to irradiation or heating. This beam passes through the specimen, which is placed on a grid. These grids can be made of metal (i.e. molybdenum, copper or aluminum) with a carbon lattice to help hold the specimen in place. This grid is placed on a movable stage to allow rotation of the sample disk to help provide a more inclusive understanding of the specimen's structure from multiple perspectives.
Depending on the sample's makeup and size, some of the electrons are scattered. These scattered electrons do not reach the detector. The transmitted electrons are focused to project an image of the specimen at the bottom of the instrument on a fluorescent screen. This screen shows a “shadow image” of the specimen with its different parts displayed in varying levels of darkness according to their density.

2.3.2.2 Electron beam and sample surface interactions

While an SEM and a TEM both use the interactions of electrons to distinguish physical properties of a specimen, they are reliant on very different methods of detection and present
different perspectives of the sample to be determined. An SEM depends on the detection of scattered electrons, whereas a TEM detects the transmitted electrons that pass through the specimen (Figure 2.11). By transmitting the electrons through the sample, a TEM can show the internal structure of the specimen rather than focusing on its surface. The diffraction of the electron beam occurs at specific angles according to the crystal structure of the sample. This diffraction of electrons is evident in the image produced by the TEM showing the crystallographic patterns within the bulk material as well as particle size and shape.

2.5 References


Koppenaal David W., Eiden Gregory C. and Barinaga Charles J. Collision and reaction cells in atomic mass spectrometry: development, status, and applications [Journal] // Journal of


Chapter Three | Assessing *Pistia stratiotes* for phytoremediation of silver nanoparticle and silver (I) contaminated waters

3.1 Abstract

To study the phytoremediation capabilities of *Pistia stratiotes* in silver nanoparticle (AgNP) and silver ion contaminated wastewaters, individual plants were grown in media spiked with different concentrations of AgNP and silver ions (0.02, 0.2, and 2 mg L\(^{-1}\)). Control experiments were carried out at the same time for comparison purposes. Visual changes in the plants were also recorded periodically during each experiment. Total silver concentrations were monitored in the media before, during, and at the termination of the experiments. In addition, analysis of total silver in plant root and leaf samples after termination were carried out to determine the effect of the different media concentrations. The results showed that *Pistia stratiotes* can survive in AgNP and ions under 0.02 mg L\(^{-1}\) and contaminants are retained within the plant. The use of *P. stratiotes* as a phytoremediator shows potential in removing heavy metal nanoparticles and is competitive in its removal of the ion counterpart. Even higher concentrations of silver, regardless of form, can be reduced to levels lower than the World Health Organization’s maximum contamination limit.

3.2 Introduction

As applications for nanoparticles gain more popularity for their inclusion in consumer products, the potential negative effects of these nanoparticles interacting with the environment become important topics of research. Silver nanoparticles (AgNPs) are known for their antibacterial capabilities, which have made their way into many commercial products such as household appliances and product coatings (Miao, *et al.*, 2010) (Siripattanakul-Ratpukdi, *et al.*, 2014). With the use of these products, the engineered nanoparticles or their daughter ions make
their way into the waterways during or after the lifetime of the product. This movement of nanoparticles and ions from consumer products to wastewater and the environment is further effected by washing and discarding of these products (Benn, et al., 2008) (Reidy, et al., 2013).

Once these nanoparticles are released into the wastewater system, the only defense between pumping nanoparticles into the environment and drinking waterways rests on the wastewater and drinking water treatment methods (Benn, et al., 2008) (Reidy, et al., 2013) (Kim, et al., 2010). Current wastewater treatment plants in the industrialized world employ extensive chemical and physical means of filtration and purification utilizing coagulation and ultraviolet sieve filtration chambers (Abbott Chalew, et al., 2013) (Hou, et al., 2012). These methods are efficient for removing most contaminants, including silver, that are present in the wastewaters and are consistent with the regulations in place. According to the World Health Organization (WHO), the maximum contamination limit (MCL) for silver is 0.1 mg L$^{-1}$. Although naturally occurring silver concentrations are generally low in surface waters, the concentration markedly increases due to runoff and wastewater from industrial and urban areas (Shafer, et al., 1998). While these standards are upheld in industrialized countries, the treatment systems are too expensive to employ and maintain for newly industrialized or lesser-developed countries that need clean water and where regulations and sustainable water treatment options are lacking (Nashaat, 2013). A solution to these expensive measures for water treatment requires an effective, low cost and easily managed approach that is useful worldwide.

Phytoremediation is a natural way to remove contaminants from a water source using plants. This process utilizes the plant’s metabolic system to remove nutrients and contaminants from their surrounding and store these in their biomass. Ideal plants for phytoremediation have large root systems, as the extent of the root length is proportional to the area that the plant is able
to interact with the contaminated water source. To ensure effective removal of metals and contaminants from a water source, the plants employed for phytoremediation must be aggressive metal and nutrient accumulators that will compete well to achieve effective contaminant removal. These plants need to quickly reproduce in order to ensure that the system for water remediation is self-sustaining, therefore reducing the expense of treatment (Harris, et al., 2008).

The plant used for this study is *Pistia stratiotes*, commonly known as water lettuce, and accepted as an invasive species of floating water plant found globally, but primarily in tropical and subtropical areas. It has a high metal tolerance with an extensive root system that aggressively accumulates contaminants and nutrients in its surrounding waters. *P. stratiotes* is very sustainable as a phytoremediator as it reproduces sexually and asexually, thereby doubling its population in a matter of weeks and decreasing the need to continually add new plants to a remediation pond (McCutcheon, et al., 2003) (Muniappan, et al., 2009). This plant is currently employed in many parts of the world for heavy metal water purification purposes and has shown promising results for the removal of silver and other metal ions from contaminated water sources (Odjegba, et al., 2004) (Gupta, et al., 2012). However, little research has been published that evaluates the effectiveness these plants have in removing metal nanoparticles from a water source in comparison to their ionic counterparts. The goal of this study is to assess the effectiveness of *P. stratiotes* in the removal of silver contaminants from a water source containing various concentrations of AgNPs and silver nitrate by utilizing inductively coupled plasma mass spectrometry. Further, we investigate *P. stratiotes* survival ability in silver contaminated waters.

**3.3 Materials and methods**

All solutions were prepared in 18MΩ/cm doubly deionized water (DDIW)
(Sybron/Barnstead, Boston, MA). All reagents were purchased from Fisher Scientific (Waltham, MA). All chemicals were used without further purification. All glassware used in experimental procedures were cleaned in a solution of 10% nitric acid and 3% hydrochloric acid, washed thoroughly with DDIW and dried before use.

3.3.1 Silver nanoparticle synthesis and characterization

AgNPs were synthesized by sodium borohydride reduction of silver nitrate (Mulfinger, et al., 2007) (Sobczak-Kupiec, et al., 2011). AgNP stock solutions were refrigerated and stored in the dark until use for experimentation. A Zetatrac particle size analyzer (Mictotrac) was used to obtain the AgNPs size distribution (Figure 3.1). The total silver concentration was determined by inductively coupled plasma mass spectrometry (ICP-MS). A FEI/Philips XL30 FEG ESEM (FEI) equipped with energy dispersive x-ray spectroscopy was used to examine the root samples after experiment termination.

![Figure 3.1. Size distribution of a typical stock solution of AgNPs achieved by dynamic light scattering.](image)

Figure 3.1. Size distribution of a typical stock solution of AgNPs achieved by dynamic light scattering.
3.3.2 Sample collection and growth conditions

Plant samples were obtained from a private pond in Cleveland, OH, and transferred to a quarantined pond before use in experimentation. Plants of similar shape and size (roots 25-30 cm in length, leaf length 5.5-7.5 cm) were selected for use and washed several times with tap water and DDIW before use. Plants were placed into three different concentrations (0.02, 0.2 and 2 mg L⁻¹) of AgNPs or silver nitrate solutions. Each concentration was done in triplicate (3 plants) and compared to a control plant placed in DDIW one hour before illumination in a 14-10 hour light-dark cycle. No additional nutrient content was added to the media solutions to reduce any precipitation or aggregation to the silver ions or nanoparticles.

The silver-containing media was contained in 500 mL containers with a hole in the lid. The media container was then placed into another box to shield the media from all light for the extent of the experiment. Roots were placed through the lid holes so that the roots were the only part of the plant to contact the media and the leaves remained above the media. Control solutions of the silver contaminated media and DDIW were placed in the same dark environment.

Water samples were taken from the control solutions and plant media at 0, 1, 2, 6, 12, 18, 24, and 36 hours and at termination (48 hours) to examine any changes in total silver concentration in the media during the experiment. Media samples were stored in a dark refrigerated container until sample preparation. Media samples were acidified and diluted to 5 mL with 2% nitric acid and 0.5% hydrochloric acid.

At the termination of each experiment, the plants were harvested and washed thoroughly with tap water first and then DDIW to remove any silver on the surface of the root. The roots were separated from the leaves and each plant component was air-dried. The dry components
were ground to a homogenous powder using a ceramic mortar and pestle before placing them into an airtight plastic container for storage until digestion. A 0.01g sample from each component was digested by hot-block with 30% nitric acid, 0.5% hydrochloric acid and 30% hydrogen peroxide until completion. Samples were diluted with DDIW to 5 mL and filtered through a 0.45-micron centrifugal filter.

### 3.3.3 Inductively coupled plasma mass spectrometry (ICP-MS)

Media and plant samples were run on an Agilent 8800 ICP-MS/MS inductively coupled plasma mass spectrometer (Agilent Technologies, Santa Clara, CA) with two mass spectrometry modes for total silver concentration by monitoring m/z 107 and 109 and utilizing 2 mL min\(^{-1}\) helium within the reaction cell. Samples were also monitored for m/z 89 and 115 for the internal standards of yttrium and indium, respectively, to compensate for instrumental drift and ensure appropriate matrix corrections. The measurement precision was determined by taking the mean of three replicates of each sample. Calibration standards from 0 to 0.1 mg L\(^{-1}\) were prepared through dilution from a stock solution with 2% v/v HNO\(_3\). To ensure accurate calibration and extraction, the National Research Council (Ottawa, Canada) drinking water standard and a secondary standard of silver (SPEX CertiPrep, Metuchen, NJ) spikes were utilized for quantification accuracy. The limit of detection (LOD) for silver was 0.08 μg L\(^{-1}\).

### 3.3.4 Data Analysis

All results are expressed as the mean ± the standard deviation of the mean. For results below the LOD, the total metal concentrations below the LOD were substituted with a value one-half the LOD. The percent recovery of the removed silver from the contaminated media found within the plant biomass was calculated by taking the total silver concentration found within the plant divided by the total silver removed from the media at the experimental termination. This
calculation of recovery helps to understand the amount of silver retained within the plant biomass.

3.4 Results and discussion

3.4.1 Visual changes observed in *Pistia stratiotes* over the experimental duration

The visual changes observed in *P. stratiotes* growing in different media over the duration of experiments are summarized in Table 3.1. *P. stratiotes* plants growing in DDIW appeared robust over the duration of the experiment with green leaves and no signs of wilting (Figure 3.2). Plants growing in 0.02 mg L$^{-1}$ of AgNPs and silver nitrate solutions were partially wilting after six hours with curled leaf tips. They remained in this condition for the entirety of the experiment. For 0.2 mg L$^{-1}$ total silver concentration, the AgNPs-containing media plant leaves appeared to wilt within 2 hours and continued to increase to a substantial wilt at 48 hours. The plants in 0.2 mg L$^{-1}$ silver nitrate containing media exhibited marginal wilting and discoloration of the leaf edges at six hours and declined in health more quickly. Additionally, after 24 hours the wilt was substantial, taking over a majority of the plant’s leaves. The plants in 2 mg L$^{-1}$ AgNP media showed limited wilting at two hours and by 24 hours, the plant leaves browned and showed substantial wilting. The plants in 2 mg L$^{-1}$ silver nitrate showed marginal wilting of the leaves at 6 hours. By 18 hours, a majority of the leaves were wilted and browned. The health of the plants in both the nanoparticle and ion contaminated media continued to deteriorate, with complete wilting at 48 hours. Upon removal from the water, the roots were no longer attached to any of the aerial parts of the plants. From the observations above, the survival of *P. stratiotes* in DDIW may be due to high accumulation of nutrients in the plant prior to experimentation, which compensates for the lack of nutrient in the DDIW (Soltan, *et al.*, 2003). The visual changes observed in the plants growing in the silver-containing media indicate that the toxicity of silver
on the plant increases with higher levels of contamination. AgNPs tend to have apparent toxic effects sooner than the silver ions; however, the silver ion toxicity caused the plants to wilt more quickly once past the initial senesce.

Table 3.1. Visual changes observed in P. stratiotes growing in different media for the duration of the experiments*.

<table>
<thead>
<tr>
<th>Media</th>
<th>Time (Hours)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>6</th>
<th>12</th>
<th>18</th>
<th>24</th>
<th>36</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDIW</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>AgNO₃</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
</tr>
<tr>
<td>AgNPs</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
</tr>
<tr>
<td>0.02 mg L⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AgNO₃</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
<td>US</td>
<td>US</td>
<td>US</td>
<td>US</td>
</tr>
<tr>
<td>AgNPs</td>
<td>H</td>
<td>H</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
<td>US</td>
<td>US</td>
</tr>
<tr>
<td>0.2 mg L⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AgNO₃</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>UM</td>
<td>UM</td>
<td>US</td>
<td>US</td>
<td>US</td>
<td>UC RL</td>
<td>UC RL</td>
</tr>
<tr>
<td>AgNPs</td>
<td>H</td>
<td>H</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
<td>US</td>
<td>US</td>
<td>US</td>
<td>UC RL</td>
<td>UC RL</td>
</tr>
</tbody>
</table>

* H, the plant appears to be healthy with green leaves, no wilting, yellowness, drying, etc. Plant survival likely due to nutrient storage in the plant; UM, the plant appears to be unhealthy and marginally wilted/shriveled of some leaves; US, the plant appears to be unhealthy with substantial wilting of the leaves and browning; UC RL, the plant is clearly unhealthy with all leaves completely wilting, along with root loss and darkening.
Figure 3.2. Depiction of each stage of a plant’s physical degradation.

Since the media solutions did not contain any additional nutritional content other than nitrates and silver, these plants may have experienced more toxic stress than if there had been nutrients available to help maintain normal internal metabolic processes and combat the destructive effects of the silver. However, addition of other nutrients to the media solutions in other studies has shown that macro-nutrient elements such as calcium and magnesium cause aggregation of AgNPs (Jin, et al., 2010) and silver ion precipitate out of solution in the presence of chlorides and sulfides (Shafer, et al., 1998). Therefore, to reduce effects of silver precipitation and alterations of nanoparticles, all solutions were prepared in DDIW.

3.4.2 Temporal concentration changes in silver media solutions

Water samples were collected periodically to identify the overall silver concentration within the plant media as a function of plant uptake. The total silver concentrations in the media solutions over time are given in Figure 3.3. The control solutions of the contaminated media retained constant silver concentration, within 5%, over the extent of the experiment. This suggests that the silver under the growth conditions did not precipitate from solution and was minimally absorbed by the container. Controlled media solutions of DDIW did not gain any silver, showing that silver is not leached from the acid washed containers or from any other external contamination in this experimental setup.
Figure 3.3. Silver concentration in plant media during the study period due to interactions with *P. stratiotes* for starting concentrations of A) 0.02, B) 0.2 and C) 2 mg L\(^{-1}\) of AgNPs (--- dashed line) and silver nitrate (— solid line). All data are the mean concentrations of three trials ± standard deviation.

The DDIW media solutions over the 48-hour experiment showed no silver in the media. Without placing silver in the media, there is no other source of silver in the experimental setup. Over the course of 48 hours, all solutions of AgNPs and silver nitrate at each concentration
decreased in every media solution, when a single plant was present within the media solution. The largest amount of silver is removed from the media in the first two hours and by six hours, the concentration of silver starts to level off at low levels in each experimental batch. The lowest concentration studied is representative of a wastewater silver level significantly lower than the WHO MCL, but at a possible concentration in a wastewater treatment plant in the United States (Shafer, et al., 1998). By the end of the experiment, the total remaining concentration of the AgNPs and silver ion contaminants were <20% of the original concentrations. When the initial contaminant concentration is twice the WHO MCL value (0.2 mg L⁻¹), the plant reduced the contamination to beneath the MCL value in the first two hours. Only when the contamination is twenty times greater than the WHO MCL does the plant appear to be unable to remove enough silver from the media within the first two hours, regardless of form. Nonetheless, the media containing 2 mg L⁻¹ silver nitrate is able to reach the MCL level at the experiment termination with only one plant present. The difficulty of removing higher levels of silver is a consequence of the decreasing plant health. The physical distress of the plant represented by wilting indicates the uptake of metals by plants is reduced (Soltan, et al., 2003). However, the metal accumulation by the plants continued throughout the duration of experiments (Gupta, et al., 2012).

3.4.3 Concentrations of silver in *Pistia stratiotes*

To further identify the location of silver accumulation by *P. stratiotes*, SEM and ICP-MS/MS analysis techniques were utilized. Scanning the surface of the plant roots with SEM equipped with energy dispersive x-ray spectroscopy at an electron beam voltage of 15 kV showed no detectable amount of silver on the surface of the roots (Table 3.2). Increasing the beam voltage to 30 kV allows the beam to penetrate further into the root and 11.6 weight percent (1.62 atomic percent) of the scanned area of the root, silver was observed. This verifies the
effectiveness of the wash method for the initial removal of silver from the outside of the root, and the majority of the silver observed in the digested samples is the silver accumulated within the plant and is not due to silver coating the root surface.

Table 3.2. Energy dispersive x-ray spectroscopy results for washing of plant roots.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Voltage (kV)</th>
<th>Weight Percent of Scanned Area (%)</th>
<th>Atomic Percent of Scanned Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unwashed Root</td>
<td>15</td>
<td>6.4</td>
<td>0.82</td>
</tr>
<tr>
<td>Washed Root</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>11.6</td>
<td>1.62</td>
</tr>
</tbody>
</table>

The total silver concentrations found in the plant biomasses are shown in Table 3.3. These data points indicate three important findings. First, the uptake of silver by plants increases with increased silver in the media. Second, the silver removed from the media is not completely retained within the plant. Third, silver is not a metabolic requirement for the plant and the silver is reliant on non-selective uptake methods or the hijacking of other metabolic processes for silver to enter the roots.
Table 3.3. Extent of silver containing media on the accumulation of silver in *P. stratiotes*. All data are the mean of three trials ± standard deviation.

<table>
<thead>
<tr>
<th>Media</th>
<th>Concentration (µg/kg of compartment)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roots</td>
<td>Leaves</td>
<td>% Recovery</td>
</tr>
<tr>
<td>0.02 mg L⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AgNO₃</td>
<td>9.49 ± 0.20</td>
<td>0.23 ± 0.05</td>
<td>87%</td>
</tr>
<tr>
<td>AgNPs</td>
<td>10.4 ± 0.06</td>
<td>0.11 ± 0.05</td>
<td>96%</td>
</tr>
<tr>
<td>0.2 mg L⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AgNO₃</td>
<td>46.7 ± 0.13</td>
<td>0.70 ± 0.04</td>
<td>49%</td>
</tr>
<tr>
<td>AgNPs</td>
<td>45.2 ± 0.16</td>
<td>1.08 ± 0.05</td>
<td>54%</td>
</tr>
<tr>
<td>2 mg L⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AgNO₃</td>
<td>101 ± 0.17</td>
<td>1.23 ± 0.05</td>
<td>15%</td>
</tr>
<tr>
<td>AgNPs</td>
<td>127 ± 0.07</td>
<td>2.71 ± 0.03</td>
<td>20%</td>
</tr>
</tbody>
</table>

Metal can accumulate in the plant by entering the root through either the extracellular (apoplastic) or intracellular (symplastic) pathway. The use of extracellular transport in the roots is limited by the cell wall’s high cation exchange capacity (Kadukova, *et al.*, 2010). This supports the results presented for silver found in the aerial compartments, which are very low for the silver nitrate containing media. The non-cationic nanoparticles are more likely translocated.
through the plant roots, as they do not have as strong of an interaction with the cell walls. The only parts of the plant to contact the media were the roots with the majority of the accumulated silver located within; trace amounts of silver were detected in the aerial components. The translocation of the silver species from roots to leaves indicates that not all the silver is bound to the root tissue, and some silver is still able to make its way into the leaves. Silver is also known to inhibit enzymes, especially those containing sulfur, thereby altering cell membrane permeability (Koontz, et al., 1980). As there were no other elements or molecules present in the media, such as nutrients, the silver does not compete to interact with the enzymes or cell membranes with the plant to transport silver through damaged cells or utilize transport proteins (Steudle, 2000) (Swanson, et al., 2013). Due to the limited availability of essential nutrients to compete with the silver for interaction within the plants, the toxic effects of silver are increased. The lack of competition for cellular interactions decreases the plant’s ability to sequester or reject toxic metals.

The 0.02 mg L\(^{-1}\) contaminated media, for both AgNPs and silver nitrate, retained 96% and 87%, respectively, of the contaminants removed from the media. At the higher concentration of silver contaminants, the total silver accumulated within the plant was far less than the reported value of silver removed from the media. As the results from Table 3.2 indicate the effectiveness of the root wash, the remaining silver removed from the media may have been attached to the surface rather than absorbed by the roots to later be rinsed away during the washing procedure.

3.5 Conclusion

*P. stratiotes* successfully accumulated a majority of the silver contaminants (nanoparticle and ions) at the lowest concentrations tested. As the media concentration of silver at 48 hours tends to be similar in concentration to the tested media at 12 hours, this shows that theoretically,
one plant in 0.02 mg L\(^{-1}\) silver contaminated media, is able to extract and retain a majority of the silver from silver ion or AgNPs in a short period of time. When projected to a large scale, this concentration of silver would be divided among multiple plants, therefore decreasing the toxic implications of silver to the plants, while increasing the possible cleanup ability and sustainability of the phytoremediation pond. Phytoremediation using \textit{P. stratiotes} is a promising method to reduce silver species to below the WHO MCL value; however, more research would be needed to assess the effectiveness of these plants for the removal of silver species when other contaminants are simultaneously present in the wastewater.

The use of \textit{P. stratiotes} for a phytoremediation pond shows promise for the removal of silver forms in a contaminated water source. Its high nutrient removal and uptake capabilities as well as its rapid growth rate and large biomass prove to be more effective than other submergent and emergent water plant species. Compared to \textit{Eichhornia crassipes}, which is a better accumulator of heavy metals, \textit{P. stratiotes} is easier to manage because of its smaller biomass, which allows it to be used in polyculture systems, while \textit{E. crassipes} are too large and aggressive for these types of phytoremediation ponds.

\section*{3.6 References}


Nashaat Nassar N. The application of nanoparticles for wastewater remediation [Book Section] //


Chapter Four | Silver nanoparticle interactions with strontium ions and the effects on phytoremediation with *Pistia stratiotes*

4.1 Abstract

The interactions between silver nanoparticles (AgNPs) and environmental ions, such as natural strontium, changed the physical and optical properties of the nanoparticles by destabilizing the electrostatic repulsion between particles to promote aggregation. The agglomeration of AgNPs by strontium ion interactions decreased the physical degradation caused by interactions between the nanoparticles and *Pistia stratiotes*, a commonly used plant for phytoremediation. By varying the concentration of strontium ions present in solution from 0.4 to 15 mg L$^{-1}$, the plants were able to remove enough silver to decrease the total silver concentration from 0.2 mg L$^{-1}$ to levels beneath the maximum contamination level given by the World Health Organization of 0.1 mg L$^{-1}$. The *P. stratiotes* retains an average of 51% of the total silver removed from the contaminated water, while retaining a majority of the silver in its roots. Strontium was removed from the contaminated water solutions at higher concentrations than seen for silver and an average of 93% of the removed strontium was retained in the plant’s biomass. AgNP and ion related reactive oxygen species production was monitored showing that the reactive oxygen species production was reduced in *P. stratiotes* leaves in waters containing strontium and calcium ions. When plant leaves were under salt stress, the AgNPs amplified the presence of reactive oxygen species produced.

4.2 Introduction

Silver nanoparticles (AgNPs) make up 50% of all nanoparticle containing consumer products on the market today (The Project on Emerging Nanotechnologies, 2013). These
products integrate the antimicrobial abilities of silver to reduce the presence and spread of bacteria and viruses. Normally, silver is not considered an environmental hazard, since silver forms precipitant with most environmental anions such as chloride and sulfate. However, the disposal of nanoparticles from consumer products increase the presence of soluble silver into environmental waters (Koontz, et al., 1980) (Miao, et al., 2010) (El Badawy, et al., 2010). The interactions between the AgNPs and environmental ions may alter physical and chemical properties of AgNPs and, ultimately, their interactions with organisms in their vicinity.

Several research articles address the interactions between AgNPs and divalent cations such as calcium and magnesium, which cause the agglomeration in high ionic strength solutions (Koontz, et al., 1980) (El Badawy, et al., 2010) (Jin, et al., 2010). Still, few studies investigate the interactions between AgNPs and naturally occurring strontium. Strontium, like calcium and magnesium, is a divalent cation that is prevalent in environmental and source waters. Unlike calcium, strontium is not a necessary nutrient for plant survival and is generally taken up by the plant at lower concentrations than calcium (Skoryna, 1981). Strontium is utilized in the plant biomass by the same metabolic pathways as calcium and may be used as a potential calcium-substitute when maintaining cellular maintenance to cellular walls and supporting normal procession of nutrients within the cell (Rediske, et al., 1953) (Roca, et al., 1995) (Swanson, et al., 2013). Despite this difference in plant uptake, natural strontium is not adequately studied for its effects on plants and nanoparticles, as it is generally considered nontoxic to most organisms even in large concentrations (Roca, et al., 1995).

For applications such as phytoremediation, which is dependent on plant health, it is essential to understand how environmental ions effect the removal of AgNPs and ultimately the sustainability of the phytoremediation system. Compared to emergent and submersent water
vegetation, top-floating water plants are more competitive in removing contaminants in larger concentrations; therefore, this research focuses on the ability of *Pistia stratiotes* to remove AgNP in strontium-rich contaminated waters (McCutcheon, *et al.*, 2003) (Muniappan, *et al.*, 2009). *P. stratiotes*, commonly referred to as water lettuce, is currently implemented for phytoremediation purposes around the world (Odjegba, *et al.*, 2004) (Gupta, *et al.*, 2012). This invasive species of aquatic plant has a large root system and is an aggressive accumulator of heavy metals that makes it ideal for removing contaminants including silver from aqueous ecosystems. *P. stratiotes* rapidly reproduces through sexual and asexual means, providing a substantial number of plants to extract contaminant from a phytoremediation pond while sustaining a large population for continual water remediation.

The previous chapter assesses *P. stratiotes* for phytoremediation of silver ions and nanoparticle removal and has shown competitive removal of both forms of silver to levels below the maximum contamination level (MCL) of 0.1 mg L$^{-1}$, as specified by the World Health Organization (WHO) (Hanks, *et al.*, 2015). The prior study shows high plant degradation when removing higher concentrations of silver forms when other environmental ions are not present.

This chapter presents research to further expand on how environmental ions, focusing on strontium, affect AgNPs and their uptake by *P. stratiotes*. By changing the physical properties of the AgNP with the addition of competing strontium ions, the plant experiences different toxic effects than when interacting alone without any uptake competition by *P. stratiotes* and its sustainability for a phytoremediation pond. AgNPs and silver ions were also monitored for production of reactive oxygen species in the plant leaves when strontium, calcium and sodium ions are present to help understand the significance of environmental ions in promotion and reduction of reactive oxygen species generation.
4.3 Materials and methods

Solutions used for this research were prepared in 18 MΩ cm\(^{-1}\) doubly deionized water (DDIW) (Sybron/Barnstead, Boston, MA). All reagents were purchased from Fisher Scientific (Waltham, MA) unless otherwise stated and were used without further purification. All glassware used for experimentation was cleaned with 10% nitric acid and 3% hydrochloric acid, washed thoroughly with DDIW and dried before use.

4.3.1 Synthesis of AgNPs and characterization

AgNPs were synthesized with silver nitrate and reduced with sodium borohydride (Mulfinger, et al., 2007). AgNP solutions were refrigerated and stored in the dark until use for experimentation. No further purification or post-modification of the nanoparticle was completed. Characterization of synthesized nanoparticle was completed for the AgNP suspension using Microtrac size analyzer (Nikkiso Co., Tokyo, Japan), which recorded the AgNP size distribution and zeta potential. Further verification of nanoparticle size and structure was provided with a FEI/Philips CM-20 transmission electron microscope (FEI Ltd., Hillsboro, Oregon). Variations in nanoparticle optical properties were observed with U-3900 Hitachi spectrometer (Hitachi Ltd., New Orleans, Louisiana) by scanning wavelengths 300-600 nm.

4.3.2 Sample collection and growth conditions

All *P. stratiotes* were obtained from a private pond in Cleveland, Ohio and transferred to a quarantine pond before use in experimentation. Plants of similar size (root length 25-30 cm, leaf length 5.5-7.5 cm) were selected and washed with tap and doubly deionized water (DDIW) before placement into contaminated test waters. Plants were placed into six different mixtures of strontium nitrate (0, 0.4, 2, 10, 15, 20 mg L\(^{-1}\)) and AgNP (0, 0.2 mg L\(^{-1}\)) contaminated water. Sodium nitrate was added to each solution to keep the nitrate concentration constant in each
water mixture and to ensure results were not distorted due to unintentional fertilization. Plants in each contaminated water concentration were done in triplicate and compared to a control plant placed in DDIW. Plants were placed in contaminated waters and DDIW one hour before illumination in a 14-10 light/dark cycle. Control solutions of contaminated waters and DDIW were placed in the same conditions without plant interactions to monitor stability under experimental conditions.

To ensure that the AgNPs did not precipitate out of solution due to photosensitivity, the contaminated waters were contained in 500 mL containers and placed in a box to shield them from light. Roots were also placed into a box to shield them from light. Roots were placed through a hole in the lid of each container and were the only plant compartment to interact with the contaminated water. Control solutions of the contaminated water and DDIW were monitored for total metal concentrations over time, in the same manner as the plant waters, at 0, 1, 2, 6, 12, 18, 24, 36 hours and at the 48-hour termination point. Once collected, the water samples were stored in dark, refrigerated containers until sample preparation. Water samples were diluted and acidified with 2% nitric acid and 0.5% hydrochloric acid. Water samples were processed in triplicate.

Each plant was removed from the water solutions at 48 hours after their introduction to the contaminated waters. When removed from the water, the plants were washed with tap and DDIW water to remove external concentrations of silver and strontium, previously shown to be an adequate wash method (Hanks, et al., 2015). The roots and leaves were separated, dried and ground into a homogenous powder with a mortar and pestle. A 0.01g sample from each plant part was digested by a hot-block with 30% nitric acid, 0.5% hydrochloric acid, and 30% hydrogen peroxide. Samples were diluted with DDIW and filtered through a 0.45 micron
centrifugal filter. Plant samples were processed in triplicate.

4.3.3 Inductively coupled plasma mass spectrometry (ICP-MS)

All samples were run on the Agilent 8800 ICP-MS/MS (Agilent Technologies, Santa Clara, CA) for total silver and strontium concentration, monitoring m/z $^{88}$Sr, $^{107}$Ag, and $^{109}$Ag using 2 mL min$^{-1}$ helium as a cell gas. Internal standards were used to monitor for instrumental drift and ensure appropriate matrix corrections with m/z $^{45}$Sc, $^{89}$Y and $^{115}$In. Calibration standards ranged from 0 to 0.1 mg L$^{-1}$ and were checked with National Research Council-Canada drinking water standard reference material (Ottawa, Canada). The extraction method was validated with a secondary standard of silver, and strontium spikes (SPEX CertiPrep, Metuchen, NJ) for extraction and quantification accuracy. The limit of detection (LOD) for silver was 0.08 μg L$^{-1}$ and 0.06 μg L$^{-1}$ for strontium.

4.3.4 Fluorescence detection

Leaves of *P. stratiotes* were collected and cleaned with tap and DDIW. Disks were punched out of the leaves with a size 2-cork borer (5 mm diameter) while avoiding major veins and placed into a beaker of DDIW over night to reduce reactive oxygen species (ROS) produced by leaf bruising. The disks were placed into a 96-well plate. Each well had 25 μL of 10 mM luminol or sodium terephthalate and 25 μL DDIW prior to standing for 30 minutes to allow the dye to equilibrate within the disk. Then, 25 μL of 0.1 μg L$^{-1}$ AgNP or AgNO$_3$ and 0.1 μg L$^{-1}$ strontium nitrate, calcium nitrate and sodium nitrate (25, 50, 75 μL) were diluted to a total volume of 150 μL with DDIW. Plates sat for four hours at room temperature to allow the production of ROS to stabilize within the plant disk. Fluorescence was detected by $\lambda_{\text{excitation}}$ = 310 nm and $\lambda_{\text{emission}}$ = 425 nm for dyes and a BioTek plate reader/ spectrometer (BioTek Instruments, Winooski, VT).
4.3.5 Data analysis

All results are expressed as the mean ± the standard deviation of the mean of three replicates unless otherwise stated. For any results below the LOD, the total elemental concentrations are listed with one-half the LOD value. LOD was calculated from seven replicates of a low level silver standard and calculated as specified by U.S. Environmental Protection Agency Method Detection Limit (MDL) in EPA method 200.8. The percent recovery of silver and strontium found within the plant biomass, which had been removed from the contaminated waters was calculated by taking the total elemental concentration from the plant divided by the total silver and strontium removed from the contaminated water at the termination of each experiment.

4.4 Results and discussion

Figure 4.1 displays the UV-Vis absorption spectra of synthesized AgNP solutions. When strontium is added to AgNP solutions, the absorbance decreases with increased strontium concentration. The increased strontium ion concentration disrupts the electromagnetic field around the nanoparticles, causing the normally observed plasmon resonance to decrease in intensity, therefore reducing the observed optical absorbance at 400 nm. The addition of strontium to AgNPs does not result in oxidation to produce silver ions (E°=-1.3 V). The AgNP and strontium interactions take place due to a physical interaction of the particles rather than a chemical alteration of silver.
**Figure 4.1.** UV-vis absorbance of 0.2 mg L\(^{-1}\) AgNPs in strontium nitrate solutions: 15 mg L\(^{-1}\) (blue), 2 mg L\(^{-1}\) (green), 0.4 mg L\(^{-1}\) (yellow), 0 mg L\(^{-1}\) strontium nitrate (red) and 20 mg L\(^{-1}\) no silver present (purple).

To determine that the cause of a decrease in absorption is due to nanoparticle size, size distributions and zeta potentials were determined by dynamic light scattering measurements (**Figure 4.2** and **Table 4.1**). The size distribution of the AgNP stock solution started at 20 ± 10 nm (also confirmed by transmission electron microscope, **Figure 4.3**). With the addition of strontium ions, the nanoparticle size distribution broadened and increased in overall size. The zeta potential indicates the magnitude of charge repulsion between particles to help understand the particle dispersion and stability within a solution. Regardless of strontium concentration, the AgNP agglomerates had negligible variation in zeta potential. The consistent zeta potentials indicated that the nanoparticle stability is not a function of strontium concentration but is due to a reduction of electrostatic repulsion surrounding the particle by strontium ions as observed by others (Jin, *et al.*, 2010) (Li, *et al.*, 2012). Despite the agglomeration of the nanoparticles by the presence of strontium, the zeta potentials of the newly formed particle clusters indicate that these clusters are stable once the solution equilibrates with the existing strontium.
Figure 4.2. Size distribution in nanometers of AgNPs (A), AgNPs and 0.4 mg L\(^{-1}\) strontium nitrate (B), AgNPs and 2 mg L\(^{-1}\) strontium nitrate (C) solutions achieved by dynamic light scattering.
Figure 4.3. Transmission electron microscope image of 20 nm AgNP starting material on carbon lace copper disk.

Table 4.1. Zeta potential of 0.2 mg L\(^{-1}\) AgNPs in strontium nitrate solutions. All data are the mean of three solutions ± the standard deviation.

<table>
<thead>
<tr>
<th>Strontium Nitrate Concentration (mg L(^{-1}))</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-16.8 ± 3.1</td>
</tr>
<tr>
<td>0.4</td>
<td>-17.6 ± 2.5</td>
</tr>
<tr>
<td>2</td>
<td>-15.4 ± 3.4</td>
</tr>
</tbody>
</table>

Before the addition of strontium to the AgNP solution, the sizes of the nanoparticles are 20 ± 10 nm in size as shown in Figure 4.3. Figure 4.4 verifies that the size of the AgNPs increase in size when in a strontium rich solution. The strontium interrupts the electrostatic double layer to allow the nanoparticles to agglomerate into large particle clusters (Jin, et al., 2010). Strontium ions’ positive charges interact with the negative charges around the AgNP reducing the repulsive forces between the nanoparticles. However, the strontium ions also cause
the nanoparticles to retain their initial spherical shape by stabilizing around the particle and acting as a bridging ion between the nanoparticles in the agglomeration. This strontium promoted silver particle agglomerates were used in the contaminated water solutions for plant experimentation.

![Image](image.png)

**Figure 4.4.** Transmission electron microscope images of AgNP agglomerates in a strontium nitrate rich solution, 200 nm scale (Left) and zoomed in on single agglomerate, 50 nm scale (Right).

### 4.4.2 Visual changes observed in *P. stratiotes* over the duration of the experiment

The visual changes observed in *P. stratiotes* growth over the duration of the experiment are summarized in Table 4.2. In the control solution of DDIW, all plants remained visually healthy over the 48 hours, as they showed no signs of physical degradation or discoloring. Plants grown in 0.2 mg L\(^{-1}\) AgNP solutions showed physical signs of poor health in the leaves, indicated by the loss of green color. The leaves were marginally wilted, and a slight discoloration at the leaf tips occurred. At termination, these plants were substantially wilted. Photographic images of each phase of the plant degradation are shown in (Hanks *et al.* 2015).

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Table 4.2. Visual changes observed in *P. stratiotes* growing in different contaminated waters for the duration of the experiments.

<table>
<thead>
<tr>
<th>Contaminated Water Solutions</th>
<th>Time (Hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>DDIW</td>
<td></td>
</tr>
<tr>
<td>0.2 mg L⁻¹ AgNP</td>
<td></td>
</tr>
<tr>
<td>0.2 mg L⁻¹ AgNP &amp; 0.4 mg L⁻¹ Sr(NO₃)₂</td>
<td></td>
</tr>
<tr>
<td>0.2 mg L⁻¹ AgNP &amp; 2 mg L⁻¹ Sr(NO₃)₂</td>
<td></td>
</tr>
<tr>
<td>0.2 mg L⁻¹ AgNP &amp; 10 mg L⁻¹ Sr(NO₃)₂</td>
<td></td>
</tr>
<tr>
<td>0.2 mg L⁻¹ AgNP &amp; 15 mg L⁻¹ Sr(NO₃)₂</td>
<td></td>
</tr>
<tr>
<td>20 mg L⁻¹ Sr(NO₃)₂</td>
<td></td>
</tr>
</tbody>
</table>

*H, the plant appeared to be healthy with green leaves; UM, the plant appeared to be unhealthy with some marginal wilting of the leaves; US, the plant appeared to be unhealthy with a substantial amount of the leaves wilted (Hanks, et al., 2015).

While physical degradation appeared for plants in the AgNP contaminated water, this effect seemed to be delayed or reduced once higher concentrations of strontium were introduced to the contaminated water solutions. At 0.4 mg L⁻¹ of strontium with the 0.2 mg L⁻¹ of AgNPs, there was no visual difference in the health of the plant over time, relative to plant health with
strontium nitrate only. When the strontium ion concentrations is 2 mg L\(^{-1}\) with the 0.2 mg L\(^{-1}\) AgNPs, the plants remained healthy until twelve hours into the experiment when partial wilting was evident. By increasing the concentrations of strontium ion to 15 mg L\(^{-1}\) in the AgNP contaminated water, the harmful effects of AgNPs are decreased or not evident in appearance within the duration of the experiment.

With silver present in the contaminated waters, the deterioration of the plant health is quickly evident. The physical signs of deteriorated health are promoted by the interactions of the AgNPs as reported in a separate study (Hanks, et al., 2015). Previously published research concludes that AgNP toxicity is dependent on particle size, and that smaller nanoparticles are more hazardous to organisms than larger sized nanoparticles (Martinez-Castanon, et al., 2008). Larger nanoparticles have a decreased toxicity toward \(P.\) stratiotes than small particles because the increased size reduces its access into organisms and severity of toxic interactions (Marchiol, et al., 2014). The aggregation affects the nanoparticle properties and toxicity by altering the nanoparticle characteristics, while also reducing the toxic dose added to the plants.

Strontium ions are known to be nontoxic and are associated with the metabolic process of calcium within plants. When calcium is not present within the environment of a plant, strontium ion, if available, takes its place and is used as a calcium-substitute to strengthen cellular walls and provide normal transport and retention of other elements within the plant (Rediske, et al., 1953) (Ophel, et al., 1970). In addition to providing a natural defense for the plant against the harmful effects of AgNPs, strontium ions interrupt the repulsive effects to the AgNP double layer, also changing the physical shape of the nanoparticles causing agglomerates to form. The reduction of the electrostatic double layer promotes the formation of larger nanoparticle agglomerates of sizes 200 nm and larger when strontium concentrations are higher, such as 10
and 15 mg L\(^{-1}\). These particle clusters are too large to access beyond the root surface into the plant roots through apoplastic pathways in the cortex of the plant, which are less than 10 nm wide transport pathways (Kim, et al., 2012).

4.4.3 Temporal concentrations of total silver and strontium investigated in the contaminated water solutions

Water samples were periodically collected to identify the overall silver and strontium concentrations in the contaminated water solutions (Figure 4.5). The control solutions of the contaminated waters retained silver and strontium with less than 5% loss of concentration over the extent of the experiment. In addition, the controlled contaminated water solutions of DDIW did not gain silver or strontium over the duration of the experiment, showing that the analytes of interest also did not leach from the containers, and there were no other forms of external contamination in this experimental setup.

Overall, the concentration of both total silver and strontium decreased over time in each of the contaminated waters. Each plant within the first 24 hours in the contaminated water solutions was able to reduce the AgNP concentrations to levels lower than the 0.1 mg L\(^{-1}\) WHO MCL. Despite the ability of \textit{P. stratiotes} to accumulate higher levels of heavy metals, silver is not a preferred element for high accumulation since it lacks specific biological processes associated with selective silver accumulation. Therefore, silver promotes degradation of plant health. The largest depletion of silver occurs in the first two hours of contact with the plant, and at six hours the silver reduction plateaus.
Figure 4.5. Silver (A) and strontium (B & C) concentration in contaminated water solutions of 0.2 mg L$^{-1}$ AgNPs (red), 0.2 mg L$^{-1}$ AgNPs and 0.4 mg L$^{-1}$ strontium nitrate (orange), 0.2 mg L$^{-1}$ AgNPs and 2 mg L$^{-1}$ strontium nitrate (purple), 0.2 mg L$^{-1}$ AgNPs and 10 mg L$^{-1}$ strontium nitrate (yellow), 0.2 mg L$^{-1}$ AgNPs and 15 mg L$^{-1}$ strontium nitrate (blue) and 15 mg L$^{-1}$ strontium nitrate (green) over time with *P. stratiotes* interactions. All data are the mean of three replicate and three trial plants ± standard deviation.

The strontium ion concentrations in the contaminated water solutions decrease quickly
with the introduction of *P. stratiotes*. This calcium-substitute is removed in the same manner as silver with the largest reduction in the contaminated water solutions taking place in the first two hours and at six-hours, the removal of strontium ion levels off. Unlike silver, strontium was removed in higher concentrations from the water solutions. The uptake of strontium ion by the plant is driven by the selective absorbance of the plant in terms of its preference for these divalent cations. However, both the AgNP and strontium removal from the water solutions continues over the duration of the experiments (Gupta, *et al*., 2012).

4.4.4 Concentrations of total silver and strontium in *P. stratiotes*

Over the duration of each experiment, the plant absorbed and retained both silver and strontium from their respective contaminated water solutions. Table 4.3 shows the extent of both elements absorbed by *P. stratiotes* over the 48-hour period. The majority of the silver and strontium are concentrated in the roots. When silver and strontium ions are absorbed into the roots, they can continue their penetration by two modes of transport: the apoplastic and symplastic pathways. The apoplastic pathway is a nonselective transport mode that allows all ions and particles to proceed further into the roots, limited only by the passage size and any interactions with the cell membranes, which line the channel. Both AgNPs and strontium ions behave in this way. The symplastic pathways are more selective, which use ion channels and protein pumps (Moyen, *et al*., 2010). Since silver is unnecessary for plant metabolism, the only way it may proceed is by commandeering another element’s protein transporter, most likely one containing sulfur (Koontz, *et al*., 1980). The strontium ion, which the plant treats similar to calcium, can utilize these ion channels more easily, since there are specific uptake pumps for calcium that regularly accumulate these elements for cellular maintenance and normal cellular processes (Moyen, *et al*., 2010).
**Table 4.3.** Extent of contamination accumulation of silver (top) and strontium (bottom) in *P. stratiotes*. Percentage recovery indicates the silver and strontium removed from the contaminated solutions and retained within the plants. All data are the mean of three trials ± the trial standard deviation.

<table>
<thead>
<tr>
<th>Contaminated Water Solutions</th>
<th>Silver Concentration (µg/kg)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roots</td>
<td>Leaves</td>
</tr>
<tr>
<td>0.2 mg L(^{-1}) AgNP</td>
<td>15.6 ± 2.5</td>
<td>1.13 ± 0.08</td>
</tr>
<tr>
<td>0.2 mg L(^{-1}) AgNP &amp; 0.4 mg L(^{-1}) Sr(NO(_3))(_2)</td>
<td>29.8 ± 1.6</td>
<td>0.13 ± 0.10</td>
</tr>
<tr>
<td>0.2 mg L(^{-1}) AgNP &amp; 2 mg L(^{-1}) Sr(NO(_3))(_2)</td>
<td>33.5 ± 1.1</td>
<td>0.09 ± 0.05</td>
</tr>
<tr>
<td>0.2 mg L(^{-1}) AgNP &amp; 10 mg L(^{-1}) Sr(NO(_3))(_2)</td>
<td>21.0 ± 1.1</td>
<td>0.30 ± 0.05</td>
</tr>
<tr>
<td>0.2 mg L(^{-1}) AgNP &amp; 15 mg L(^{-1}) Sr(NO(_3))(_2)</td>
<td>25.6 ± 1.3</td>
<td>0.26 ± 0.11</td>
</tr>
<tr>
<td>20 mg L(^{-1}) Sr(NO(_3))(_2)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contaminated Water Solutions</th>
<th>Strontium Concentration (µg/kg)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roots</td>
<td>Leaves</td>
</tr>
<tr>
<td>0.2 mg L(^{-1}) AgNP</td>
<td>169 ± 22</td>
<td>34.6 ± 21.5</td>
</tr>
<tr>
<td>0.2 mg L(^{-1}) AgNP &amp; 0.4 mg L(^{-1}) Sr(NO(_3))(_2)</td>
<td>184 ± 12</td>
<td>50.1 ± 11.9</td>
</tr>
<tr>
<td>0.2 mg L(^{-1}) AgNP &amp; 2 mg L(^{-1}) Sr(NO(_3))(_2)</td>
<td>1130 ± 190</td>
<td>76 ± 35</td>
</tr>
<tr>
<td>0.2 mg L(^{-1}) AgNP &amp; 10 mg L(^{-1}) Sr(NO(_3))(_2)</td>
<td>5310 ± 330</td>
<td>278 ± 29</td>
</tr>
<tr>
<td>0.2 mg L(^{-1}) AgNP &amp; 15 mg L(^{-1}) Sr(NO(_3))(_2)</td>
<td>8880 ± 300</td>
<td>1600 ± 120</td>
</tr>
</tbody>
</table>
As mentioned previously, a majority of the silver is retained in the plant’s roots. However, some silver is able to reach the plant leaves. For plants in the 0.2 mg L\(^{-1}\) AgNP contaminated water without strontium, the silver concentration is higher in the leaves compared to those contaminated water solutions containing strontium ion, meaning that the translocation of silver is reduced for one or more of the following reasons. First, the larger nanoparticle size promoted by the strontium mediated agglomeration causes the particles to have difficulty penetrating smaller channels between cells or in entering cellular pumps (Kim, et al., 2012). Secondly, the strontium ion competes with silver to interact with the cell membranes, meaning that strontium blocks some of the silver’s activity with enzymes and in uptake channels. Finally, since the plant prefers strontium to silver for metabolic maintenance and regulation, the plant is able to maintain processes that are able to sequester silver in the roots to reduce further penetration into the plant biomass. Despite the challenges for silver to interact with the cellular processes when strontium ion is present, silver is still absorbed and retained at similar concentrations with only AgNPs in solution.

As seen from the water results in Figure 4.5, at the conclusion of the experiments a greater concentration of strontium ion was overall removed from the contaminated water solutions than silver. The plant’s preference for strontium as a substitute for calcium, leads to a greater accumulation of strontium ions within the plant’s biomass than is found for silver. A majority of the strontium resides in the plant roots, which is also observed for silver. As the concentration of strontium increases, the mass recovery of the absorbed strontium ion in the
plant biomass is greater, and the plant shows lesser amounts of wilting and discoloration in the leaves since strontium ions are accumulated within the plants as a calcium substitute to support natural biological functions such as strengthening the cell walls. It is apparent that the difference between the uptake of AgNPs and strontium shows the selectivity of the plant for specific elements. For the plants used in the 20 mg L\(^{-1}\) strontium containing water, a recovery of 128% was observed, as the plants in this batch may have held more strontium initially than the control plants used in comparison. The silver and strontium concentrations not recovered in the plant biomass were attached to the outside of the roots and proven in previous research to be removed from the root surface when washed (Hanks, et al., 2015).

4.4.5 ROS production

AgNPs have been found to enhance luminol fluorescence and chemiluminescence by acting as a catalyst with hydrogen peroxide to form other reactive oxygen species such as hydroxyl radical and superoxide that also react with luminol (Panzarasa, 2014). Figure 4.6 shows the fluorescence emission intensity for AgNPs in solution with hydrogen peroxide and luminol, indicated by the blue line. When AgNPs are in solution with strontium nitrate, the intensity of the luminol fluorescence (green dotted line) is much lower than when strontium is absent from solution. Apparently, strontium not only causes aggregation of AgNPs, but also decreases their catalytic interactions with hydrogen peroxide. Therefore, with strontium ion present in AgNP solutions, the signal produced by luminol reacting with hydrogen peroxide is no longer amplified by the AgNP degradation products reacting with hydrogen peroxide since this reaction is quenched. With strontium ion and AgNPs in solution, the intensity observed for hydrogen peroxide and luminol is the same as the intensity observed for hydrogen peroxide and luminol without any AgNPs present. AgNP and luminol concentrations remain the same in each
solution and the strontium ions stop the catalytic process between AgNPs and hydrogen peroxide. The overall fluorescence intensities of strontium containing solutions may be compared to each other, but in comparing AgNP solutions to those with silver nitrate, they may vary slightly with intensity. Therefore, only similar concentrations and silver forms are compared.

Figure 4.6. Fluorescence intensity of AgNPs with hydrogen peroxide and luminol (blue), strontium nitrate, AgNPs, hydrogen peroxide, and luminol (- - - dotted green) and only hydrogen peroxide and luminol (black).

Figure 4.7 shows the fluorescence intensities for ROS production of hydrogen peroxide, superoxide radical, and hydroxide ion promoted by silver interactions within *P. stratiotes* leaves. The production of these ROS is in agreement with work previously published (Cash, *et al.*, 2007) (Jones, *et al.*, 2011). Both strontium nitrate and calcium nitrate were tested to verify results, since they both are divalent cations and share the same metabolic pathways within the plant. Calcium has the same ability to agglomerate AgNPs (E= -1.27v) as strontium ions (E= -1.3v) and does not chemically alter the AgNPs.
Figure 4.7. Effect of 10 µg L\(^{-1}\) silver nitrate (A) and 10 µg L\(^{-1}\) AgNP with calcium (gray) and strontium (black) on the production of ROS in *P. stratiotes* leaf disks. All data are the mean of 30 trials ± the standard deviation.

When 30 µg L\(^{-1}\) strontium and calcium ions are present alone in solution with the leaf disk, they produce similar fluorescence intensities. Calcium is known to activate ROS generation within the cell’s mitochondria due to a close relationship between the oxidative and calcium dependent pathways (Kreslaviski, *et al.*, 2012). As strontium ion interacts with the plant’s metabolic system, it utilizes the calcium uptake pathways and transport proteins. This shared use of the same metabolic pathways for strontium and calcium ions should show similar changes in ROS production when these compounds are present with the different silver forms. In silver
nitrate solutions, the production of ROS remains constant when strontium and calcium ions are present. Despite the increase of both strontium and calcium ion concentrations, the production of ROS is not dependent on the concentration of these divalent cations.

For the AgNP solutions tested for ROS, the fluorescence signal declines with increased divalent cation concentration, indicating that the agglomeration of AgNPs, as discussed above, does have an impact on the ROS produced. These results suggest that the toxicity of the nanoparticles decreases with increased elemental interactions by calcium and strontium ions, allowing the plant to remain green and healthy over time. Decreasing AgNP toxicity with increased levels of divalent cations is dependent on the AgNP size and catalytic activity. The strontium and calcium ions reduce the charge dependent double layer around the nanoparticle, which would normally repel the particles from each other, therein forming larger particles, unable to penetrate further into the plant because of the reduced surface area, thereby mitigating the toxicity. This decrease of available surface area to the nanoparticle also allows for a change in the catalytic properties. Not only does the catalytic reaction utilizing AgNPs to convert hydrogen peroxide into other ROS promote intensity amplification, but the reaction also creates more ROS mediated damage within the plant. While hydrogen peroxide generation within the plant is exacerbated by the presence of nanoparticles, the onset of more ROS produced from the catalytic reaction and the oxidation of the nanoparticles into silver ions causes a higher incidence of silver toxicity than with the plant interacting only with the silver ions.
Figure 4.8. Effect of 10 μg L⁻¹ silver nitrate (A) and 10 μg L⁻¹ AgNP with sodium nitrate on the production of ROS in *P. stratiotes* leaf disks. All data are the mean of 30 trials ± the standard deviation of all trials.

When sodium is present in solution with AgNPs, there is not a physical change in AgNP shape or size (E⁰ = -3.51v) (Figure 4.8). The production of ROS is instead driven by the presence of sodium. While sodium is normally used within the plant for building proteins and photosynthesis, the generation of salt stress due to excess sodium ions disturbs the capacity of cells to extract water and inhibits nutrient uptake (Abogadallah, *et al.*, 2010). Salt stress increases the ROS production within the leaves. As the sodium ion concentration increased in the
presence of silver ions, the observed fluorescence intensity increased linearly. This implies that the ROS production observed by the luminol is driven by the sodium ion concentration within the plant leaf. AgNP solutions with sodium also follow this increased salt stress increased with higher sodium ion concentrations. However, the stress is exacerbated by the presence of AgNPs. The ROS produced due to the AgNP’s catalytic abilities promotes more ROS within the plant than when it is compared with the high sodium ion solution without AgNPs.

Sodium terephthalate also was used to detect the production of hydroxyl radical. There were no detectable amounts of fluorescence produced when using sodium terephthalate. This indicates that hydroxyl radicals are not produced in large amounts via promotion by any form of silver, when in solution with strontium nitrate, calcium nitrate and sodium nitrate.

4.5 Conclusion

With the presence of divalent cations such as those from strontium ion in AgNP contaminated solutions, the aggregation of the AgNPs influences the toxic effects on *P. stratiotes*. With increased strontium ion concentrations, the plant remains healthy and green, while still able to remove silver to a lower concentration than the WHO MCL, within 12 hours from introduction to the contaminated waters. The strontium ion uptake is greater than the silver uptake, which allows the plant to sustain normal metabolic functions. The strontium ion uptake also altered physical characteristics of the AgNPs to decrease any catalytic interactions with the ROS produced. However, when AgNPs are present and the plant is under salt stress, ROS production can be amplified by AgNP and hydrogen peroxide degradation into other ROS, causing more damage to the plant. Overall, the phytoremediation pond is more sustainable with divalent cations when working to remove AgNPs than without these ions present in the pond water, as they help to reduce the production of ROS mediated damage, even though *P. stratiotes*
are still able to remove AgNPs just as effectively without divalent cations present.

4.6 References


Jin Xue [et al.] High-Throughput Screening of Silver Nanoparticle Stability and Bacterial Inactivation in Aquatic Media: Influence of Specific Ions [Journal] // Environmental


McCUTCHEON Steve C. and Schnoor Jerald L. Phytoremediation: Transformation and control of


Chapter Five | Silver nanoparticle and silver ion removal using point-of-use filtration media

5.1 Abstract

With silver nanoparticles (AgNPs) and silver ions utilized in consumer products, these contaminants are becoming prevalent in source waters. In an effort to identify point-of-use filtration media that would be promising in reducing silver forms for drinking water, 0.2 and 1.5 mg L\(^{-1}\) silver nitrate and AgNP contaminated waters were passed through single medium gravity filters of activated carbon, silica, ceramic sphere and KDF-55 grains. Total silver concentration was monitored in effluent waters to identify which filters reduce silver concentration in the water under the 0.1 mg L\(^{-1}\) World Health Organization maximum contaminant level for drinking water. Activated carbon and KDF-55 are the only media able to reduce 0.2 mg L\(^{-1}\) contaminants concentrations for both silver forms. However, all filtration media were unable to successfully reduce silver forms at contaminant level 1.5 mg L\(^{-1}\) under the tested conditions.

5.2 Introduction

Finding efficient methods of heavy metals from a contaminated water source is crucial for achieving clean drinking water. Heavy metals tend to accumulate in living organisms and cause health disorders and diseases (Ahamed, et al., 2010) (Kim, et al., 2010) (Greulich, et al., 2011). Unlike organic contaminants, heavy metals do not degrade and are not easily removed from the water system. For this reason, most large-scale water treatment facilities rely on coagulation to chemically precipitate metals from the source water and on sieve filtration to mechanically remove metal particles (Abbott Chalew, et al., 2013) (Li, et al., 2013) (Sun, et al., 2013). However, these methods are expensive and complicated to employ in individual homes for personal use.
Instead, many personal water filtration systems rely on in-line or gravity filtration systems. These point-of-use filtration systems are placed just before the tap or used directly before direct exposure to the individual. Point-of-use filtration systems like the ones previously mentioned are the last resort to remove contaminants before direct ingestion or through secondary exposure by cooking and cleaning by the end user. The filter media utilized in these filtration systems need to have good permeability, they need to be hard and durable, be free of impurities and be insoluble in the water they are filtering (Agency, 1995).

Common point-of-use filtration removal systems utilize various types of media to remove contaminants. For gravity type filtration systems, the filter medium can be mechanically or chemically driven to remove contaminants. The most common methods of contaminant removal by filtration media rely on mechanical remove large particulates, adsorption, absorption and redox or chemical reactions to produce safe drinking water (Agency, 1995). The porous filter medium allows more surface area to interact with contaminants in the water and creates smaller pathways to mechanically remove particulates within the medium. Therefore, it is important to assess the capabilities of each filter type to identify the best filter medium to help remove or reduce specific contaminants.

One of the heavy metals that is gaining momentum as a hazard in drinking water is silver. Silver, although very toxic, was previously not considered a hazard because it is easily precipitated out of the environment with environmental ions causing the overall silver levels to be low enough that it was not considered a threat (Cong, et al., 2011). While silver ions are easily removed from drinking water sources through natural interactions with ions like chloride and sulfates, silver nanoparticles (AgNPs) pose a new threat to drinking water. AgNPs are the transitional phase between the ion and bulk forms of silver. Nanoparticles
are between 0 and 100 nm in size and have a large surface area that can amplify the intensity of chemical properties. For AgNPs, the antibacterial abilities of silver tend to promote more intense interactions with the surrounding micro- and macro-organisms (Kim, et al., 2012). When the AgNP is oxidized by surrounding conditions such as temperature, chemical interactions or pH, the nanoparticle becomes a Trojan horse by releasing silver ions (Kittler, et al., 2010) (Park, et al., 2010). However, AgNPs may be altered to have stabilizing surface coatings that help the nanoparticles in conditions that would normally induce oxidation or aggregation and therefore allow the AgNPs to remain as a threat to drinking water sources. The World Health Organization (WHO) has placed the maximum contamination level (MCL) at 0.1 mg L\(^{-1}\) in drinking water as higher concentrations promote kidney damage and argyria (Cong, et al., 2011).

Silver contamination in natural waters continues to increase due to the use and disposal of silver containing consumer products. This mass usage of silver products causes the environmental waters around industrial and urban areas to be 20% higher in silver concentrations (Abbott Chalew, et al., 2013). Products such as clothing, paints and even water purification devices are employing silver ions and AgNPs to reduce or eliminate bacterial growth (Benn, et al., 2008) (Rai, et al., 2009) (Ahamed, et al., 2010). These consumer products continue to grow in popularity, causing AgNPs to be the most widely applied nanoparticle material, making up 50% of the entire consumer product market for nanoparticles (The Project on Emerging Nanotechnologies, 2013).

This research assesses the removal capabilities of activated carbon, silica, ceramic spheres, and KDF-55 filtration media for the removal of silver ions and nanoparticles from contaminated water. Assessment is made by monitoring the total silver introduction into the filter and the carry over once the silver contaminants are no longer present in the water passing
through the filter media below the WHO MCL of 0.1 mg L\(^{-1}\). All media analysis was accomplished by scanning electron microscope and the water concentrations were monitored with inductively coupled plasma mass spectrometry.

5.3 Methods and materials

All solutions were prepared in 18 MΩ cm\(^{-1}\) doubly deionized water (DDIW) (Sybron/Barnstead, Boston, MA). All chemicals unless otherwise stated were purchased from Fisher Scientific (Watham, MA) and were used without further purification. All glassware used for experimentation was acid washed in 10% nitric acid, washed thoroughly with DDIW and dried before use.

5.3.1 AgNP synthesis and characterization

AgNPs were synthesized by reducing silver nitrate with sodium borohydride. No additional modifications to the nanoparticles were completed. AgNP solutions were refrigerated and stored in the dark until further use in experimentation. For characterization, synthesized nanoparticle solutions were analyzed for AgNP size distribution and zeta potential using Microtrac size analyzer (Nikkiso Co., Tokyo, Japan) and U-3900 Hitachi spectrometer (Hitachi Ltd., New Orleans, LA). The average size distribution of the AgNP solutions was 20 ± 8 nm.

5.3.2 Filtration of contaminated water setup and sampling

The filter media tested were coconut shell activated carbon (Botl Inc., Canada), silica (E.M. Science, Gibbstown, NJ), ceramic sphere and rough KDF-55 bulk media (PureWater Site, Sandpoint, ID). All filtration media was tested without chemical cleaning. Filters were made of single media composition in a gravity filter with the total volume of 14.2 cm\(^3\). The inlet water reservoir holds 25 mL, and the outlet filter water holds up to 50 mL. All DDIW and Ag contaminated waters were pH neutral and 21°C in temperature.
Testing of each filtration medium was completed in the following sequential steps:

1) Rinse filtration medium with 50 mL of DDIW to remove any dust and particulates available on the granular surface and collect effluent for analysis.

2) Introduce 25 mL of silver contaminants to the filter bed and collect effluent for analysis.

3) Introduce 25 mL of the same silver contaminant to the filter bed and collect effluent for analysis.

4) Rinse filtration bed with 25 mL of DDIW and collect effluent for analysis.

5) Rinse filtration bed with 25 mL of DDIW and collect effluent for analysis.

6) Rinse filtration bed with 50 mL of DDIW and collect effluent for analysis.

Each medium composition was completed in triplicate for each contaminated water concentration and silver form. Each collection point post filter water was also collected in triplicate for analysis of total silver concentration by inductively coupled plasma mass spectrometry (ICP-MS).

At the end of experimentation with each filter, the filter media was oven dried at 120°C until dry. Media was then stored in an airtight container until analysis with scanning electron microscope (SEM) equipped with X-ray desorption spectroscopy (EDS).

### 5.3.3 Inductively Coupled Plasma Mass Spectrometry Analysis

All samples were analyzed on the Agilent 8800-QQQ ICP-MS (Agilent Technologies, Santa Clara, CA) for total silver concentration. Silver was monitored using m/z $^{107}$Ag and $^{109}$Ag at 3 mL/min of helium. An internal standard of $^{89}$Y and $^{115}$In were used to monitor instrumental drift and ensure consistency in matrix makeup. Calibration standards were used to tune the ICP-MS with a range of 0 to 0.1 mg L$^{-1}$ and verified with the drinking water standard reference material from the National Research Council-Canada (Ottawa, Canada) for quantification.
accuracy. The limit of detection (LOD) of silver was 0.08 μg L⁻¹.

5.3.4 Scanning Electron Microscope Analysis

A Philips XL30 environmental scanning electron microscope field emission gun (FEI, Hillsboro, OR) equipped with TEAM™ energy dispersive X-ray spectroscopy analysis system (EDAX Inc., Mahwah, NJ) was used in this research in environmental mode. A dry sample of filtration media was analyzed before and after use to remove AgNPs from 1.5 mg L⁻¹ contaminated waters. Chemical compositions of filtration media were analyzed with EDS to identify the surface elemental composition. Images acquired with SEM were used to understand the particle size, pore size, and surface structure.

5.3.5 Data Analysis

All results are reported as the mean of all replicates ± the standard deviation of replicates. The samples less than the LOD were reported as half of the LOD value. All recoveries for silver contaminant found in filtered water are the sum of all concentration values collected at each volume and divided by the total concentration in the contaminated water. Averaging relative dimensions (length, width and diameter) of filtration media from SEM images accomplished particle and pore size measurements.

5.4 Results and Discussion

5.4.1 Coconut Shell Activated Carbon

The most common point-of-use filtration media is activated carbon. Activated carbons are able to absorb organic and inorganic contaminants and are more commonly used to reduce any flavor or odor in the water (Agency, 1995) (Sutherland, 2008). In addition to the high surface area, activated carbon can chemically interact with heavy metals in the water. Most carbon materials like activated carbon are known to have high adsorption capabilities towards metal ions
The pores of the carbon trap large particles and increase the available surface for contaminants to interact. Table 5.1 shows the overall size distribution of the activated carbon grains, average pore diameter and elemental composition of the activated carbon used for experimentation. The carbon has a rough exterior with many cavities and pathways for particles and ions in the water to filter (Figure 5.1).

Table 5.1. Activated carbon physical properties and chemical composition.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Grain Length:</td>
<td>614 ± 132 μm</td>
</tr>
<tr>
<td>Average Grain Width:</td>
<td>419 ± 183 μm</td>
</tr>
<tr>
<td>Average Pore Diameter:</td>
<td>5.85 ± 6.05 μm</td>
</tr>
<tr>
<td>Weight of Media Bed:</td>
<td>7.51 g</td>
</tr>
<tr>
<td>Elemental Composition (Weight %):</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>93.2%</td>
</tr>
<tr>
<td>O</td>
<td>5.53%</td>
</tr>
<tr>
<td>Al</td>
<td>0.48%</td>
</tr>
</tbody>
</table>

Figure 5.1. SEM image of activated carbon grains (Left) and zoomed in image of grain pore size and surface texture (Right).
When a silver ion solution of 0.2 mg L\(^{-1}\) is introduced to the activated carbon bed, the silver concentration is decreased to 88.3 mg L\(^{-1}\) in the effluent (Figure 5.2). The bed is able to retain a majority of the introduced contaminant and is not released in the DDIW wash after the contaminant introduction. Even when the silver ion contamination level is at 1.5 mg L\(^{-1}\), the carbon retains 92.0% of contaminant. Thus, the contamination is easily removed by the filtration medium and is no longer a threat. This data agrees with previously published research about silver ion adsorption by activated carbon (Jia, et al., 2003) (Lopez-Ramon, et al., 2003) (Song, et al., 2011).

The catalytic ability of activated carbon to reduce heavy metals is due to unpaired electron sites on the crystalline edges (Jia, et al., 2003). These edge regions are where the oxygen functional groups are located. Previous studies have shown that carbon surfaces are made up of graphene layer surfaces and oxygen containing functional groups on the edges (Jia, et al., 2003). The most common oxygen functional groups on the carbon surface are lactone, carboxyl, phenol/quinine, carbonyl and ether groups (Srivastava, et al., 2008). Furthermore, quinine functional groups are considered responsible for the activated carbon reactivity along with lactones that participate in redox reactions. The carboxyl groups are responsible for adsorption of heavy metal ions, such as silver, by participating in ion exchange mechanisms (Jia, et al., 2003).

For AgNP removal, activated carbon does not retain enough contaminant concentration in the effluent water to be under the WHO MCL. Only 36.7% of the 0.2 mg L\(^{-1}\) and 10.1% of the 1.5 mg L\(^{-1}\) is retained within the activated carbon filtration bed. The AgNPs continue to elute when the column is rinsed with DDIW. This continued elution of AgNPs during the bed rinse indicates that the AgNPs are moving though the filter bed through the pores and different
pathways between the carbon grains to achieve varying retention times. In addition, if any AgNPs were weakly adsorbed to the surface or trapped in the pores of the carbon, they would have a longer retention time in the filtration bed. Therefore, this filtration medium is applicable for silver ion removal even in high concentrations, but not for the removal of higher concentrations of AgNPs. The chemical interaction between the silver ions and the functional groups on the activated carbon is much more effective than the mechanically reliant removal of AgNPs.

![Graph showing silver concentration in effluent water from activated carbon filter bed system for 0.2 mg L$^{-1}$ (A) and 1.5 mg L$^{-1}$ (B) contaminant level of AgNPs (- - -) and AgNO$_3$ (——). All results are presented as the mean of three replicate filters ± the standard deviation.]

**Figure 5.2.** Silver concentration in effluent water from activated carbon filter bed system for 0.2 mg L$^{-1}$ (A) and 1.5 mg L$^{-1}$ (B) contaminant level of AgNPs (- - -) and AgNO$_3$ (——). All results are presented as the mean of three replicate filters ± the standard deviation.

### 5.4.2 Silica

Silica sand filtration is commonly used for rapid and slow rate filters. Silica medium is extremely common because it is cheap to obtain and easy to back flush the medium to rejuvenate the filtration bed (Logsdon, 2008). Silica is mechanically driven for filtration purposes and is
effective for removing large particulates such as dirt and rust. This research utilizes a smaller grain silica in an effort to trap the AgNPs mechanically, as they are not chemically active with the silica (Reddy, et al., 2014) (Table 5.2). The smooth surface of the silica does not provide additional pathways via pores for water to move through the bed (Figure 5.3). Therefore, the water can only move between the grains.

Table 5.2. Silica physical properties and chemical composition.

<table>
<thead>
<tr>
<th></th>
<th>Average Grain Length: 112 ± 34.9 μm</th>
<th>Average Grain Width: 57.1 ± 19.8 μm</th>
<th>Average Pore Diameter: Not Applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elemental Composition</td>
<td>Si (38.8%)</td>
<td>O (52.6%)</td>
<td>C (8.03%)</td>
</tr>
<tr>
<td>(Weight %)</td>
<td></td>
<td></td>
<td>Al (0.57%)</td>
</tr>
<tr>
<td>Weight of Media Bed</td>
<td>7.48 g</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The silica grains were able to retain 6.72% of the silver ion contaminant at 0.2 mg L⁻¹ (Figure 5.4). At the 1.5 mg L⁻¹ contamination level, the silica grains retained 7.45% of the silver ions. This removal of ionic silver is extremely low and may have resulted from interactions with the trace levels of carbon and aluminum available on the silica surface for redox interactions. Other articles have indicated that the removal of low levels of heavy metals is due to impurities in the sand (Reddy, et al., 2014). Most all the ion contaminant is quickly eluted in the first 25 mL wash of DDIW. The nanoparticle retention is much lower with 1.29% and 3.01% at 0.2 and 1.5 mg L⁻¹, respectively. The nanoparticles act in the same manner as the ion contaminants through the silica filtration bed.
Figure 5.3. SEM image of silica grains (Left) and zoomed in image of surface texture (Right).

Figure 5.4. Silver concentration in effluent water from silica filter bed system for 0.2 mg L\textsuperscript{-1} (A) and 1.5 mg L\textsuperscript{-1} (B) contaminant level of AgNPs (\textendash \textendash) and AgNO\textsubscript{3} (\textemdash\textemdash). All results are presented as the mean of three replicate filters \(\pm\) the standard deviation.

As shown here, silica or silicon dioxide is chemically inert and is only able to
mechanically remove particulates larger than the pores between granulates. Since silica employed systems do not provide chemical means of removing contaminants, this filtration medium would require the use of another method to remove heavy metal contamination in the water and should not be used as a standalone method to remove either ion or nanoparticle forms (Reddy, et al., 2014). Other than relying on mixed bed systems to remove contaminants, many new forms of coated silica sands are proposed for removal of contaminants by chemically activating the surface of the sand to react with specific organic or inorganic compounds in the water (Tzvetkova, et al., 2010). In addition to the use of silica form mechanical filtration, many slow-sand filters promote biological film growth to mediate the removal of heavy metals because the silica is inert. Unfortunately, the use of biofilms to remove silver reduce or altogether stop the biofilm growth because silver is a strong antimicrobial (Miao, et al., 2010) (Cong, et al., 2011) (Kim, et al., 2012) (Reidy, et al., 2013).

5.4.3 Ceramic Sphere

Ceramic filtration is one of the oldest filtration techniques due to the longevity of the medium and its simple application. These filters are particularly effective in systems under high fluid temperatures and corrosive materials that would normally cause physical destruction to other filtration media forms (Sutherland, 2008). Ceramics rely on the micropores created when the clay is heated in a kiln and any moisture left in the clay evaporates. This creates random pores and channels throughout the ceramic. Variations in pore size derive from the type of clay utilized and the moisture content at the time of baking (Benfer, et al., 2004).
Table 5.3. Ceramic sphere physical properties and chemical composition.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Grain Diameter:</td>
<td>2.75 ± 0.39 mm</td>
</tr>
<tr>
<td>Average Pore Length:</td>
<td>19.3 ± 11.4 μm</td>
</tr>
<tr>
<td>Average Pore Width:</td>
<td>6.22 ± 3.64 μm</td>
</tr>
<tr>
<td>Weight of Media Bed:</td>
<td>21.0 g</td>
</tr>
</tbody>
</table>

Elemental Composition (Weight %):
- O (51.7%)
- Zn (19.2%)
- Si (14.6%)
- Mg (6.09%)
- C (3.66%)
- S (2.17%)
- Ca (1.25%)
- Al (1.07%)

The ceramic spheres used for filtration contained the largest grain size of all bed media tested (Table 5.3). Figure 5.5 shows a rough surface with large fissures of varying length covering the ceramic. These cracks work mechanically, like the pores on activated carbon, to remove particulate contaminants and increase the surface area to interact. In addition to physical means of removal, this ceramic was coated with magnesium, calcium and silicone oxides to promote redox reactions on the ceramic surface (Table 5.3).

When silver ion contaminants are introduced to the ceramic sphere bed, 61.1% is retained from the 0.2 mg L\(^{-1}\) contamination level, reducing the silver concentration to a safe drinking level under 0.1 mg L\(^{-1}\). At the higher contamination of 1.5 mg L\(^{-1}\), 64.9% was retained; however, this is not a high enough retention concentration to reduce the silver below the WHO MCL. The ion removal of the ceramic surface is promoted by the magnesium and calcium oxides participating in redox reactions.
The AgNP contaminants were not as effectively removed from the inlet water as the ions. 26.7% was retained by the ceramic medium at the contamination level of 0.2 mg L\(^{-1}\). Unfortunately, this retaining efficiency for ceramic was not efficient in reducing the contamination to safe drinking levels. At 1.5 mg L\(^{-1}\), the sphere bed retained 10.02% of the nanoparticle contamination. The ceramic is unable to remove enough silver to produce safe drinking water. In addition, both forms of silver elute in the 50 mL of rinse at higher concentrations than any of the tested filtration bed material, indicating they are loosely retained in the medium and will elute gradually in the next 50 mL of water passing through the bed.

Even though the ceramic was chemically altered to promote redox reactions with contaminants, it was incapable of removing enough silver at higher concentrations for either form. At the lower concentration level, the ceramic was only successful at removing lower level contamination of silver ions. Therefore, this medium does not provide the ideal removal
capabilities for a silver contaminated water source. Instead, a mixed media bed or a layered bed may promote more reduction of silver forms. This is implicated by the large channels for water to pass through between the spheres compared to the other medium that the grains sit flush to each other.

![Figure 5.6](image)

**Figure 5.6.** Silver concentration in effluent water from ceramic ball filter bed system for 0.2 mg L\(^{-1}\) (A) and 1.5 mg L\(^{-1}\) (B) contaminant level of AgNPs (— —) and AgNO\(_3\) (——). All results are presented as the mean of three replicate filters ± the standard deviation.

### 5.4.4 KDF-55

KDF-55 is a 50% copper 50% zinc alloy filtration medium designed to remove heavy metal contaminants with its redox active surface (KDF Fluid Treatment, 2003). This type of medium is suggested for use before other forms of filtration medium in order to promote longevity of the filtration beds and reduce the growth of bacteria (KDF Fluid Treatment, 2003). KDF-55 is recyclable and therefore more environmentally friendly than some of the other
filtration media that are reliant on biofilms such as silica beds or that become biohazard material after use, as in the case for activated carbon.

**Table 5.4.** KDF-55 physical properties and chemical composition.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Grain Length</td>
<td>2.55 ± 1.54 mm</td>
</tr>
<tr>
<td>Average Grain Width</td>
<td>95.2 ± 64.4 μm</td>
</tr>
<tr>
<td>Average Valley Length</td>
<td>5.36 ± 2.69 μm</td>
</tr>
<tr>
<td>Average Valley Width</td>
<td>1.33 ± 0.71 μm</td>
</tr>
<tr>
<td>Weight of Media Bed</td>
<td>40.4 g</td>
</tr>
<tr>
<td><strong>Elemental Composition</strong></td>
<td></td>
</tr>
<tr>
<td><strong>(Weight %):</strong></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>(28.0%)</td>
</tr>
<tr>
<td>C</td>
<td>(25.3%)</td>
</tr>
<tr>
<td>Cu</td>
<td>(23.4%)</td>
</tr>
<tr>
<td>O</td>
<td>(14.8%)</td>
</tr>
<tr>
<td>Al</td>
<td>(8.48%)</td>
</tr>
</tbody>
</table>

KDF-55 comes in varying granular sizes, allowing smaller granular sizes to nestle between larger alloy grains (Table 5.4). The grains are valley like channels that do not traverse through the alloy, but amplify the surface area on top of each alloy grain (Figure 5.7). This foiled surface and inconsistent grain size allow varying pathways to form between the tightly packed bed. Copper and zinc are redox active with silver ions and the presence of zinc or copper oxides may promote the chemical activity with AgNPs.
KDF-55 was the most promising filtration medium to remove low levels of silver forms. At 0.2 mg L\(^{-1}\), KDF-55 retained 75.9% silver ions and 74.9% AgNPs. This medium has the highest retention out of all tested media for AgNPs. Even at the highest concentration of 1.5 mg L\(^{-1}\), KDF-55 retained 41.9% of the AgNP contaminant. This removal was not high enough to produce safe drinking water, but it has better removal capabilities than any other filter bed for AgNPs. At 1.5 mg L\(^{-1}\) silver ion contaminated waters, the removal was less efficient with a 37.5% retention capacity.
Figure 5.8. Silver concentration in effluent water from KDF-55 filter bed system for 0.2 mg L\(^{-1}\) (A) and 1.5 mg L\(^{-1}\) (B) contaminant level of AgNPs (---) and AgNO\(_3\) (-----). All results are presented as the mean of three replicate filters ± the standard deviation.

In a KDF-55 medium filtration bed, the lower contaminations of silver forms are reduced to safe drinking levels. Higher contamination levels are less successfully reduced than at the lower level contamination, but KDF-55 is still the most promising for AgNP removal. While this medium is not as widely applied to filtration systems, it is a promising technique to reduce heavy metal contamination in drinking water.

5.5 Conclusion

This research indicates that the removal capabilities of single medium filtration beds with a 14.2 cm\(^3\) volume are not capable of removing high concentrations of AgNPs or silver ions. Activated carbon is the only filtration medium to reduce ~90% of the 0.2 and 1.5 mg L\(^{-1}\) silver ion contaminated waters to safe levels below the WHO MCL. At 0.2 mg L\(^{-1}\) contamination
concentration of silver ions and AgNPs, activated carbon and KDF-55 were the only tested single medium beds to reduce both silver forms to safe drinking levels below 0.1 mg L⁻¹. KDF-55 was more promising for nanoparticle removal whereas silver ion removal was better with activated carbon. Therefore, lower silver contamination is manageable by common filtration media, but for higher contamination levels, techniques such as coagulation (Sun, et al., 2013), flocculation (Shafer, et al., 1998), and phytoremediation (Hanks, et al., 2015) may prove to be more efficient removal techniques.

5.6 References


Srivastava Vimal Chandra, Mall Indra Deo and Mishra Indra Mani Adsorption of toxic metal...


Chapter Six | Conclusion and Future Work

This dissertation covers the remediation of silver ion and AgNP contaminated waters by phytoremediation and point-of-use filtration media to produce safe drinking water. Both methods of water remediation were successful in reducing the concentration of silver forms in water.

Phytoremediation with *P. stratiotes* showed competitive results for the removal of both silver forms at 0.2 mg L\(^{-1}\) concentration to safe drinking levels below the WHO MCL within the first hour of contact. At the higher concentration level of 2 mg L\(^{-1}\), the plants did not remove enough silver in the first several hours, but were able to produce safe drinking water within 24 hours of exposure. The plants underwent higher intensity physical degradation with higher concentrations of silver forms present in the media as the silver went unopposed within the plant for cellular interactions.

When strontium was present in solution with AgNPs, the nanoparticles underwent agglomeration due to a disruption of electrostatic repulsion between particles mediated by strontium’s divalent cationic charge. This interaction allowed for the formation of larger particle agglomerates and a wider distribution of particle sizes to be present in solutions tested by *P. stratiotes*. The plants interacting with these strontium rich solutions showed a reduction of physical deterioration due to an increase of strontium concentration. Despite the change in plant health and strontium concentration, *P. stratiotes* remained consistent in removing AgNP contaminants to safe drinking levels and internal accumulation by the plant of total silver removed from the contaminated waters. However, less silver was translocated to the leaves when strontium was present, as strontium competed for cellular interactions within the plant and the AgNP size increase reduced the continued penetration through smaller channels within and
between cell membranes.

In order to identify the toxicity of silver forms within *P. stratiotes* plant leaves, ROS generation was observed using fluorescent detection. When in solution with silver ions and divalent cations (calcium and strontium), ROS production remained consistent despite variation in the cation concentration. However, solutions containing silver nanoparticles (AgNPs) with the divalent cations showed a reduction of detected ROS. Divalent cations also quenched side reactions between hydrogen peroxide and AgNPs that produce other ROS such as superoxide radical. Nonetheless, when *P. stratiotes* leaves were in solutions of sodium and silver ions, the ROS production was a linear relationship to the concentration of sodium. Conversely, sodium solutions with AgNPs promoted higher intensities of ROS production than the plants would experience under the same sodium concentrations without AgNPs present. Overall, *P. stratiotes* was effective in phytoremediation of silver ion and nanoparticle contaminated waters to produce safe drinking water. The toxic production of ROS was shown to vary depending on what silver form and environmental ions were present.

Point-of-use filtration media for gravity filtration of AgNP and ion contaminants were also examined. Activated carbon and KDF-55 proved to remove high enough concentrations of both silver forms at the contamination level of 0.2 mg L\(^{-1}\). When higher concentrations of 1.5 mg L\(^{-1}\) silver forms are present, all filtration media proved to be incapable of removing ample silver concentrations to provide safe drinking water. The analysis of silica proved that mechanical removal was not enough to reduce the silver forms to safe drinking levels. The ceramic also showed difficulty in removing silver at concentration level 0.2 mg L\(^{-1}\), showing that even chemically treated media may not provide enough removal capacity or chemical activity to produce safe drinking water. Therefore, media bed size, composition, and concentration of
contaminant introduced to the filtration system mediate the efficiency to remove enough silver forms.

Future studies involving phytoremediation with *P. stratiotes* would further investigate protein signaling within the plant to identify proteins with silver affinities as well as any inhibition of normal protein functions. In addition, quantification of ROS production with ROS specific detection dyes would provide further understanding of ROS production when AgNPs and ions are present in the plant with environmental ions. Furthermore, point-of-use filtration media would be analyzed for contaminant filtration bed capacity and bed size to remove silver forms. Further analysis of other filtration media, such as cation and ion exchange resins, may prove to have higher capacities for chemical removal of silver ions and nanoparticles for clean drinking water. For all types of water remediation, variations in nanoparticle coatings (citrate, PVP), water composition (salt content, pH) and variation of metal nanoparticles and metal ions (ZnO2, TiO2, Au) may be applied to provide a more inclusive understanding of heavy metal nanoparticle and ion removal for safe drinking water.
Appendix 1: Cloud Point Extraction

Extraction and speciation techniques

For many real world samples, the determination of trace analytes at low concentrations can be challenging and is often a problem faced by analytical chemists. To solve this problem, chemists use extraction techniques to isolate the target analytes from the sample matrix. This in turn can reduce, control or eliminate matrix interferences, but also presents an opportunity to pre-concentrate select analytes to levels determinable by analytical instrumentation.

Cloud point extraction

Cloud point extraction (CPE) is an extraction process that pre-concentrates a select species of interest (i.e. molecule, ion, or nanoparticle). This is completed by utilizing a phase separation, induced by heat, to concentrate the target species into the surfactant phase. This includes separating different forms of complexes such as metal nanoparticles from their ion counterparts. CPE is used in situations where the analyte of interest’s concentration is lower than the detection limits in its current state, which would also require a technique to help concentrate the material for analysis. This technique does not use highly toxic chemical solutions such as those used for chromatographic techniques. The surfactants used in CPE are non-flammable, effective at low concentrations and present low volatility, which minimize risks in the extraction process (Ojeda, et al., 2012). The first published process for CPE was in 1976 using Triton X-100 to extract Ni (II) with 1-2-thiazolylazo-2-naphthol as the ligand (Mirua, et al., 1976). Since then, applications continue to grow targeting separations of metals, proteins, organic molecules, and nanomaterials. More information on CPE may be found in the cited references (Paleologos, et al., 2005) (Kilinc, et al., 2009) (Ojeda, et al., 2012) (Hartmann, et al., 2014).
Theory

CPE is an easy extraction and pre-concentration procedure to complete (Figure A1). Typically, a nonionic surfactant is added to an aqueous sample containing the interested species. The concentration of the chosen surfactant must be over the critical micelle concentration (CMC) of the detergent. This allows the surfactant to organize and form micelles, an aggregate of detergent molecules. To allow for metal ion extraction, a chelating agent is required. The chelating agent facilitates a partitioning of metal ions to the micelle by forming a hydrophobic complex with the metal. The separation takes place when the solution is heated to its cold point temperature (CPT). Once the CPT is reached, the solution becomes cloudy and separates into the aqueous phase and the surfactant rich phase. The aqueous phase contains salts and a low concentration of the surfactant under the CMC level. Below this layer is the surfactant rich phase that contains a majority of the detergent. This phase is significantly smaller in volume than the initial sample allowing the target species to accumulate and concentrate. Upon cooling below the CPT and by mixing, this phase separation may be reversed. Optimizing CPE methods involve choosing appropriate concentrations and type of detergent, ionic strength, chelating agent, temperature, pH, and equilibrium time. This is normally completed using systematic optimization by trial and error.
Figure A1. A scheme showing the extraction of a target analyte by CPE.

Surfactants

There are several types of surfactants that are used for CPE to isolate analytes of interest. These surfactants are listed in Figure A2. Usually, the detergents used for CPE are nonionic; however, mixtures of nonionic with cationic and anionic surfactants are also effective (Ojeda, et al., 2012). The use of nonionic surfactants are more commonly used for CPE, as they are inexpensive, readily available, and have low CPTs and CMCs. These surfactants have hydrophobic tails made of long hydrocarbon chains, which may branch and contain aromatic rings. The head of these compounds contain a hydrophilic polar group (i.e. alcohols, ethers) (Figure A3). When these molecules are present in solutions at concentrations higher than the CMC, the surfactant molecules in the solution will form micelles. The hydrocarbon chains orient themselves at the micelle core with the hydrophilic end oriented toward the water. Nonpolar analytes solubilize within the micelle core. When the solution reaches above the CPT, the micelle aggregates forming a separate layer with the analyte of interest sequestered in this phase.
For this dissertation, experimentation was completed with Triton X-114 (Figure A4).

Triton X-114 CMC value is 0.2 mM with a CPT of 23°C.
Figure A4. Chemical structure of Triton X-114, n=7-8.

Materials and methods

All chemicals were purchased from Fisher Scientific unless otherwise stated. Dietary supplements were purchased from Amazon.com and stored in the conditions suggested by the manufacturer. Dilutions and solutions were made with doubly deionized water (DDIW). All glassware was acid washed in 10% nitric acid and 3% hydrochloric acid to avoid contamination.

Cloud point extraction and sample makeup

4.425 grams of sample was placed in a glass vial. 0.4 grams of Triton X-100, 0.1 gram of sodium terephthalate and adjusted to pH of 3 with nitric acid. Samples were mixed and heated to 75°C for 1.5 hours. Supernatant and detergent phase were separated and 0.1 gram of each phase was placed into a glass digestion vial. To digest each phase, 30% nitric acid, hydrochloric acid and hydrogen peroxide were added to each vial with an internal standard solution of Y and In and heated. After the digestion, samples were diluted to 5 grams with DDIW.

Inductively coupled plasma mass spectrometry analysis

All samples were analyzed via the Agilent 8800 ICP-MS using 2 mL/min of helium gas flow. All samples were analyzed for Y^{89}, Ag^{107}, Ag^{109}, and In^{115} for silver content and instrumental drift by referencing the internal standard spike. Calibration curve of 0-100 ug/L was used for quantization purposes and verified using the drinking water standard reference material from the National Research Council-Canada, elemental silver spike from Spex
CertiPrep and in-house synthesized AgNPs for extraction and digestion efficiency. All data analysis was completed using the Agilent Mass Hunter programming.

**Results and discussion**

**Cloud Point Extraction of Colloidal Supplement**

With the introduction of silver dietary supplements, the user is exposed to larger concentrations of silver. The human body under normal conditions is able to remove a majority of silver ingested due to the hydrochloric acid in the stomach that causes the silver to become insoluble and no longer a threat to the individual. However, dietary supplements using higher concentrations of silver in various forms expose the human body to higher concentrations and may result in kidney damage, complications with other medications currently used, and in extreme cases argyria (bluish-gray discoloration of large areas of skin especially when exposed to sunlight) (Reidy, et al., 2013).

Silver dietary supplements do not always inform the user of its composition on its packaging. These supplements can contain various concentrations of silver ions, AgNPs, and silver particles suspended in solution. **Table A1** indicates the silver form concentration of AgNPs and silver ions in some of the silver supplements available for purchase. Those labeled as elemental silver supplements do contain mostly silver ions. The supplements labeled as colloidal vary in concentrations of silver ion and silver particles. The solutions do not contain just one form of silver.
Table A1. AgNP and silver ion concentrations for silver dietary supplements. All data is presented as the mean percentage of three replicates ± standard deviation.

<table>
<thead>
<tr>
<th>Brand</th>
<th>Advertised to Contain</th>
<th>Other Ingredients</th>
<th>% Ag Particle</th>
<th>% Ag Ion</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultra Pure</td>
<td>Elemental</td>
<td>Distilled Water</td>
<td>10.58% ± 2.25%</td>
<td>86.5% ± 1.29%</td>
<td>76.9% ± 6.86%</td>
</tr>
<tr>
<td>Peaceful Mountain</td>
<td>Elemental</td>
<td>Peppermint Oil</td>
<td>0.13% ± 4.68%</td>
<td>88.7% ± 2.65%</td>
<td>82.9% ± 8.69%</td>
</tr>
<tr>
<td>Heritage Store</td>
<td>Colloidal</td>
<td>Atomidine (Iodine)</td>
<td>75.6% ± 3.98%</td>
<td>29.7% ± 3.91%</td>
<td>87.7% ± 8.77%</td>
</tr>
<tr>
<td>Natural Path Silver Wings</td>
<td>Colloidal Distilled Water</td>
<td></td>
<td>58.75% ± 2.20%</td>
<td>65.9% ± 7.11%</td>
<td>104.0% ± 3.98%</td>
</tr>
</tbody>
</table>

References


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reference materials by cloud point extraction - atomic absorption spectrometry


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