ABSTRACT

ALICEA, ANNA. Phytotoxicity of Silver Nanoparticles to Hydroponic Hybrid Poplar and Willow Cuttings. (Under the direction of Elizabeth Guthrie Nichols.)

AgNPs (silver nanoparticles) are currently used in several industries and are also frequent constituents in common household products. Due to their prevalence, AgNPs have the capacity to enter all environmental compartments. In this study, the phytotoxicity and bioaccumulation of bulk Ag (silver), AgNPs, and PVP-AgNPs (polyvinylpyrrolidone silver nanoparticles) to *Populus deltoides x Populus nigra- OP367 and Salix nigra* tree cuttings was examined in hydroponic solutions. Hybrid poplar cuttings exposed to 50 mg/L PVP-AgNP had significantly less biomass than other treatments ($p = 0.01$). No other significant differences in biomass were observed. Bulk Ag treatments reduced water use below 20% NRT (normalized relative transpiration) in both willows and poplars. EC_{50} (Effective concentrations at 50%) were calculated using wilt as a response. AgNPs were found to be most toxic in poplar cuttings (1.95 mg/L EC_{50}) followed by bulk Ag in both willow and poplars (9.72 mg/L EC_{50}). The distribution of Ag in tree tissues was also examined. The roots were the primary sites for Ag bioaccumulation in poplar and willow cuttings and were statistically different from bioaccumulation in leaves ($p = 0.04$) and shoots ($p = 0.03$). The bioaccumulation of bulk Ag by trees was also greater than AgNPs and PVP-AgNPs, suggesting that dissolution of Ag ions governs uptake. Significant differences in bioaccumulation were not observed between tree species ($p = 0.23$).

Phytotoxicity of Silver Nanoparticles to Hydroponic Hybrid Poplar and Willow Cuttings

by
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DEDICATION

This thesis is dedicated to my husband for his endless patience, support, and encouragement throughout this project.

BIOGRAPHY

Anna Barnes is the daughter of Michael & Angela Barnes of Selma, NC. Anna obtained her high honors diploma from North Johnston High School in 2007. She graduated cum laude in 2010 with a Bachelors of Science in Environmental Technology and minor in Environmental Toxicology from North Carolina State University.

Her aspirations of science grew in high school while taking courses in environmental science. Anna's ultimate career objective is to investigate the impact of innovative technologies and chemical compounds to the environment.

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PHYTOTOXICITY OF SILVER NANOPARTICLES TO HYDROPONIC WILLOW AND POPLAR TREES

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ABSTRACT

AgNPs (silver nanoparticles) are currently used in several industries and are also frequent constituents in common household products. Due to their prevalence, AgNPs have the capacity to enter all environmental compartments. In this study, the phytotoxicity and bioaccumulation of bulk Ag (silver), AgNPs, and PVP-AgNPs (polyvinylpyrrolidone silver nanoparticles) to *Populus deltoides x Populus nigra- OP367 and Salix nigra* tree cuttings was examined in hydroponic solutions. Hybrid poplar cuttings exposed to 50 mg/L PVP-AgNP had significantly less biomass than other treatments ($p = 0.01$). No other significant differences in biomass were observed. Bulk Ag treatments reduced water use below 20% NRT (normalized relative transpiration) in both willows and poplars. EC_{50} (Effective concentrations at 50%) were calculated using wilt as a response. AgNPs were found to be most toxic in poplar cuttings (1.95 mg/L EC_{50}) followed by bulk Ag in both willow and poplars (9.72 mg/L EC_{50}). The distribution of Ag in tree tissues was also examined. The roots were the primary sites for Ag bioaccumulation in poplar and willow cuttings and were statistically different from bioaccumulation in leaves ($p = 0.04$) and shoots ($p = 0.03$). The bioaccumulation of bulk Ag by trees was also greater than AgNPs and PVP-AgNPs, suggesting that dissolution of Ag ions governs uptake. Significant differences in bioaccumulation were not observed between tree species ($p = 0.23$).

Keywords: silver nanoparticles, phytotoxicity, NRT, absolute transpiration

COMPETING FINANCIAL INTEREST DECLARATION

The authors declare they have no actual or potential competing financial interest.

ABBREVIATIONS

NPs Nanoparticles

AgNPs Silver Nanoparticles

PVP-AgNPs Polyvinylpyrrolidone Silver Nanoparticles

NRT Normalized Relative Transpiration

EC50 Effective Concentrations at 50%

QC Quality Control

NOM Natural Organic Material

ICP-MS Inductively Coupled Plasma- Mass Spectrometer

TiO_2 NPs Titanium Oxide Nanoparticles

DLS Dynamic Light Scattering

%RSD Percent Relative Standard Deviation

INTRODUCTION

Silver nanoparticles (AgNPs) are nanoscale clusters of metallic silver atoms and are currently the most prevalent metallic nanoparticle (NP) in industry [1, 2]. AgNPs are in high demand due to their high reactivity, unique physicochemical properties, and antibacterial capabilities [2, 3]. Compared to bulk materials, AgNPs have a larger surface area to mass ratio which increases reactivity [4]. AgNPs are currently used in several industries such as medicine, cosmetology, electronics, and textiles [5-9]. They are also frequent constituents in common household products such as laundry additives, cleaners, contraceptives, and food supplements [1, 10]. Current sources of AgNP contamination in wastewater include photographic facilities, smelters, mines, and biosolids [11, 12]. Because silver nanoparticles are widely used in industrial products, their presence in soil, air, and water is possible [2, 3, 13, 14]. AgNPs have the potential to enter the environment through non-point sources or point sources and to be transported, transformed, or accumulated within higher organisms or humans [13].

AgNPs released into the environment can impact lower trophic levels first and are known to be toxic to fungi, viruses, and algae [1]. At higher trophic levels, AgNPs can impair osmoregulation in fish gills [15] and dramatically reduce mitochondrial function and cell viability in mammalian cells [16]. A recent study showed that gold NPs biomagnified within hornworms after the worms consumed tobacco leaves containing 5-15 nm gold NPs [17]. Collectively, these studies suggest potential biomagnification of AgNPs within the terrestrial food web from primary producers to consumers.

The toxicity of AgNPs to soil organisms has recently been studied. AgNPs at 1,000

mg/kg dry soil caused total reproductive failure in earthworms [18]. Toxicity was related to the release of Ag^+ ions into soil from AgNPs. In a separate study, soil type was found to be more critical than particle size in determining the accumulation of AgNPs in earthworms [19]. The authors contribute the difference in accumulation between soils to lower pH and organic content in the sandy loam [19]. Other key bacterial activities in soil are expected to be impeded by exposure to AgNPs. Microbial denitrification in soil may also be altered and, consequently, key soil processes fundamental to terrestrial plant function can be impaired by AgNP exposure [20].

Ionic strength, pH, and the presence of natural organic matter (NOM) affect both AgNP bioavailability and AgNP uptake by terrestrial plants [20]. In general, NOM can affect the behavior of NPs by altering their surface charge to prevent aggregation. Musante and White (2010) found that 50 mg/L humic acid reduced the ionic Ag content of bulk and NP solutions. The 50 mg/L humic acid additions enhanced uptake in plant shoots by 5.1 and 2.4 fold respectively, after exposure to 100 and 500 mg/L AgNPs. In a separate study, Badawy *et al.* (2010) found that uncoated, citrate, and borohydride coated AgNPs aggregated at higher ionic strengths (100 mM sodium nitrate) and/or pH. In contrast, polyvinylpyrrolidone NPs (PVP-AgNPs) lacked interaction to changes in ionic strength and pH due to steric stabilization. Therefore, PVP-AgNPs are expected to be immediately bioavailable to plants. Uncoated AgNPs are expected to aggregate in solution due to greater particle to particle interaction [21].

The concentration of AgNPs in solution also governs their behavior. Particles are

expected to aggregate at higher concentrations, which may decrease bioavailability of AgNPs to terrestrial plants. Seeger *et al.* (2008) found no difference in absolute transpiration after exposing willow cuttings to titanium oxide NPs (TiO_2) between 0-100 mg/L. Microscopical imaging curves demonstrated that particle sizes of 25 nm and 100 nm were very similar between 0-100 mg/L, demonstrating the need for more research highlighting the physico-chemical properties of NPs in solution at different concentrations [22].

The uptake, translocation, and accumulation of AgNPs into plants depend on plant species and maturity [2]. Recently, hydroponic ryegrass [23], flax [23], barley [23], zucchini [24], and onion [25] seedlings exhibited impaired germination, reduced root length, lowered biomass, and reduced transpiration after exposure to AgNPs. Phytotoxicity was measured in ryegrass, flax, and barley as a function of particle size. The germination rate of barley decreased after exposure to AgNPs (20 nm) and AgNPs (5 nm) at concentrations of 10-100 mg/L. Flax and ryegrass germination rates were unaffected by AgNP particle size. Reduced shoot length was observed after exposure to AgNPs (10 nm) in flax and barley. Similarly, reduced shoot length was also observed to AgNPs (20 nm) in barley and ryegrass. Flax was unaffected by larger AgNPs (20 nm). The phytotoxicity of AgNPs zucchini seedlings at 500 and 100 mg/L was found to decrease biomass by 57% or 41% respectively in a separate study [24]. Shoot concentrations of AgNPs were 4.7 times greater than corresponding bulk Ag materials, suggesting that AgNPs are more bioavailable [24]. Kumari *et al*. (2009) found that AgNPs (<100 nm) are cytotoxic to onion bulbs. Decreased mitosis and disturbed metaphase was observed at 50 mg/L. Complete disintegration of cell walls was observed at 100 mg/L

AgNPs.

Once plants are exposed, NPs may enter the cell wall directly and interfere with metabolic processes such as water and nutrient transport [20]. Navarro *et al*. (2008) hypothesized that cell walls are typically between 5 to 20 nm. NPs less than 5 nm translocate readily through pores of plant cell walls while particles up to 20 nm can move between cells through the plasmodesmata [2]. NPs may interact with external root sources to reduce root hydraulic conductivity. Reduced root hydraulic conductivity can lead to xylem embolism, leaf growth inhibition, stomatal closure, and consequently, wilting and desiccation [26]. Asli and Neumann (2009) showed that TiO_2 NPs (30 nm) inhibited maize leaf growth and transpiration due to the reduction of hydraulic conductivity. This physical phytotoxicity involved the blocking of intercellular spaces in the cell wall or symplastic connections between cells. The observed phytotoxicity was similar to water stress.

Previous phytotoxicity studies using AgNPs provide limited information to Ag toxicity because of the incomplete vascular development of the exposed plants [4], and germination rates and root elongation assays have been shown to be poor indicators of phytotoxicity [4, 24]. In this study, tree cuttings were used to examine the phytotoxicity and uptake of AgNPs. Hybrid poplar (*Populus deltoides x Populus nigra-OP367*) and black willow (*Salix nigra*) cuttings were used due to their rapid growth and their ability to sequester contaminants in biomass [27, 28]. Particle size, surface coating, and bulk materials were evaluated to determine the bioavailability and phytotoxicity of Ag.

For this study, phytotoxicity was measured using water usage and biomass as

endpoints for tree cuttings with developed root and leaf biomass [29]. Water use between larger plants is highly variable; therefore, absolute transpiration and normalized relative transpiration were calculated. A mass balance of Ag was determined to account for Ag recovery through the hydroponic system and to determine the uptake and distribution of AgNPs between tree tissues (roots, shoots, and leaves). Silver concentrations normalized to plant tissue mass were determined to evaluate the distribution of Ag in roots, shoots, and leaves among silver treatments and between tree species.

MATERIALS AND METHODS

REAGENTS

Aqueous silver nanoparticles (AgNP, uncoated, 25 nm diameter) were purchased from Melorium Technologies, Inc. (Rochester, New York, USA). The reference material was provided as 3.5 g in 30 mL of deionized water. The pH was measured to be 5.6 ± 0.56. Polyvinylpyrrolidone coated silver nanoparticles (PVP-AgNPs, 25 nm diameter) were obtained from the United States Environmental Protection Agency (Cincinnati, OH, USA) in an aqueous solution. The pH was also measured at 5.6 ± 0.56. Silver nitrate (Bulk Ag, 1,000 mg/L, $AgNO_3$) reference standard material was purchased from Fisher Scientific (Fair Lawn, NJ, USA) in 10% (v/v) nitric acid at pH 2.5. Sodium hydroxide (1.0 M NaOH) was also purchased from Fisher Scientific (Fair Lawn, NJ, USA) to buffer bulk Ag stock solutions between pH 5.6 and 6.2. Concentrated nitric acid (HNO_3, 75% v/v) (Plasma Pure Plus, Champlain, NY, USA) and concentrated hydrochloric acid (HCl, 75% v/v) (Plasma Pure Plus, Champlain, NY, USA) were used for acid digestions. Ionic Grow 3-1-5 premium plant

nutrient solution (Hydrodynamics International, Lansing, Mi, USA) was used to propagate tree cuttings as a stock solution for toxicity testing. Due to possible aggregation of particles at higher ionic strengths, the recommended mix was diluted by 75%. Rooting hormone was purchased to encourage root development (TechPac, LLC., Lexington, KY, USA). Deionized water was used in all stock solutions (Pure Flow Inc., Graham, NC, USA).

PREPARATION OF HYDROPONIC EXPOSURE SOLUTIONS

All glassware was acid washed in 20% HNO_3 (v/v), rinsed in deionized water (Pure Flow Inc., Graham, NC, USA), and covered with parafilm (American Can Company, CT, USA) for storage. During experiments, cross contamination was limited by changing pipette tips in between treatments and limiting air exposure to samples. Bulk Ag, AgNP, PVP-AgNP, and no Ag (control) were diluted with 25% Ionic Grow solutions to concentrations of 0, 10, 20, 50, and 100 mg/L and stored in the dark. Stock solutions were prepared within 24 hours of exposure by serial dilution. Exact volumes of solutions were added via pipette to acid digestion tubes as 30 or 50 mL. No characterization data was obtained prior to exposure. The pH of stock solutions was measured using an Accumet basic AB15 pH meter (Fisher Scientific, Fair Lawn, NJ, USA). Concentrations of Ag in stock solutions were quantified in triplicate by ICP-MS.

TREES

Tree cuttings (approximately 25 cm in length, 15 mm in diameter) were obtained from two trees in North Carolina. Cuttings were obtained from one hybrid poplar tree (*Populus deltoides x Populus nigra, OP367*) and one black willow tree (*Salix nigra*).

Cuttings were placed in tap water with rooting hormone after harvesting and placed under 14 hour lights for eight weeks or until substantial roots and leaves developed. After successful rooting was observed, tree cuttings were transferred into Ionic Grow (100% of recommended mix solutions) and tap water for approximately four weeks to encourage leaf and shoot growth.

Prior to exposure, cuttings were randomized and placed into groups (n=3 or n=4). Baseline water use without treatment solutions was measured for all cuttings under 14 hour lights for 48 hours. Tree cuttings were transferred to 50 mL acid digestion tubes and cuttings were allowed to acclimate for 48 hours in 25% Ionic Grow or calcium nitrate. Calcium nitrate additions were added to lower concentrations (10, 20, and 50 mg/L) of bulk Ag ($AgNO_3$) stock solutions to account for the fertilization effect of added nitrate provided by 100 mg/L bulk Ag solutions. The mass of each apparatus (digestion tube, tree cutting, and solution) was weighed every 24 hours, for a total of 48 hours, on an analytical balance. Reductions in mass were attributed to water use by tree cuttings. Each apparatus was capped and wrapped with aluminum foil to prevent the evaporation of solutions and to prevent light induced toxicity to the roots. A set of control trees (n=3; dead cuttings) were found to absorb less than 1 ml of solution in acid digestion tubes after a seven day trial.

PHYTOTOXICITY TESTS

After baseline water use was evaluated, tree cuttings were removed from control solutions and the mass of each cutting was measured to determine the initial wet biomass.

Cuttings were then placed in treatments solutions with either 50 mL of uncoated AgNPs and Bulk Ag or 30 mL PVP-AgNPs. Each digestion tube wrapped in aluminum foil. Water use was monitored every 24 hours, for a total of 72 hours by measuring the mass of the apparatus (digestion tube, tree, and solution); solutions were not refilled. After 72 hours of exposure, tree cuttings were taken out of their respective solutions, blotted dry, and a final wet biomass was obtained. Each cutting was rinsed and dissected into roots, shoots, or leaves with a scalpel. Remaining Ionic Grow and Ag solutions from each cutting were homogenized in the acid digestion tube to re-suspend particles. Aliquots of post-exposure solutions were then transferred into 20 mL plastic scintillation vials (Fisher Scientific, Fair Lawn, NJ, USA) and were immediately frozen to -20°C. Tissues were frozen to -20°C and then freeze dried at -107°C (Virtis Sentry Lyophilizer, Gardiner, NY, USA).

Tree tissues were digested on a hot plate (Precision Scientific, Chicago, IL, USA) with concentrated HNO_3 and HCl for 6 hours at 95°C using EPA Method 3050B [30]. The extract was cooled then filtered using 0.45 um cellulose acetate syringe filters (Whatman, Piscataway, NJ, USA) and BD Luer-Lok ™ Tip syringes (Franklin Lakes, NJ, USA). Silver concentrations in extracts were analyzed by Inductively Coupled Plasma- Mass Spectroscopy (ICP-MS) using EPA Method 6020A [31]. Standard operating conditions are listed in Appendix A. All stock solutions and post exposure solutions were analyzed directly by ICP-MS.

DATA ANALYSIS

Water usage by trees was analyzed as absolute transpiration and normalized relative

transpiration. Absolute transpiration quantifies the rate of water usage per tree in grams per hour. Normalized Relative Transpiration (NRT) normalizes treatments to the initial transpiration and transpiration of non-exposed control trees. NRT eliminates variations due to differences in initial size and growth of tree cuttings during assay [29]. Using this formula, control trees are always 100% NRT. Treatments under 100% represent inhibition or declination of water use. NRT was calculated using the formula below:

$$\text{NRT}\ (\%)\ (C,t)=\frac{\frac{1}{n}\times\sum_{i=1}^{n} T_i\ (C,t)/T_i(C,0)}{\frac{1}{m}\times\sum_{j=1}^{m} T_j\ (0,t)/T_j(0,0)} \qquad [29]$$

where C is concentration (milligrams per liter), t is time period (hour, 0-72),T is absolute transpiration (grams per hour), i is replicate 1, 2, …, *n* and j is control 1, 2, …, *m*.

All statistical analyses were performed using SAS v 9.2 Enterprise 4.2. The normality of raw data was tested using the Shapiro Wilkes Test. Statistical differences in absolute transpiration after 72 hours of exposure was determined using least square means ANOVA analysis. Statistical significance between wet biomass before and after exposure treatments was determined using the two-way Student's t test ($p < 0.05$). Effective concentrations at 50% for wilting were calculated using probit analysis [32]. Statistical differences between tree tissues and among tree species were also determined using two-factor ANOVA followed by two-way Student's t test ($p < 0.05$).

QUALITY CONTROL AND QUALITY ASSURANCE

Deionized water and Ionic Grow solutions were analyzed by ICP-MS and no silver

was detected (Table 1, instrument detection limit). Calcium nitrate solutions were found to contain 38 ± 4.4 µg/L Ag. Reagent blanks contained 40.1 ± 2.74 µg/L Ag. Reagent blanks contained higher than normal levels for trace metal analysis due to the common memory effect of Ag analysis via ICP-MS. Matrix spikes at 10 µg/ml (High Purity™ Standards, Charleston, SC, USA) were used to determine if the digestion method for tree tissues was appropriate for quantification. The percent recovery of Ag^+ from digestion was 102% ± 5.43 on average (n=3). Terbium was used as an internal standard (Internal Standard Multi-Element Mix 2, 40 µg/mL, VHG Labs, Manchester, NH, USA) for all samples (n = 261). The percent relative standard deviation (% RSD) for all samples was less than 18.7. Percent error was less than 12% for all samples. Other qualitative results are listed in Table 1. The ICP-MS detection limit, an average of the standard deviations of twenty one samples of 25% Ionic Grow solution in deionized water, was determined to be 0.004 to 0.013 µg/L. Approximately 5% of total samples (n=12) were analyzed in duplicates.

RESULTS AND DISCUSSION

EFFECT OF SILVER TO WATER USAGE

The rate of water use in treatment trees (bulk Ag, AgNPs, PVP-AgNPs) was measured relative to control trees (no silver) before and after treatments. Both hybrid poplar and willow cuttings exposed to 100, 50, 20, and 10 mg/L bulk Ag demonstrated statistically significant reductions in absolute transpiration relative to the control after 72 hours of exposure (Table 2). AgNPs did not significantly change absolute transpiration when compared to the controls for any concentration and either species. The absolute transpiration

of willow cuttings was unaffected after exposure by PVP-AgNPs. Interestingly, hybrid poplar cuttings exposed to 20 mg/L PVP-AgNPs used more water than control trees.

The absolute transpiration of willow cuttings exposed to TiO_2 NPs was measured in a separate hydroponic study [22]. Variation in absolute transpiration was evident over time, but significant differences were not observed in comparison to control trees. TiO_2 uptake into willows was limited due to TiO_2 NP agglomeration in solution. Stampoulis *et al.* (2009) found that exposure to < 50 mg/L AgNPs did not impact the transpiration volume of zucchini seedlings. Similar results were obtained in the present study where AgNPs had no effect to willow or poplar cuttings from 10-100 mg/L. Exposure to 20 mg/L PVP-AgNPs, however, demonstrated significant differences in willows compared to controls. Significant differences in absolute transpiration could be attributed to increased water use. Higher concentrations of AgNPs (100, 500, and 1,000 mg/L) reduced the transpiration volume of zucchini seedlings by 41%, 78%, and 79% respectively. Results were significantly different from control and bulk Ag ($p < 0.05$). Bulk Ag at all concentrations (100, 500, and 1,000 mg/L) also did not significantly affect transpiration volume [24]. In a similar study, authors also found that 100 and 500 mg/L bulk Ag had no impact on transpiration in yellow squash seedlings [33]. These results were inconsistent with the present study in that significant differences in absolute transpiration were observed in between both tree species between 10-100 mg/L.

NRT was calculated from the absolute transpiration of tree cuttings to account for differences in transpired volume of individual cuttings and control trees (Figure 1). NRT

was impaired below 20% in all bulk Ag treatments in both willows and poplar tree cuttings. Wilting of leaves in both trees was immediately observed after 24 hours. Decreased NRT was observed for poplar cuttings exposed to AgNPs for all concentrations. PVP-AgNPs also demonstrated a negative dose response for poplar cuttings, particularly at 50 mg/L. Wilting continued to occur in poplars through the 72 hour exposure duration.

AgNPs at all concentrations increased NRT after 48 hours of exposure in willow cuttings. In a separate study, exposure to 0, 1, 10 and 100 mg/L TiO_2 (25 nm) slightly reduced NRT in willow cuttings. The effect was weaker for larger sized TiO_2 NPs, suggesting that larger NPs were less bioavailable. NRT increased above controls between 0 and 50 mg/L TiO_2 (100 nm) [22]. Similar results were found in the present study in willows after exposure to AgNP and PVP-AgNPs. The increase in NRT was consistent through the 72 hour exposure duration. Other literature concerning NRT and NP exposure is currently limited.

EFFECT OF AG ON TREE BIOMASS

Differences between wet biomass were measured before and after experiments and are provided in Supporting Documents (Appendix B). Hybrid poplar cuttings exposed to 50 mg/L PVP-AgNP demonstrated significant decline in biomass (Student's t test, $p < 0.01$) after the 72 hour dosing period; wilt was observed in 100% of tree cuttings after 24 hours of exposure. As previously noted, NRT was reduced to <50% for poplar cuttings exposed to 50 mg/L PVP-AgNP.

Table 1. Results of Quality Control (QC) analyses

Sample Type	Mean[a] Ag^+ content (μg/L)	RSD[b] (%)	Accuracy (%)	Relative Percent Difference (%)
Instrument detection limit[c], n=21	0.008 ± 0.004	10 ± 4.5	----	---
Reagent blank[d],n=3	40 ± 2.7	2.3 ± 1.6	----	---
Matrix spike[e], n=3	10200 ± 543	1.0 ± 0.67	102 ± 0.543	---
Analytical Duplicates, n=12	----	----	---	2.5 ± 0.052
Calcium nitrate solution[f], n=3	38 ± 4.4	15 ± 0.30	----	---
Terbium internal standard, n=261	---	<10	---	---

[a]Mean ± one standard deviation

[b]Relative standard deviation between samples

[c]Seven samples measured on three separate days in 25% Ionic Grow solution diluted with deionized water

[d]Blank samples for HNO_3 and HCl used in the digestion process

[e]Ag single element standard (10 μg/mL) used in the digestion process

[f]100 mg/L $Ca(NO_3)_2$ in 25% Ionic Grow solution

No significant differences in biomass were observed in all other treatments and concentrations as observed in Table 2, although NRT declines were evident for bulk Ag, AgNP exposure in poplars, and PVP-AgNP exposure in poplars. Similar results were found during a 15 day hydroponic assay where <100, 500, and 1,000 mg/L bulk Ag had no impact on zucchini biomass [24].

Exposure to AgNPs at higher concentrations (1,000 and 500 mg/L) proved to be toxic by reducing zucchini biomass by 71% and 57%, respectively. The authors cited that exposure to 10 mg/L bulk Ag closely resembled plant growth, even though potassium nitrate controls were employed to control for fertilization [24]. In a similar study, 100 and 500 mg/L bulk Ag was found to have no impact on the biomass of hydroponic squash seedlings. However, exposure to 500 mg/L AgNP reduced biomass by 83%. Reductions in biomass from AgNP treatments were significantly different from control and bulk Ag ($p = <0.001$) [33]. Contrasting results were found in the present study where poplar cuttings exposed to 50 mg/L PVP-AgNPs demonstrated significant increase in biomass.

According to absolute transpiration and NRT data, significant reductions in biomass were expected for bulk Ag treatments in both willows and poplars, while biomass increase might be expected for AgNPs and PVP-AgNPs since NRT increased. No significant differences in biomass were evident for these treatments. Water use data, in these cases, may indicate impending plant stress but longer exposure durations would be required to observe changes in biomass. Seventy-two hours of exposure to tree cuttings prohibits complete

desiccation of plant tissues. In this study, foliar wilt was immediately observed, but accounted for less than 10% of the dry weight of these trees on average. Previous studies noting statistically significant differences to plant biomass after exposure to AgNPs were performed over seven or fifteen days [24, 33].

UPTAKE OF AG INTO TREE TISSUES

The bioavailability and fate of Ag between tree tissues was examined (Table 4). Roots were the primary sites for Ag bioaccumulation in poplar and willow cuttings and were statistically different from concentrations in leaves (Student's t test, $p = 0.04$) and shoots (Student's t test, $p = 0.03$). Concentrations of Ag in roots ranged from 5.84 to 4,910 μg Ag/g and were higher in bulk Ag treatments than PVP-AgNPs (Student's t test, $p = 0.04$) and AgNPs (Student's t test, $p = 0.05$). Concentrations of bulk Ag in roots were an order of magnitude higher than roots in other treatments.

Bioaccumulation of Ag into trees was dependent upon concentrations of exposure solutions and solution type (Figure 2). Bulk Ag was more bioavailable to trees than AgNPs (Student's t test, $p = 0.03$). Significant differences in bioaccumulation between trees exposed to PVP-AgNPs and AgNPs were not observed, which suggests that the bioavailability of AgNP uptake is independent of surface coating (Student's t test, $p = 0.44$). Differences in bioaccumulation of Ag between tree species were insignificant (Student's t test, $p = 0.23$).

Higher accumulation of Ag from bulk Ag was consistent with impaired NRT and absolute transpiration. NRT and absolute transpiration was reduced in all concentrations from bulk Ag exposure in both tree species. Because greater bioaccumulation of Ag was

Table 2. Mean absolute transpiration (grams/hour ± 1 standard deviation) of cuttings after 72 hours of exposure to silver solutions

Tree	Treatment	100 mg/L	50 mg/L	20 mg/L	10 mg/L	Control[a]
Willow	Bulk Ag[b]	0.041 ± 0.027[A]	0.060 ± 0.028[A]	0.041 ± 0.016[A]	0.080 ± 0.067[A]	0.21 ± 0.069
Willow	AgNP[c]	0.11 ± 0.087	0.21 ± 0.073	0.10 ± 0.038	0.28 ± 0.16	0.17 ± 0.060
Willow	PVP-AgNP[d]	NT	0.049 ± 0.20	0.036 ± 0.17[Ae]	0.068 ± 0.085	0.091 ± 0.069
Poplar	Bulk Ag[b]	0.096 ± 0.020[A]	0.088 ± 0.047[A]	0.067 ± 0.026[A]	0.093 ± 0.063[A]	0.41 ± 0.19
Poplar	AgNP[c]	0.26 ± 0.16	0.19 ± 0.11	0.36 ± 0.37	0.18 ± 0.065	0.37 ± 0.27
Poplar	PVP-AgNP[d]	NT	0.10 ± 0.040	0.10 ± 0.070	0.17 ± 0.080	0.29 ± 0.027

[a]Control= no treatment (n=4)

[b]Silver nitrate (n=4)

[c]Silver nanoparticles (25 nm)(n=4)

[d]Polyvinylpyrrolidone silver nanoparticles (25 nm)(n=3)

[e]Statistical significance due to the enhancement of absolute transpiration

[A]Statistical significance using lease square means analysis comparing the effect of each concentration of silver or silver nanoparticle relative to the control or no dose treatment ($p < 0.05$).

NT = Not tested

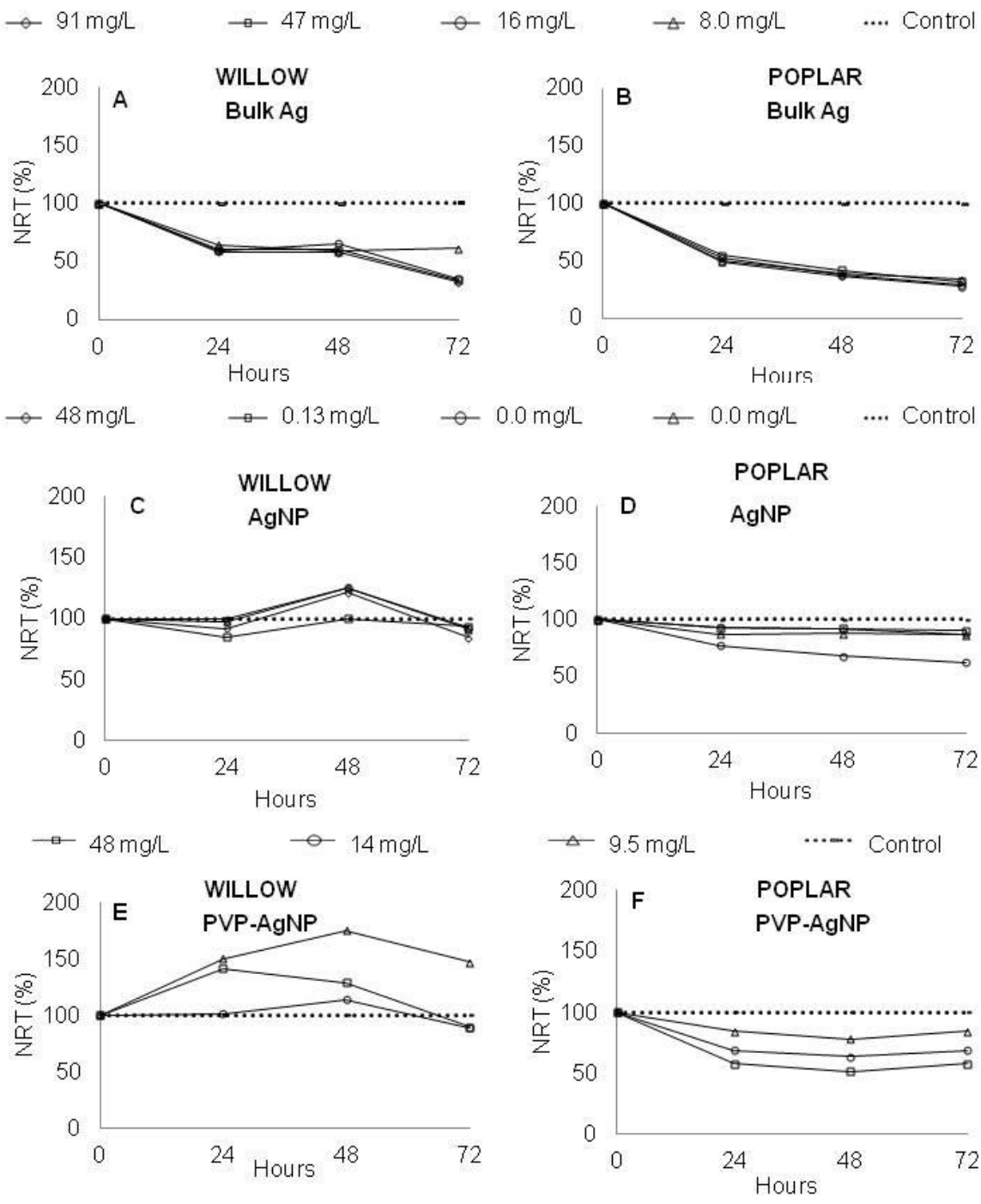

Figure 1. Percent Normalized Relative Transpiration (NRT %) for willows and poplars with and without silver and silver nanoparticles.

found in the roots of both species, water transport via roots may have been limited. Significant reductions in biomass were expected, but was not observed due to short exposure duration.

Differences in the absolute transpiration of poplars and willows were statistically insignificant from AgNP exposure. AgNPs decreased NRT in poplars but increased NRT in willows. Although AgNPs were expected to agglomerate, they are a potential source of ionic Ag in the hydroponic system [20, 24, 34, 35]. Exposure to PVP-AgNPs demonstrated insignificant affects to absolute transpiration, but decreased NRT in poplar cuttings. Biomass reduction was evident after exposure to 50 mg/L PVP-AgNPs.

Musante and White (2010) found that Ag content in stems of yellow squash seedlings were unaffected by particle size, concentration, or solution type [33]. Our results agree with these findings in that particle size was independent of accumulation. Solution type was important in this study for trees exposed to bulk Ag bioaccumulated significantly more Ag that AgNPs. Greater bioaccumulation in trees was observed between treatments with higher concentrations, but statistical significance was not observed. Contrastingly, Stampoulis *et al.* (2009) found the uptake of AgNPs into plant shoots of zucchini seedlings was 4.7 times higher than bulk Ag at similar concentrations [24]. In a separate study, Ag accumulation in duckweed increased significantly with higher concentrations of AgNPs and bulk Ag. Similar to the results in this study, bulk Ag was significantly more bioavailable to duckweed tissues than AgNPs [36].

The mass and distribution of Ag in tree tissues (roots, shoots, and leaves) was

Table 3. P-values ($p < 0.05$) from Student's t test (two-way) comparing differences in mean biomass before and after exposure to cuttings

	Willow					Poplar				
	100 mg/L	50 mg/L	20 mg/L	10 mg/L	0 mg/L	100 mg/L	50 mg/L	20 mg/L	10 mg/L	0 mg/L
Bulk Ag[a]	0.81	0.78	0.77	0.59	0.92	0.12	0.42	0.071	0.51	0.59
AgNP[b]	0.85	0.81	0.52	0.10	0.97	0.85	0.81	0.52	0.10	0.66
PVP-AgNP[c]	NT	0.57	0.36	0.92	0.94	NT	**0.010**[A]	0.83	0.61	0.94

[a]Silver nitrate (n=4)

[b]Silver nanoparticles (25 nm)(n=4)

[c]Polyvinylpyrrolidone silver nanoparticles (25 nm)(n=3)

[A]Statistical significant increase in biomass of PVP-AgNPs to Poplar cuttings

NT = Not tested

determined (Table 5 and 6). Ag was detected well above background levels in all tissues of tree cuttings in all treatments, which confirms uptake and translocation through the hydroponic system. Aliquots of stock solutions and post exposure solutions were analyzed without digestion and are listed in Appendix C. Greater amounts of Ag were found in post-exposure solutions in the hydroponic system, suggesting dissolution or aggregation of particles. Low mass balance recoveries could be explained through the formation of insoluble salts with ionic grow solutions or HCl; ionic Ag^{+} forms more insoluble salts than any other metal at room temperature [37]. Musante and White (2010) found that the ionic Ag concentrations in 100 mg/L bulk Ag and AgNP stock solutions was 180 μg/L and 1,800 μg/L. At 500 mg/L, the Ag^{+} ionic concentration in bulk Ag and AgNP stock solutions was 840 μg/L and 3,700 μg/L [33]. These authors noted poor recoveries of stock solutions due to the oxidation with halides, which ultimately can cause precipitation of particles, and thus, lower ionic levels in solution. AgNP uptake in tree cuttings was limited, possibly due to agglomeration of particles in hydroponic solutions. Dynamic light scattering (DLS) confirmed that AgNPs gradually aggregated in ¼ Hoagland media within seven hours in a separate study [38]. The same authors also found that elemental Ag^{0} increased with higher exposure concentrations in both bulk Ag and AgNPs. More characterization work is needed to determine the molecular interactions of reagents in their amended solutions.

DIFFERENCES BETWEEN TREE SPECIES

Effective concentrations at 50% were calculated using the number of wilted trees per treatment after 72 hours of exposure (Table 6). AgNPs were found to be more toxic to poplar

cuttings than bulk Ag or PVP-AgNPs by a factor of nine. Willows showed not toxic response to AgNPs. PVP-AgNPs were the least toxic having an EC_{50} of 18.2 mg/L in poplars. Willows showed not toxic response to PVP-AgNPs. Poplar and willow tree cuttings were equally affected by bulk Ag, having an EC_{50} of 9.72 mg/L. Differences in species were observed, with poplars being more sensitive to AgNPs (1.95 EC_{50}).

Stampoulis *et al.* (2009) found that the effective concentration to reduce zucchini seedling biomass and/or transpiration was 2 orders of magnitude lower for AgNPs than bulk Ag. Several studies found contrasting results. Yin *et al.* (2011) found that EC_{50} of bulk Ag was more toxic than AgNPs in duckweed, in terms of EC50 [36]. Similar EC_{50} results were obtained in a study where bulk Ag was 20 times more toxic to green algae compared to bulk Ag [10]. Also, based on EC_{50} values, Navarro *et al.* (2008) found that toxicity was 18 times higher for bulk Ag than AgNP. Our results agree with Stampoulis *et al.* (2009) in that EC_{50} were one order of magnitude lower in AgNPs than bulk Ag.

CONCLUSION

This study investigated the phytotoxicity and uptake of AgNPs in hybrid poplar and black willow tree cuttings. Based on water usage data, phytotoxicity was not based on plant-NP physical interactions. Cuttings were found to exhibit temporary water stress after exposure to AgNPs, but the effects were more profound in bulk Ag treatments. Bulk Ag bioaccumulation in plants was also greater than AgNP and PVP-AgNPs. Uptake in bulk Ag treatments increased with increasing concentrations. These results suggest that the dissolution of Ag^{+} ions are more important than particle size in determining the phytotoxicity

Table 4. Mean silver concentrations (µg Ag /g dry weight) of tree tissues

Species	Treatment	Estimated Exposure Concentration (mg/L) of [Ag^+]	Roots[a]	Shoots	Leaves
Willow	Bulk Ag[b]	100	**4910 (5330)^{A,B}**	15.1 (15.6)	735 (651)
		10	**4100 (6800)^{A,B}**	42 (18)	74 (54)
Willow	AgNP[b]	100	**350 (400)^{A}**	7.5 (9.9)	12 (1.1)
		10	**87 (84)^{A}**	0.69 (0.46)	1.6 (1.6)
Willow	PVP-AgNP[b]	50	**697 (227)^{A}**	2.47 (2.03)	17.3 (18.9)
		10	**130 (87)^{A}**	9.5 (9.6)	4.1 (2.5)
Poplar	Bulk Ag[b]	100	**1620 (2620)^{A,B}**	13.5 (21.4)	24.0 (28.0)
		10	**1300 (2100)^{A,B}**	3.3 (1.4)	5.8 (4.2)
Poplar	AgNP[c]	100	**210 (180)^{A}**	2.2 (0.67)	21 (23)
		10	**12 (3.5)^{A}**	1.2 (1.6)	0.53 (0.05)
Poplar	PVP-AgNP[d]	50	**150 (220)^{A}**	15 (8.2)	4.1(1.8)
		10	**29 (24)^{A}**	8.2 (6.1)	3.8 (1.3)

[a]Two-way ANOVA analysis without replication followed by two-way Student's t test analysis ($p < 0.05$)

[b]Silver nitrate (n=4)

[c]Silver nanoparticles (25 nm)(n=4)

[d]Polyvinylpyrrolidone silver nanoparticles (25 nm)(n=3)

[A]Statistical significance of Ag bioaccumulation in roots relative to other tissues

[B]Statistical significance of Ag bioaccumulation in roots of bulk Ag relative to AgNP and PVP-AgNP roots

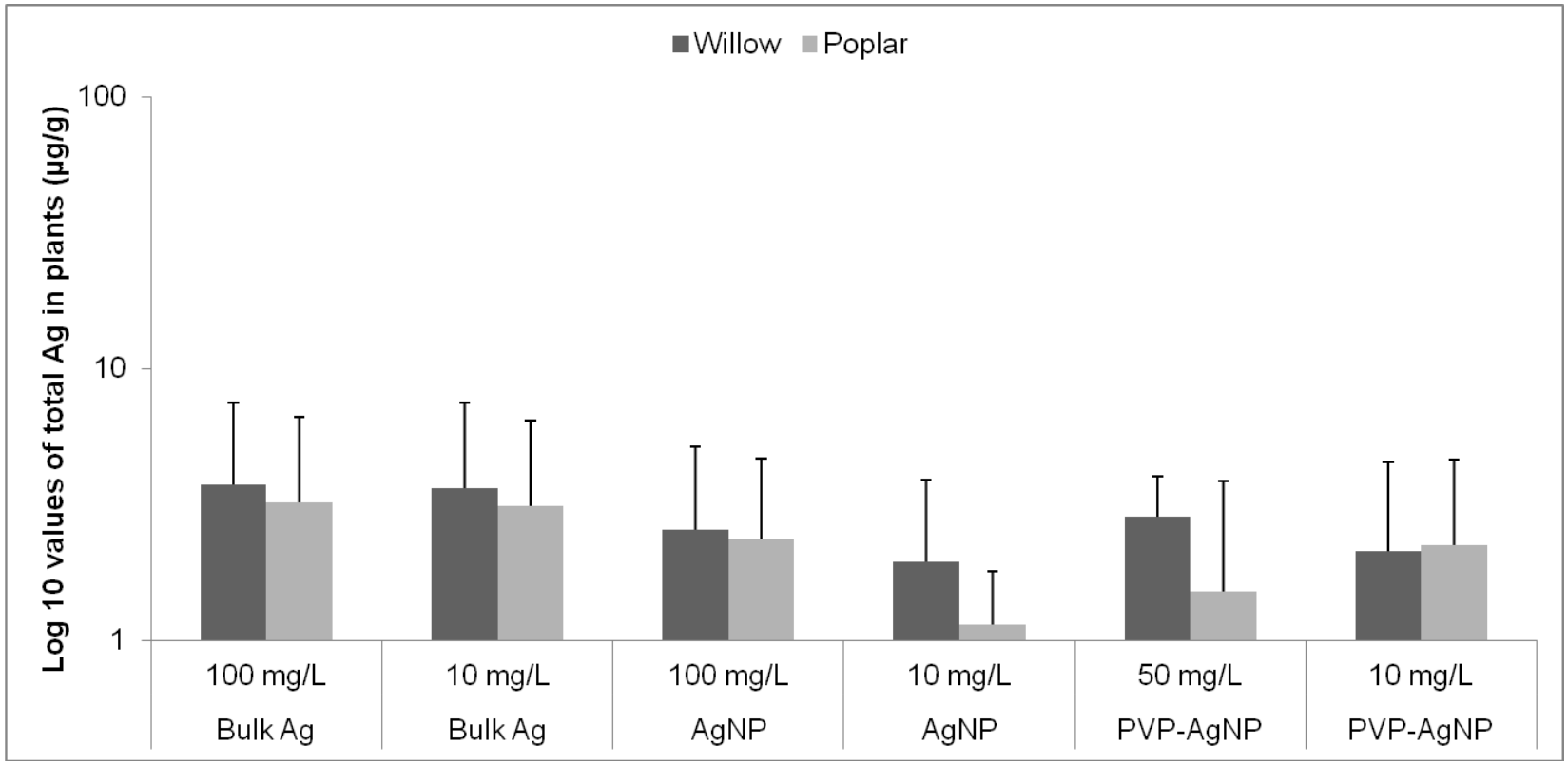

Figure 2. Mean silver concentrations (µg Ag/g plant mass ± one standard deviation) between trees for each treatment. Values are shown as log values for clarity.

to tree cuttings.

Although Ag is a non-essential mineral, plants still have the capacity to accumulate trace quantities. The effectiveness of bioaccumulation is plant specific and will depend on several factors such as sequestration into tissues, the efficiency of xylem loading and transport, and the mobilization and uptake of Ag from soil [39].

Terrestrial plants that accumulate large quantities of Ag are potentially valuable for future environmental remediation projects. Differences in bioaccumulation of Ag between tree species were insignificant. Willows, however, were able to bioaccumulate Ag without showing signs of phytotoxicity, demonstrating their potential use in phytoremediation projects. Literature is lacking on how plant species and environmental factors will collectively affect the accumulation of NPs.

This study was the first to examine impacts of AgNPs to tree cuttings. More research is needed to investigate the effects of AgNPs under realistic exposure of exposure solutions and to distinguish uptake between ionic Ag and AgNPs.

Currently, AgNP toxicity studies together on terrestrial plants are restricted to hydroponic settings, short exposure durations, pre-mature developmental stages, and primarily food crops. These studies have revealed that several factors influence toxicity, but general trends across studies are not evident. Although hydroponic studies are helpful in tracking the fate and transport of AgNPs through the plant system, terrestrial plants exist in more complex soil/water systems. More environmentally relevant exposure settings are

Table 5. Mass balance[a] of silver in black willow cuttings after 72 hours of exposure

		Mass of Ag (µg)						
Treatment	C_{added}[b]	$Sol_{, initial}$[c]	Roots	Shoots	Leaves	$Sol_{, final}$[d]	Total	Recovery (%)
Bulk Ag	100	2330 (2020)	23.0 (9.41)	12.7 (11.6)	15.7 (15.3)	2780 (816)	2830 (828)	121 (35.5)
	10	402 (1.49)	39.5 (33.3)	45.1 (39.2)	0.91 (0.38)	92.0 (37.7)	177 (66.0)	44.1 (16.5)
	0	5.2 (3.2)	0.87 (0.58)	3.5 (2.3)	1.5 (0.94)	0.18 (0.05)	7.0 (2.8)	---
AgNP	100	1980 (305.7)	3.56 (3.88)	19.0 (28.1)	0.68 (0.46)	258 (57.2)	281 (44.6)	14.2 (2.26)
	10	14 (1.6)	1.5 (1.3)	0.90 (0.58)	0.23 (0.11)	3.7 (2.3)	6.5 (2.1)	48 (15)
	0	0.71 (0.31)	0.24 (0.11)	0.57 (0.31)	0.20 (0.13)	0.26 (0.02)	1.7 (1.3)	---
PVP-AgNP	50	1060 (534.1)	21.7 (6.99)	15.7 (12.0)	10.8 (9.44)	616 (394)	664 (374)	62.5 (35.2)
	10	286 (27.6)	5.03 (3.98)	4.83 (3.63)	1.2 (0.57)	127 (109)	138 (106)	48.3 (36.9)
	0	0.42 (0.18)	0.29 (0.11)	0.27 (0.16)	0.39 (0.19)	1.5 (0.25)	2.5 (0.16)	---

[a]Values are the mean of three or four replicates. Standard deviation is shown in brackets. The method detection limit is 0.01 µg/L.

[b]C_{added} is the theoretical concentration of stock solution.

[c]Mass of Ag in original stock solutions

[d]Mass of Ag in post exposure solutions

Table 6. Mass balance[a] of silver data to hybrid poplar cuttings after 72 hours of exposure

		Mass of Ag (μg)						
Treatment	C_{added}[b]	$Sol_{, initial}$[c]	Roots	Shoots	Leaves	$Sol_{,final}$[d]	Total	Recovery (%)
Bulk Ag	100	2330 (2020)	77.4 (128)	23.8 (39.2)	6.23 (6.85)	1640 (900)	1750 (886)	74.9 (38.0)
	10	402 (1.49)	19.3 (28.1)	5.44 (1.72)	1.37 (0.29)	72.0 (28.8)	98.0 (58.5)	24.3 (14.6)
	0	5.1 (3.2)	3.1 (3.1)	1.2 (0.73)	1.3 (1.2)	0.32 (0.070)	6.0 (4.0)	---
AgNP	100	1980 (306)	14.1 (17.5)	2.35 (0.282)	8.92 (12.2)	199 (215)	225 (211)	11.4 (10.7)
	10	14 (1.6)	0.69 (0.070)	4.1 (6.5)	0.21 (0.020)	4.6 (2.7)	9.7 (9.3)	70 (68)
	0	0.71 (0.31)	0.54 (0.42)	0.64 (0.29)	0.31 (0.12)	0.07 (0.02)	1.6 (0.58)	---
PVP-AgNP	50	1060 (534)	10.1 (15.5)	29.1 (19.1)	1.68 (1.00)	1060 (60.4)	1100 (31.20)	103 (2.94)
	10	290 (27.6)	1.9 (1.4)	14 (9.3)	1.6 (0.73)	110 (47.0)	120 (43.3)	43 (15)
	0	0.42 (0.18)	0.34 (0.16)	0.33 (0.17)	0.12 (0.10)	0.29 (0.27)	1.1 (0.30)	---

[a]Values are the mean of three or four replicates. Standard deviation is shown in brackets. The method detection limit is 0.01 μg/L.

[b]C_{added} is the theoretical concentration of stock solutions.

[c]Mass of Ag in original stock solutions

[d]Mass of Ag in post exposure solutions

essential such as the use of humic acids and other microbial organisms. Studies involving NP exposure to plants in soil needed higher concentrations to demonstrate significant effects, suggesting that several metabolic activities are involved in NP uptake [23, 40]. Longer exposure durations are needed to determine if plants can recover from water related phytotoxicity. Studies based on plant defense mechanism to AgNPs periodically through its life cycle would be more appropriate in predicting toxicity. Lastly, building connections between the characteristics of NPS (surface coating, surface area, particle size) with phytotoxicity is needed.

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Table 7. Effective concentrations at 50% (EC_{50}) at which poplar and willow cuttings wilt

Tree	Treatment	EC_{50} (mg/L)[a]
Willow	Bulk Ag[b]	9.72
Willow	AgNP[c]	NT
Willow	PVP-AgNP[d]	NT
Poplar	Bulk Ag[b]	9.72
Poplar	AgNP[c]	1.95
Poplar	PVP-AgNP[d]	18.2

[a]Probit analysis method

[b]Silver nitrate (n=4)

[c]Silver nanoparticles (25 nm)(n=4)

[d]Polyvinylpyrrolidone silver nanoparticles (25 nm)(n=3)

Note: NT = Non-toxic

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APPENDICES

APPENDIX A

Inductively coupled plasma- mass spectrometry operating conditions

RF power (W)	1450
Argon plasma gas (L/min)	13
Argon aux gas (L/min)	1
Argon neb gas (L/min)	0.73
Operating vacuum (Torr)	4.9×10^{-7}
Cones	Nickel
Nebulizer	Glass expansion conikal u-series
Sample uptake (ml/min)	0.5
Quad mode	Peak jumping, 50 ms dwell
Acquisition	2 s, five cycles, 4 replicates
Rinse	60 s, 1% HNO_3
Internal standard	40 µg/L Terbium

APPENDIX B

Normalized biomass of tree cuttings before and after exposure

Treatment	Tree ID	Pre-exposure biomass (g)	Post-exposure biomass (g)	Normalized Biomass (g)	Normalized Percent Difference (g)
Control	W10	8.10	8.00		0.970
	W17	7.50	7.40		0.970
	W26	15.2	14.7		4.87
	W28	10.3	10.00	10.3	2.92
100 mg/L Ag	W3	2.10	1.80		7.36
	W23	2.50	2.40		2.45
	W1	6.10	5.80		7.36
	W15	5.60	4.90	4.08	17.2
50 mg/L Ag	W5	3.60	3.20		6.43
	W8	4.50	4.00		8.03
	W24	5.30	4.80		8.03
	W27	11.5	10.1	6.23	22.5
20 mg/L Ag	W2	3.30	2.70		9.92
	W7	4.10	3.50		9.92
	W21	10.7	9.60		18.2
	W13	6.10	5.60	6.05	8.26
10 mg/L Ag	W30	3.60	3.20		9.47
	W25	3.40	3.00		9.47
	W18	3.30	2.90		9.47
	W14	6.60	5.50	4.23	26.0

Control	P19	5.50	4.70		12.3
	P26	7.40	6.60		12.3
	P6	4.80	4.40		6.15
	P14	8.30	7.70	6.50	9.23
100 mg/L Ag	P22	4.80	4.10		10.8
	P30	5.60	4.40		18.5
	P1	8.30	5.50		43.2
	P8	7.20	5.50	6.48	26.3
50 mg/L Ag	P23	10.2	8.00		33.2
	P24	6.80	5.50		19.6
	P13	4.00	3.30		10.6
	P2	5.50	3.90	6.63	24.2
20 mg/L Ag	P16	8.50	6.00		36.4
	P11	7.20	5.50		24.7
	P17	6.90	4.30		37.8
	P15	4.90	3.60	6.88	18.9
10 mg/L Ag	P27	7.60	4.90		44.1
	P20	3.70	2.80		14.7
	P25	4.90	4.30		9.80
	P9	8.30	8.10	6.13	3.27
Control	W14	4.10	4.10		0.00
	W15	7.10	7.10		0.00
	W25	4.10	3.90		4.15
	W16	4.00	4.00	4.83	0.00
100 mg/L AgNP	W5	18.5	17.6		10.8
	W8	6.30	6.00		3.61

	W20	3.30	3.20		1.20
	W1	5.10	4.90	8.30	2.41
50 mg/L AgNP	W2	5.40	5.10		6.22
	W17	4.60	4.60		0.00
	W13	6.70	6.70		0.00
	W24	2.60	2.50	4.83	2.07
20 mg/L AgNP	W6	7.70	7.60		1.90
	W23	2.70	2.60		1.90
	W9	8.10	7.30		15.2
	W18	2.60	2.60	5.28	0.00
10 mg/L AgNP	W22	4.10	4.20		1.44
	W21	7.20	7.40		2.88
	W12	6.40	6.30		0.0100
	W19	10.0	10.0	6.95	1.44
Control	P9	11.7	8.90		34.2
	P2	5.10	4.90		2.44
	P3	8.80	9.20		4.88
	P16	7.20	6.60	8.20	7.32
100 mg/L AgNP	P11	6.90	6.70		2.61
	P22	10.3	10.6		3.91
	P18	9.10	8.20		11.7
	P4	4.40	3.70	7.68	9.12
50 mg/L AgNP	P12	6.00	5.50		6.97
	P23	5.20	5.00		2.79
	P21	7.60	7.10		6.97
	P10	9.90	9.60	7.18	4.18

20 mg/L AgNP	P25	14.3	11.1		32.7
	P15	7.10	5.40		17.4
	P7	11.4	11.2		2.04
	P6	6.40	4.30	9.80	21.4
10 mg/L AgNP	P20	6.30	5.30		15.2
	P1	6.80	6.20		9.13
	P14	6.90	6.60		4.56
	P24	6.30	5.70	6.58	9.13
Control	W18	9.00	8.80		3.35
	W15	3.00	2.80		3.35
	W11	5.90	5.70	5.97	3.35
50 mg/L PVP-AgNP	W19	17.9	16.9		5.74
	W5	19.1	18.4		4.02
	W10	15.3	13.7	17.4	9.18
20 mg/L PVP-AgNP	W3	17.7	16.8		7.50
	W7	7.00	6.30		5.83
	W13	11.3	10.4	12.0	7.50
10 mg/L PVP-AgNP	W1	3.30	3.20		1.78
	W14	3.30	2.70		10.7
	W9	10.3	9.90	5.63	7.10
Control	P9	4.60	4.40		3.35
	P5	7.60	7.60		0.00
	P14	5.70	5.60	5.97	1.68
50 mg/L PVP-AgNP	P18	9.10	6.20		32.7
	P13	8.80	5.40		38.4
	P17	8.70	7.30	8.87	15.8

20 mg/L PVP-AgNP	P4	3.30	3.00		3.81
	P8	13.8	11.9		24.2
	P10	6.50	5.90	7.87	7.63
10 mg/L PVP-AgNP	P1	5.10	4.80		3.10
	P2	11.4	9.80		16.6
	P12	12.5	9.70	9.67	29.0

APPENDIX C

Undigested concentrations of Ag in stock solutions

Silver (Ag^+) type	Theoretical [Ag^+] in solution (mg/L)	Actual [Ag^+] calculation (mg/L)	Percent Recovery (%)
AgNP	0	0.0075	-
AgNP	0	0.020	-
AgNP	0	0.015	-
Bulk Ag	0	0.065	-
Bulk Ag	0	0.068	-
Bulk Ag	0	0.18	-
PVP-AgNP	0	0.0075	-
PVP-AgNP	0	0.020	-
PVP-AgNP	0	0.015	-
AgNP	10	0.31	3.1
AgNP	10	0.27	2.7
AgNP	10	0.24	2.4
Bulk Ag	10	8.0	80.0
Bulk Ag	10	8.0	80.0
Bulk Ag	10	8.1	81
PVP-AgNP	10	11	110
PVP-AgNP	10	9.1	91
PVP-AgNP	10	8.9	89
AgNP	20	ND	0.0
AgNP	20	ND	0.0
AgNP	20	ND	0.0
Bulk Ag	20	16	80.0
Bulk Ag	20	17	85

Bulk Ag	20	17	85
PVP-AgNP	20	14	70.0
PVP-AgNP	20	14	70.0
PVP-AgNP	20	14	70.0
AgNP	50	0.12	0.24
AgNP	50	0.15	0.30
AgNP	50	0.14	0.28
Bulk Ag	50	48	96
Bulk Ag	50	47	94
Bulk Ag	50	46	92
PVP-AgNP	50	46	110
PVP-AgNP	50	49	61
PVP-AgNP	50	48	41
AgNP	100	54.1	54
AgNP	100	39.4	39
AgNP	100	50.7	51
Bulk Ag	100	90	90
Bulk Ag	100	88	88
Bulk Ag	100	95	95