Environmental Science & Technology

Article

pubs.acs.org/est

Phytostimulation of Poplars and *Arabidopsis* Exposed to Silver Nanoparticles and Ag^+ at Sublethal Concentrations

Jing Wang,[†,⊥] Yeonjong Koo,[‡,⊥] Anne Alexander,[§,⊥] Yu Yang,[†] Samantha Westerhof,[§] Qingbo Zhang,[∥] Jerald L. Schnoor,[§] Vicki L. Colvin,[∥] Janet Braam,[‡,*] and Pedro J. J. Alvarez[†,*]

[†]Department of Civil & Environmental Engineering, Rice University, Houston, Texas 77005, United States

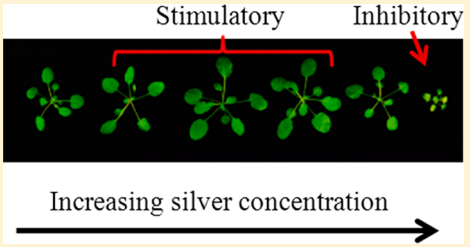
[‡]Department of Biochemistry & Cell Biology, Rice University, Houston, Texas 77005, United States

[§]Department of Civil & Environmental Engineering, University of Iowa, Iowa City, Iowa 52242, United States

[∥]Department of Chemistry, Rice University, Houston, Texas 77005, United States

Supporting Information

ABSTRACT: The increasing likelihood of silver nanoparticle (AgNP) releases to the environment highlights the importance of understanding AgNP interactions with plants, which are cornerstones of most ecosystems. In this study, poplars (*Populus deltoides* × *nigra*) and *Arabidopsis thaliana* were exposed hydroponically to nanoparticles of different sizes (PEG-coated 5 and 10 nm AgNPs, and carbon-coated 25 nm AgNPs) or silver ions (Ag^+, added as $AgNO_3$) at a wide range of concentrations (0.01 to 100 mg/L). Whereas all forms of silver were phytotoxic above a specific concentration, a stimulatory effect was observed on root elongation, fresh weight, and evapotranspiration of both plants at a narrow range of sublethal concentrations (e.g., 1 mg/L of 25 nm AgNPs for poplar). Plants were most susceptible to the toxic effects of Ag^+ (1 mg/L for poplar, 0.05 mg/L for *Arabidopsis*), but AgNPs also showed some toxicity at higher concentrations (e.g., 100 mg/L of 25 nm AgNPs for poplar, 1 mg/L of 5 nm AgNPs for *Arabidopsis*) and this susceptibility increased with decreasing AgNP size. Both poplars and *Arabidopsis* accumulated silver, but silver distribution in shoot organs varied between plant species. *Arabidopsis* accumulated silver primarily in leaves (at 10-fold higher concentrations than in the stem or flower tissues), whereas poplars accumulated silver at similar concentrations in leaves and stems. Within the particle subinhibitory concentration range, silver accumulation in poplar tissues increased with exposure concentration and with smaller AgNP size. However, compared to larger AgNPs, the faster silver uptake associated with smaller AgNPs was offset by their toxic effect on evapotranspiration, which was exerted at lower concentrations (e.g., 1 mg/L of 5 nm AgNPs for poplar). Overall, the observed phytostimulatory effects preclude generalizations about the phytotoxicity of AgNPs and encourage further mechanistic research.



INTRODUCTION

Silver nanoparticles (AgNPs) are currently the most widely commercialized nanomaterial.[1] Both coated and uncoated AgNPs are produced by various companies.[2] The low manufacturing costs and broad-spectrum antimicrobial properties of AgNPs make their use increasingly common in household antiseptic sprays, food packaging, clothes, and antimicrobial coatings of medical devices.[3−8] Of the 1317 nanotechnology-enabled consumer products available in 2011, 313 products contained AgNPs compared to 91 products incorporating the second most popular class, carbon-based nanomaterials.[1] The widespread use of AgNPs has increased the likelihood of accidental or incidental releases to the environment underscoring the need to assess their fate and potential impacts to ecosystem health.

Material flow analysis suggests that the majority of AgNPs released from consumer products enter sewer systems and wastewater treatment plants, where they are eventually incorporated into sewage sludge and remain in the biosolids that are applied to agricultural fields.[9−12] Recent studies of silver speciation in wastewater treatment plants[12,13] and freshwater mesocosms[14] suggest that sulfidation (i.e., Ag_2S formation) is common, which can limit bioavailability and toxicity. AgNPs are known to be potentially toxic to bacteria,[15,16] algae,[17,18] human cells,[19,20] and animal cells.[21] However, compared to the now hundreds of articles addressing AgNPs aquatic toxicity, there are many fewer studies that consider how AgNPs interact with terrestrial plants.[17,22−31] This is a critical knowledge gap because plants are cornerstones of most ecosystems and influence the overall fate and impact of many environmental pollutants.[32] Of particular relevance is the need for quantitative characterization of silver uptake by plants as a function of AgNP properties (e.g., nanoparticle size and Ag^+ dissolution kinetics) to enable predictions about environmental consequences of the diverse AgNP forms and to determine whether AgNPs accumulate in edible portions of the plant, which could lead to trophic transfer.

Received: January 28, 2013
Revised: April 18, 2013
Accepted: April 18, 2013
Published: May 1, 2013

   dx.doi.org/10.1021/es4004334 | Environ. Sci. Technol. 2013, 47, 5442−5449

The potential phytotoxicity of AgNPs has been investigated in a few studies, and negative or inconsequential effects were reported after short-term exposure. For example, citrate-coated AgNPs inhibited *Arabidopsis thaliana* seedling root elongation with a linear dose response from 67 to 535 μg/L after 2 week exposure but seed germination was not affected.[24] Similar effects were observed for *Lolium multiflorum*, with inhibition of plant growth on agar medium becoming more pronounced as the concentration of (gum arabic-coated) AgNPs increased from 1 to 40 mg/L.[22] Similar AgNPs at 5 or 10 mg/L also reduced duckweed biomass after 72 h hydroponic exposure.[25] Plants in the environment are likely to be exposed to much lower AgNP concentrations (e.g., the occurrence of AgNPs in surface waters was estimated at about 0.1 ng/L in the US and 0.8 ng/L in Europe[9]), but the effect of AgNPs at such lower concentrations or after long-term exposure has received limited attention. Furthermore, whereas uptake studies have been conducted with several types of nanoparticles,[33−38] internalization, and translocation by plants as a function of AgNP properties and exposure concentrations remain poorly understood. AgNPs have been detected inside *Arabidopsis* root cell walls and plasmodesmata; however, whether particles were taken up intact or some formed within the plant from assimilated Ag^+ released by AgNPs is unclear.[24,39−42]

This study characterizes the effects of AgNPs on two plant species belonging to different genera − *Arabidopsis* and *Populus* (i.e., poplar trees) − to discern species-specific variability and commonalities in plant response. Both *Arabidopsis* and poplar are common model plants for studies of basic plant biology and of toxicity, bioaccumulation and translocation of heavy metals and organic contaminants. To shed light on the effects of AgNPs on plant physiology over a broad range of exposures, plants were exposed to AgNPs of various sizes and to a wide range of concentrations (0.01 to 100 mg/L). Phytotoxicity was assessed by measuring effects on shoot and root growth, biomass accumulation, and evapotranspiration relative to unexposed controls. To assess the role of released Ag^+, we also characterized the dissolution of AgNPs in the hydroponic exposure medium and included analysis of plant responses to the presence of Ag^+ (added as $AgNO_3$). Through these efforts, we characterize more broadly dose−response relationships and total silver bioaccumulation among different plant compartments.

MATERIALS AND METHODS

Preparation and Characterization of AgNPs. Amorphous-carbon-coated 25 nm AgNPs, which represent commercially available AgNPs with (thus) a higher probability of release, were bought from Novacentrix (Austin, TX). Polyethylene glycol-thiol (PEG)-coated 10 and 5 nm AgNPs were synthesized in the lab to obtain a narrow size distribution at relatively small sizes as described previously.[43] The PEG coating of custom-made AgNPs enhances their stability in water. Commercial AgNP powder and concentrated (23 mg/L) custom-made AgNP water suspensions were covered with aluminum foil and stored in anaerobic chambers (4% H_2, 4% CO_2, 92% N_2). TEM images showed that the Novacentrix 25 nm (27.3 ± 5.6 nm) AgNPs were nonuniform in size and aggregated together after 15 day incubation in $^1/_4$ strength Hoagland solution (pH 6.8),[44] whereas the lab-generated 10 nm (10.4 ± 1.7 nm) and 5 nm (5.1 ± 0.3 nm) AgNPs were uniform and did not significantly aggregate after 1 month incubation in $^1/_4$ strength Hoagland solution (Figure S1 of the Supporting Information). The main equilibrium speciation of dissolved silver in this medium were free Ag^+ (94%) and AgCl (3.8%) at 0.01 to 1 mg/L as determined by Visual MINTEQ (Table S1 of the Supporting Information).

***Arabidopsis* Growth and Treatment.** *Arabidopsis thaliana* (ecotype Col-0) seeds were sterilized, as described previously[45] and sown singly in Araponics[46] seedholders filled with sterile 0.8% agar (1.01614, EMD Millipore chemicals, Darmstadt, Germany) in $^1/_4$ strength Hoagland solution (pH 6.8).[44] Each seedholder was placed at the top of a 14 mL polypropylene tube (352059, Falcon, Franklin Lakes, NJ) containing 13 mL of sterile $^1/_4$ strength Hoagland solution with or without AgNPs or $AgNO_3$ (S7276, Sigma-Aldrich, St. Louis, MO) as indicated. The tubes were covered with foil to minimize light exposure. Racks holding the seedholders and tubes were placed under a transparent cover for two days, and the seedlings were allowed to grow under 16 h photoperiods, with 50 μmol·m^{-2}·s^{-1} at 22 °C. Under these conditions, seedling roots elongated through the agar plug and emerged below into the liquid medium within 10 days and the seedlings had developed two pairs of true leaves. At approximately 6 weeks of hydroponic growth using this system, flowers developed and seeds were produced. All plants were grown without silver until indicated ages, and then seedholders harboring plants were transferred to new tubes containing Ag^+ or AgNPs for the indicated number of days as described in each figure legend.

Poplar Growth and Treatment. Nine-inch cuttings (Segal Ranch, WA) from male clones of adult imperial Euro-americana hybrid poplar trees (*Populus deltoides* × *nigra*, DN-34) were fitted into predrilled screw caps. The interface of the cutting and the cap was sealed with 100% silicone sealant. Cuttings were grown in $^1/_4$ strength Hoagland solution (pH 6.8)[44] at 25 °C with 16 h photoperiods and 85 to 100 μmol·m^{-2}·s^{-1}. Fresh nutrient solution was supplied twice a week. After 4 weeks of growth, healthy poplars (trees with similar stem length, root length, and leaf number, and with healthy roots) were moved into the hydroponic exposure reactor, a 500 mL screw-top glass bottle modified with a screw-top side arm as the sampling port. The reactors were wrapped with aluminum foil to prevent algae growth. The total volume of $^1/_4$ strength Hoagland solution in each reactor was 400 mL. The medium contained no additional supplements (controls), or AgNPs or $AgNO_3$, as indicated in text. DI water was added daily, except every fifth day, to replace lost solution. Every fifth day, $^1/_4$ strength Hoagland solution instead of water was added to compensate for nutrient loss.

Evapotranspiration was determined by monitoring weight loss of the reactors. Cumulative evapotranspiration for each tree was determined as the sum of the daily evapotranspiration during the 11 day exposure, which is a typical exposure period for phytotoxicity studies.

Silver Concentration Measurements. Solution samples (12 mL) were taken from poplar hydroponic solutions amended with 10 nm AgNPs or Ag^+ (at 1 mg/L) on day 0, 1, 2, 5, and 11 after thoroughly mixing. Total silver concentration (both AgNPs and released Ag^+) in solution was analyzed by inductively coupled plasma-mass spectrometry using an Elan 9000 apparatus (ICP-MS, PerkinElmer, Waltham, MA) after digestion with 70% HNO_3 digestion at 90 °C for 2 h. To quantify total silver accumulation in the plant, different plant tissues were separated after exposure without washing and dried at over 80 °C for at least 2 days prior to grinding with mortar and pestle at room temperature. For root tissue, measurements include the total amount of silver adsorbed on

the root surface plus accumulated within the root. After weighing, tissue powders were digested with 67−70% Trace-Metal grade nitric acid (A509, Fisher Scientific, Pittsburgh, PA) at over 70 °C overnight. After cooling, an equal volume of 30% hydrogen peroxide was added and the mixture was incubated at over 70 °C for at least 2 h. The remaining solution was filtered with 0.2 μm sterile syringe filters (28145, VWR International, Radnor, PA) after diluting with deionized water and analyzed with ICP-MS.

Statistical Analysis. Whether differences in plant responses among treatments were significant was determined by one-way analysis of variance (ANOVA) followed by a post hoc multiple comparison test at the 95% significance level.

RESULTS AND DISCUSSION

AgNPs Enhanced Poplar and *Arabidopsis* Growth at Some Sublethal Concentrations. We first examined the overt growth effects of commercially available 25 nm AgNPs on both poplar and *Arabidopsis*, as the commercial AgNPs are better candidates for environmental release than our custom-made AgNPs. Exposure to low concentrations of AgNPs enhanced poplar evapotranspiration and biomass growth, whereas high concentrations exerted toxicity. Specifically, AgNPs at sublethal concentrations (1 mg/L, or about 1/100th of the inhibitory level) increased poplar evapotranspiration by 42% compared to unexposed controls, whereas high concentrations (100 mg/L) significantly decreased evapotranspiration by 87% ($p < 0.05$, part A of Figure 1). Compared to control poplars, root and stem biomass were increased by 63% and 46% respectively after exposure to these AgNPs at 1 mg/L for 11 days, but high concentrations (100 mg/L) reduced the fresh weights of roots, stems, and leaves by 87%, 42%, and 81%, respectively ($p < 0.05$, part B of Figure 1). Similarly, although *Arabidopsis* growth in agar medium was strongly inhibited at 100 mg/L of 25 nm AgNPs, 1 mg/L enhanced growth, resulting in plants with more extensively expanded leaves (part A of Figure S2 of the Supporting Information).

Because this is the first report of a stimulatory effect of AgNPs on plant growth, we further investigated this phenomenon using custom-made AgNPs of uniform size, which are less prone to aggregate and precipitate. Furthermore, the well-defined coating of the custom-made AgNPs, which is similar to those used in previous studies,[47] enabled experiments to test for potential effects of the coating itself.

PEG-coated 10 nm AgNPs and Ag^+ were tested for physiological effects on poplars. Exposure to 0.1 mg/L of 10 nm AgNPs significantly enhanced poplar evapotranspiration by 43% ($p < 0.05$, part A of Figure 2). The final fresh weight of roots, stems, and leaves was also increased relative to the control poplars by 48%, 50%, and 39%, respectively ($p < 0.05$, part B of Figure 2). None of these stimulatory effects were

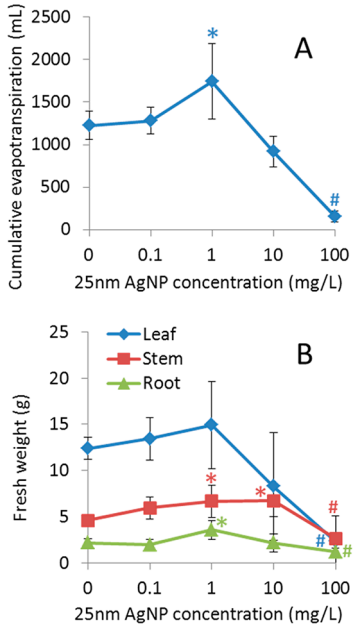


Figure 1. Effects of 25 nm AgNPs on poplar cumulative evapotranspiration (A) and fresh weight (B) of leaf, stem, and root after 11 day hydroponic exposure. Asterisks (*) denote statistically significant ($p < 0.05$) phytostimulation compared to untreated controls. Hash signs (#) denote statistically significant ($p < 0.05$) inhibition. Error bars represent ± one standard deviation from the mean of 8 replicates.

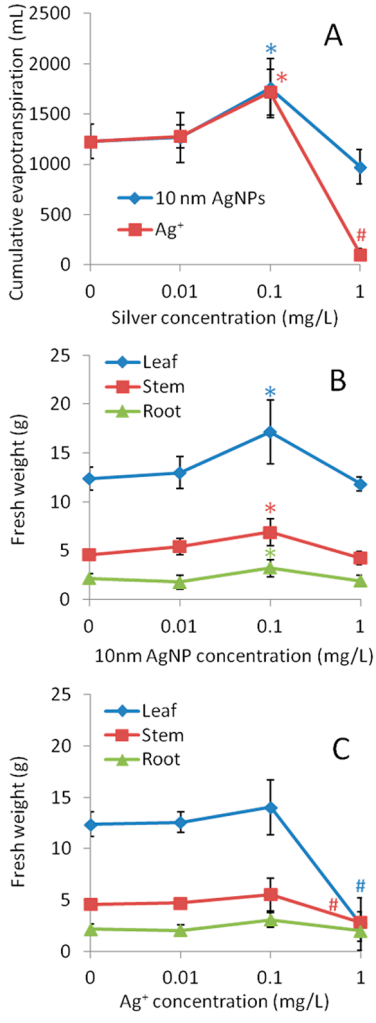


Figure 2. Effects of 10 nm AgNPs and Ag^+ on poplar cumulative evapotranspiration (A), 10 nm AgNPs on poplar fresh weight (B), and Ag^+ on poplar fresh weight (C) after 11 day hydroponic exposure. Asterisks (*) denote statistically significant ($p < 0.05$) phytostimulation compared to untreated controls. Hash signs (#) denote statistically significant ($p < 0.05$) inhibition. Error bars represent ± one standard deviation from the mean of 8 replicates.

dx.doi.org/10.1021/es4004334 | Environ. Sci. Technol. 2013, 47, 5442−5449

observed at 1 mg/L. Ag^+ exerted stronger toxicity on poplar growth than AgNPs, although it had similar stimulatory effects on evapotranspiration at sublethal concentrations (part A of Figure 2). Exposure to PEG alone (at an equivalent concentration to that present in the stimulatory 0.1 mg/L 10 nm AgNP dose) did not significantly enhance evapotranspiration (Figure S4 of the Supporting Information). Thus, the stimulatory effects of AgNPs were not due to the PEG coating. Ag^+ significantly decreased poplar leaf (by 78%) and stem (by 37%) fresh weight at 1 mg/L ($p < 0.05$, part C of Figure 2), and poplars died after 11 day exposure. But at 0.1 mg/L, cumulative transpiration significantly increased by 40% ($p < 0.05$, part A of Figure 2). Ag^+ did not significantly increase the plant fresh weight (part C of Figure 2).

Growth enhancing and toxic effects (at higher concentrations) were also apparent for *Arabidopsis* exposed to PEG-coated AgNPs (5 and 10 nm) or Ag^+. Moderate increases in overall rosette size and coloration were seen at 0.01 mg/L Ag^+ and 0.05 mg/L AgNPs (5 and 10 nm), whereas the rosette size became progressively smaller at higher concentrations, especially at 1 mg/L (part A of Figure 3). Root growth rate was completely inhibited at 1 mg/L of AgNPs ($p < 0.05$, part B of Figure 3); however, 0.01 to 0.05 mg/L of 5 nm AgNPs or 0.01 to 0.02 mg/L of 10 nm AgNPs increased root growth by 38% and 20%, respectively ($p < 0.05$, part B of Figure 3). Ag^+ also enhanced root growth at concentrations below 0.02 mg/L, but concentrations above 0.05 mg/L dramatically impaired root growth ($p < 0.05$, part B of Figure 3); similar effects of silver salts have been reported previously.[48] Plant shoot growth in response to AgNPs was similar to that seen in root growth assays (part C of Figure 3). Maximal shoot weight was obtained when plants were grown in the presence of 0.05 mg/L of 5 nm AgNPs or 0.01 mg/L of Ag^+; toxicity was apparent when plants were grown in the presence of 1 mg/L 5 nm AgNPs, or >0.1 mg/L Ag^+ ($p < 0.05$, part B of Figure 3). Only when grown in the presence of 1 mg/L of Ag^+ or AgNPs, *Arabidopsis* leaves showed signs of chlorosis and chlorophyll content was reduced to approximately 20% of the chlorophyll level found in *Arabidopsis* grown in the absence of silver ($p < 0.05$, part A of Figure S5 of the Supporting Information). Anthocyanin accumulation, an additional sign of stress, was increased more than 5-fold in *Arabidopsis* grown with either 1 mg/L of Ag^+ or AgNPs over that present in *Arabidopsis* grown without silver ($p < 0.05$, part B of Figure S5 of the Supporting Information). Similar growth enhancing and toxic effects were observed when *Arabidopsis* seedlings were grown on solid agar medium supplemented with the various silver additives ($p < 0.05$, Figure S3 of the Supporting Information). Overall, Ag^+ exerted similar effects as AgNPs (phytostimulatory at low doses and toxic at higher doses) at similar or much lower concentrations, possibly because of more efficient uptake (Figure 5 and 6).

It is unlikely that this stimulatory effect was due to suppression of potential pathogens because of the relatively short duration of exposure and because the plants were grown pest-free for 1 month prior to exposure. Furthermore, although we cannot rule out a potential stimulatory effect due to the proprietary coating of the commercial 25 nm AgNPs, no role for the PEG coating on the custom-made AgNPs was detectable. Although further studies are required to discern the specific underlying phytostimulation mechanisms, the literature provides some insights into possible modes of action. AgNPs have been reported to stimulate the photosynthetic system of cyanobacteria (*Synechocystis* sp. PCC6803) due to

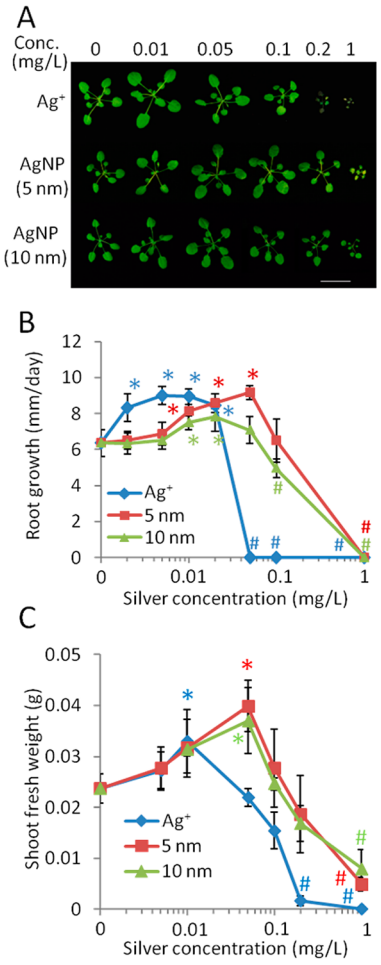


Figure 3. Effects of Ag^+ and 5 and 10 nm AgNPs on *Arabidopsis* overall shoot phenotype (A), root elongation (B), and shoot fresh weight (C). Photographs (A) show *Arabidopsis* plants exposed to Ag^+ or AgNPs for two weeks. Asterisks (*) denote statistically significant ($p < 0.05$) phytostimulation compared to untreated control, and hash signs (#) denote statistically significant ($p < 0.05$) inhibition. Error bars represent ± one standard deviation from the mean of 6 (B) or 3 (C) replicates.

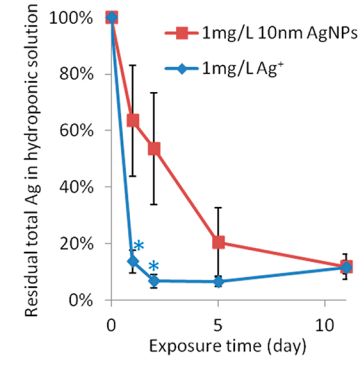


Figure 4. Decrease in total silver concentration remaining in the poplar hydroponic solution compared to day 0 (C_0 =1 mg/L). Asterisks (*) denote statistically significant ($p < 0.05$, *t* test) decrease of the total silver residual percentage in Ag^+ hydroponic solution compared to that in the 10 nm AgNPs solution on the same day. Error bars represent ± one standard deviation from the mean of 8 replicates.

plasmon resonance.[49] Other metallic nanopaticles have also been reported to exert biostimulation through different

dx.doi.org/10.1021/es4004334 | *Environ. Sci. Technol.* 2013, 47, 5442−5449

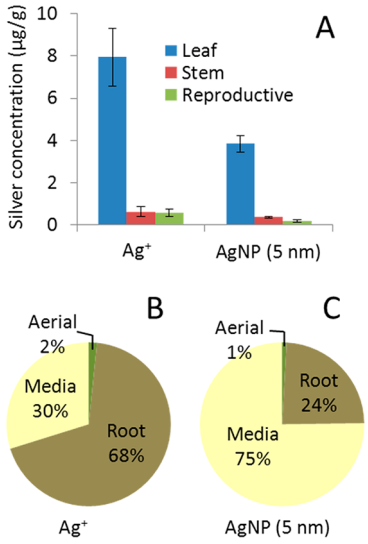


Figure 5. Silver accumulation in *Arabidopsis*. Silver concentration in 6 week old plant shoot organs after 3 day hydroponic exposure to 1 mg/L Ag^+ or 5 nm AgNPs (A). Reproductive refers to flower, silique, and floral bud organs. Error bars represent ± one standard deviation from the mean of 6 replicates. The distribution of silver in 6 week old plants after 3 days of exposure to 1 mg/L Ag^+ (B) or 5 nm AgNPs (C) is depicted. Aerial includes the combined amount detected in leaf, stem, and reproductive organs; $n = 3$. Differences in distribution percentages are statistically significant ($p < 0.05$, t test) (Table S4 of the Supporting Information).

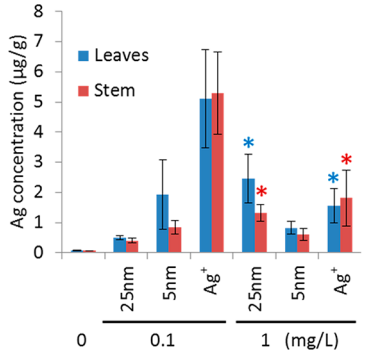


Figure 6. Total silver concentrations in poplar leaves and stems after 7 day hydroponic exposure. Asterisks (*) denote statistically significant ($p < 0.05$, t test) change of accumulated Ag concentration in 1 mg/L silver treated poplar tissues compared to that in the same silver treatments at 0.1 mg/L. Error bars represent ± one standard deviation from the mean of 8 replicates.

mechanisms. TiO_2 nanoparticles stimulated spinach growth apparently by promoting light energy utilization efficiency during photosynthesis (5 μM)[50] and by enhancing active carbon fixation through up-regulation of the Rubisco activase gene (0.25%).[51−53] However, the contribution of dissolved titanium, which also has a phytostimulatory effect,[54] was not considered in these studies. In a separate study, 20 nm Al_2O_3 NPs increased Lemna minor growth at 1000 mg/L by possibly enhancing photosynthetic efficiency,[55] whereas dissolved Al^{3+} (added as $Al_2(SO_4)_3$) inhibited growth at 0.81 mg/L. These latter studies underscore the importance to consider the effect of released metal ions.

Released Ag^+ from AgNPs may act through known effects of silver on plants. Silver blocks ethylene receptor function[56−58] and enhances efflux of the auxin indole-3-acetic acid from roots.[59] Low levels of silver in growth media have been reported to enhance *Arabidopsis* root elongation whereas high levels inhibit root growth.[59] Therefore, released Ag^+ may affect phytohormone levels and function to confer the observed plant growth effects (Figures 1 and 3). Alternatively, this response could be related to hormesis, which is a biphasic dose response in which the presence of low doses of toxicants can activate repair mechanisms that overcompensate for the exposure.[60−62] Accordingly, hormesis is characterized by low-dose stimulation and high-dose inhibition and has been demonstrated to occur in various biological models with different chemical agents.[63] Whether hormesis is part of a global metabolic response to stress exerted by AgNPs or other nanomaterials, and the detailed mode of action, remain to be determined.

Effects of Released Ag^+. The extent to which the observed effects were exerted mainly by Ag^+ released from the AgNPs or to nanoparticle-specific effects is unclear. The continuous dissolution of AgNPs in the growth medium, as well as the potential formation of nanoparticles from Ag^+ on root surfaces[24,39] and in planta,[39,40,42] make it difficult to differentiate ion from particle effects. As mentioned above, Ag^+ is known to affect plant growth by altering perception and/or responses to phytohormones.[56−59,64] However, some nanoparticle-specific effects were likely exerted. For example, exposure to 5 nm AgNPs at 1 mg/L (including 0.12 mg/L of released Ag^+ in the hydroponic solution) decreased poplar fresh weight significantly (Figure S8 of the Supporting Information). Yet, exposure to Ag^+ alone at 0.1 mg/L, which is similar to the amount released in the former treatment, had a stimulatory effect (part C of Figure 2). Similarly, poplar growth was enhanced in the presence of 10 nm AgNPs at 0.1 mg/L (1.6 μg/L released Ag^+) (part A of Figure 2) or 25 nm AgNPs at 1 mg/L (11 μg/L released Ag^+) (part A of Figure 1), but the measured Ag^+ in these hydroponic solutions was too low to account for the observed stimulatory effects.

Silver Uptake by Plants during Hydroponic Growth. Both poplar and *Arabidopsis* removed Ag^+ faster than PEG-coated AgNPs at equivalent concentrations (e.g., 1 mg/L) from the hydroponic solutions indicating faster uptake of Ag^+. Total silver concentration in poplar hydroponic solution decreased much faster when present as Ag^+ (1 mg/L) than as 10 nm AgNPs at the same concentration, especially on day 1 and day 2 ($p < 0.05$) (Figure 4), despite the inhibitory effect of Ag^+ on evapotranspiration at this concentration (part A of Figure 2). Similarly, *Arabidopsis* accumulated more silver when present in the hydroponic solutions at 1 mg/L as Ag^+ (70% of the added silver) than as 5 nm AgNPs (20%) (Figure 5).

Both Ag^+- and AgNP-exposed *Arabidopsis* had the highest accumulation of silver associated with roots (parts B and C of Figure 5), similarly to that reported for *Lolium multiflorum*.[22] The darker color of *Arabidopsis* roots exposed to 1 mg/L 5 nm AgNPs (which is a sublethal dose) suggests that much of the NPs were adsorbed to the root surface (Figure S6 of the Supporting Information).

The mass of silver accumulated in poplar tissues cannot be explained solely by uptake of Ag^+ released in the hydroponic solution (Table S3 of the Supporting Information), which suggests specific AgNP effects including possible nanoparticle uptake as suggested by other studies.[24,31] Among shoot organs, *Arabidopsis* leaves were the most prominent site for silver accumulation in plants exposed to Ag^+ or 5 nm AgNPs, with 10-fold higher accumulation than in the stem or flower tissues (part A of Figure 5). In contrast, total silver concentrations in

the leaves and stems of poplars exposed to Ag^+ or AgNPs (25 and 5 nm) were statistically indistinguishable (Figure 6). Therefore, the distribution of silver accumulation in plant aerial parts was species dependent.

Effect of Nanoparticle Size. Particle size has been reported to affect AgNP toxicity[65−67] with smaller particles typically offering a larger specific surface area that enables more interaction with cells[65] as well as faster Ag^+ release due to enhanced curvature.[66] Even though differences in NP coatings in this study could confound the toxicity comparisons across the tested particle sizes, the smaller 5 and 10 nm AgNPs (with higher specific surface area) dissolved faster in $^1/_4$ strength Hoagland solution (Table S2 of the Supporting Information) and also exerted higher toxicity to both *Arabidopsis* and poplar than the 25 nm AgNPs at the same exposure concentration.

Total silver accumulation by poplars was influenced by both particle size and hydroponic concentration. Within the subinhibitory concentration range, Ag^+ was likely more easily taken up than AgNPs, as indicated by significantly ($p < 0.05$) higher total silver accumulation in plant tissues at 0.1 mg/L (Figure 6). Furthermore, smaller particles generally resulted in higher silver accumulation, although it is not clear whether this reflects easier direct translocation or faster release (and subsequent uptake) of Ag^+ or both. For poplars exposed to 0.1 mg/L, the highest accumulation of silver occurred for treatments with Ag^+, followed by treatments with 5 nm AgNP, and the lowest extent of accumulation was observed in trees exposed to 25 nm AgNPs (Figure 6). This is consistent with the lower stimulatory concentration of 5 or 10 nm AgNPs (0.1 mg/L) on poplar growth compared to 25 nm AgNPs (1 mg/L) (Figures 1 and 2), which might be due to the easier silver accumulation (and/or faster dissolution) with smaller AgNPs. For a given type of AgNP within their subinhibitory domain, silver accumulation increased with exposure concentration and evapotranspiration. For poplars exposed to 25 nm AgNPs, total silver accumulation in leaves increased significantly ($p < 0.05$, t test) from 0.5 ± 0.06 μg/g for the 0.1 mg/L dose to 2.2 ± 0.72 μg/g for the 1 mg/L dose (Figure 6). However, when evapotranspiration was inhibited at higher silver concentrations, silver accumulation decreased. For instance, tissue accumulation of silver in plants exposed to 1 mg/L Ag^+ was significantly ($p < 0.05$, t test) less than that of plants exposed to 0.1 mg/L Ag^+ (Figure 6) likely due to the inhibitory effects exerted by the higher concentration (part A of Figure 2). The higher accumulation of silver in poplars exposed to 25 nm AgNP compared to 5 nm AgNPs (both at 1 mg/L) was likely due to the stimulatory effect on evapotranspiration by 25 nm AgNPs (part A of Figure 1) as well as the inhibitory effect of 5 nm AgNPs (Figure S8 of the Supporting Information) at this concentration. Thus, compared to larger AgNPs, the greater silver accumulation associated with smaller AgNPs can be offset by the toxic effect exerted at lower concentrations.

Implication for Environmental Impact of AgNPs. Previous studies reported the phytotoxicity of AgNPs at relatively high concentrations, which was corroborated by this study. Nevertheless, the observed stimulation at sublethal concentrations precludes generalizations about the phytotoxicity of NPs. Whether this phenomenon can be exploited to enhance primary productivity requires better quantitative understanding of the mechanism underlying potential photosynthesis enhancement or hormetic effects. The similar effects of AgNPs and Ag^+ on both poplars and *Arabidopsis*, and the difficulty to separate AgNPs from Ag^+ that is constantly released during exposure, preclude us from discerning whether AgNPs exerted particle-specific toxicity; the main critical effector could have been Ag^+. Similar inferences have been made in recent studies that demonstrated that the antibacterial activity of AgNPs was solely due to released Ag^+.[47] Regardless of phytotoxicity mechanisms, the accumulation of silver in plant tissue and the plant-species-dependent distribution in shoot organs calls for further study to quantify the translocation and accumulation of AgNPs in edible parts of important food crops.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Details on experimental procedures and analytical methods, TEM images and AgNP dissolution data, additional data on *Arabidopsis* growth and chlorophyll measurements, dissolved silver speciation modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: alvarez@rice.edu; tel: (713)348-5903 (P.J.J.A.); e-mail: braam@rice.edu; tel: (713)348-4277 (J.B.).

Author Contributions

⊥These cofirst authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the National Science Foundation (CMMI-1057906). We thank Hema Puppala for synthesizing AgNPs for preliminary experiments and discussing AgNP properties, and Carolina Avendano for helpful discussions.

■ REFERENCES

(1) The project on emerging nanotechnologies. http://www.nanotechproject.org/inventories/consumer/analysis_draft/.

(2) Nanowerk: nanomaterial database. http://www.nanowerk.com/phpscripts/n_element_results.php?element=Silver&x=52&y=15.

(3) Quadros, M. E.; Marr, L. C. Silver nanoparticles and total aerosols emitted by nanotechnology-related consumer spray products. *Environ. Sci. Technol.* **2011**, *45* (24), 10713−10719.

(4) Faunce, T.; Watal, A. Nanosilver and global public health: international regulatory issues. *Nanomedicine* **2010**, *5* (4), 617−632.

(5) Benn, T. M.; Westerhoff, P. Nanoparticle silver released into water from commercially available sock fabrics. *Environ. Sci. Technol.* **2008**, *42* (11), 4133−4139.

(6) Capek, I. Preparation of metal nanoparticles in water-in-oil (w/o) microemulsions. *Adv. Colloid Interface Sci.* **2004**, *110* (1−2), 49−74.

(7) Frattini, A.; Pellegri, N.; Nicastro, D.; Sanctis, O. d. Effect of amine groups in the synthesis of Ag nanoparticles using aminosilanes. *Mater. Chem. Phys.* **2005**, *94* (1), 148−152.

(8) Fabrega, J.; Zhang, R.; Renshaw, J. C.; Liu, W.-T.; Lead, J. R. Impact of silver nanoparticles on natural marine biofilm bacteria. *Chemosphere* **2011**, *85* (6), 961−966.

(9) Gottschalk, F.; Sonderer, T.; Scholz, R. W.; Nowack, B. Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, fullerenes) for different regions. *Environ. Sci. Technol.* **2009**, *43* (24), 9216−9222.

(10) Blaser, S. A.; Scheringer, M.; MacLeod, M.; Hungerbühler, K. Estimation of cumulative aquatic exposure and risk due to silver: Contribution of nano-functionalized plastics and textiles. *Sci. Total Environ.* **2008**, *390* (2−3), 396−409.

(11) Mueller, N. C.; Nowack, B. Exposure modeling of engineered nanoparticles in the environment. *Environ. Sci. Technol.* **2008**, *42* (12), 4447−4453.
(12) Kaegi, R.; Voegelin, A.; Sinnet, B.; Zuleeg, S.; Hagendorfer, H.; Burkhardt, M.; Siegrist, H. Behavior of metallic silver nanoparticles in a pilot wastewater treatment plant. *Environ. Sci. Technol.* **2011**, *45* (9), 3902−3908.
(13) Kim, B.; Park, C.-S.; Murayama, M.; Hochella, M. F. Discovery and characterization of silver sulfide nanoparticles in final sewage sludge products. *Environ. Sci. Technol.* **2010**, *44* (19), 7509.
(14) Lowry, G. V.; Espinasse, B. P.; Badireddy, A. R.; Richardson, C. J.; Reinsch, B. C.; Bryant, L. D.; Bone, A. J.; Deonarine, A.; Chae, S.; Therezien, M.; Colman, B. P.; Hsu-Kim, H.; Bernhardt, E. S.; Matson, C. W.; Wiesner, M. Long-term transformation and fate of manufactured Ag nanoparticles in a simulated large scale freshwater emergent wetland. *Environ. Sci. Technol.* **2012**, *46* (13), 7027.
(15) Morones, J. R.; Elechiguerra, J. L.; Camacho, A.; Holt, K.; Kouri, J. B.; Ramirez, J. T.; Yacaman, M. J. The bactericidal effect of silver nanoparticles. *Nanotechnology* **2005**, *16* (10), 2346−2353.
(16) Pal, S.; Tak, Y. K.; Song, J. M. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. *Appl. Environ. Microbiol.* **2007**, *73* (6), 1712−1720.
(17) Navarro, E.; Piccapietra, F.; Wagner, B.; Marconi, F.; Kaegi, R.; Odzak, N.; Sigg, L.; Behra, R. Toxicity of silver nanoparticles to *Chlamydomonas reinhardtii*. *Environ. Sci. Technol.* **2008**, *42* (23), 8959−8964.
(18) Miao, A.-J.; Luo, Z.; Chen, C.-S.; Chin, W.-C.; Santschi, P. H.; Quigg, A., Intracellular uptake: A possible mechanism for silver engineered nanoparticle toxicity to a freshwater alga *Ochromonas danica*. *PLoS One* **2010**, *5*, (12).
(19) Jiang, W.; Kim, B. Y. S.; Rutka, J. T.; Chan, W. C. W. Nanoparticle-mediated cellular response is size-dependent. *Nat. Nanotechnol.* **2008**, *3* (3), 145−150.
(20) AshaRani, P. V.; Mun, G. L. K.; Hande, M. P.; Valiyaveettil, S. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano* **2009**, *3* (2), 279−290.
(21) Hussain, S. M.; Hess, K. L.; Gearhart, J. M.; Geiss, K. T.; Schlager, J. J. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. *Toxicol. Vitro* **2005**, *19* (7), 975−983.
(22) Yin, L. Y.; Cheng, Y. W.; Espinasse, B.; Colman, B. P.; Auffan, M.; Wiesner, M.; Rose, J.; Liu, J.; Bernhardt, E. S. More than the ions: The effects of silver nanoparticles on *Lolium multiflorum*. *Environ. Sci. Technol.* **2011**, *45* (6), 2360−2367.
(23) Gubbins, E. J.; Batty, L. C.; Lead, J. R. Phytotoxicity of silver nanoparticles to *Lemna minor* L. *Environ. Pollut.* **2011**, *159* (6), 1551−1559.
(24) Geisler-Lee, J.; Wang, Q.; Yao, Y.; Zhang, W.; Geisler, M.; Li, K.; Huang, Y.; Chen, Y.; Kolmakov, A.; Ma, X. Phytotoxicity, accumulation and transport of silver nanoparticles by *Arabidopsis thaliana*. *Nanotoxicology* **2012**, DOI: 10.3109/17435390.2012.658094.
(25) Jiang, H.-S.; Li, M.; Chang, F.-Y.; Li, W.; Yin, L.-Y. Physiological analysis of silver nanoparticles and $AgNO_3$ toxicity to *Spirodela polyrhiza*. *Environ. Toxicol. Chem.* **2012**, *31* (8), 1880−1886.
(26) El-Temsah, Y. S.; Joner, E. J. Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil. *Environ. Toxicol.* **2012**, *27* (1), 42−49.
(27) Stampoulis, D.; Sinha, S. K.; White, J. C. Assay-dependent phytotoxicity of nanoparticles to plants. *Environ. Sci. Technol.* **2009**, *43* (24), 9473−9479.
(28) Patlolla, A. K.; Berry, A.; May, L.; Tchounwou, P. B. Genotoxicity of silver nanoparticles in *Vicia faba*: a pilot study on the environmental monitoring of nanoparticles. *Int. J. Environ. Res. Public Health* **2012**, *9* (5), 1649−1662.
(29) Barrena, R.; Casals, E.; Colon, J.; Font, X.; Sanchez, A.; Puntes, V. Evaluation of the ecotoxicity of model nanoparticles. *Chemosphere* **2009**, *75* (7), 850−857.
(30) Kumari, M.; Mukherjee, A.; Chandrasekaran, N. Genotoxicity of silver nanoparticles in *Allium cepa*. *Sci. Total Environ.* **2009**, *407* (19), 5243−5246.
(31) Dimkpa, C. O.; McLean, J. E.; Martineau, N.; Britt, D. W.; Haverkamp, R.; Anderson, A. J. Silver nanoparticles disrupt wheat (*Triticum aestivum* L.) growth in a sand matrix. *Environ. Sci. Technol.* **2012**, *47* (2), 1082−1090.
(32) McCutcheon, S. C.; Schnoor, J. L. *Phytoremediation: Transformation and Control of Contaminants*. Wiley Interscience: New York, 2003; p 987.
(33) Sabo-Attwood, T.; Unrine, J. M.; Stone, J. W.; Murphy, C. J.; Ghoshroy, S.; Blom, D.; Bertsch, P. M.; Newman, L. A. Uptake, distribution and toxicity of gold nanoparticles in tobacco (*Nicotiana xanthi*) seedlings. *Nanotoxicology* **2012**, *6* (4), 353−360.
(34) Zhu, H.; Han, J.; Xiao, J. Q.; Jin, Y. Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. *J. Environ. Monit.* **2008**, *10* (6), 713−717.
(35) Hischemoeller, A.; Nordmann, J.; Ptacek, P.; Mummenhoff, K.; Haase, M. In-vivo imaging of the uptake of upconversion nanoparticles by plant roots. *J. Biomed. Nanotechnol.* **2009**, *5* (3), 278−284.
(36) Nedosekin, D. A.; Khodakovskaya, M. V.; Biris, A. S.; Wang, D.; Xu, Y.; Villagarcia, H.; Galanzha, E. I.; Zharov, V. P. In vivo plant flow cytometry: a first proof-of-concept. *Cytometry, Part A* **2011**, *79A* (10), 855−865.
(37) Wang, Z.; Xie, X.; Zhao, J.; Liu, X.; Feng, W.; White, J. C.; Xing, B. Xylem- and phloem-based transport of CuO nanoparticles in maize (*Zea mays* L.). *Environ. Sci. Technol.* **2012**, *46* (8), 4434−4441.
(38) Kurepa, J.; Paunesku, T.; Vogt, S.; Arora, H.; Rabatic, B. M.; Lu, J.; Wanzer, M. B.; Woloschak, G. E.; Smalle, J. A. Uptake and distribution of ultrasmall anatase TiO_2 Alizarin red S nanoconjugates in *Arabidopsis thaliana*. *Nano Lett.* **2010**, *10* (7), 2296−2302.
(39) Gardea-Torresdey, J. L.; Gomez, E.; Peralta-Videa, J. R.; Parsons, J. G.; Troiani, H.; Jose-Yacaman, M. Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. *Langmuir* **2003**, *19* (4), 1357−1361.
(40) Harris, A.; Bali, R. On the formation and extent of uptake of silver nanoparticles by live plants. *J. Nanopart. Res.* **2008**, *10* (4), 691−695.
(41) Kumar, V.; Yadav, S. K. Plant-mediated synthesis of silver and gold nanoparticles and their applications. *J. Chem. Technol. Biotechnol.* **2009**, *84* (2), 151−157.
(42) Marchiol, L. Synthesis of metal nanoparticles in living plants. *Ital. J. Agronomy* **2012**, *7* (3), e37.
(43) Hiramatsu, H.; Osterloh, F. E. A simple large-scale synthesis of nearly monodisperse gold and silver nanoparticles with adjustable sizes and with exchangeable surfactants. *Chem. Mater.* **2004**, *16* (13), 2509−2511.
(44) Hoagland, D. R.; Arnon, D. I. The water-culture method for growing plants without soil. *Circular. California Agricultural Experiment Station* **1950**, *347*, (2nd edit).
(45) Tsai, Y.-C.; Koo, Y.; Delk, N. A.; Gehl, B.; Braam, J. Calmodulin-related CML24 interacts with ATG4b and affects autophagy progression in Arabidopsis. *Plant J.* **2013**, *73* (2), 325−335.
(46) Tocquin, P.; Corbesier, L.; Havelange, A.; Pieltain, A.; Kurtem, E.; Bernier, G.; Périlleux, C. A novel high efficiency, low maintenance, hydroponic system for synchronous growth and flowering of *Arabidopsis thaliana*. *BMC Plant Biol.* **2003**, *3* (1), 2.
(47) Xiu, Z.-m.; Zhang, Q.-b.; Puppala, H. L.; Colvin, V. L.; Alvarez, P. J. J. Negligible particle-specific antibacterial activity of silver nanoparticles. *Nano Lett.* **2012**, *12* (8), 4271−4275.
(48) Strader, L. C.; Beisner, E. R.; Bartel, B. Silver ions increase auxin efflux independently of effects on ethylene response. *Plant Cell* **2009**, *21* (11), 3585−90.
(49) Govorov, A. O.; Carmeli, I. Hybrid structures composed of photosynthetic system and metal nanoparticles: plasmon enhancement effect. *Nano Lett.* **2007**, *7* (3), 620−625.
(50) Su, M.; Liu, H.; Liu, C.; Qu, C.; Zheng, L.; Hong, F. Promotion of nano-anatase TiO_2 on the spectral responses and photochemical

activities of D1/D2/Cyt b559 complex of spinach. *Spectrochim. Acta, Part A* **2009**, *72* (5), 1112−1116.
(51) Ma, L.; Liu, C.; Qu, C.; Yin, S.; Liu, J.; Gao, F.; Hong, F. Rubisco activase mRNA expression in spinach: modulation by nanoanatase treatment. *Biol. Trace Elem. Res.* **2008**, *122* (2), 168−178.
(52) Gao, F.; Hong, F.; Liu, C.; Zheng, L.; Su, M.; Wu, X.; Yang, F.; Wu, C.; Yang, P. Mechanism of nano-anatase TiO_2 on promoting photosynthetic carbon reaction of spinach - inducing complex of Rubisco-Rubisco activase. *Biol. Trace Elem. Res.* **2006**, *111* (1−3), 239−253.
(53) Zheng, L.; Hong, F.; Lu, S.; Liu, C. Effect of nano-TiO_2 on strength of naturally aged seeds and growth of spinach. *Biol. Trace Elem. Res.* **2005**, *104* (1), 83−91.
(54) Hruby, M.; Cigler, P.; Kuzel, S. Contribution to understanding the mechanism of titanium action in plant. *J. Plant Nutr.* **2002**, *25* (3), 577.
(55) Juhel, G.; Batisse, E.; Hugues, Q.; Daly, D.; van Pelt, F. N. A. M.; O'Halloran, J.; Jansen, M. A. K. Alumina nanoparticles enhance growth of *Lemna minor*. *Aquat. Toxicol.* **2011**, *105* (3−4), 328−336.
(56) Rodriguez, F. I.; Esch, J. J.; Hall, A. E.; Binder, B. M.; Schaller, G. E.; Bleecker, A. B. A copper cofactor for the ethylene receptor ETR1 from *Arabidopsis*. *Science* **1999**, *283* (5404), 996−998.
(57) Zhao, X. C.; Qu, X.; Mathews, D. E.; Schaller, G. E. Effect of ethylene pathway mutations upon expression of the ethylene receptor ETR1 from Arabidopsis. *Plant Physiol.* **2002**, *130* (4), 1983−1991.
(58) Binder, B. M.; Rodriguez, F. I.; Bleecker, A. B.; Patterson, S. E. The effects of group 11 transition metals, including gold, on ethylene binding to the ETR1 receptor and growth of *Arabidopsis thaliana*. *FEBS Lett.* **2007**, *581* (26), 5105−5109.
(59) Strader, L. C.; Beisner, E. R.; Bartel, B. Silver ions increase auxin efflux independently of effects on ethylene response. *Plant Cell* **2009**, *21* (11), 3585−3590.
(60) Stebbing, A. Hormesis—the stimulation of growth by low levels of inhibitors. *Sci. Total Environ.* **1982**, *22* (3), 213−234.
(61) Calabrese, E.; Baldwin, L. Defining hormesis. *Hum. Exp. Toxicol.* **2002**, *21* (2), 91−97.
(62) Calabrese, E. J. Hormesis: why it is important to toxicology and toxicologists. *Environ. Toxicol. Chem.* **2009**, *27* (7), 1451−1474.
(63) Calabrese, E. J.; Baldwin, L. A. Hormesis: the dose-response revolution. *Annu. Rev. Pharmacol. Toxicol.* **2003**, *43* (1), 175−197.
(64) Beyer, E. M., Jr A potential inhibitor of ethylene action in plants. *Plant Physiol.* **1976**, *58* (3), 268−271.
(65) Park, M.; Neigh, A. M.; Vermeulen, J. P.; de la Fonteyne, L. J. J.; Verharen, H. W.; Briede, J. J.; van Loveren, H.; de Jong, W. H. The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles. *Biomaterials* **2011**, *32* (36), 9810−9817.
(66) Sotiriou, G. A.; Pratsinis, S. E. Antibacterial activity of nanosilver ions and particles. *Environ. Sci. Technol.* **2010**, *44* (14), 5649−5654.
(67) Lee, C. W.; Mahendra, S.; Zodrow, K.; Li, D.; Tsai, Y. C.; Braam, J.; Alvarez, P. J. J. Developmental phytotoxicity of metal oxide nanoparticles to *Arabidopsis thaliana*. *Environ. Toxicol. Chem.* **2010**, *29* (3), 669−675.