Assay-Dependent Phytotoxicity of Nanoparticles to Plants

DIMITRIOS STAMPOULIS,*,† SAION K. SINHA,‡ AND JASON C. WHITE*,§

Department of Biology and Environmental Science and Department of Physics, University of New Haven, 300 Boston Post Road, West Haven, Connecticut 06516, and Department of Analytical Chemistry, The Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, Connecticut 06504

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The effects of five nanomaterials (multiwalled carbon nanotubes [MWCNTs], Ag, Cu, ZnO, Si) and their corresponding bulk counterparts on seed germination, root elongation, and biomass of *Cucurbita pepo* (zucchini) were investigated. The plants were grown in hydroponic solutions amended with nanoparticles or bulk material suspensions at 1000 mg/L. Seed germination was unaffected by any of the treatments, but Cu nanoparticles reduced emerging root length by 77% and 64% relative to unamended controls and seeds exposed to bulk Cu powder, respectively. During a 15-day hydroponic trial, the biomass of plants exposed to MWCNTs and Ag nanoparticles was reduced by 60% and 75%, respectively, as compared to control plants and corresponding bulk carbon and Ag powder solutions. Although bulk Cu powder reduced biomass by 69%, Cu nanoparticle exposure resulted in 90% reduction relative to control plants. Both Ag and Cu ion controls (1−1000 mg/L) and supernatant from centrifuged nanoparticle solutions (1000 mg/L) indicate that half the observed phytotoxicity is from the elemental nanoparticles themselves. The biomass and transpiration volume of zucchini exposed to Ag nanoparticles or bulk powder at 0−1000 mg/mL for 17 days was measured. Exposure to Ag nanoparticles at 500 and 100 mg/L resulted in 57% and 41% decreases in plant biomass and transpiration, respectively, as compared to controls or to plants exposed to bulk Ag. On average, zucchini shoots exposed to Ag nanoparticles contained 4.7 greater Ag concentration than did the plants from the corresponding bulk solutions. These findings demonstrate that standard phytotoxicity tests such as germination and root elongation may not be sensitive enough or appropriate when evaluating nanoparticle toxicity to terrestrial plant species.

Introduction

In 2005, total global investment in nanotechnologies exceeded $4 billion, and the estimated annual value for nanotechnology-related products is expected to reach $1 trillion by 2015 (*1*). The manufacture and use of particulate material in the size range of a few nanometers (nm) is the driving force behind this growth (*2*). The extremely small size, structure, and surface characteristics of nanoparticles result in unique physicochemical properties not observed with larger or bulk particles of the same material. For example, materials with dimensions less than 5 nm exhibit unique electronic states, magnetic/optical properties, and catalytic reactivities that differ from corresponding atomic and bulk scale counterparts (*3*). In addition, insoluble substances can exhibit drastically enhanced solubility when the particle size is less than 100 nm. Nanoparticles also have a greater surface area than larger particles with equivalent mass, yielding a greater proportion of atoms on the surface relative to the interior of the structure and resulting in higher surface reactivity (*4*). Surfactants and other additives can modify the surface active properties of nanomaterials and may prevent particle aggregation (*5*). Such effects can be particularly important for hydrophobic particles such as carbon nanotubes and fullerenes.

The U.S. EPA organizes engineered nanomaterials into four categories, including (1) carbon-based materials in tubes, spheres, or ellipsoids; (2) metal-based materials such as Au, Ag, but also including metal oxides and quantum dots; (3) dendrimers or nanosized polymers; and (4) composites integrating nanoparticles with other bulk scale materials. Hundreds of nanotechnology-based products are currently in the marketplace, with common applications being found in electronics, optics, food packaging, textiles, medical devices, cosmetics, water treatment technology, fuel cells, catalysts, biosensors, and components for environmental remediation (*6*−*8*). For example, nanoscale zerovalent iron can be used to detoxify halogenated molecules such as polychlorinated biphenyls or to reduce nitrates in groundwater (*9*). The antimicrobial properties of Ag nanoparticles have found use in a range of products, including textiles, bandages, air filters, and vacuum cleaners. Carbon-based nanomaterials are integrated into plastics, catalysts, battery/fuel cell electrodes, water purification systems, orthopedic implants, adhesives/composites, and electronics (*10*).

Nanoparticulate matter can be produced by naturally occurring processes such as volcanic activity, fire, and erosion; as such, organisms have long been exposed to and have evolved with these materials. However, the current magnitude of exposure and the unique nature of engineered particulates warrant caution. Manufactured nanoparticles can enter the environment unintentionally through atmospheric emissions, domestic wastewater, agriculture, and accidental release during manufacture/transport; or through intentional releases such as during remediation efforts (*11*). The interaction and impact of nanomaterials, with their unique physical and chemical properties, on living systems has only recently been explored (*12*). Obviously, some direct data on human response to nanoparticle exposure has been acquired (*13*). A range of species have been investigated in nanotoxicology studies, including bacteria (*14, 15*), algae (*16*), invertebrates such as nematodes and crustaceans (*17, 18*), and vertebrates such as fish and rats (*19, 20*). However, this literature is far from complete and is plagued by shortcomings, with many studies failing to directly compare bulk and nanoparticle toxicity for a given material. In terms of ecotoxicity, there has been significantly greater focus on aquatic rather than terrestrial species, and very little work has focused on terrestrial plants. Some studies have reported the toxic effects of nanoparticles on the germination and/or root growth of some plant species (*21, 22*). A recent study

* Corresponding author e-mail: Jason.White@ct.gov; phone: 203-974-8523; fax: 203-974-8502.
† Department of Biology and Environmental Science, University of New Haven.
‡ Department of Physics, University of New Haven.
§ The Connecticut Agricultural Experiment Station.

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(*23*) showed that select nanoparticles can be absorbed, translocated, and accumulated within tissues of pumpkin plants.

The current study focuses on comparing the effects of five types of commonly used nanoparticles (multiwalled carbon nanotubes [MWCNTs], Ag, Cu, Si, and Zn oxide) to their corresponding bulk material counterparts on germination, root elongation, and biomass of the agricultural plant *Cucurbita pepo* (zucchini). In this preliminary nanotoxicology study, initial concentrations of 1000 mg/L were chosen to ensure observation of relevant phytotoxic responses. In addition, the effect of nanoparticle or bulk Ag concentration (0−1000 mg/L) on zucchini biomass, transpiration, and Ag content was determined in a dose−response study. Assessing the impacts of nanoparticles on agricultural plants will provide insight into the risk of ecological exposure to these materials, as well as to the potential for human exposure through food chain contamination.

Experimental Section

Chemicals. The nanoparticles and corresponding bulk materials used were acquired commercially and used as purchased. The particles were MWCNTs, Ag, Cu, Si, and Zn oxide; information on these materials can be found in Supporting Information Table 1. The MWCNTs were produced through a high-yield catalytic process based on chemical vapor deposition (CVD) that results in a high degree of purity (>99%), low concentration of residual catalyst, and absence of amorphous carbon. Their number of walls ranged from 3 to 15. A 10.1-cm Si wafer was pulverized with a mortar and pestle. Hoagland Solution and sodium dodecyl sulfate (SDS) were purchased from MP Biomedicals (Solon, OH). Nanoparticle or bulk material solutions were prepared at 1000 mg/L in 25% Hoagland solution with 0% or 0.2% SDS. Solutions were sonicated for 20 min and manually agitated prior to use. As this study was a straightforward comparison of phytotoxicity from exposure to equivalent nanoparticle and bulk particle treatments, no further information on particle aggregation/dissolution was obtained.

Seeds. Zucchini seeds (*Cucurbita pepo* cv Costata Romanesco) were purchased from Johnny's Selected Seeds (Albion, ME). Seeds were soaked in 10% sodium hypochlorite for 10 min and were then rinsed thoroughly with reverse osmosis (RO) water. The seeds were air-dried for several days and were then stored at room temperature.

Germination and Root Elongation Assays. The effect of nanoparticles or corresponding bulk materials on seed germination and root elongation was determined. These assays were conducted with R.O. water amended with 0% or 0.2% SDS to promote particle dispersion. For the elongation assay, seeds were pregerminated and those with a radical of approximately 0.5 mm were selected. Three individually marked seeds (germination) or emerging seeds (elongation) were placed in Petri dishes (35 mm × 10 mm); each dish was amended with 3 mL of nanoparticle or bulk material solution (1000 mg/L) with or without 0.2% SDS. There were five replicate dishes for each treatment. The dishes were closed and placed on an orbital shaker operating at 50 rpm and 28 °C for 5 days (elongation) or 12 days (germination). The solution was replenished as needed with the appropriate nanoparticle or bulk particle containing solution so as to avoid exposure concentration dilution.

Hydroponic Biomass Assays. A batch hydroponic experiment was conducted to determine the effect of nanoparticles and corresponding bulk materials on the biomass of zucchini seedlings. Seeds were added to moist germination paper and 4-day-old seedlings were subsequently added to 8 mL amber vials containing 7.5 mL of 25% Hoagland solution. The seedlings were placed in a growth room maintained at 25 °C with a 12 h photoperiod of 200 μmol m^{-2} s^{-1} photosynthetic active radiation (PAR) for 14 days. Seedlings were then transferred to 40-mL amber vials containing 39 mL of solution amended with either the nanoparticles or corresponding bulk materials at 1000 mg/L. There were six replicate plants per treatment. The plants were then returned to the growth room, and the biomass was monitored during a subsequent 15-day exposure period. The solution was replenished as needed with the appropriate nanoparticle or bulk particle containing solution so as to avoid exposure concentration dilution.

For particles showing significant differences in plant toxicity between bulk and nanomaterials (Ag and Cu), experiments were run to differentiate soluble ion phytotoxicity from that of the elemental nanoparticles. Seven-day-old seedlings were transferred to 40-mL amber vials containing 39 mL of solution amended with 1.0, 10, 100, or 1000 mg/L $AgNO_3$ or $Cu(NO_3)_2 \cdot 5H_20$ in 25% Hoagland solution. Additional KNO_3 controls were included to address potential enhanced growth from added nitrate. Also, solutions of Ag and Cu nanoparticles (1000 mg/L in 25% Hoagland solution) were added to 50 mL Teflon centrifuge tubes and were spun at 2000 rpm for 5 min. The elemental nanoparticles settled, and the ion containing supernatants were collected for use as a growth medium (referred to as "Cu or Ag NP supernatant"). The seedlings were incubated as above, and biomass was measured daily for 15 days. There were five seedlings per treatment.

Ag Dose-Uptake Assay. On the basis of the large difference in phytotoxicity observed between bulk and nanoparticulate Ag in the hydroponic biomass assay, the effect of Ag nanoparticles or bulk powder at 0, 1.0, 10, 50, 100, 500, and 1000 mg/L on zucchini biomass and transpiration volume was determined. Seeds were added to moist germination paper and 4-day-old seedlings were subsequently added to 40 mL amber vials containing 39 mL of 25% Hoagland Solution amended with bulk or nanoparticle Ag at 0−1000 mg/L. There were six replicate plants per treatment. The plants were placed in a greenhouse at 22−28 °C with ambient light. The biomass and transpiration volume (determined by mass change of solution) were measured during the 17 day exposure period. The solution was replenished as needed with the appropriate nanoparticle or bulk particle containing solution so as to avoid exposure concentration dilution. At 17 days, stems were severed with a razor blade at least 4 cm above the level of solution so as to acquire tissues never in direct contact with nanoparticle or bulk Ag. The shoots were oven-dried at 100 °C for 72 h and digested on a hot block with concentrated HNO_3 for 1 h at 115 °C. The digested plant tissues were analyzed by inductively coupled plasma mass spectroscopy (ICP-MS) for Ag content.

Results and Discussion

Germination Assay. The effect of 1000 mg/L nanoparticles or corresponding bulk material on the germination of zucchini (*C. pepo*) seeds was determined (Table 1). In the first assay, solutions were amended with the surfactant sodium dodecyl sulfate (0.2%) to facilitate particle dissolution. The percent germination in the RO water and RO water + SDS (0.2%) were 90% and 60%, respectively; these values are significantly different (Student *t* test; $p < 0.05$). Similarly, the time to 66% germination (2 of 3 seeds for a given replicate dish) was 3.0 and 5.5 days for the RO water and RO water + SDS (0.2%), respectively (significantly different at $p < 0.05$). Because of the reduction in germination rate in the presence of the surfactant, the RO water + SDS was used as the control against which the nanoparticles and their corresponding bulk materials were statistically evaluated. In general, no further reductions in germination were observed in the various treatments. The exception was silicon nanoparticles, where germination was completely inhibited; although these values are statistically different from the unamended control, there

TABLE 1. Percent Germination of Zucchini Seeds Exposed to Nanoparticles and Corresponding Bulk Materials[a]

Trial One (SDS)

RO water[b]	RO water+ SDS	AC[c]	MWCNT[c]	ZnO powder	ZnO 5 nm	ZnO 10 nm	Si powder	Si 100 nm	Cu powder	Cu 50 nm	Ag powder	Ag 100 nm
90 a (0.05)	60 A[d] (0.07)	60 Aa[e] (0.17)	40 Aa (0.11)	67 Aa (0.13)	86 Aa (0.07)	67 Aa (0.08)	40 ABa (0.11)	0 Ba (NA)	40 Aa (0.07)	53 Aa (0.08)	67 Aa (0.15)	33 Aa (0.15)

Trial Two (No SDS)

RO water	RO water+ SDS	AC	MWCNT	ZnO powder	ZnO 5 nm	ZnO 10 nm	Si powder	Si 10 nm	Cu powder	Cu 50 nm	Ag powder	Ag 100 nm
87 Aa (0.08)	NA	67 Aa (0.21)	87 Ab (0.08)	80 Ab* (0.08)	67 Ab* (0.13)	87 Aa (0.11)	93 Ab (0.07)	80 Ab (0.08)	73 Ab* (0.13)	80 Aa (0.13)	93 Aa (0.07)	87 Ab (0.08)

[a] Trial one included the surfactant sodium dodecyl sulfate (SDS, 0.2%) in all treatments to facilitate particle dispersion; trial 2 did not use the surfactant. Values represent % germination after 4 days. Values in parentheses represent standard errors. [b] For trial one, all treatments except RO water included SDS. [c] AC = activated carbon, MWCNT = multiwalled carbon nanotubes. [d] Within a row, values followed by different capital letters are significantly different by one-way ANOVA followed by a Dunns multiple comparison test involving three treatments (trial 1, RO water + SDS, a nanomaterial, and the corresponding bulk material; trial 2, RO water, a nanomaterial, and the corresponding bulk material). [e] Within a column, values followed by different lowercase letters are significantly different (Student *t* test, $p < 0.05$; * indicates difference at $p < 0.10$).

is no difference from the silicon bulk material (40% germination). The average time to 66% germination across the nanoparticle and corresponding bulk materials was 6.1 and 7.5 days. These values are not significantly different from each other or from the control, although this analysis excludes the Si nanoparticles which had 0% germination.

In trial two, which lacked the confounding effect of the surfactant, none of the investigated nanoparticles or corresponding bulk materials at 1000 mg/L had a statistically significant impact on seed germination. The average percent germination across all treatments was 82% (±9.0%). The time to 66% germination was not different across treatments and averaged 3.4 days (±0.68 days). Our observation that, in the absence of SDS, nanoparticles had little impact on seed germination agrees with Lin and Xing (*22*), who evaluated the impact of five nanoparticles on the germination of six plant species. Although corresponding bulk materials were not evaluated in that study, the authors noted only two instances of reduced germination out of 30 treatments. Cucumber was the most closely related plant to zucchini (same family) in the Lin and Xing (*22*) study; even at concentrations twice that of our work, none of the five nanoparticles resulted in phytotoxicity. Conversely, Zheng et al. (*24*) evaluated the effects of nanoparticulate (5 nm) and bulk TiO_2 on spinach seed germination; however, in their study, seeds were presoaked in nanoparticle or bulk TiO_2 solution for 48 h prior to planting in perlite growth media. Under these conditions, the authors noted that while nanoparticle exposure significantly increased germination at their lower concentrations (250−4000 mg/L), higher levels of exposure (6000−8000 mg/L) had the opposite effect.

The finding that seed germination was reduced in the presence of SDS clearly indicates surfactant-mediated phytotoxicity. The literature on the ability of surfactants to emulsify cell membranes and other lipid-containing cellular constituents is mature, and the precise mechanisms are well characterized (*25*−*27*). Clearly, the use of surfactants to facilitate nanoparticle dispersion is useful but must be employed cautiously and with the inclusion of appropriate controls. In the current study, when comparing within individual particle treatments, SDS reduced germination in the following instances: MWCNT, ZnO powder, ZnO (5 nm) nanoparticles, Si power, Si nanoparticles, Cu powder, Cu nanoparticles, and Ag nanoparticles. Clearly, any discussion of mechanisms for reduced germination is confounded by the 30% reduction caused by SDS alone. However, one could speculate on potential additive effects in certain treatments where reductions in germination are greater than 30%. For example, the reduction in germination in the SDS-MWCNT, -Si nanoparticle, and -Ag nanoparticle treatments were 47%, 80%, and 54%, respectively; however, further investigation is necessary to statistically evaluate these trends.

Root Elongation Assay. The effect of 1000 mg/L nanoparticles or corresponding bulk material on the root elongation of pregerminated zucchini (*C. pepo*) seeds over 5 days was determined in the presence or absence of 0.2% SDS. For the purposes of data analysis, an individual nanoparticle treatment was statistically evaluated only against the corresponding bulk material and the control. In the first trial (surfactant), the root elongation in the RO water and RO water + SDS (0.2%) were 13 and 3.4 mm, respectively; these values are significantly different (Mann−Whitney Rank sum test; $p < 0.001$). Since the surfactant reduced root growth, the RO water + SDS was used as the control against which the nanoparticles and bulk materials were statistically evaluated. No further reductions in root growth were observed in the various treatments. On day 3, both bulk and nanoparticle Ag treatments had significantly greater root growth than the controls but that difference disappeared by the following day (data not shown).

In the absence of SDS, the average root length of untreated control seeds after 5 days reached 24 mm. All treatments except Cu had no significant impact on root elongation; the average lengths for nanoparticles and corresponding bulk materials at day 5 were 23 and 27 mm, respectively. Cu nanoparticles resulted in a significantly decreased root length after 24 h; at day 5, the average root lengths of the unamended controls, Cu powder, and Cu nanoparticles were 24, 16, and 5.7 mm, respectively (one-way ANOVA followed by Student−Newman−Keuls multiple comparison test) (Supporting Information Figure 1). These findings agree with those of Yang and Watts (*21*), who determined the impact of alumina nanoparticles on the root elongation of corn, cucumber, cabbage, and carrot. Although 20 and 200 mg/L alumina nanoparticles did not impact the root growth of the plants, 2000 mg/L resulted in a 13% reduction in root length across all four species. However, the authors did not compare these findings to the impact of bulk alumina. Similarly, Lin and Xing (*22*) investigated the impact of five nanoparticles on the root elongation of radish, rape, ryegrass, lettuce, corn, and cucumber. Again, the authors did not include the corresponding bulk counterparts, but MWCNT, Al_2O_3, and Al nanoparticles did not impact root elongation at 2000 mg/L. Conversely, ZnO nanoparticles at 2000 mg/L dramatically

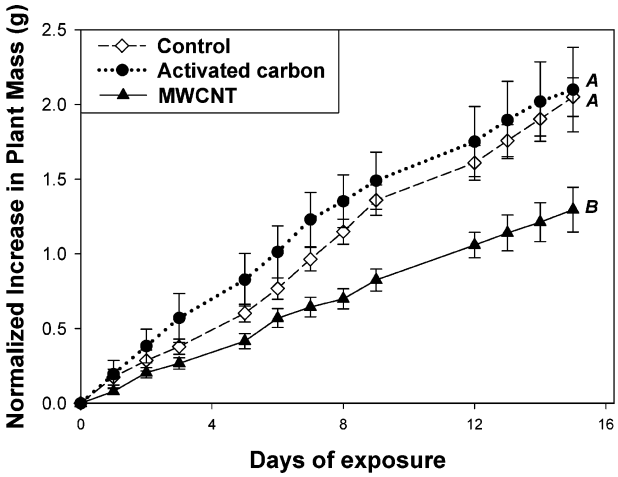

FIGURE 1. Effect of activated carbon powder and MWCNT on zucchini biomass. Curves displaying different numbers are significantly different (one-way ANOVA on slopes of lines regressed through replicate data followed by Student−Newman−Keuls multiple comparison test). Error bars represent standard errors of the mean.

reduced root growth for all five species. In our study, no statistical differences were observed between the control, ZnO powder, and ZnO nanoparticles. This difference may be due to the higher particle concentration used by Lin and Xing, the different plant species used, and also the high replicate variability within our ZnO treatments. Cañas et al. (*28*) exposed six crop species to carbon nanotubes and also reported inherently high variability among replicates of a given treatment. Again, the authors of this study did not include a corresponding non-nanoparticle carbon control, but they did note that impact of nanotube exposure on root growth was species-specific and that functionalization of the material generally reduced toxicity.

Given the above discussion on the impact of anionic surfactants on seed germination, it is not surprising that SDS similarly reduced root elongation. However, when comparing an individual treatment in the presence and absence of SDS, the surfactant reduced root elongation in the following instances: Ag powder, Ag nanoparticles, Si powder, Si nanoparticles, and Cu powder. Given the 73% reduction in root length caused by the surfactant alone, the mechanism for reduced germination among the various treatments may have little to do with particle size.

Hydroponic Biomass Assay. The effect of 1000 mg/L nanoparticles or corresponding bulk material on the biomass of zucchini (*C. pepo*) seedlings grown under batch hydroponic conditions was determined. The results fall into three general categories. First, the impact of nanoparticles on plant growth is not significantly different from the corresponding bulk material (Supporting Information Figure 2). The biomass of plants exposed to Si nanoparticles or bulk Si powder were not significantly different from the control plants. Conversely, all ZnO treatments reduced zucchini biomass by 78−90% relative to the controls (significant at $p < 0.01$), but there were no differences between the nanoparticles and bulk material. These findings are in partial agreement with Lin and Xing (*30*), where ryegrass was exposed for 12 days to ZnO nanoparticles at 0−1000 mg/L. The authors observed significant reductions in shoot mass at solution concentrations as low as 0.05 mg/L and 50% reduction at 1000 mg/L. Although no corresponding bulk ZnO was investigated, the authors did include $ZnSO_4 \cdot 7H_2O$ as a source of Zn^{2+} and determined that the phytotoxicity observed with the ZnO nanoparticles could not be fully explained by dissolution to the free ion.

Second, as shown in Figures 1 and 2A, the bulk material has no significant impact on plant biomass relative to the

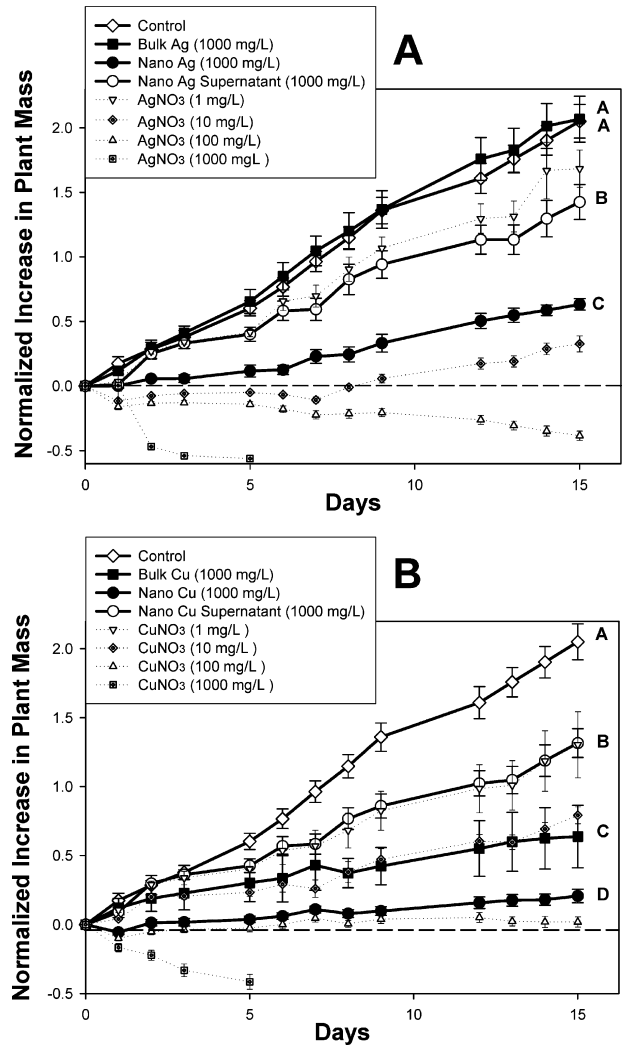

FIGURE 2. Effect of Ag powder or Ag nanoparticles (A) and Cu powder or Cu nanoparticles (B) on zucchini biomass under hydroponic conditions. Dissolved ion controls were included, as were controls consisting of the supernatant of centrifuged nanoparticle solutions. Within a graph, curves displaying different letters are significantly different (one-way ANOVA on slopes of lines regressed through replicate data followed by Student−Newman−Keuls multiple comparison test). Error bars represent standard errors of the mean.

controls, but the corresponding nanoparticle does reduce plant growth. MWCNT and Ag nanoparticle exposure resulted in 38% and 69% reductions, respectively, in zucchini biomass relative to the control and corresponding bulk materials. These results disagree with those of Zheng et al. (*24*), who showed that, at concentrations of 250−4000 mg/L, exposure to nanoparticle TiO_2 for 48 h prior to planting in clean media significantly increased seedling growth whereas the corresponding bulk material had little impact. However, the alternative exposure method and given that at higher concentrations (8000 mg/L) both bulk and nanoparticle TiO_2 reduce plant growth make comparison with the current study problematic. Exposure to $AgNO_3$ at 10 mg/L most closely approximates plant growth upon exposure to Ag nanoparticles. Exposure to the supernatant of a 1000 mg/L Ag nanoparticle solution results in significantly greater plant growth than that observed with the noncentrifuged nanoparticle solution, clearly indicating that increased Ag ion dissolution from the nanoparticles only partially explains the observed phytotoxicity.

Third, as shown in Figure 2B, Cu powder significantly reduced plant biomass relative to the controls, but exposure to Cu nanoparticles resulted in even greater toxicity. Copper powder and nanoparticles reduced zucchini biomass by 69% and 90%, respectively, relative to untreated control plants. These findings are in partial agreement with those of Lee et al. (*31*), who exposed bean and wheat seedlings to Cu nanoparticles (0− 1000 mg/L) for 48 h. Although no corresponding bulk Cu was included, which is obviously significant given our data, the authors did note 40% reduction in the biomass of both species at 200 mg/L and 80% reduction at 1000 mg/L. Plant growth in the Cu nanoparticle solution most closely tracks with exposure to 10 mg/L $Cu(NO_3)_2 \cdot 5H_20$. Similar to Ag, exposure to the supernatant 1000 mg/L Cu nanoparticle solution results in significantly greater plant growth than that observed with the noncentrifuged nanoparticle solution, suggesting direct phytotoxic effects from the elemental nanoparticles.

Ag Dose−Uptake Assay. The biomass and transpiration volume of zucchini exposed to bulk or nanoparticle Ag at 0−1000 mg/L was determined. Bulk Ag at all concentrations had no impact on zucchini biomass or transpiration volume; control plants and plants exposed to bulk Ag (all concentrations) had an average mass of 4.4 g (wet) (±0.95) and transpired an average of 70 mL (±14) of solution over 17 days. Exposure to Ag nanoparticles at 1000 and 500 mg/L reduced zucchini biomass by 71% and 57% respectively, as compared to unamended controls and corresponding bulk Ag treatments (significantly different at $p < 0.05$) (Supporting Information Figure 3). Exposure at 100 mg/L or lower did not significantly impact zucchini biomass. The transpiration volume of plants exposed to Ag nanoparticles at 100, 500, and 1000 mg/L were reduced by 41%, 78%, and 79%, respectively (significantly different from control and corresponding bulk material at $p < 0.05$) (Figure 3). Exposure to Ag at concentrations of 50 mg/L or lower did not impact zucchini transpiration volume. The Ag content of shoots from nanoparticle or bulk Ag solutions is shown in Figure 4. The Ag content of plants grown in unamended Hoagland's solution was 22 μg/kg. At all levels except 1 mg/L, the Ag concentration in plants exposed to nanoparticles was significantly greater than those exposed to bulk Ag powder (Student *t* test, $p < 0.05$). On average, the nanoparticle solutions yielded shoots with 4.7-fold greater Ag content than present in plants grown in the corresponding levels of bulk element.

Information on the uptake of nanoparticles by plants, as well as on the potential mechanisms of phytotoxicity, remains largely unknown. Clearly nanoparticle chemical structure, size, shape, and surface area significantly impact their behavior and fate in the environment, as well as interactions with biota. Metals may release ions that result in toxicity; differences between nanoparticle and corresponding bulk material phytotoxicity could be due to greater ion release from the nanomaterials. In this study, increased release of Cu and Ag ions from the nanopowders appears to have been partially responsible for the observed toxicity (Figures 2−4). This is further supported by the greater shoot Ag levels in plants exposed to the nanoparticles as compared to the Ag powder. However, exposure to the elemental Ag nanoparticles seems to result in at least half of the observed phytotoxicity. Ratte (*31*) notes that the toxicity of Ag to plant species varies, with sensitive cultivars being impacted at aqueous concentrations as low as 75 mg/L. Conversely, in sludges spiked with photographic Ag waste at 120 mg/kg, no impact on the growth of several agricultural species was noted. In the case of bulk and potentially nanoparticle Ag, toxicity is clearly related to availability of the ion. Similarly, El-Ghamery et al. (*32*) reported that Zn^{2+} phytotoxicity includes decreased or stunted growth; in the current study, such symptoms were observed in plants exposed to bulk and nanoparticle ZnO.

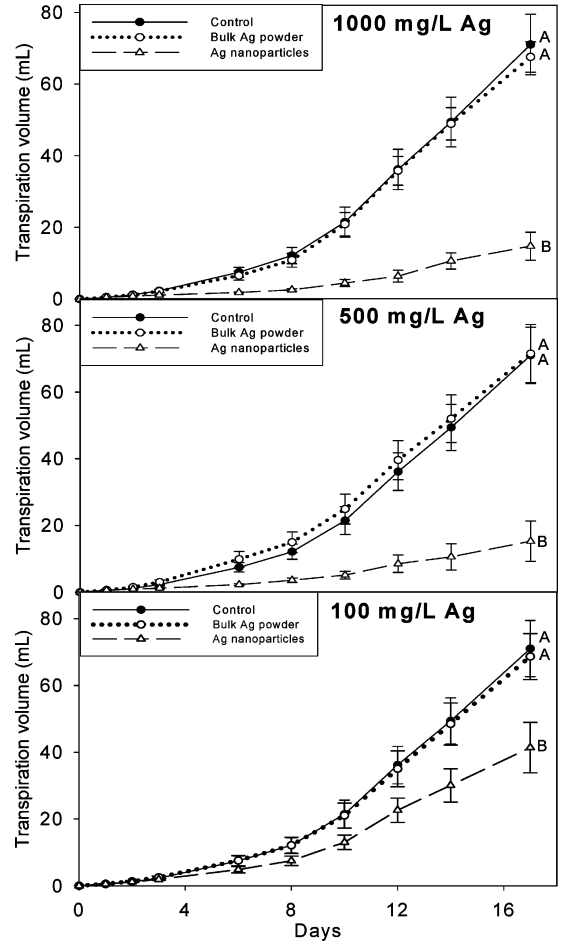

FIGURE 3. Impact of bulk or nanoparticle (<100 nm) Ag on the transpiration volume of zucchini grown hydroponically. Ag was at 0, 100, 500, or 1000 mg/L. Curves followed by different letters are significantly different (data was log-transformed and a one-way ANOVA was performed on the slopes of lines regressed through replicate data followed by Student−Newman−Keuls multiple comparison test). Error bars represent standard errors of the mean.

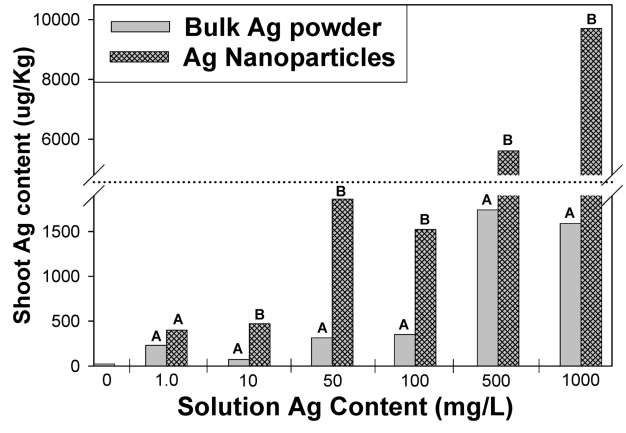

FIGURE 4. Shoot Ag content of plants not directly exposed to nanoparticle or bulk Ag solutions. At a given Ag solution concentration, bars with different letters are significantly different (Student *t* test).

However, Lin and Xing (*22*) reported that ZnO nanoparticle-induced toxicity resulted not only from the dissolution of ions into the nutrient solution but also something specific to the mere presence of nanoparticles. Although in a later study, the same authors (*29*) reported little or no Zn

translocation into rye plants that were exposed to ZnO nanoparticles. In this instance, potential mechanisms of toxicity could include increased reactive oxygen species formation upon contact and subsequent lipid peroxidation of cellular membranes.

The literature does report uptake of some metal nanoparticles and of carbon nanotubes in bacterial and mammalian cells, but data on plant species is generally lacking (*34*). Zhu et al. (*23*) watered pumpkin plants with a solution containing 500 mg/L Fe_3O_4 nanoparticles. Although no corresponding bulk material was included and the exposed plants displayed no visible signs of toxicity, the authors did report magnetometric detection of root to shoot translocation of the nanoparticles. Lee et al. (*31*) did measure Cu nanoparticle accumulation and phytotoxicity in bean and wheat. Interestingly, the authors report that released Cu ions had little impact on the plants and that the dose-dependent toxicity was specifically due to nanoparticle accumulation within cells. Conversely, Cañas et al. (*29*) reported that plants exposed to carbon nanotubes had visible layered sheets of the nanomaterial on the outer surfaces of exposed roots but that no visible uptake had occurred during the 48 h trial. Current investigations are focused on elucidating potential mechanisms of toxicity, including uptake and disposition of nanoparticles within various agricultural plant species.

Although nanoparticulate matter is a natural component of the environment, the recent dramatic increase in manufactured nanomaterials has certainly altered the nature and magnitude of environmental exposure. For terrestrial plants, the impacts of these exposures remain largely unknown. The current study shows that two of the most commonly employed phytotoxicity tests largely fail to demonstrate any appreciable effects of nanoparticle exposure on a model agricultural species. However, two week hydroponic experiments yield dramatically different results, with exposure to MWCNT, Ag, and Cu nanoparticles resulting in significantly decreased biomass relative to controls or corresponding bulk materials. Increased ion dissolution from the nanomaterials only partially explains the observed phytotoxicity. In a dose−response study, zucchini shoots indirectly exposed to Ag nanoparticles contained 4.7 greater Ag concentrations than did the plants from the corresponding bulk solutions. The effective concentration to reduce zucchini biomass and/or transpiration is at least 2 orders of magnitude lower for the nanoparticles than for bulk Ag powder. Clearly, more work needs to be done to clarify the ecotoxicological effects of nanoparticle exposure in soils and under field conditions, as well as to characterize potential risk associated with food chain contamination through agricultural species.

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Supporting Information Available

Additional table and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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