

# Toxicity of Silver and Copper to *Cucurbita pepo*: Differential Effects of Nano and Bulk-Size Particles

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**ABSTRACT:** The phytotoxicity of bulk and nanoparticle Cu and Ag was directly compared. NP Ag reduced biomass and transpiration by 66–84% when compared with bulk Ag. The Ag ion concentration was 4.4–10-times greater in NP than bulk particle solutions. The Cu ion concentration was 1.4–4.4-times greater in bulk than NP amended solutions. Humic acid (50 mg/L) decreased the ion content of bulk Cu solution by 38–42% but increased ion Cu content of NP solutions by 1.4–2.9 times. Bulk and NP Cu were highly phytotoxic; growth and transpiration were reduced by 60–70% relative to untreated controls. NP Cu phytotoxicity was unaffected by solution type, but humic acid (50 mg/L) completely alleviated phytotoxicity caused by bulk Cu. The data demonstrate differential toxicity of Ag NP relative to bulk Ag. The finding that humic acid and solution chemistry differentially impact bulk and NP behavior highlights the importance of evaluating nanoparticles under environmentally relevant conditions. © 2010 Wiley Periodicals, Inc. *Environ Toxicol* 27: 510–517, 2012.

**Keywords:** nanoparticles; phytotoxicity; Ag; Cu; engineered nanomaterials; nanotoxicology

## INTRODUCTION

The term nanotechnology describes a rapidly developing discipline involving the characterization and use of structures, devices, and systems with size ranging between 1 and 100 nm. As of 2005, the total global investment in nanotechnologies exceeded \$4 billion; by 2015, the annual value for all nanotechnology-related products will be in excess of \$1 trillion (Roco, 2005; Aitken et al., 2006). Although nanoparticulate matter can be produced by naturally occurring processes such as volcanic activity, fire, and erosion, engineered nanomaterials (NM) differ in a number of key respects from those produced unintentionally. Engineered particles are monodispersed/homogeneous, often with regular conformation, and designed to have specific surface

characteristics that may vary greatly from those of the corresponding bulk material.

For example, nanoparticles have higher reactivity due to a greater proportion of atoms on the surface relative to the interior of the structure (Handy et al., 2008a,b). As such, insoluble substances can exhibit drastically enhanced solubility when the particle size is less than 100 nm. In addition, materials with dimensions less than 5 nm exhibit unique magnetic/optical properties, electronic states, and catalytic reactivities that differ from corresponding bulk materials (Auffan et al., 2009).

As of November 2009, in excess of 1000 nanotechnology products were on the market. Common applications include electronics, optics, textiles, medical devices, cosmetics, food packaging, water treatment technologies, fuel cells, catalysts, biosensors, and components of environmental remediation (Roco, 2003; Zhang and Elliot, 2006; Klaine et al., 2008). For example, nanoparticulate Ag has well-known antimicrobial properties that have directly led to incorporation into products ranging from bandages to socks to vacuum cleaners (Geranio et al., 2009). Carbon-based NM such as single/multiwalled nanotubes are

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currently integrated into products such as plastics, orthopedic implants, electronics, catalysts, battery/fuel cell electrodes, water purification systems, and adhesives/composites (Klaine et al., 2008). Nanoscale zero-valent iron is currently being used in groundwater to detoxify halogenated pollutants or reduce nitrates (Zhang and Elliot, 2006). Other NM applications include drug delivery agents, components on DNA chips, and composites to recognize and attach to diseased/cancerous cells for targeted treatment and cell destruction (Handy et al., 2008a,b; Klaine et al., 2008).

Given the current and future widespread usage of NM, release to the environment is inevitable. Release could occur at any point of the NM lifecycle, including atmospheric emissions, domestic wastewater, agriculture, remediation, and accidental release during manufacture/transport (Zhang and Elliot, 2006; Klaine et al., 2008). The effects and toxicity of NM on living systems have only recently been explored (Nowack and Bucheli, 2007; Gottschalk et al., 2009); nanotoxicology studies have looked at a number of species, including bacteria (Johansen et al., 2008; Jiang et al., 2009), algae (Wang et al., 2008), nematodes (Wang et al., 2009), crustaceans (Heinlaan et al., 2008), fish (Griffit et al., 2008), and rats (Elgrabi et al., 2008). However, this literature is far from complete, and most studies have failed to directly compare bulk and nanoparticulate behavior for a given material. In addition, the vast majority of this research has taken place under highly controlled laboratory conditions; very little is known about the fate, transport, and behavior of NM under environmentally relevant conditions. Last, with regard to ecotoxicology, the focus has been on aquatic rather than terrestrial species, and little work has addressed terrestrial plants (Blinova et al., 2010). Some studies have investigated the phytotoxicity, as measured by germination and/or root elongation, of select nanoparticles to a small number of plant species (Yang and Watts, 2005; Lin and Xing, 2007, 2008). A recent study (Zhu et al., 2008) suggested that nanoparticles can be absorbed and accumulated within tissues of pumpkin plants. Stampoulis et al. (2009) showed that while germination and root elongation were inadequate measures for NP phytotoxicity, 14-day hydroponic assays demonstrated clear biomass reductions for MWCNTs, as well as for NP Ag and Cu, when compared with corresponding bulk materials or contaminant-free controls.

The current study directly compares the effects of Ag and Cu NPs to their corresponding bulk material counterparts on the biomass and transpiration of the agricultural plant *Cucurbita pepo* ssp *ovifera* (squash) under hydroponic conditions. The initial elemental concentrations were 0, 100, and 500 mg/L, and the aqueous media were Hoagland's solution with or without 50 mg/L humic acid. In addition, the Ag and Cu content of solutions and plant shoot tissues was determined by inductively coupled plasma mass spectroscopy (ICP-MS). Characterizing the interactions of nanoparticles with agricultural species 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.

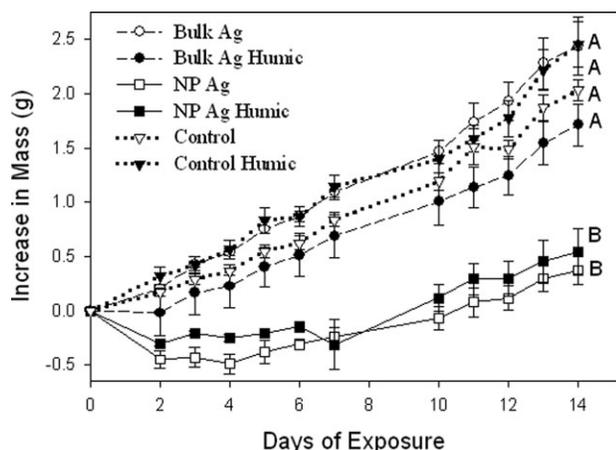
## EXPERIMENTAL

### Seeds and Chemicals

Yellow squash seeds (*Cucurbita pepo* subspecies *ovifera* cv Yellow crookneck) were purchased from Johnny's Selected Seeds (Albion, ME). The Cu bulk, as well as the Ag nanoparticles and bulk material, was acquired from Sigma-Aldrich (St. Louis, MO) and used as purchased. The Cu NPs were purchased from Melorium Technologies (Rochester, NY). The Cu and Ag NP dimensions were <50 and <100 nm, respectively. Hoagland's solution and humic acid were purchased from Fisher Scientific (Pittsburgh, PA). Nanoparticle or bulk material solutions were prepared at 0, 100, or 500 mg/L in 25% Hoagland's solution with 0 or 50 mg/L humic acid. Solutions were manually agitated before use. As this study was a direct comparison of phytotoxicity from exposure to equivalent nano- (less than 100 nm) and non-nanoparticle treatments in the presence or absence of humic acid, no further information of particle aggregation/dissolution was obtained.

### Hydroponics Assay

A batch hydroponic experiment was conducted to determine the effect of NP and bulk Ag and Cu on the biomass and transpiration of squash seedlings. Seeds were pregerminated in moistened germination paper, and 7-day-old seedlings were added to 8-mL amber vials containing 7.5 mL of 25% Hoagland's solution. The seedlings were placed in a growth room at 25°C with a 12-h photoperiod for 7 days. Seedlings were then transferred to 40 mL amber vials containing 39 mL of Hoagland's solution with or without 50 mg/L humic acid. The solutions had previously been amended with either the NP or bulk Ag or Cu at 0, 100, or 500 mg/L. There were eight replicate plants per treatment. The plants were returned to the growth room; biomass and transpiration (determined by mass change of solution) were monitored during a 14-day exposure period. As the elements were added to solution at 2.5–4 orders of magnitude above the measured aqueous ion content, the vials were replenished as needed with the appropriate (humic vs. non-humic) element-free containing solution. At 14 days, stems were severed with a razor blade at least 4 cm above the solution level so as to acquire tissues never in direct contact with nano- or bulk particles. The shoots were oven-dried at 100°C for 72 h and digested on a hot block with concentrated HNO<sub>3</sub> for 1 h at 115°C. The digested plant tissues were analyzed by ICP-MS for Ag or Cu content. In addition, the aqueous Ag and Cu concentration in the 0, 100,



**Fig. 1.** Biomass of squash exposed to bulk or NP Ag at 500 mg/L in Hoagland solution (25%) with or without humic acid (50 mg/L). Error bars represent standard error ( $n=8$ ). Curves followed by different letters are significantly different (one-way analysis of variance [ANOVA] on the slopes of lines regressed through replicate data followed by a Student Newman Keuls multiple comparison test).

and 500 mg/L stock solutions, with and without humic acid, was determined. Portions of the original stock solutions were removed and centrifuged at 3000 rpm for 10 min. An aliquot of these solutions was removed and acidified to contain 5% (v/v) of concentrated  $\text{HNO}_3$ , and their Ag and Cu concentrations were determined by ICP-MS. Because of high-Ag NP phytotoxicity during the 14-day assay, insufficient biomass was obtained for elemental analysis. As a result, a second hydroponic assay with Ag exposure was executed under identical conditions to that above except that the duration was 120 h.

### Statistical Analysis

The statistical significance of differences in NP and bulk particle content of solutions or plant tissues was determined by a Student's  $t$  test (Sigma Stat 3.0). The statistical significance of differences in plant biomass or transpiration between control, bulk particle exposed-, or NP-exposed plants was determined by a one-way analysis of variance on the slopes of lines regressed through replicate data followed by a Student Newman-Keuls multiple comparison test (Sigma Stat 3.0).

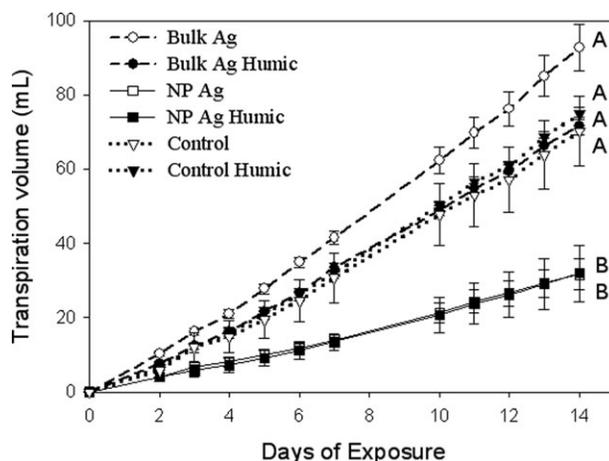
## RESULTS

### Silver Exposure

The Ag concentration in the Hoagland's solution with 50 mg/L humic acid was  $3.2 \mu\text{g/L}$ ; unamended Hoagland's solution had nondetectable levels of Ag. At an initial level of

100 mg/L, the ionic Ag concentration in bulk and NP solutions were 180 and  $1800 \mu\text{g/L}$  (significantly different;  $t = 212$ ,  $p < 0.001$ ). In the presence of 50 mg/L humic acid, the ionic Ag content of the bulk and NP solutions was 180 and  $1700 \mu\text{g/L}$ ; these values are significantly different from each other ( $t = 398$ ,  $p < 0.001$ ) but not from the corresponding humic acid-free solutions. Similarly, at 500 mg/L, the ionic Ag concentration in bulk and NP solutions was 840 and  $3700 \mu\text{g/L}$  (significantly different;  $t = 25.3$ ,  $p < 0.001$ ). In the presence of 50 mg/L humic acid, the aqueous ionic Ag content of the bulk and NP solutions was 470 and  $3600 \mu\text{g/L}$ , respectively. These values are significantly different from each other ( $t = 28.8$ ,  $p < 0.001$ ); in addition, humic acid significantly decreased ionic Ag content in the bulk solution (500 mg/L;  $t = 77.8$ ,  $p < 0.001$ ).

The biomass and transpiration volume of squash exposed to bulk or NP Ag at 0, 100, or 500 mg/L in the presence or absence of 50 mg/L humic acid were determined. Bulk Ag at 100 and 500 mg/L had no impact on the biomass or transpiration volume of the plants (Figs. 1 and 2). Similarly, 50 mg/L humic acid did not affect bulk Ag phytotoxicity. Control plants and plants exposed to bulk Ag (all concentrations) had an average mass of 4.1 g (wet weight) ( $\pm 0.78$ ) and transpired an average of 76 mL ( $\pm 16$ ) of solution over 14 days. Exposure to 500 mg/L Ag NP in Hoagland's solution amended with or without humic acid reduced squash biomass by 74 and 83%, respectively, when compared with unamended controls and corresponding bulk Ag treatments (Fig. 1). The biomass reductions caused by the NPs are significantly different from the control or bulk Ag values ( $F = 22.1$ ,  $p < 0.001$ ); the attenuated decrease



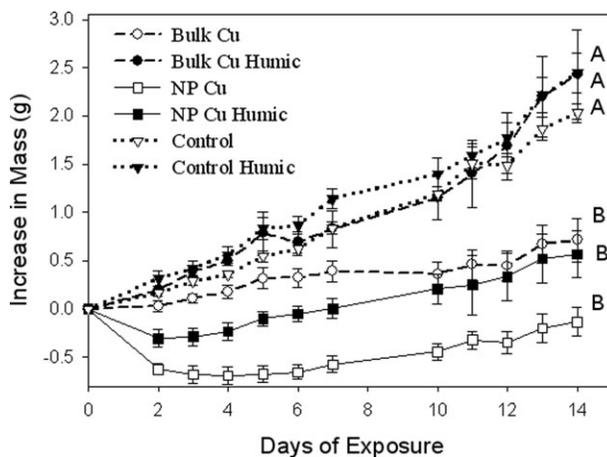
**Fig. 2.** Transpiration of squash exposed to bulk or NP Ag at 500 mg/L in Hoagland solution (25%) with or without humic acid (50 mg/L). Error bars represent standard error ( $n=8$ ). Curves followed by different letters are significantly different (one-way analysis of variance [ANOVA] on the slopes of lines regressed through replicate data followed by a Student Newman Keuls multiple comparison test).

in NP phytotoxicity caused by humic acid is not significant. Although the trends for the biomass of squash exposed to 100 mg/L Ag NP were similar to those at the 500 mg/L exposure level, none of the treatments yielded differences of statistical significance. The volume of solution transpired by plants exposed to Ag NP at 500 mg/L in Hoagland's solution with or without humic acid was reduced by 56 and 61%, respectively (significantly different from control and corresponding bulk material at  $p = 0.04$ ,  $F = 18.9$ ; Fig. 2). Again, although NP exposure reduced transpiration, the attenuated decrease caused by humic acid was insignificant. Exposure to Ag NP 100 mg/L did not impact squash transpiration volume.

Because of Ag NP phytotoxicity and overall lower levels of uptake, insufficient biomass existed for the determination of elemental content of the shoot tissues. As such, a second hydroponic experiment was initiated according to the identical experimental design with the exception that the Ag exposure was for 120 h. In plants grown in unamended Hoagland's solution with or without 50 mg/L humic acid, there were nondetectable levels of Ag shoot tissues. The Ag content of shoots exposed to Ag-containing solutions ranged from 0.34 to 2.6  $\mu\text{g/g}$  (wet weight), but these values were largely unaffected by particle size, concentration, or solution type. In Hoagland's solution, the Ag shoot content in bulk and NP treatments at 100 mg/L was 0.80 and 0.34  $\mu\text{g/g}$  (wet weight), respectively (not significantly different) and at 500 mg/L, these values were 2.6 and 0.33  $\mu\text{g/g}$ , respectively (not significantly different;  $t = 1.58$ ,  $p = 0.19$ ). The presence of 50 mg/L humic acid had no impact on shoot Ag content in bulk solution. However, humic acid increased the plant shoot Ag content in 100 and 500 mg/L Ag NP solutions by 5.1 and 2.4-fold, respectively. In Hoagland's solution amended with humic acid, the Ag shoot content in bulk and NP treatments at 100 mg/L was 0.62 and 1.7  $\mu\text{g/g}$  (wet weight), respectively (significantly different,  $t = 3.24$ ,  $p = 0.032$ ), and at 500 mg/L, these values were 1.0 and 0.78  $\mu\text{g/g}$ , respectively (not significantly different).

## Copper Exposure

The Cu concentration in the Hoagland's solution with and without 50 mg/L humic acid was 5.6 and 3.1  $\mu\text{g/L}$  (significantly different;  $t = 36.5$ ,  $p = <0.001$ ). At an initial level of 100 mg/L, the ionic Cu concentration in bulk and NP solutions was 1540 and 1100  $\mu\text{g/L}$  (significantly different;  $t = 11.2$ ,  $p = <0.001$ ). In the presence of 50 mg/L humic acid, the ionic Cu content of the bulk and NP solutions was 950 and 1500  $\mu\text{g/L}$ , respectively. These values are not only significantly different from each other ( $t = 12.3$ ,  $p = <0.001$ ) but are also different from the corresponding humic acid-free solutions ( $t = 7.77$ ,  $p = 0.001$ ). Similarly, at 500 mg/L, the ionic Cu concentration in bulk and NP solutions was 1400 and 320  $\mu\text{g/L}$  (significantly different;  $t = 93.9$ ,  $p =$

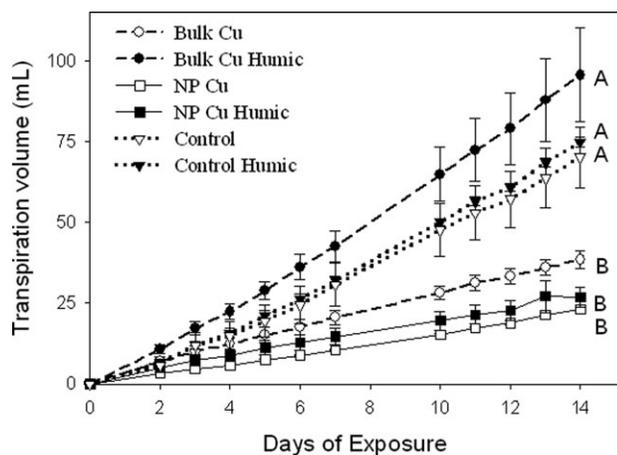


**Fig. 3.** Biomass of squash exposed to bulk or NP Cu at 100 mg/L in Hoagland solution (25%) with or without humic acid (50 mg/L). Error bars represent standard error ( $n = 8$ ). Curves followed by different letters are significantly different (one-way analysis of variance [ANOVA] on the slopes of lines regressed through replicate data followed by a Student Newman Keuls multiple comparison test).

$<0.001$ ). In the presence of 50 mg/L humic acid, the aqueous Cu content of the bulk and NP solutions was 880 and 940  $\mu\text{g/L}$ , respectively. These values are significantly different from each other ( $t = 5.99$ ,  $p = 0.004$ ); in addition, the ionic Cu content in the bulk and NP solutions is significantly different based on the presence or absence of humic acid ( $t = 35.7$ ,  $p = <0.001$ ).

The observation that ionic Cu content in solution at 100 mg/L NP was significantly greater than that at 500 mg/L was assumed to be experimental error. However, these findings were confirmed when new Cu NP solutions were prepared in 25% Hoagland's solution. Ten days after solution preparation, the ionic Cu content of NP solutions prepared at initial concentrations of 100, 250, 500, and 1000 mg/L was 980, 690, 330, and 160  $\mu\text{g/L}$ , respectively (all values significantly different,  $F = 9190$ ,  $p = <0.001$ ).

The biomass and transpiration volume of squash exposed to bulk or NP Cu at 0, 100, or 500 mg/L in the presence or absence of 50 mg/L humic acid was determined. Bulk Cu at both exposure levels significantly reduced the biomass and volume of solution transpired by the plants (Fig. 3). The plant mass after 14 days of exposure to bulk Cu at 100 and 500 mg/L was 3.0 and 2.6 g, respectively. The increase in plant mass for these treatments during the 14 days exposure period was reduced by 65 and 74% relative to untreated control plants (significantly different,  $F = 17.9$ ,  $p = <0.001$ ). However, the presence of 50 mg/L humic acid completely alleviated the phytotoxicity of bulk Cu at both exposure concentrations. In the presence of humic acid, the biomass of plants exposed to bulk Cu at 100 and 500 mg/L was 4.8 and 5.0 g, respectively. These values are statistically equivalent to the untreated control



**Fig. 4.** Transpiration of squash exposed to bulk or NP Cu at 500 mg/L in Hoagland solution (25%) with or without humic acid (50 mg/L). Error bars represent standard error ( $n = 8$ ). Curves followed by different letters are significantly different (one-way analysis of variance [ANOVA] on the slopes of lines regressed through replicate data followed by a Student Newman Keuls multiple comparison test).

plants and represent 60 and 92% increases, respectively, in normalized growth (i.e., growth from day 0 of the exposure period) when compared with plants exposed to bulk Cu in the absence of humic acid. Similar results were observed for transpiration volume; plants exposed to bulk Cu at 500 mg/L transpired an average of 39 mL or 45% less volume than untreated control plants. At 100 mg/L Cu, plants transpired 50 mL, but this value was not significantly different from the controls or 500 mg/L exposure level. However, the presence of 50 mg/L humic acid completely alleviated the observed phytotoxicity; transpiration volumes at 100 and 500 mg/L averaged 86 and 96 mL, respectively. These values are statistically equivalent to untreated control plants and represent 1.7 and 2.5-fold increases in transpiration volume relative to plants exposed in the absence of humic acid.

Exposure to Cu NPs also resulted in statistically significant ( $F = 27.1$ ,  $p = <0.001$ ) decreases in plant biomass and transpiration volume relative to untreated control plants (Fig. 4). The biomass of plants exposed to 100 and 500 mg/L Cu NPs was 2.5 and 1.9 g, respectively; these values represent 93 and 99% reductions in normalized plant growth relative to untreated controls. Similarly, the transpiration volume of plants exposed to 100 and 500 mg/L Cu NP averaged 29 and 23 mL, respectively. Again, these values represent 59 and 67% reductions in transpiration relative to untreated plants. However, the reductions in biomass and transpiration caused by Cu NPs are statistically equivalent to that observed with the bulk Cu particles. Unlike bulk Cu particles, the presence of humic acid resulted in slight but statistically insignificant alleviation of the phytotoxicity caused by Cu NP exposure. For example, the volume of solution transpired by plants exposed to 100 and 500 mg/L Cu NP

in the presence of humic acid was 36 and 27 mL, respectively; the biomass of exposed plants was 2.8 and 2.1 g, respectively. None of these values are statistically different from that of plants exposed to Cu NP in the absence of humic acid.

The Cu content of control plants exposed to Hoagland's solution with or without 50 mg/L humic acid was 0.48 and 0.35  $\mu\text{g/g}$  (wet weight; not significantly different). The Cu content of shoots exposed to Cu-containing solutions was significantly greater than control plants ( $F = 28.7$ ,  $p = <0.001$ ) and ranged from 2.8 to 4.8  $\mu\text{g/g}$ . However, these values were unaffected by particle size, concentration, or solution type. In Hoagland's solution, the Cu shoot content in bulk and NP treatments at 100 mg/L was 3.2 and 3.9  $\mu\text{g/g}$  (wet weight), respectively (not significantly different), and at 500 mg/L, these values were 3.8 and 4.8  $\mu\text{g/g}$ , respectively (not significantly different). The presence of 50 mg/L humic acid had no impact on shoot Cu content in bulk or NP solutions. In Hoagland's solution amended with humic acid, the Cu shoot content in bulk and NP treatments at 100 mg/L was 3.1 and 2.8  $\mu\text{g/g}$  (wet weight), respectively (not significantly different), and at 500 mg/L, these values were 3.9 and 3.8  $\mu\text{g/g}$ , respectively (not significantly different).

## DISCUSSION

The literature on NP interactions with terrestrial plant species is rather limited, with most phytotoxicity studies focusing on seed germination and root elongation. Lin and Xing (2007) observed that five different NP at 2000 mg/L had little impact on the germination of six plant species. Similarly, Stampoulis et al. (2009) noted that zucchini seed germination was unaffected by bulk or NP Ag, Cu, Si, MWCNTs, or ZnO at 1000 mg/L. Alternatively, Yang and Watts (2005) showed that the root elongation of four agricultural crops was unaffected by alumina NP at 2–200 mg/L. However, at 2000 mg/L, root length and development of all species were reduced by 13%, although no direct comparison to bulk alumina particles was made. Lin and Xing (2008) also measured the root elongation of six agricultural crops in the presence of five NPs at 2000 mg/L. The authors observed that while MWCNT,  $\text{Al}_2\text{O}_3$ , and Al NPs had no impact root elongation, ZnO nanoparticles dramatically reduced root growth for all five species, although corresponding bulk materials were not evaluated. Stampoulis et al. (2009) showed that of bulk or NP Ag, Cu, Si, MWCNTs, and ZnO; only Cu NPs significantly reduced zucchini root growth. Canas et al. (2008) reported that the impact of carbon nanotubes at 9–1,750 mg/L on the root growth of six crop plants was species-specific, with NP exposure inhibiting root elongation in some species (tomato) but enhancing root growth (onion, cucumber) or having no effect in others (cabbage, carrots). Interestingly, the authors note that NP functionalization

with poly-3-aminobenzene-sulfonic acid significantly impacted phytotoxicity.

Very little work has been done to assess NP uptake by plants or to determine the impact of these materials on overall growth or broader physiological parameters. Lee et al. (2008) observed that Cu NP exposure for 48 h to bean and wheat seedlings reduced plant biomass by 40% at 200 mg/L and 80% at 1000 mg/L. Lin and Xing (2008) exposed rye plants to ZnO NPs and reported negligible Zn in the shoots that could be attributed to the nanoparticle. Similarly, Canas et al. (2008) reported that although carbon nanotube exposure resulted in phytotoxic effects on several plant species, the NM formed layered sheets on the outer root surfaces, and no visible uptake was evident. Conversely, Lin et al. (2009) confirmed the uptake, translocation, and inheritance of  $C_{70}$  by rice using Fourier transform-Raman and infrared (IR) spectroscopy. Similarly, Zhu et al. (2008) noted that pumpkin plants watered with  $Fe_3O_4$  NP-containing (500 mg/L) solution displayed no visible phytotoxicity, but root-to-shoot NP translocation was detected magnetometrically.

The finding that 500 mg/L Ag NPs were significantly more phytotoxic to squash than the corresponding bulk treatment agrees with our previous findings (Stampoulis et al., 2009). We observed that Ag NP exposure resulted in significant biomass and transpiration effects on zucchini seedlings at concentrations as low as 100 mg/L, but that bulk Ag powder at similar concentrations had no impact on plant growth. In the current study, Ag content of the stems was unaffected by particle size, concentration, or solution type. These findings disagree with Stampoulis et al. (2009), where the zucchini stem Ag content was 4.7-times greater if the exposure solution contained NP Ag when compared with an equivalent concentration of bulk metal. A number of differences in experimental design between the current study and that of Stampoulis et al. (2009) make direct comparison difficult, but differences in plant selection and exposure conditions are clearly important. The nonsignificant effect of humic acid on Ag NP phytotoxicity is somewhat surprising. Others have observed that natural organic matter and humic acid (100 mg/L) both increase the stability of MWCNT and  $Al_2O_3$  NP solutions (Ghosh et al., 2008; Lin and Xing, 2008). Such increased stability should promote the extent of NP-root surface interactions. Lin et al. (2009) observed that dissolved organic matter promoted the uptake of  $C_{70}$  by rice. However, analysis of the aqueous solutions in the current study showed that 50 mg/L humic acid either had no effect (500 mg/L) or slightly decreased (100 mg/L) Ag NP dissolution.

The finding of equivalent phytotoxicity of Cu NP and bulk material at 100 and 500 mg/L partially agrees with the findings of Stampoulis et al. (2009). In that study, bulk and NP Cu at 1000 mg/L reduced zucchini biomass by 69 and 90%, respectively. These toxicity values were significantly different but at the lower initial concentrations used in the current study, it is not surprising to see the particle-size

based difference disappear. This is supported by Cu content in the shoots of exposed plants; the concentration was unaffected by particle size, solution content, or initial concentration. However, further discussion of the Cu content of the plants is somewhat confounded by the unusual phenomenon of decreasing Cu ion in solution with increasing initial NP concentration. As indicated earlier, the mechanisms responsible for this effect are currently under investigation; however, this phenomenon is likely the result of Cu oxidation in solution. This reaction will subsequently reduce oxygen and consume protons from solution, resulting in an increasing pH. At increasing pH, the ionic Cu in solution will then precipitate as Cu phosphates, carbonates, and hydroxides (Ziemniak et al., 1992). Interestingly, at higher initial Cu concentrations in solution, this reaction will proceed more quickly, resulting in higher pH values, greater Cu precipitation, and ultimately lower ionic levels in solution. It is also noteworthy that this reaction is much greater for the Cu NPs than for the bulk Cu powder; this is clearly a function of the increased surface area-to-volume and reactivity for the nanoparticles.

It is also notable that this phenomenon is partially minimized by the presence of humic acid. The observation that 50 mg/L humic acid completely alleviated the phytotoxicity of bulk Cu but had no effect on Cu NP reductions in biomass or transpiration is interesting. Clearly, the loss of toxicity of the bulk Cu is the result of metal ion complexation with the dissolved humic acid structure. Humic acids are known to contain an array of phenolic and carboxylic acid functional groups that significantly complex many ions, with a primary consequence being effects on plant nutrient fate and availability (Piccolo, 2002). Pandey et al. (1999) used IR spectroscopy to show that the carboxylic functional groups of humic acid were the primary site of copper ion complexation. The explanation for the particle size dependent-nature of this reaction in our study is unknown and is the topic of ongoing investigations.

The current study reports that humic acid and solution chemistry differentially impact bulk and nanoparticle dissolution and phytotoxicity for Cu and Ag. These findings are in agreement with Ghosh et al. (2008) and Domingos et al. (2009), where the stability of carbon nanotube,  $Al_2O_3$ , and  $TiO_2$  NP suspensions was shown to be significantly affected by humic acid, fulvic acid, and pH. Alternatively, Slaveykova and Startchev (2009) noted that humic acid had little impact on the stability or transformation of coated quantum dots. To further complicate matters, Fabrega et al. (2009) reported that the stability of Ag NP suspensions was significantly increased by humic acid presence but that these changes only impacted toxicity to bacteria at pH 9.

Given the dramatic increase in nanoparticle utilization in the marketplace, the release of these materials to the environment appears inevitable. Unfortunately, information on fate and effects of nanoparticles is almost completely lacking. The findings of the current study are significant in that

Ag nanoparticles were found to be more phytotoxic (as measured by plant growth and transpiration) than bulk Ag powder. In addition, the paucity of environmentally based fate and transport data on engineered NM is of significant concern in that this lack of knowledge completely prevents meaningful and accurate determination of NP exposure, hazard, and risk. This report highlights the need for more intense evaluation of nanoparticle fate and effects under environmentally relevant conditions. The findings of differential NP toxicity and behavior are particularly disconcerting given the increasing use of engineered nanoparticles in pesticides and fertilizers, resulting in potential food chain contamination and an uncharacterized pathway of human exposure to these materials. Future investigations in this laboratory and others are seeking to more fully evaluate the level of exposure and risk associated with these processes.

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