Environmental Science & Technology

Article

pubs.acs.org/est

Impact of Ag Nanoparticle Exposure on *p,p′*-DDE Bioaccumulation by *Cucurbita pepo* (Zucchini) and *Glycine max* (Soybean)

Roberto De La Torre-Roche, [†] Joseph Hawthorne, [†] Craig Musante, [†] Baoshan Xing,[‡] Lee A. Newman,[§] Xingmao Ma,[∥] and Jason C. White*[,†]

[†]Department of Analytical Chemistry, The Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, Connecticut 06504, United States

[‡]Stockbridge School of Agriculture, University of Massachusetts, Amherst, Massachusetts 01003, United States

[§]Department of Environmental and Forest Biology, SUNY—College of Environmental Science and Forestry, Syracuse, New York 13210, United States

[∥]Department of Civil and Environmental Engineering, Southern Illinois University Carbondale, Carbondale, Illinois 62901, United States

Supporting Information

ABSTRACT: The effect of nanoparticle (NP), bulk, or ionic Ag exposure on dichlorodiphenyldichloroethylene (*p,p′*-DDE; DDT metabolite) accumulation by *Glycine max* L. (soybean) and *Cucurbita pepo* L. (zucchini) was investigated. The plants were grown in 125-mL jars of vermiculite amended with 500 or 2000 mg/L of bulk or NP Ag; ion controls at 5 and 20 mg/L were established. During 19 d of growth, plants were amended with solution containing 100 ng/mL of *p,p′*-DDE. Total shoot *p,p′*-DDE levels in non-Ag exposed *G. max* and *C. pepo* were 500 and 970 ng, respectively; total root DDE content was 13 700 and 20 300 ng, respectively. Ag decreased the *p,p′*-DDE content of *G. max* tissues by up to 40%, with NP exposure resulting in less contaminant uptake than bulk Ag. Total Ag content of exposed *G. max* ranged from 50.5 to 373 μg; NP-exposed plants had 1.9−2.2 times greater overall Ag than corresponding bulk particle treatments and also significantly greater relative Ag transport to shoot tissues. Bulk and NP Ag at 500 mg/L suppressed DDE uptake by *C. pepo* by 21−29%, although Ag exposure at 2000 mg/L had no impact on contaminant uptake. Similar to *G. max*, *C. pepo* whole plant Ag content ranged from 50.5 to 182 μg, with tissue element content generally being greater for NP exposed plants. These findings show that the Ag may significantly alter the accumulation and translocation of cocontaminants in agricultural systems. Notably, the cocontaminant interactions vary both with Ag particle size (NP vs bulk) and plant species. Future investigations will be needed to clarify the mechanisms responsible for the cocontaminant interactions and assess the impact on overall exposure and risk.

INTRODUCTION

It is now widely recognized that, at particle sizes under 100 nm, materials acquire unique chemical and physical properties that often differ dramatically from corresponding bulk characteristics.[1−3] Although many of these novel properties arise from the greatly increased surface area to volume ratio at this scale, the ability to engineer or design specific nanomaterials is also possible.[3] Unique properties at the nanometer scale can include altered reactivity, solubility, and conductivity, as well as altered optical or magnetic characteristics.[4] Consequently, the development and use of engineered nanomaterials (NM) has increased exponentially, achieving a projected $1 trillion value by 2015.[3] The Project on Emerging Nanotechnologies [5] currently lists more than 1317 products as "nanotechnology-based" in its consumer products inventory, including textiles, biosensors, water treatment technology, fuel cells, agents for environmental remediation, and food packaging. Notably, significant nanotechnology use has begun in agricultural applications, ranging from crop improvement applications to chemical delivery agents to nanoemulsions and preservatives in pesticide/fertilizer formulations.[4,6] Chinnamuthu and Boopathi[7] describe the increased activity and stability of nanocides; products in which the pesticidal molecule is encapsulated within a nanomaterial. Corredor et al.[8] explored the use of nanomaterials as chemical delivery agents and described the penetration of carbon-coated Fe nanoparticles (NP) into plants and specifically, vascular tissues. Similarly, carbon nanotubes have been shown to deliver molecules directly to specific cellular organelles.[9]

In spite of the increases in engineered nanomaterial use, a thorough understanding of the implications of that usage and of

Received: October 12, 2012
Revised: December 18, 2012
Accepted: December 19, 2012
Published: December 19, 2012

 © 2012 American Chemical Society

the potentially unique toxicity profile of these materials does not exist. In a recent review of published data on food-related NM usage, Card et al. [10] concluded that insufficient information exists to adequately assess risk. Although there is a growing body of literature investigating the toxicity of specific nanomaterials to select receptors under defined laboratory conditions,[11−13] an understanding of the environmental fate and in situ toxicity of these materials on biota and ecosystems is sorely lacking. This lack of understanding is particularly disconcerting in agriculture where nanoparticle contamination of the food chain may represent a significant and uncharacterized pathway of human exposure to these materials.[14−16] In addition to direct toxicity to and accumulation by crop plants, the interaction of nanomaterials with cocontaminants and other chemical constituents (pesticides, fertilizers) in agroecosystems may also impact food safety.[16] Recent work has shown that, in a model system (vermiculite), fullerene exposure may significantly increase the accumulation of pesticide residues by food crops [16] but similar enhanced cocontaminant uptake may not occur upon fullerene exposure in soil.[17] The current study investigates the impact of NP, bulk, and ionic Ag on the uptake and translocation of a common agricultural cocontaminant, *p,p′*-DDE (persistent and estrogenic metabolite of DDT), by *Glycine max* (soybean) and *Cucurbita pepo* (zucchini). In addition, the accumulation and translocation of Ag by the two plants was evaluated.

EXPERIMENTAL SECTION

Analytes and Plants. Crystalline *p,p′*-DDE (DDE) and *o,p′*-DDE were acquired from the Environmental Protection Agency (EPA) National Pesticide Standard Repository (Fort Meade, MD). Bulk Ag (99.99% purity; <250 μm) was acquired from Strem Chemicals Inc. (Newburyport, MA); $AgSO_4$ was acquired from Fisher Scientific (Pittsburgh, PA); NP Ag (99.99% purity; <20 nm) was obtained from US Research Nanomaterials, Inc. (Houston, TX). To determine particle characteristics in water, 500- and 2000 mg/L Ag NP solutions were prepared, sonicated for 10 min, and left on the bench overnight. The solutions were then centrifuged at 3000 rpm for 10 min and a portion of the supernatant was removed for particle size and ζ-potential determination (Malvern, Nano Series ZS90). The average particle size and ζ-potential values were 68 nm and −31.1 mV at 2000 mg/L and 91 nm and −21.5 mV at 500 mg/L. Efforts to characterize Ag NP particle size within vermiculite by a range of rinsing and centrifugation techniques were unsuccessful due to large instrumental response from the Ag-free vermiculite controls. *Cucurbita pepo* L. (cv Black Beauty) seeds were obtained from Seedway (Hall, NY); *Glycine max* L. seeds were obtained from Johnny's Selected Seeds (Albion, ME). The seeds were pregerminated in uncontaminated vermiculite for 5−7 days prior to transplanting for the exposure assay.

Exposure Assay. Clear 125 mL jars (Fisher Scientific, Pittsburgh PA) were amended with 12 g of dry vermiculite (approximately 80 mL). Twenty or 40 mg of bulk or NP Ag were added to the vermiculite to achieve concentrations of 500 and 2000 mg/L (based on 40 mL of added solution). Ag ion (in the form of $AgSO_4$) was added as a powder to yield concentrations of 5 and 20 mg/L. The jars were capped and shaken vigorously to ensure thorough mixing of contents. Select *C. pepo* and *G. max* seedlings were gently planted in the vermiculite (one plant per jar). The planted jars were then amended with 40 mL of 25% Hoagland's solution (MP Biomedical) containing 100 μg/L DDE, yielding Ag bulk and NP concentrations of 500 or 2000 mg/L, and Ag ion concentrations of 5 or 20 mg/L. In total, there were eight treatments for each plant species: 1. DDE alone (no Ag); 2. DDE + 500 mg/L bulk Ag; 3. DDE + 500 mg/L NP Ag; 4. DDE + 5 mg/L Ag ion; 5. DDE + 2000 mg/L bulk Ag; 6. DDE + 2000 mg/L NP Ag; 7. DDE + 20 mg/L Ag ion; 8. negative control (no DDE, no Ag). Ten replicate plants were grown for each treatment. The plants were incubated in a growth room under ambient and supplemental fluorescent lighting (60 μE/m^2·s) at approximately 22 °C and were top watered as needed with the appropriate solutions according to the above treatments for a 19-d growth period.

Vegetation Extraction and Digestion. At harvest, replicate plants were separated into roots, stems and leaves, and the mass of each tissue was determined. Roots were rinsed in tap water to remove vermiculite before weighing. To quantify DDE content, replicate tissues were extracted by a QuEChERS method based on Anastassiades et al.[18] Briefly, up to 15 g (wet weight) of sample was added to a 50 mL centrifuge tube containing 15 mL of acetonitrile and 100 μL of *o,p′*-DDE (from a 10 μg/mL solution) was added as an internal standard. The tubes were placed on a wrist-action shaker for 10 min. Six grams of magnesium sulfate and 1.5 g of sodium acetate were added to each tube, which were then centrifuged at 3000 rpm for 10 min. Plastic centrifuge tubes (15 mL) were amended with 1.5 g of magnesium sulfate and 500 mg of primary and secondary amine (PSA). Toluene (2 mL) was added to wet the powders and 10 mL of sample extract were transferred to each tube. The tubes were shaken by hand for 30 s and centrifuged at 3000 rpm for 10 min. Each extract (6 mL) was concentrated to 1 mL under nitrogen. The samples were then transferred to chromatography vials for storage at −4 °C until analysis. To determine Ag content in the replicate tissues, vegetation samples were oven-dried at 100 °C for 72 h and digested for 1 h on a hot block with concentrated HNO_3 at 115 °C. The digests were analyzed by inductively coupled plasma mass spectroscopy (ICP-MS)(Agilent 7500ce) for Ag content (mass 107).

Lipid Peroxidation. Lipid peroxidation in *G. max* (roots, shoots) and *C. pepo* (roots, stems, leaves) was measured by the TBARS assay.[19] Malondialdehyde, which forms during fatty acid degradation and is indicative of lipid peroxidation, was determined as a function of treatment.

Chemical Analysis. A 1000 mg/L stock of *p,p′*-DDE in 2,2,4-trimethylpentane was diluted to prepare calibration standards of 10−500 ng/mL, each of which were amended with 100 ng/mL *o,p′*-DDE as an internal standard. The concentration of *p,p′*-DDE in the vegetation was determined on an Agilent (Avondale, PA, USA) 6890 gas chromatograph (GC) with a ^{63}Ni microelectron capture detector (ECD) by internal standard calibration. An Equity-5 (Supelco, Bellefonte, PA, #28089-U) GC column (30 m × 0.25 mm × 0.25 μm) was used and the oven program was as follows: 75 °C initial temperature ramped at 20 °C/min to 217 °C, then ramped at 0.25 °C/min to 219 °C, then ramped at 15 °C/min to 280 °C with a final hold time of 2.0 min. The injection port was maintained at 250 °C and a 2-μL splitless injection was used. The carrier gas over the column was He. The ECD was maintained at 300 °C and the makeup gas to the detector was 5% CH_4 in Ar at 60 mL/min. The detection limit of DDE was approximately 1 ng/mL injected.

Table 1. Wet Biomass (g) of *G. max* and *C. pepo* Tissues Exposed to NP, Bulk, or Ionic Ag during Growth

plant	control	DDE alone	5 ion	20 ion	500 bulk	2000 bulk	500 NP	2000 NP
G. max								
leaf	2.1 A[a]	2.5 B	1.9 A	1.5 C	2.1 A	1.9 AC	1.9 AC	1.6 C
stem	2.8 A	2.4 B	1.9 C	1.7 C	2.0 C	1.7 CD	1.8 CD	1.5 D
root	1.5 A	3.0 B	2.4 B	1.9 AC	2.9 B	2.6 BC	2.7 B	2.3 BC
total	6.4 A	7.8 B	6.2 ACD	5.1 C	7.0 D	6.3 AD	6.3 AD	5.4 AC
C. pepo								
leaf	8.3 A	7.0 BC	8.7 A	6.6 C	8.4 A	7.7 AB	8.2 A	7.7 AB
stem	5.9 A	5.2 AB	5.8 A	4.4 B	5.7 A	5.1 AB	5.6 AB	4.8 AB
root	2.7 AB	2.3 A	3.6 CD	3.1 BC	3.2 BCD	3.8 D	3.3 BCD	3.6 CD
total	17 AB	15 AC	18 B	14 C	17 B	17 AB	17 AB	16 AB

[a]Within a row, values followed by different letters are significantly different (one way ANOVA with a Student−Newman−Keuls Multiple Comparison Test at $p < 0.05$).

Statistical Analysis. At harvest, four replicate plants were extracted for DDE content; three were digested for Ag content, and the remaining plants were used in the lipid peroxidation assay. All values of DDE or Ag content are expressed on a dry weight basis. A one-way ANOVA followed by a Student−Newman−Keuls (SNK) Multiple Comparison Test ($p < 0.05$) was used to determine all differences of statistical significance among treatments.

RESULTS AND DISCUSSION

DDE Exposure and Plant Mass. *G. max* plants were each irrigated with an average of 257 mL (194−277 mL) during the 19-d of growth, equating to a DDE exposure of 25.7 μg of DDE (19.4−27.7 μg). Replicate *C. pepo* plants were amended with an average of 389 mL (326−413 mL) of solution, yielding an average exposure of 38.9 μg of DDE (32.6−41.3 μg). Individual replicate irrigation volumes varied based on plant size, growth rate, and transpiration volume.

At harvest, *G. max* total plant mass ranged from 3.67 to 8.35 g (wet mass) and differed significantly as a function of treatment (Table 1). DDE-exposed plants in the absence of Ag displayed the greatest plant mass. Ag exposure to 20 mg/L ionic Ag decreased total plant biomass by 20.7%, whereas plants exposed to no Ag (DDE alone) and 500 mg/L bulk Ag had significantly greater biomass than controls. However, Ag exposure significantly decreased stem biomass in all treatments. Conversely, Ag exposure increased plant root biomass. These trends were particularly evident when relative mass per tissue compartment was calculated. The control stem biomass compartment was 44% of the total mass; Ag exposure decreased that tissue to 27−34%, with greater Ag exposure resulting in greater loss in stem mass. Conversely, the relative root mass of control plants was 23% but, with Ag exposure, increased to 37−44%, with greater increases at higher Ag exposure levels.

C. pepo total wet mass ranged from 10.9 to 19.6 g (wet mass) and varied significantly as a function of treatment (Table 1). Similar to *G. max*, exposure to 20 mg/L ionic Ag resulted in a reduction (17.2%) in total *C. pepo* biomass. Interestingly, the trends noted for *G. max* of decreasing stem mass and increasing root mass upon Ag exposure were also evident for *C. pepo*, although the statistical significance of these effects was less pronounced. The stem mass of control plants was 5.93 g (35% of total mass) but declined insignificantly to 4.75 g (30%) at 2000 mg/L Ag NP exposure. Conversely, Ag exposure did significantly increase *C. pepo* root mass. The control root tissue mass was 2.65 g (16%) but increased significantly with Ag exposure to 3.83 g (23%) upon exposure to 2000 mg/L bulk Ag.

The lack of negative effects of DDE on tissue or whole plant biomass of *G. max* or *C. pepo* was expected, as exposure to similar concentrations in a range of media have produced no observable phytotoxicity.[16,20] However, exposure to higher concentrations of Ag did result in moderate phytotoxicity; these findings are in general agreement with the literature. Stampoulis et al.[14] reported that, upon exposure to NP Ag at 1000 mg/L under hydroponic conditions, *C. pepo* biomass was reduced by 75% as compared to controls and to plants exposed to bulk Ag powder. Musante and White[21] observed similar increased phytotoxicity of NP Ag relative to bulk element for a separate subspecies of *C. pepo* under hydroponic conditions. In the current study, the biomass of plants exposed to NP Ag was consistently less than that of plants exposed to corresponding bulk material, but these effects were rarely of statistical significance. Kumari et al.[22] exposed *Allium cepa* root tip cells to Ag NPs (25−100 mg/L) and noted that increasing Ag NP exposure resulted in decreases in the mitotic index, as well as inducing several types of chromosomal aberration. However, as no bulk material or ion controls were included, linking these effects specifically to NP-exposure is somewhat difficult. Yin et al.[23] exposed *Lolium multiflorum* to NP and ionic Ag and noted altered root tissue and cellular morphology upon exposure to the nanoparticle but no such effects upon exposure to the ionic form. Perhaps more importantly, the authors reported greater phytoxicity at 6 nm Ag particles than equivalent exposure at 25 nm. The reasons for the general lack of particle size specific toxicity in the current experiment are unknown but may be the result of the growth media (vermiculite; a hydrous silicate clay) used and the resulting impacts on element availability. Recently, Lee et al. [24] described concentration-dependent phytotoxicity of *Phaseolus radiatus* upon exposure to 0−40 mg/L Ag NPs in agar, but in a soil test with the NPs at 0−2000 mg/kg, particle bioavailability was reduced and no negative effects on plant growth were observed.

The observation of decreasing relative stem mass and increasing root biomass across both species with increasing Ag exposure is interesting and agrees with the literature. Wang et al.[25] noted that exposure to CuO nanoparticles increased the root diameter of *Zea mays*, whereas equivalent bulk and ion treatments exerted no such effect. Similar observations of root morphological changes were noted for *Arabidopsis thaliana* upon exposure to NP TiO_2 and fullerenes.[26,27] The mechanism seems to be one of stress response related to altered deposition of cellulose fibers in exposed root cells. Notably, in our study

Table 2. Total DDE Content (ng; Based on Dry Mass) of *G. max* and *C. pepo* Leaf, Stem, and Root Tissues Exposed to NP, Bulk, or Ionic Ag during Growth

plant	control	DDE alone	5 ion	20 ion	500 bulk	2000 bulk	500 NP	2000 NP
G. max								
leaf	25 A[a]	85 B	97 C	67 D	110 E	53 F	49 F	41 F
stem	5.3 A	410 B	390 B	81 C	280 D	47 C	140 E	180 E
root	2.9 A	14 000 B	11 000 C	11 000 BCD	13 000 BC	9900 DE	9300 E	8500 E
C. pepo								
leaf	14 A	57 B	83 C	63 D	110 E	89 C	88 C	130 F
stem	22 A	920 B	940 B	640 C	1100 D	740 E	550 F	320 G
root	840 A	20 000 B	26 000 C	22 000 BC	16 000 D	22 000 BC	15 000 D	22 000 BC

[a]Within a row, values followed by different letters are significantly different (one way ANOVA with a Student−Newman−Keuls Multiple Comparison Test at $p < 0.05$).

the impact on root tissues was induced by Ag exposure, regardless of particle size (NP vs bulk) or form (elemental vs ionic).

Lipid Peroxidation. Malondialdehyde (MDA) formation is indicative of lipid degradation and was determined in the control and treated tissues of the *G. max* and *C. pepo* (Table S1, Supporting Information). Although MDA content of *G. max* roots were unaffected by treatment, peroxidation in shoot tissues did vary significantly. Exposure to NP Ag at 500 and 2000 mg/L resulted in significant increases (54−75%) in MDA formation (significant at $p < 0.05$); bulk Ag at 2000 mg/L yielded a 36% increase in MDA production. Ionic Ag had no impact on shoot peroxidation whereas bulk Ag at 500 mg/L actually resulted in lower MDA content. For *C. pepo*, MDA production by root tissues was either unaffected by treatment or actually declined significantly relative to unexposed tissues (Table S1, Supporting Information). Conversely, peroxidation in *C. pepo* stems was significantly increased by exposure to 5 mg/L Ag ions, as well as bulk Ag at 2000 mg/L and NP Ag at 500 mg/L. MDA production by *C. pepo* leaves was unaffected by treatment.

The mechanisms of nanoparticle toxicity to plants remains an area of active research. It is clear that many elements, whether in nanoparticle, bulk, or ionic form, can induce the formation of reactive oxygen species with subsequent lipid peroxidation.[28] One measure of lipid peroxidation is ion leakage to the surrounding solution; Wang et al.[25] observed that CuO NP exposure induced significantly greater K^+ leakage out of *Z. mays* roots than did exposure to bulk CuO or ionic Cu. Alternatively, De La Torre et al.[16] reported that K^+ leakage, as well as total ion release, as measured by conductivity, of several plants was unaffected by exposure to C_{60} fullerenes. However, in partial agreement with the current study, the authors[16] showed that while MDA formation in *G. max* shoots was indeed increased upon C_{60} treatment, *C. pepo* root and shoot membrane integrity was not compromised by nanomaterial exposure.

DDE Content. Comparison of DDE concentrations in individual tissues is confounded by the fact that watering volumes, and resulting DDE exposure, varied among individual replicates due to plant mass and transpiration differences. Consequently, average DDE concentrations were converted to absolute amounts (ng) by multiplying by dry mass within plant species and values were normalized based on volume of exposure/watering solution, as described in De La Torre et al.[16] The DDE content of *G. max* tissues and whole plants is shown in Table 2 and Figure 1, respectively. For *G. max*, leaf DDE levels in exposed plants were relatively low, ranging from 41.3 to 107 ng. The leaf DDE content of *G. max* not exposed

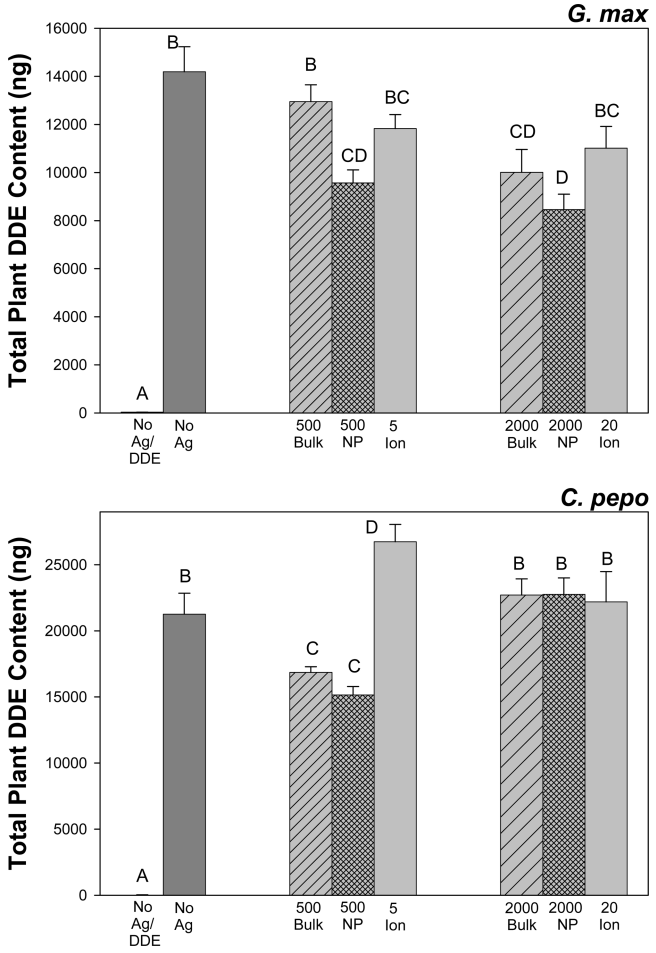

Figure 1. Whole plant DDE content (ng; based on dry mass) of *G. max* (top) or *C. pepo* (bottom) exposed to bulk, NP, or ionic Ag during growth. Within a plant species, values followed by different letters are significantly different (one way ANOVA with a Student−Newman−Keuls Multiple Comparison Test at $p < 0.05$.

directly to DDE in the irrigation water was 24.5 ng, and likely the result of aerial contaminant deposition. Although bulk Ag at 500 mg/L and ionic Ag at 5 mg/L slightly increased DDE levels, in general, exposure to Ag resulted in significantly lower (22−52%) amounts of the organochlorine in leaf tissues. The stem DDE content of control plants was 410 ng and other than 5 mg/L ion, Ag exposure significantly decreased DDE content (33−88%), with higher levels of the metal generally resulting in lower organochlorine content. Although less dramatic than the stems, DDE content of root tissues also tended to decrease

dx.doi.org/10.1021/es3041829 | Environ. Sci. Technol. 2013, 47, 718−725

Table 3. Total Ag Content (μg; Based on Dry Mass) of *G. max* and *C. pepo* Leaf, Stem, and Root Tissues Exposed to NP, Bulk, or Ionic Ag during Growth

plant	control	DDE alone	5 ion	20 ion	500 bulk	2000 bulk	500 NP	2000 NP
G. max								
leaf	0.66 A[a]	0.77 A	1.2 A	1.1 A	2.8 B	3.3 B	6.1 C	22 D
stem	0.47 A	0.41 A	1.1 A	0.89 A	3.2 B	3.2 B	11 C	21 D
root	0.04 A	0.17 A	48 B	110 C	93 C	190 D	200 D	330 E
C. pepo								
leaf	0.12 A	0.19 B	1.4 B	1.8 B	1.7 B	20 C	5.4 D	7.9 E
stem	0.64 A	0.49 A	5.1 BC	3.9 B	7.3 D	23 E	6.2 C	20 F
root	0.04 A	0.47 A	44 B	74 C	99 D	110 D	170 E	140 F

[a]Within a row, values followed by different letters are significantly different (One way ANOVA with a Student−Newman−Keuls Multiple Comparison Test at $p < 0.05$).

with Ag exposure; however, the reductions at 500 mg/L bulk Ag and 20 mg/L ionic Ag were insignificant. The whole plant DDE content of non-Ag exposed plants was 14 190 ng (Figure 1); Ag exposure at 2000 mg/L bulk-, 500 mg/L NP-, and 2000 mg/L NP Ag resulted in 30, 34, and 40% reductions in whole plant contaminant content, respectively. Interestingly, at 500 mg/L Ag, the effect on DDE content in leaf, stem, root, as well as the whole plant values, were significantly different between the bulk and NP treatments, with the nanoparticles suppressing uptake to a significantly greater extent. The statistical significance of this particle size specific effect largely disappears at the higher exposure level. Ag at the lower concentrations had no effect on the relative distribution of DDE among the shoots and roots of *G. max*. However, at 2000 mg/L bulk Ag and 20 mg/L ionic Ag, the relative amount of DDE in the root tissue increased by 2.2−2.6%, with parallel decreases in the relative shoot amounts.

The total DDE content of *C. pepo* tissues and whole plants is shown in Table 2 and Figure 1, respectively. The leaf DDE content of *C. pepo* not exposed directly to DDE was 14.0 ng and likely the result of air to leaf deposition. Although ionic Ag at 20 mg/L had no effect on DDE levels, exposure to Ag in all other treatments resulted in significantly greater (31−55%) organochlorine content in the leaf tissues. The stem DDE content of control plants was 915 ng; the 500 mg/L bulk and 5 mg/L ion treatments had no effect on contaminant content in stems. However, Ag exposure in the other treatments significantly decreased DDE content (19−65%), with higher levels of the metal generally resulting in lower organochlorine content. Notably, the suppression in DDE uptake was significantly greater for the nanoparticle exposure than for the corresponding bulk Ag treatments. In the root tissue, 500 mg/L Ag (bulk and NP) significantly suppressed DDE accumulation, but at higher exposure levels, contaminant content was unaffected. A similar pattern is evident in the whole plant DDE levels (Figure 1); notably, particle-size-dependent effects on contaminant uptake are generally not evident at the higher exposure concentration. Similar to the case of *G. max*, Ag at the higher exposure concentrations increased the relative amount of DDE in the root tissue (1.0−2.7%) with parallel decreases in the relative shoot amounts. Perhaps more interesting is the particle size specific effects on relative DDE distribution; for both exposure concentrations, NP Ag yielded significantly greater amounts of contaminant in the roots and less in the shoot (1.7−2.7% differences; significant at $p < 0.05$).

Directly comparing the DDE content between *C. pepo* and *G. max* is confounded by the different plant biomass and total irrigation volume, which resulted in species-specific DDE exposure levels. However, although the DDE exposure of *C. pepo* was 1.5 times greater than that of *G. max*, the contaminant concentrations of control (no Ag) *C. pepo* roots and stems were 2.0 and 3.9 times greater than that of *G. max*. The observation of greater organic contaminant accumulation by *C. pepo* is in agreement with the literature, although the mechanism responsible for this phenomenon remains unknown.[20,29,30] The finding of general suppression of DDE uptake in the presence of Ag, even when controlled for biomass effects at the higher exposure levels, and the fact that this suppression was of a greater magnitude with NP exposure is interesting. The available literature assessing the impact of nanomaterials on the accumulation and toxicity of cocontaminants by biota is rather limited. A number of recent studies have investigated the impact of carbon nanomaterials on organic cocontaminant uptake or toxicity by nonplant species.[31−33] Results from these studies vary, ranging from reduced toxicity/uptake in the presence of the carbon nanomaterial to no effect and, in some cases, to enhanced activity. With regard to plants, Ma and Wang[34] observed that fullerene coexposure increased trichloroethylene uptake by poplar under hydroponic conditions. Notably, previous work from our group under conditions similar to that of the current study showed the opposite effect with C_{60}; in that study, fullerene exposure significantly increased DDE bioaccumulation by *G. max*, *C. pepo*, and *S. lycopersicum*.[16]

The physiological impacts of bulk, NP, and ionic Ag exposure on the two plant species, as well as the subsequent impacts on DDE accumulation processes, are unknown. Hydrophobic contaminants such as DDE may enter plant tissues by passive diffusion through the hydrophobic membranes or potentially by unique transport proteins.[35,36] However, with the exception of *G. max* shoots at select exposures, membrane integrity in the current study appeared to be largely unaffected by Ag. Berger et al.[35] recently showed that aquaporins may play a role in the uptake of chlordane by *C. pepo*; in that study, plants exposed to hydrogen peroxide, which closes aquaporins, accumulated less chlordane and, upon removal of the peroxide from solution, contaminant levels again rose. Notably, Ag exposure has been shown to be an effective inhibitor of aquaporins in both plant and animal cells.[37] In preliminary batch hydroponic studies addressing aquaporins, Ag NP exposure at 500 mg/L for 14 d decreased DDE shoot content in *C. pepo* and *G. max* by 49 and 40%, respectively (Supporting Information Table 2). Ag exposure had no impact on root or shoot lipid peroxidation of either species (data not shown), and although *G. max* biomass and transpiration was significantly decreased by NP exposure, *C. pepo* growth was unaffected. Although not

definitive, these findings suggest a potential role of aquaporin activity in the decrease of DDE uptake upon Ag coexposure; additional work is underway to fully characterize this relationship.

Ag Content. The Ag content of *G. max* and *C. pepo* tissues was determined; for ease of comparison, element concentration was multiplied by plant biomass to yield absolute amounts. The Ag content of *G. max* tissues as well as whole plant values are shown in Table 3 and Figure 2, respectively. Plants not directly exposed to Ag did have trace quantities of the element detected, with values ranging from 0.04 to 0.77 μg. The leaf Ag content of exposed plants ranged from 1.2 to 22 μg but for the bulk and ion treatments; higher exposure levels did not result in greater Ag leaf concentrations. Conversely, plants exposed to 2000 mg/L of the NP contained 3.7 times more Ag than did leaves of plants exposed to 500 mg/L. Perhaps more interestingly, NP exposure resulted in 2.2−6.8 fold greater Ag leaf content than did the corresponding bulk treatments. The overall amount of Ag in *G. max* stems, as well as the pattern among the different treatments, was identical to that of the leaves. Bulk and ion tissue Ag levels did not increase with exposure concentration, and NP exposure yielded significantly greater element tissue amounts (3.4−6.6 times) than the corresponding bulk exposures. In the roots, higher exposure levels did yield significantly greater Ag content. Overall Ag root content ranged from 50.5 to 330 μg, with NP exposures yielding 1.8−2.2 times more element in the tissues than the corresponding bulk treatments. However, no effort was made to differentiate surface adsorbed Ag from absorbed element. Whole plant Ag content for *G. max* is shown in Figure 2 and displays an identical pattern to that observed in the root tissues. Whole plant Ag levels ranged from 50.5 to 373 μg, with higher exposure levels yielding greater element content and with NP treatments having significantly greater Ag (1.9−2.2 times) than the corresponding bulk treated plants. In terms of relative Ag distribution within the plants, the root tissues contained between 88 and 98% of the total element present. Importantly, the NP exposure at 500 and 2000 mg/L yielded significantly greater Ag translocation to the shoot compartment than did the corresponding bulk treatments. Alternatively, the ion treatments resulted in greater relative retention of Ag within the root system.

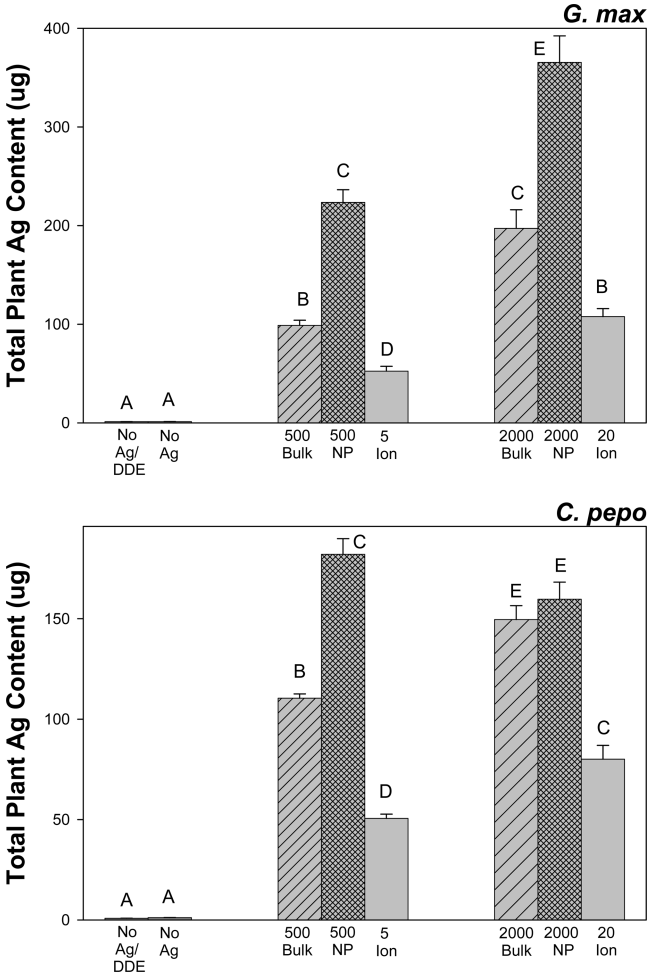

Figure 2. Whole plant Ag content (μg; based on dry mass) of *G. max* (top) or *C. pepo* (bottom) exposed to bulk, NP, or ionic Ag during growth. Within a plant species, values followed by different letters are significantly different (one way ANOVA with a Student−Newman−Keuls Multiple Comparison Test at $p < 0.05$.

The Ag content of *C. pepo* tissues as well as whole plant values are shown in Table 3 and Figure 2, respectively. Similar to the case of *G. max*, *C. pepo* plants not directly exposed to Ag did have trace quantities of the element, ranging from 0.04 to 1.2 μg. The leaf Ag content of exposed plants ranged from 1.4 to 20 μg, but for the bulk and NP treatments, higher exposure levels resulted in greater Ag leaf amounts. Although NP exposure at 500 mg/L resulted in significantly greater Ag leaf content than did the corresponding bulk treatments, the opposite was true at 2000 mg/L. The *C. pepo* stem Ag content ranged from 5.1 to 22.7 μg, and again, excluding the ion treatments, greater Ag exposure concentration resulted in significantly higher stem element content. Unlike the case of *G. max* stems, NP treatments had significantly lower Ag content than did bulk exposed tissues. However, these trends were not observed in the root tissue, where content ranged from 50.5 to 182 μg. In the roots, higher Ag exposure concentrations did not yield greater Ag content and NP exposed plants possessed greater element content than the corresponding bulk treatments. Whole plant Ag content of *C. pepo* plants is shown in Figure 2. At 500 mg/L, the Ag content of NP-exposed plants was 41% higher than that of the corresponding bulk treatments (significant at $p < 0.05$). Although a similar trend was evident at 2000 mg/L, the differences were not statistically significant. Notably, Ag content in the bulk particle and ion treatments increased with exposure concentration but that is not the case for the NP-exposed plants. In terms of relative Ag distribution, *C. pepo* root tissues contained 71−94% of the total element present. Importantly, unlike the case of *G. max*, NP exposure at 500 and 2000 mg/L yielded significantly less Ag translocation to the stem compartment than did the corresponding bulk treatments.

The literature on the uptake and distribution of nanoparticles within the plant tissues is not extensive, but some of the findings of the current study are in line with published work.[4,15,38] Perhaps the most significant finding is that the form of Ag significantly impacted overall element uptake. For *G. max*, NP exposure resulted in 1.8−6.6 times more tissue Ag than did the corresponding bulk material exposure. For *C. pepo*, this pattern of greater NP uptake held at 500 mg/L but disappeared at the higher exposure level. Notably, the ion exposure levels at 5 mg/L and 20 mg/L were consistently the lowest among the different treatments. The ion amount

released from the elemental particles in vermiculite is unknown; Musante and White[21] measured 1−3% ion dissolution from NP Ag under aqueous conditions and that served as the basis for ion concentrations tested in the current study. The phytotoxicity observed at the 20 mg/L level slightly confounds total Ag content comparison for this treatment, but when considering the Ag concentration (ng/g), the elemental content of the tissues in the ion treatments were still well below the bulk or NP exposure levels.

These findings support a growing body of evidence suggesting greater uptake and translocation of select elemental nanoparticles as compared to corresponding bulk or ion controls. Stampoulis et al.[14] observed that, under hydroponic conditions, exposure to NP Ag at 0−1000 mg/L resulted in tissue shoot levels in *C. pepo* that were on average 4.7-times greater than that observed with corresponding bulk particles. This is in partial agreement with the *C. pepo* findings at 500 and 2000 mg/L in the current study, although the use of vermiculite as a growth medium and the higher exposure concentration may explain these differences. Similarly, Wang et al.[25] observed significantly greater accumulation of CuO NP in corn grown hydroponically relative to appropriate controls. Recently, Larue et al.[39] observed that at particle sizes above 140 nm, TiO_2 uptake into wheat roots did not occur whereas smaller particle sizes were readily accumulated. Interestingly, the authors note that particle sizes below 36 nm were able to reach the stele, thereby becoming available for transfer to shoot tissues, whereas those between 36 and 140 nm were restricted to the parenchyma cells. Hawthorne et al.[40] noted that NP Si was accumulated at levels 5.6−6.5 times greater in *C. pepo* shoots than that observed with the corresponding bulk particles. Interestingly, in that study NP Au was accumulated to a lesser extent than bulk particles but upon addition of humic acid to the exposure solution, NP Au uptake values increased 5-fold whereas the bulk levels rose by only 80%. Conversely, Musante and White[21] noted that under hydroponic conditions, the accumulation of Ag and Cu by yellow squash (*C. pepo* subspecies *ovifera*; zucchini is *C. pepo* subspecies *pepo*) when exposed to 0−500 mg/L did not vary with particle size. The reasons for these varied reports in the literature are not known but the dynamic process of nanoparticle aggregation and subsequent dissolution and/or disaggregation is likely impacted by media type, as well as by root exudates and microbial processes. Clearly, the understanding of nanoparticle uptake and disposition within plants is an active area of research and a mechanistic understanding of the key processes operating under realistic exposure regimes (soil, multiple receptors) will likely be necessary prior to adequate assessment of exposure and risk.

The rapid and increasing use of nanomaterials in consumer products that directly (pesticides, fertilizers) or indirectly (biosolids) impact agriculture presents exposure and risk scenarios that have not been fully considered. The interaction of nanomaterials with coexisting contaminants and other agrichemicals remains almost completely unexplored. The current study demonstrates that the uptake and translocation of DDE, a widespread persistent and estrogenic pollutant, into two food crops was generally suppressed upon coexposure to Ag and that element interactions with membrane transport proteins may be involved. Importantly, there were species specific differences with regard to these effects and also as a function of the form of Ag exposure; nanoparticle vs bulk particle vs ion. The effects of nanomaterial exposure of food crops, as well as the potential risk associated with food chain contamination by nanomaterials, is a subject of concern and active investigation.

■ ASSOCIATED CONTENT

Supporting Information

Additional tables and information about the experimental design. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 203-974-8523. Fax: 203-974-8502. E-mail: Jason.White@ct.gov.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank USDA-AFRI (#2011-67006-30181) for funding and Terri Arsenault, John Ranciato, Dr. Helmi Hamdi, and Kittipath Prapayotin-Riveros (CAES) for technical assistance.

■ REFERENCES

(1) Handy, R. D.; Owen, R.; Valsami-Jones, E. The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. *Ecotox* **2008**, *17*, 315−325.

(2) Roco, M. C. Environmentally responsible development of nanotechnology. *Environ. Sci. Technol.* **2005**, *39*, 106A−112A.

(3) Roco, M. C.; Mirkin, C. A.; Hersam, M. C. *Nanotechnology Research Directions for Societal Needs in 2020*; Springer, Science Policy Reports: New York. 2011.

(4) Peralta-Videa, J. R.; Zhao, L.; Lopez-Moreno, M. L.; de la Rosa, G.; Hong, J.; Gardea-Torresdey, J. L. Nanomaterials and the environment: A review for the biennium 2008−2010. *J. Hazard. Mater.* **2011**, *186* (1), 1−15.

(5) Project on Emerging Nanotechnologies. http://www.nanotechproject.org/ (accessed June 1, 2012).

(6) Mukal, D.; Sexena, N.; Dwivedi, P.D.. Emerging trends of nanoparticles application in food technology: Safety paradigms. *Nanotoxicology* **2009**, *3* (1), 10−18.

(7) Chinnamuthu, C. R.; Boopathi, P. M. Nanotechnology and agroecosystem. *Madras Agric. J.* **2009**, *96*, 1−6.

(8) Corredor, E.; Testillano, P. S.; Coronado, M.-J.; Gonzalez-Melendi, P.; Fernandez-Pacheco, R.; Marquina, C.; Ibarra, M. R.; de la Fuente, J. M.; Rubiales, D.; Perez-de-Luque, A.; Risueno, M.-C. Nanoparticle penetration and transport in living pumpkin plants: In situ subcellular identification. *BMC Plant Biol.* **2009**, *9*, 45−46.

(9) Liu, Q.; Chen, B.; Wang, Q.; Shi, X.; Xiao, Z.; Lin, J.; Fand, X. Carbon nanotubes as molecular transporters for walled plant cells. *Nano Lett.* **2009**, *9*, 1007−1010.

(10) Card, J. W.; Jonaitis, T. S.; Tafazoli, S.; Magnuson, B. A. An appraisal of the published literature on the safety and toxicity of food related nanomaterials. *Crit. Rev. Toxicol.* **2011**, *41*, 20−49.

(11) Panacek, A.; Prucek, R.; Safarova, D.; Dittrich, M.; Richtrova, J.; Benickova, K.; Zboril, R.; Kvitek, L. Acute and chronic toxicity effects of silver nanoparticles (NPs) on *Drosophila melanogaster*. *Environ. Sci. Technol.* **2011**, *45*, 4974−4979.

(12) Zhang, J.; Wages, M.; Cox, S. B.; Maul, J. D.; Li, Y.; Barnes, M.; Hope-Weeks, L.; Cobb, G. P. Effect of titanium dioxide nanomaterials and ultraviolet light coexposure on African clawed frogs (*Xenopus laevis*). *Environ. Toxicol. Chem.* **2012**, *31*, 176−183.

(13) Unrine, J. M.; Shoults-Wilson, W. A.; Zhurbich, O.; Bertsch, P. M.; Tsyusko, O. V. Trophic transfer of Au nanoparticles from soil along a simulated terrestrial food chain. *Environ. Sci. Technol.* **2012**, *46*, 9753−9760.

(14) Stampoulis, D.; Sinha, S. K.; White, J. C. Assay-dependent phytotoxicity of nanoparticles to plants. *Environ. Sci. Technol.* **2009**, *43*, 9473−9479.
(15) Rico, C. M.; Majumdar, S.; Duarte-Gardea, M.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Interaction of nanoparticles with edible plants and their possible implications in the food chain. *J. Agric. Food. Chem.* **2011**, *59* (8), 3485−3498.
(16) De La Torre-Roche, R.; Hawthorne, J.; Deng, Y.; Xing, B.; Cai, W.; Newman, L. A.; Wang, C.; Ma, X.; White, J. C. Fullerene-enhanced accumulation of *p,p′*-DDE in agricultural crop species. *Environ. Sci. Technol.* **2012**, *46*, 9315−23.
(17) Kelsey, J. W.; White, J. W. Effect of C60 fullerenes on the accumulation of weathered *p,p′*-DDE by plant and worm species under single and multi-species conditions. *Environ. Toxicol. Chem.* In press.
(18) Anastassiades, M.; Lehotay, S. J.; Stajnbaher, D.; Schenck, F. J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. *J. AOAC Int.* **2003**, *86*, 412−431.
(19) Jambunathan, N. Determination and detection of reactive oxygen species (ROS), lipid peroxidation, and electrolyte leakage in plants. In *Plant Stress Tolerance, Methods in Molecular Biology, Vol. 639*; Springer: New York, 2010; Ch.18, pp 291−297.
(20) White, J. C. Inheritance of *p,p′*-DDE Phytoextraction ability in hybridized *Cucurbita pepo* cultivars. *Environ. Sci. Technol.* **2010**, *44*, 5164−5169.
(21) Musante, C.; White, J. C. Toxicity of silver and copper to *Cucurbita pepo*: Differential effects of nano and bulk size particles. *Environ. Toxicol.* **2012**, *27*, 510−517.
(22) Kumari, M.; Mukherjee, A.; Chandrasekaran, N. Genotoxicity of silver nanoparticles in *Allium cepa*. *Sci. Total Environ.* **2009**, *407*, 5243−5246.
(23) Yin, L.; Cheng, Y.; 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*, 2360−2367.
(24) Lee, W.-M.; Kwak, J. I.; An, Y.-J. Effect of silver nanoparticles in crop plants *Phaseolus radiatus* and *Sorghum bicolor*: Media effect on phytotoxicity. *Chemosphere* **2012**, *86*, 491−499.
(25) Wang, Z.; Xie, X.; Zhao, J.; Li, 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*, 4434−4441.
(26) Wang, S.; Kurepa, J.; Smalle, J. A. Ultra-small TiO_2 nanoparticles disrupt microtubular networks in *Arabidopsis thaliana*. *Plant Cell Environ.* **2011**, *34*, 811−820.
(27) Liu, Q.; Zhao, Y.; Wan, Y.; Zheng, J.; Zhang, X.; Wang, C.; Fang, X.; Lin, J. Study of the inhibitory effect of water-soluble fullerenes on plant growth at the cellular level. *ACS Nano* **2010**, *4*, 5743−5748.
(28) Nel, A.; Xia, T.; Mädler, L.; Li, N. Toxic potential of materials at the nanolevel. *Science* **2006**, *311*, 622−627.
(29) White, J. C.; Wang, X.; Gent, M. P. N.; Iannucci-Berger, W.; Eitzer, B. D.; Schultes, N. P.; Arienzo, M.; Mattina, M. I. Subspecies-level variation in the phytoextraction of weathered *p,p′*-DDE by *Cucurbita pepo*. *Environ. Sci. Technol.* **2003**, *37*, 4368−4373.
(30) Lunney, A. I.; Zeeb, B. A.; Reimer, K. J. Uptake of weathered DDT by vascular plants: Potential for phytoremediation. *Environ. Sci. Technol.* **2004**, *38*, 6147−6154.
(31) Park, J.-W.; Henry, T. B.; Menn, F.-M.; Compton, R. N.; Sayler, G. No bioavailability of 17-α ethinylestradiol when associated with nC_{60} aggregates during dietary exposure in adult male zebrafish (*Danio rerio*). *Chemosphere* **2010**, *81*, 1227−1232.
(32) Baun, A.; Sorensen, S. N.; Rasmussen, R. F.; Hartmann, N. B.; Koch, C. B. Toxicity and bioaccumulation of xenobiotic organic compounds in the presence of aqueous suspensions of aggregates of nano-C_{60}. *Aquatic Toxicol.* **2007**, *86*, 379−387.
(33) Hu, X.; Liu, J.; Zhou, Q.; Lu, S.; Liu, R.; Sui, L.; Yin, D.; Mayer, P.; Jiang, G. Bioavailability of organochlorine compounds in aqueous suspensions of fullerene: evaluated with medaka (*Oryzias latipes*) and negligible depletion solid phase extraction. *Chemosphere* **2010**, *80*, 693−700.
(34) Ma, X.; Wang, C. Fullerene nanoparticles affect the fate and uptake of trichloroethylene in phytoremediation systems. *Environ. Eng. Sci.* **2010**, *27*, 989−992.
(35) Berger, W. A.; Mattina, M. I.; White, J. C. Effect of hydrogen peroxide on the uptake of chlordane by *Cucurbita pepo*. *Plant Soil* **2012**, DOI: 10.1007/s11104-012-1207-6.
(36) Chhikara, S.; Paulose, B.; White, J. C.; Parkash Dhankher, O. Understanding the physiological and molecular mechanism of persistent organic pollutant (POP) uptake and detoxification in cucurbit species (zucchini and squash). *Environ. Sci. Technol.* **2010**, *44*, 7295−7301.
(37) Niemietz, C. M.; Tyerman, S. D. New potential inhibitors of aquaporins: Silver and gold compounds inhibit aquaporins of plant and human origin. *FEBS Lett.* **2002**, *531*, 443−447.
(38) Miralles, P.; Church, T. L.; Harris, A. T. Toxicity, uptake, and translocation of engineered nanomaterials in vascular plants. *Environ. Sci. Technol.* **2012**, *46*, 9224−9239.
(39) Larue, C.; Laurette, J.; Herlin-Boime, N.; Khodja, H.; Fayard, B.; Flank, A.-M.; Brisset, F.; Carriere, M. Accumulation, translocation, and impact of TiO_2 nanoparticles in wheat (*Triticum aestivum* ssp.): Influence of diameter and crystal phase. *Sci. Total Environ.* **2012**, *431*, 197−208.
(40) Hawthorne, J.; Musante, C.; Sinha, S. K.; White, J. C. Accumulation and phytotoxicity of engineered nanoparticles to *Cucurbita pepo*. *Int. J. Phytorem.* **2012**, *14*, 429−442.