Assay-Dependent Phytotoxicity of Nanoparticles To Plants

J. C. White[a], D. Stampoulis[b], and S.K. Sinha[b]

[a]Head, Department of Analytical Chemistry, The Connecticut Agricultural Experiment Station

[b]Department of Biology and Environmental Science,University of New Haven

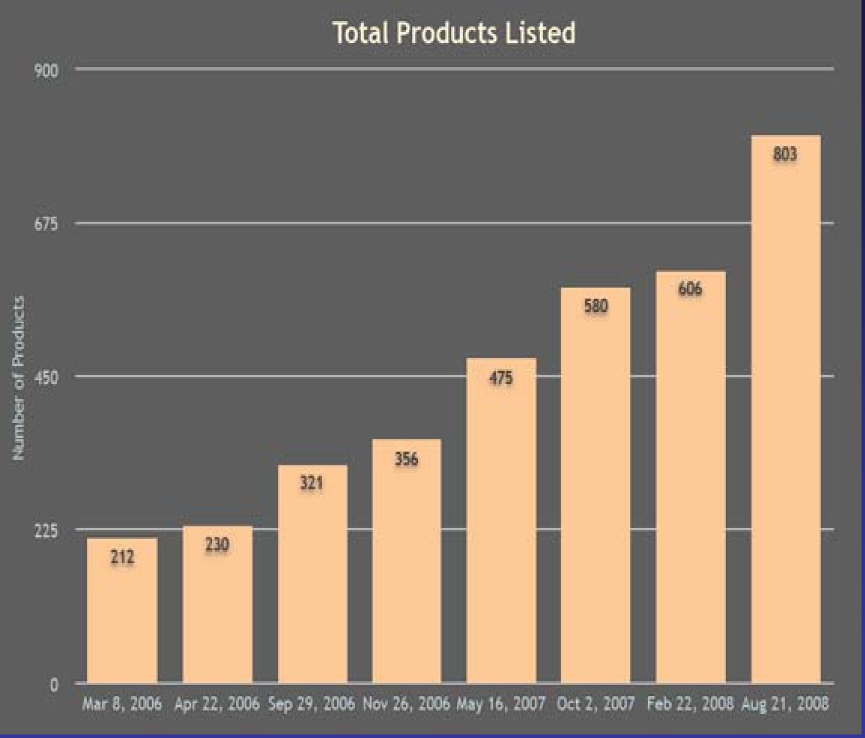
Properties of Nanoparticles (NP)

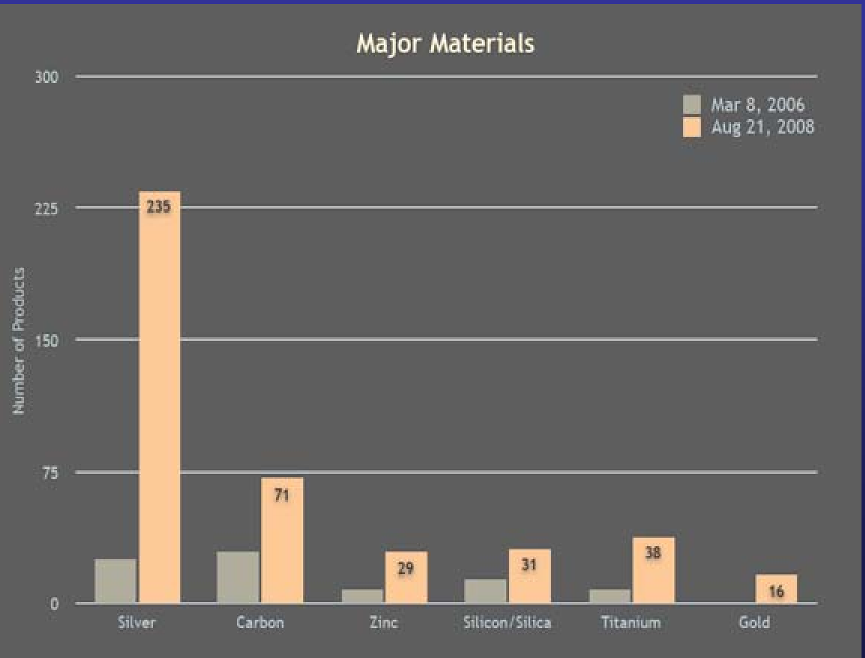
- **Generic**
  - A. High surface area/volume ratio
  - B. Differential properties as a matter of scale
  - C. High surface reactivity

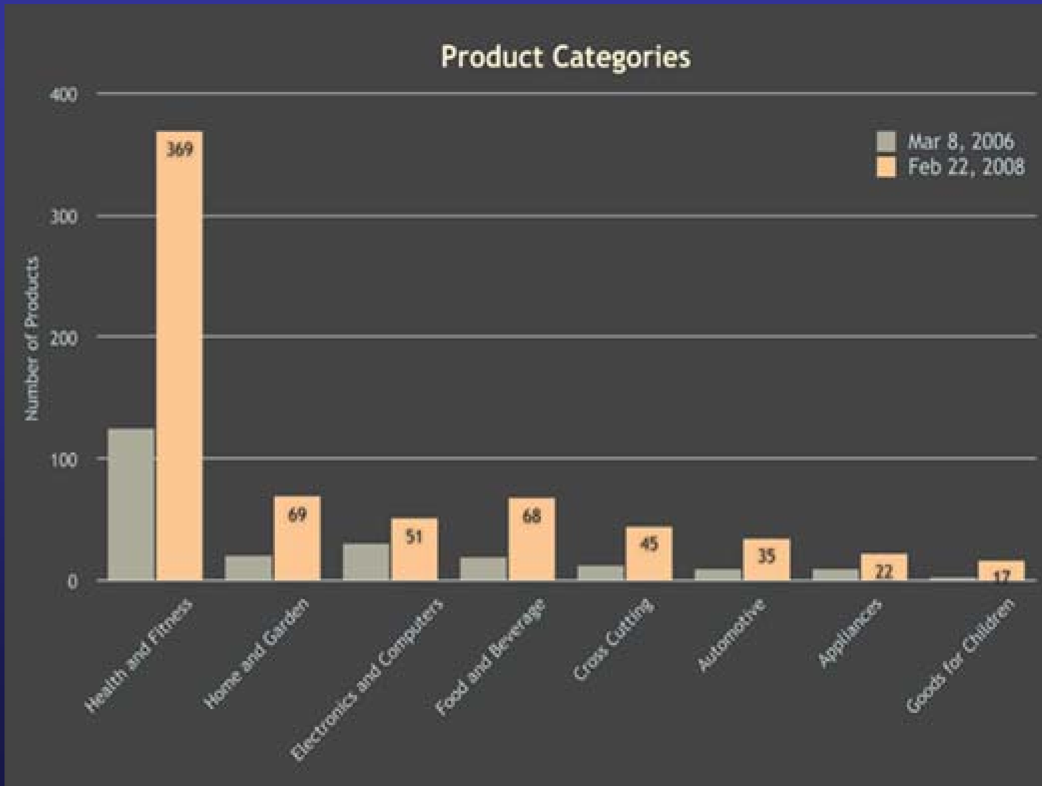
- **Material-dependent**

Changes in properties		
	Bulk-scale	**Nano-scale**
Si	Insulator	Conductive
Cu	Malleable and ductile	Stiff
TiO_2	White color	Colorless
Au	Chemically inert	Chemically active

Nanotechnology-based products







However…

- The benefits of nanotechnology are accompanied by potential risks, the extent of which we do not yet fully appreciate.

- Numerous concerns have been raised that the same attributes of NPs that make them attractive, may lead to new and unusual risks to the human health and the environment.

- Little is known about NP biological and environmental fate.

- If differential toxicity is observed, should nanoparticle Ag should be regulated differently than bulk Ag?

NPs possess toxic potential

Nanotoxicology: the field of science which looks at the potential for nanotechnology to cause adverse effects

Preliminary studies: A range of species have been investigated:

- Bacteria (Jiang et al., 2009; Johansen et al., 2008)
- Algae (Wang et al., 2008)
- Invertebrates such as nematodes and crustaceans (Wang et al., 2009; Heinlaan et al., 2008)
- Vertebrates such as fish and rats (Griffitt et al., 2008; Elgrabli et al., 2008)

However, most look only at NPs (no bulk material comparison)

NP-induced phytotoxicity

- Data on NP toxicity to plants is almost non-existent.
- There are a handful of studies assessing the effects of specific NPs on germination and root elongation

  (Lin and Xing, 2008; Yang and Watts, 2005; Canas *et al*. 2008; Zhu *et al*. 2008).
- This literature is plagued by shortcomings (failure to compare bulk- and nanoparticle-induced toxicity for each tested material).

Experimental Design

- Determine the impact of engineered nanomaterials on zucchini:
  - Seed germination
  - Emerging seed root elongation
  - Biomass under hydroponic conditions
- Used 5 commonly employed nanomaterials and evaluated them against corresponding bulk materials
  - Ag, Cu, Si, ZnO, MWCNTs

Seeds

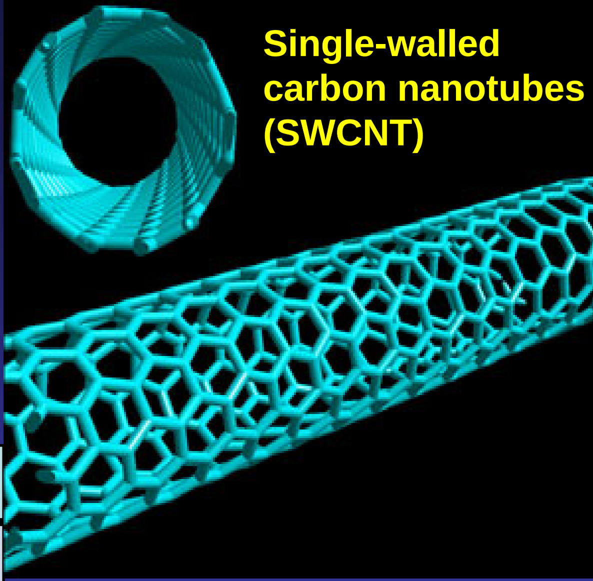
- Initially soaked in 10% sodium hypochlorite for 10 min.
- Rinsed with reverse osmosis (R.O.) water
- Air-dried and stored in plastic bags at room temperature

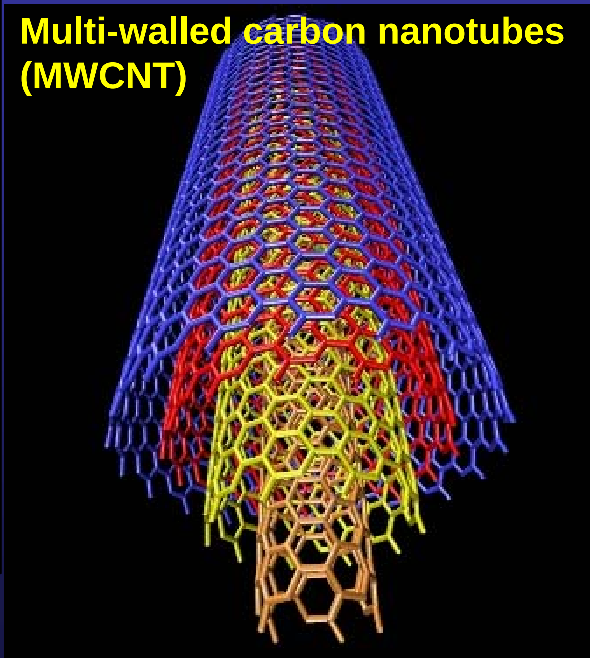
Particle suspensions

- 2 methods of achieving dispersion
  - Surfactant [SDS at 0.2%]
  - Ultrasonic vibration
- Suspensions were made with R.O. water or Hoagland solution, depending on the phytotoxicity assay.
- All suspensions (NP or bulk) were at 1000 mg/L.
- Suspensions in amber jars
- Agitated before application

Characteristics of NPs used in the study

Particle	Size	Purity
MWCNTs	Outer mean diameter : 13-16 nm Outer diameter distribution : 5-20 nm Inner mean diameter : 4 nm Inner diameter distribution : 2-6 nm Length : 1-10 μm	>99%
ZnO	Diameter : 5 nm/10 nm	99.99%
Si	Diameter < 100 nm (average ~ 50 nm)	≥98%
Cu	Diameter : < 50 nm	99.8%
Ag	Diameter < 100 nm	99.5%





Percent germination- zucchini seeds exposed to NPs and corresponding bulk materials (1000mg/L). Trial one included the surfactant SDS (0.2%) to facilitate particle dispersion; trial 2 had no SDS. Values represent % germination after 4 days.

Trial One (SDS)												
RO water[a]	RO water+ SDS	AC	MW-CNT	ZnO powd.	ZnO 5 nm	ZnO 10 nm	Si powd.	Si 100 nm	Cu powd.	Cu 50 nm	Ag powd.	Ag 100 nm
90	60 A	60 A	40 A	67 A	86 A	67 A	40 AB	0 B	40 A	53 A	67 A	33 A
Trial Two (no SDS)												
RO water	RO water+ SDS	AC	MW-CNT	ZnO powd	ZnO 5 nm	ZnO 10 nm	Si powd.	Si 100 nm	Cu powd.	Cu 50 nm	Ag powd.	Ag 100 nm
87 A	NA	67 A	87 A	80 A	67 A	87 A	93 A	80 A	73 A	80 A	93 A	87 A

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).

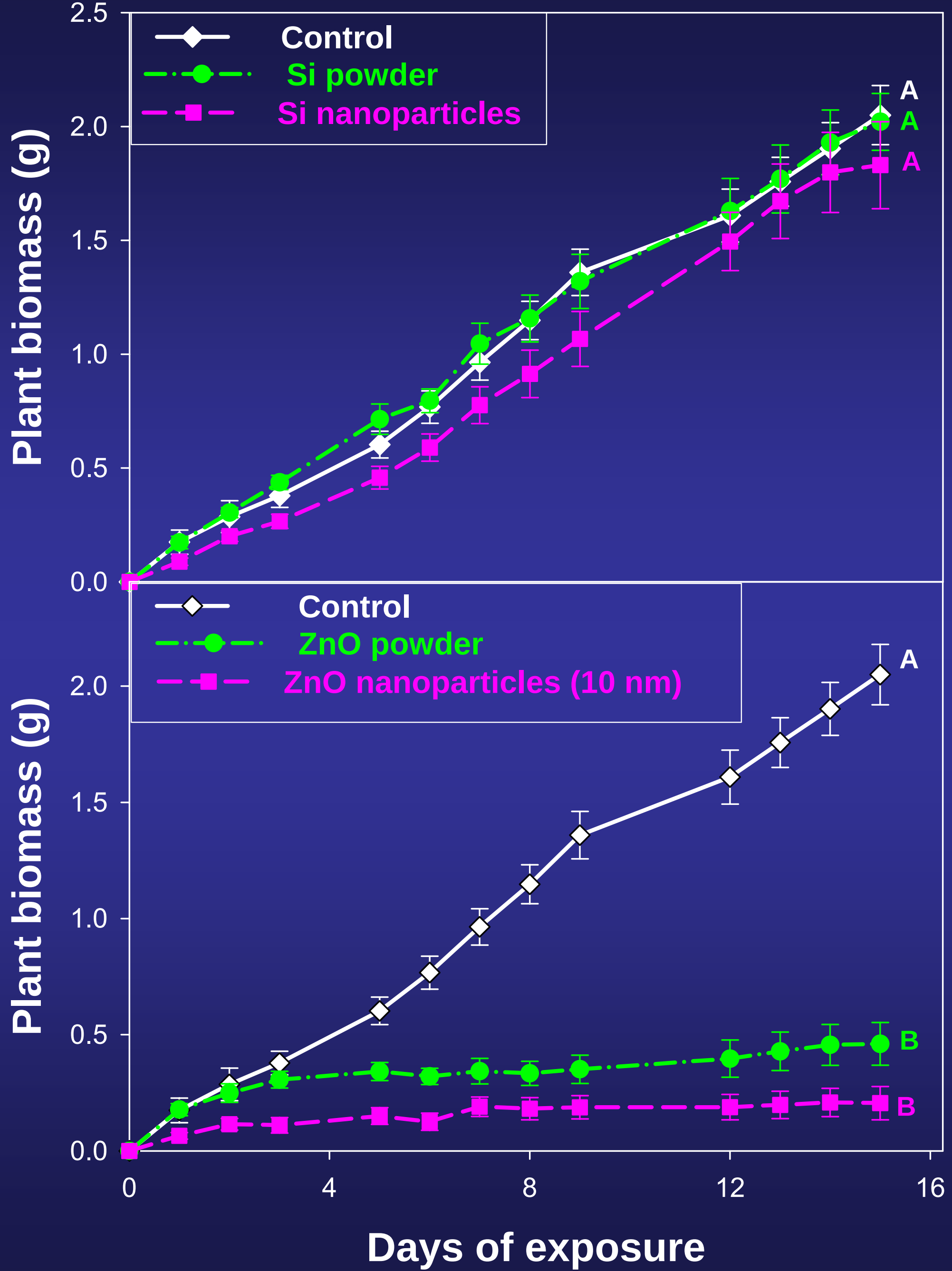
Root elongation- Effects of 1000 mg/L suspensions of NPs and their corresponding bulk materials on root elongation (mm). Numerical values represent the average root lengths of 15 seeds. SDS (0.2%) was used for particle dispersion.

	Day 1	Day 2		Day 3		Day 4	
DIW water + SDS	2.9	3.1	A	3.3	A	3.4	A
Ag Bulk	3.4	3.9	A	4.2	B	4.3	A
Ag Nano	6.0	6.3	A	6.4	C	6.5	A
DIW + SDS	2.9	3.1	A	3.3	A	3.4	A
Si Bulk	5.5	6.0	A	6.2	A	6.4	A
Si Nano	4.5	4.8	A	4.9	A	5.2	A
DIW + SDS	2.9	3.1	A	3.3	A	3.4	A
Cu Bulk	4.2	4.6	A	4.8	A	4.9	A
Cu Nano < 50 nm	6.0	6.8	A	6.9	A	7.1	A
Cu Nano 10 nm	5.9	6.5	A	6.5	A	6.8	A
DIW + SDS	2.9	3.1	A	3.3	A	3.4	A
ZnO Bulk	5.8	6.6	A	6.7	A	6.8	A
ZnO 10nm	5.6	5.8	A	6.1	A	6.3	A
ZnO 5nm	5.8	6.2	A	6.6	A	7.1	A
DIW + SDS	2.9	3.1	A	3.3	A	3.4	A
Act. Carbon	5.5	5.9	A	6.0	A	6.3	A

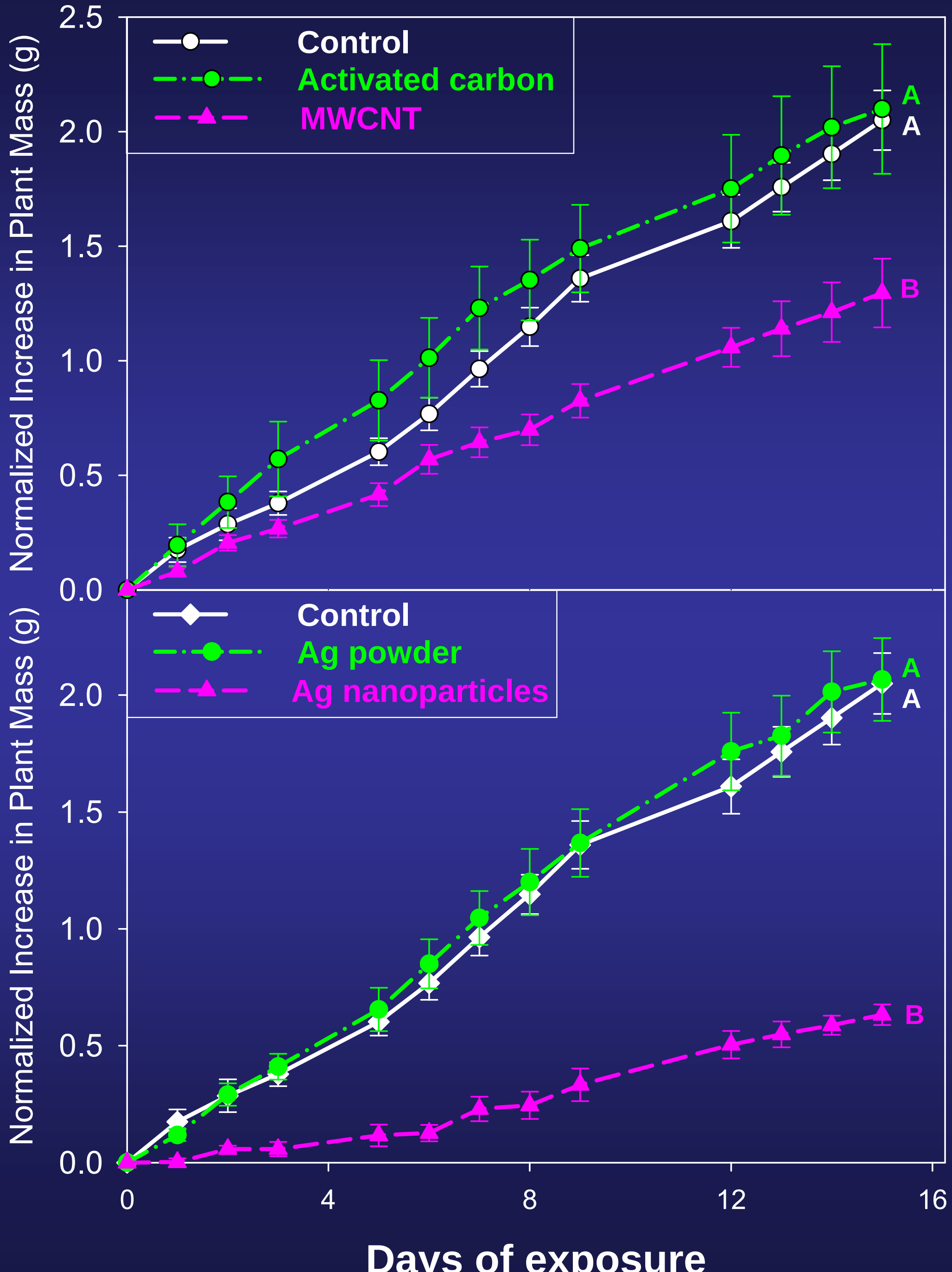
Root elongation- Effects of 1000 mg/L suspensions of NPs and their corresponding bulk materials on root elongation (mm). Numerical values represent the average root lengths of 15 seeds. No SDS was used.

	Day 1	Day 2		Day 3		Day 4		Day 5		Day 6	
DIW water	0.5	3.9	A	9.7	A	20.7	A	22.8	A	24.3	A
Ag Bulk	0.5	5.4	A	14.5	A	34.4	A	40.6	A	42.9	A
Ag Nano	0.5	4.9	A	12.3	A	27.1	A	33.1	A	36.2	A
DIW	0.5	3.9	A	9.7	A	20.7	A	22.8	A	24.3	A
Si Bulk	0.5	5	B	12.6	A	26.8	A	28.4	A	31.7	A
Si Nano	0.5	4.2	A/B	11.8	A	28.1	A	40.1	A	45.1	A
DIW	0.5	3.9	A/B	9.7	A	20.7	A	22.8	A	24.3	A
Cu Bulk	0.5	4.9	A	8.5	A	12.8	A	14	A	15.9	A
Cu Nano	0.5	2.9	B	3.8	B	4.3	B	4.7	B	5.7	B
DIW	0.5	3.9	A	9.7	A	20.7	A	22.8	A	24.3	A
ZnO Bulk	0.5	4	A	8.2	A/B	8.6	A/B	10	B	13	A
ZnO 10nm	0.5	3.9	A	7.5	A/B	8.5	A/B	9.5	B	12.1	A
ZnO 5nm	0.5	3.3	A	5.3	B	5.8	B	6.9	B	8.5	A
DIW	0.5	3.9	A	9.7	A	20.7	A	22.8	A	24.3	A
Act. Carbon	0.5	4.2	A	10.2	A	12.5	A	14.4	A	20.2	A
MWCNTs	0.5	3.7	A	7.3	A	8.7	A	11.2	A	13.9	A

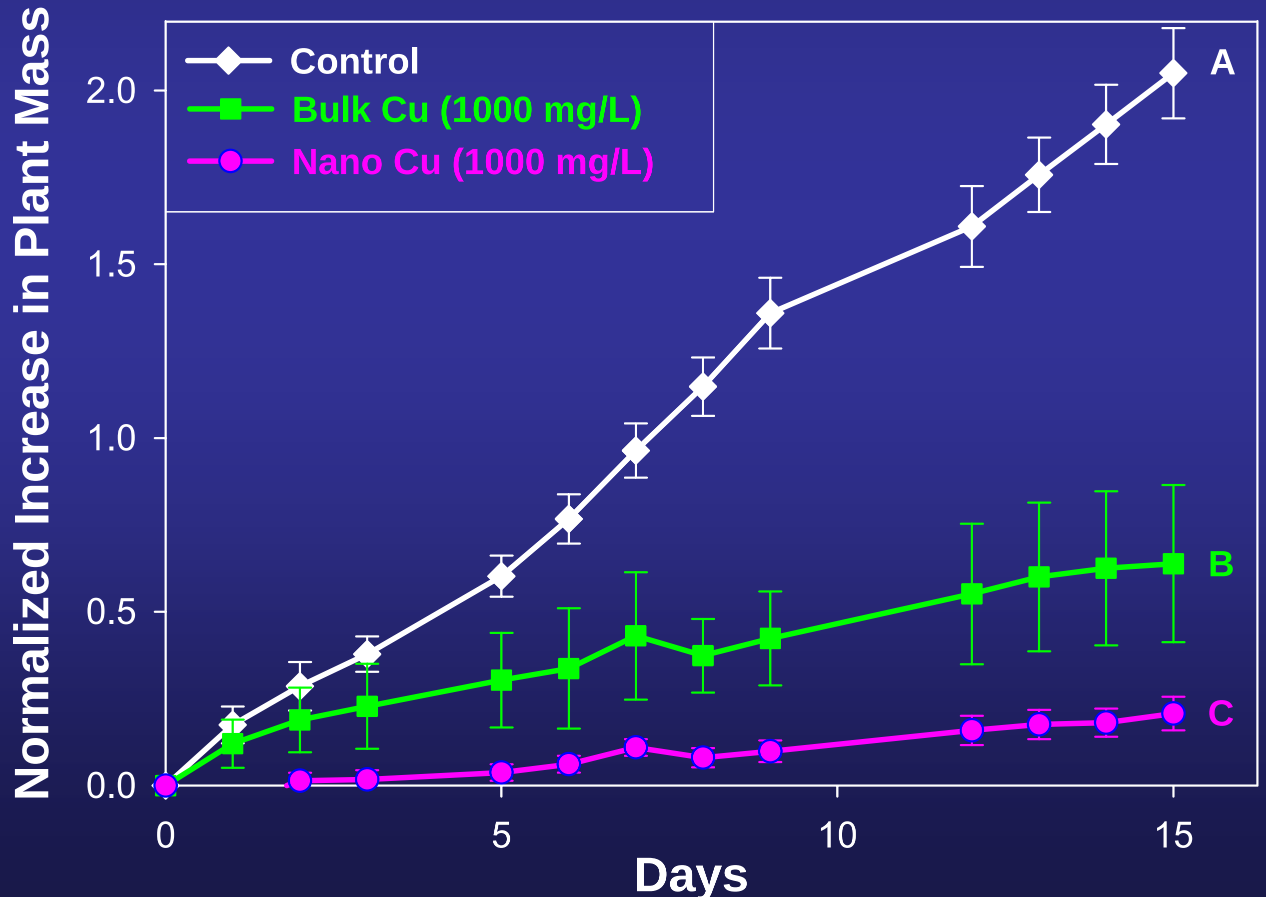
Effect of Si or Si nanoparticles (top) or ZnO powder or ZnO nanoparticles (bottom) on zucchini biomass under hydroponic conditions.



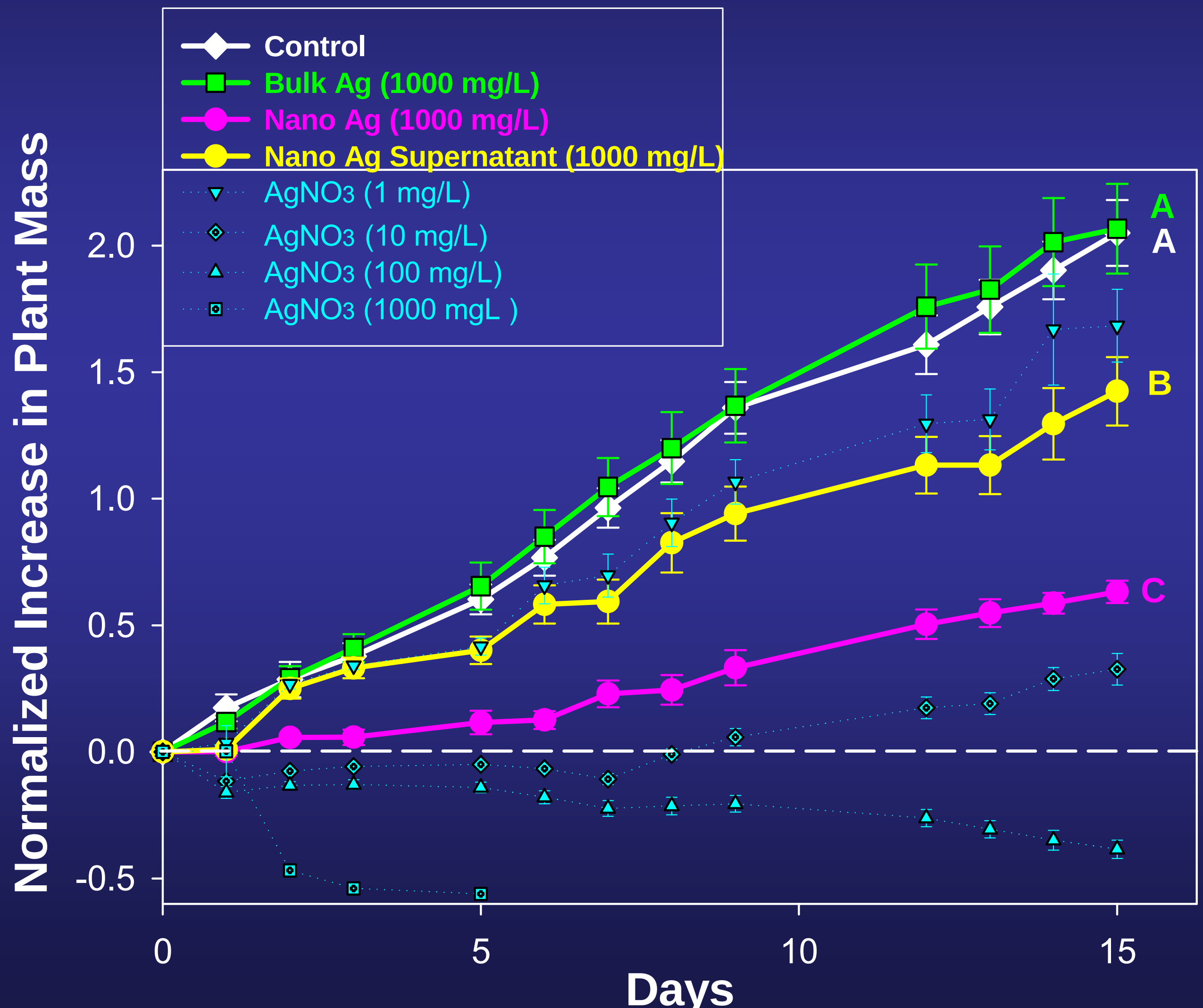
Effect of activated carbon or MWCNTs (top) or Ag powder or nanoparticles (bottom) on zucchini biomass under hydroponic conditions.

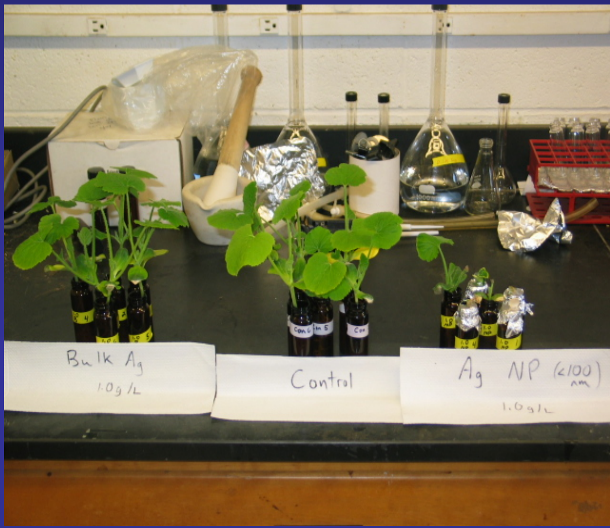


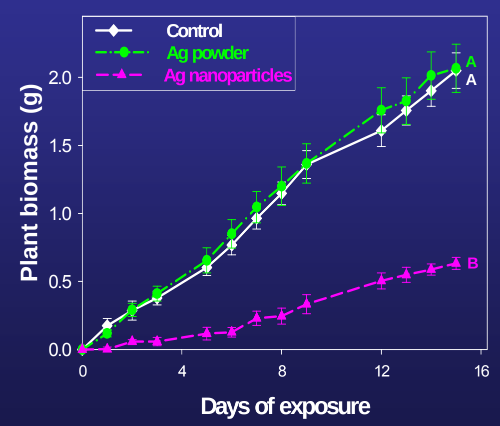
Effect of Cu powder or nanoparticles on zucchini biomass under hydroponic conditions.



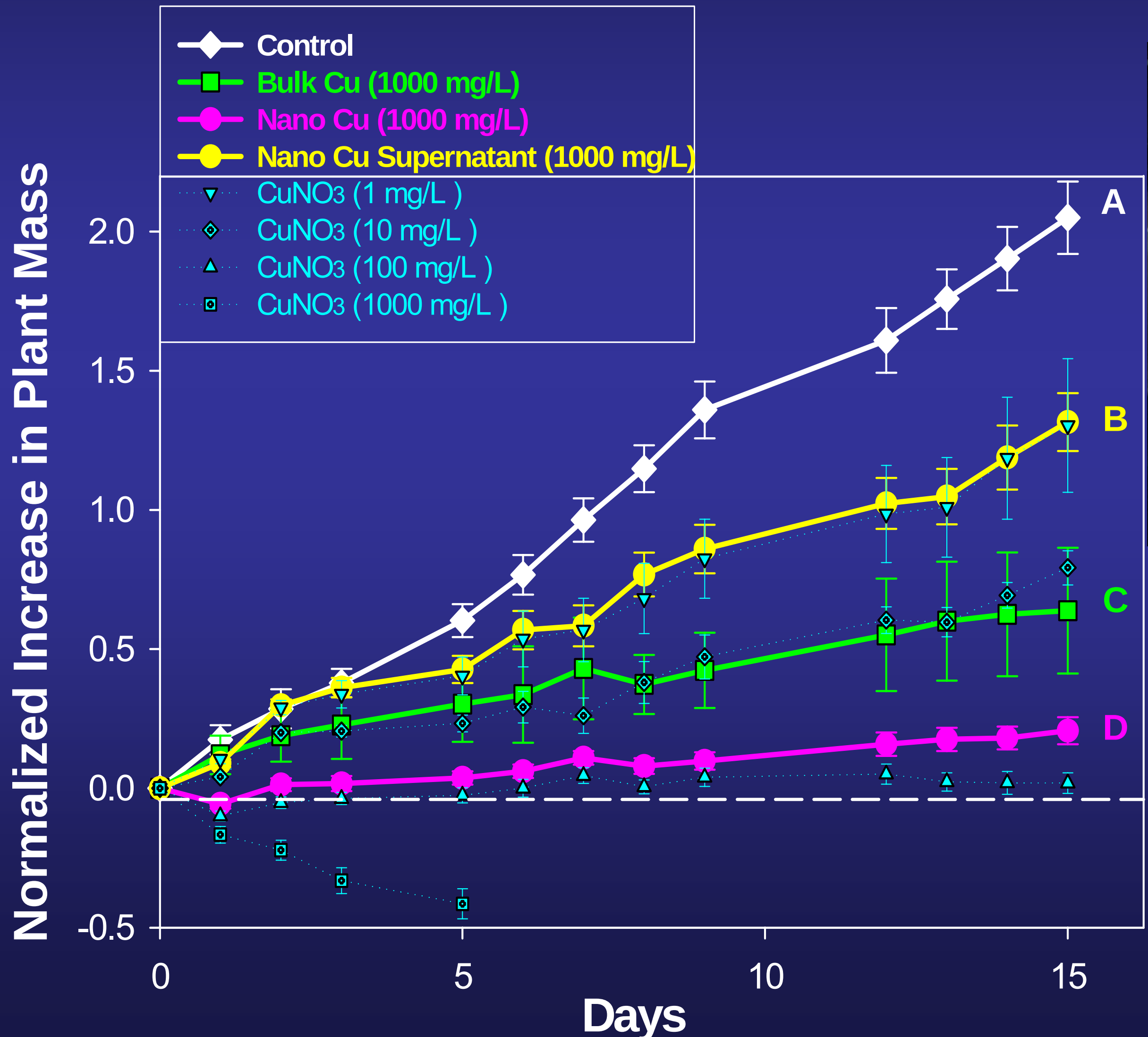
Effect of Ag powder or nanoparticles on zucchini biomass under hydroponic conditions. Dissolved ion controls were included, as were controls consisting of the supernatant of centrifuged nanoparticle solutions.

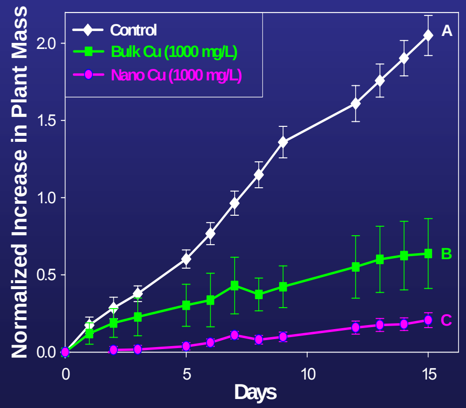






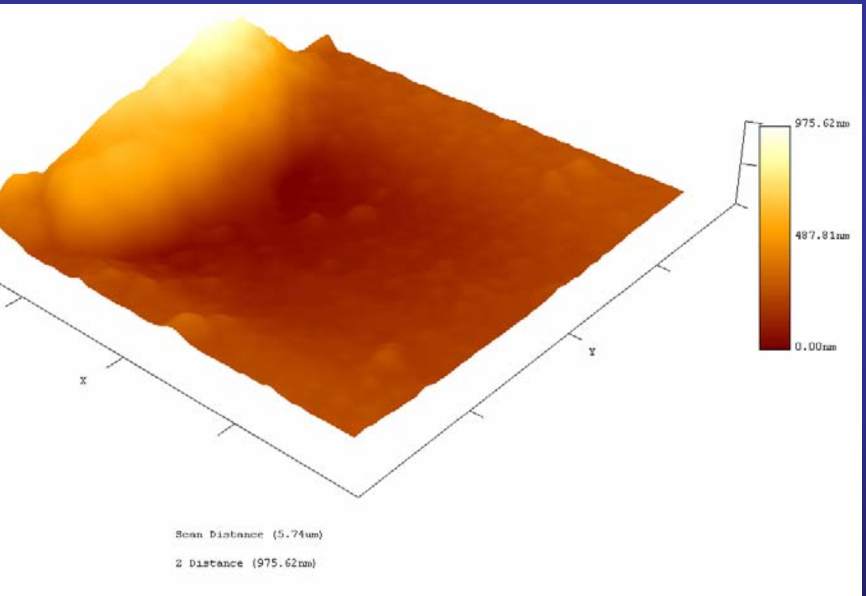
Effect of Cu powder or nanoparticles on zucchini biomass under hydroponic conditions. Dissolved ion controls were included, as were controls consisting of the supernatant of centrifuged nanoparticle solutions.





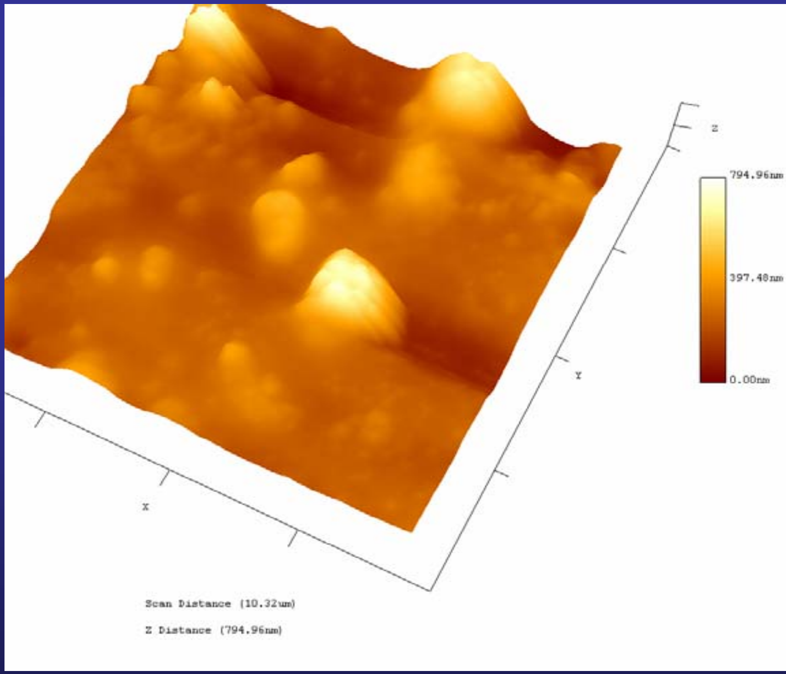
Microscopy

- The presence of long, thin and cylindrical-like features found within the plant tissues (not directly exposed to MWCNTs) indicate the potential uptake and translocation of MWCNTs via the xylem or phloem.



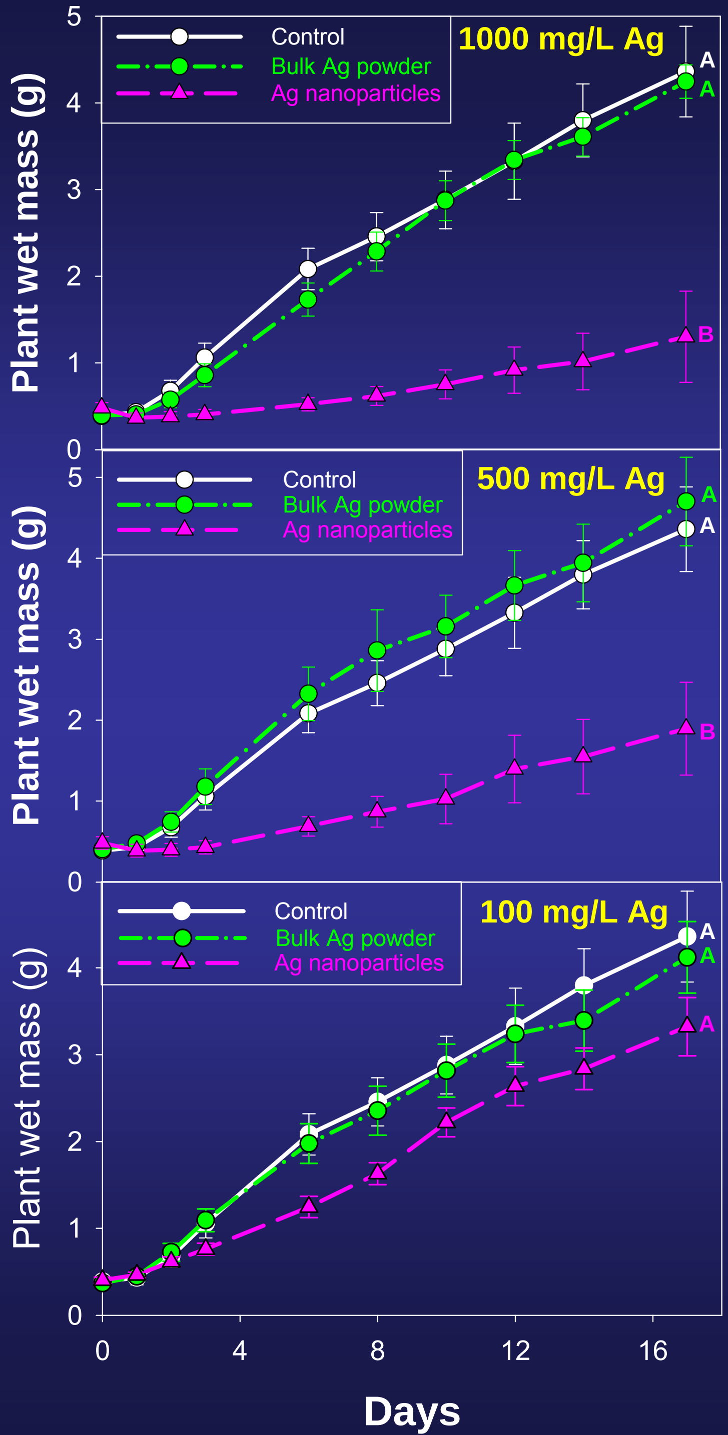
AFM image of a zucchini tissue that had never been exposed to MWCNTs

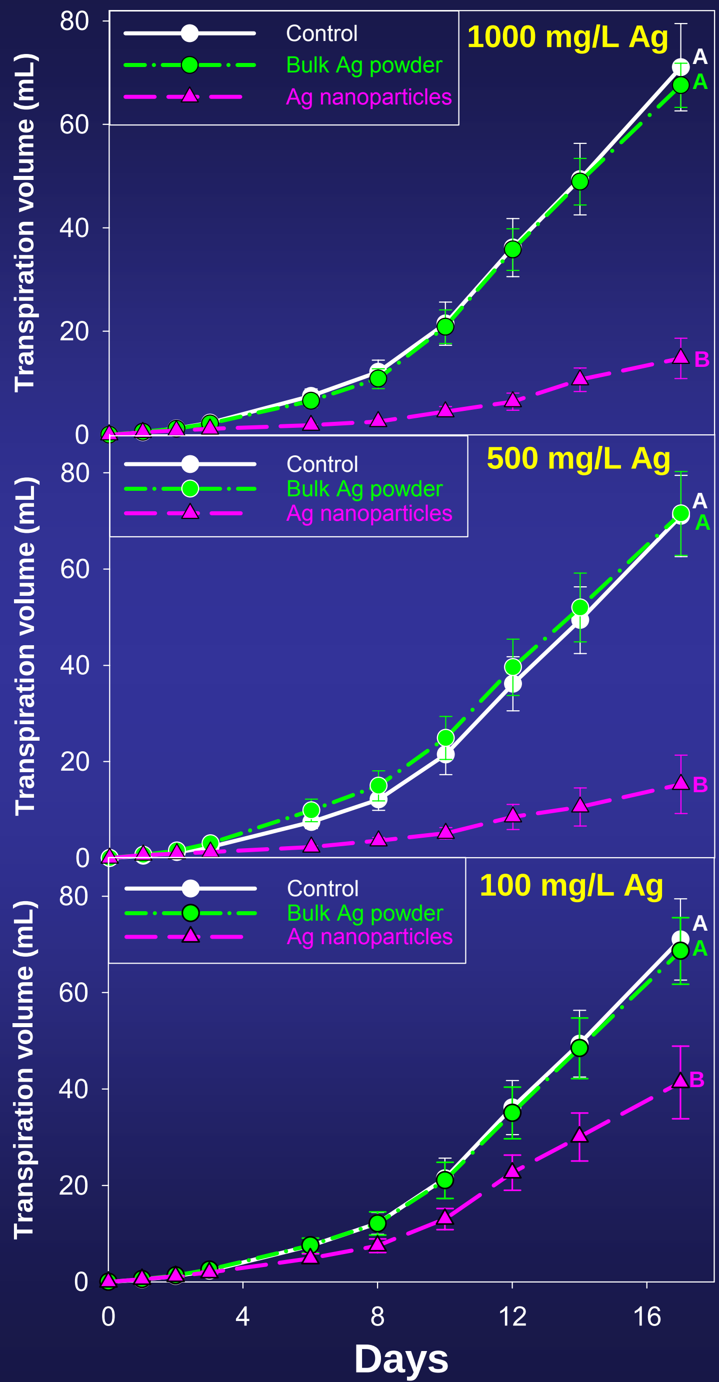
The area scanned is a square with a 10 µm side.



AFM image of a zucchini tissue indirectly exposed to MWCNTs

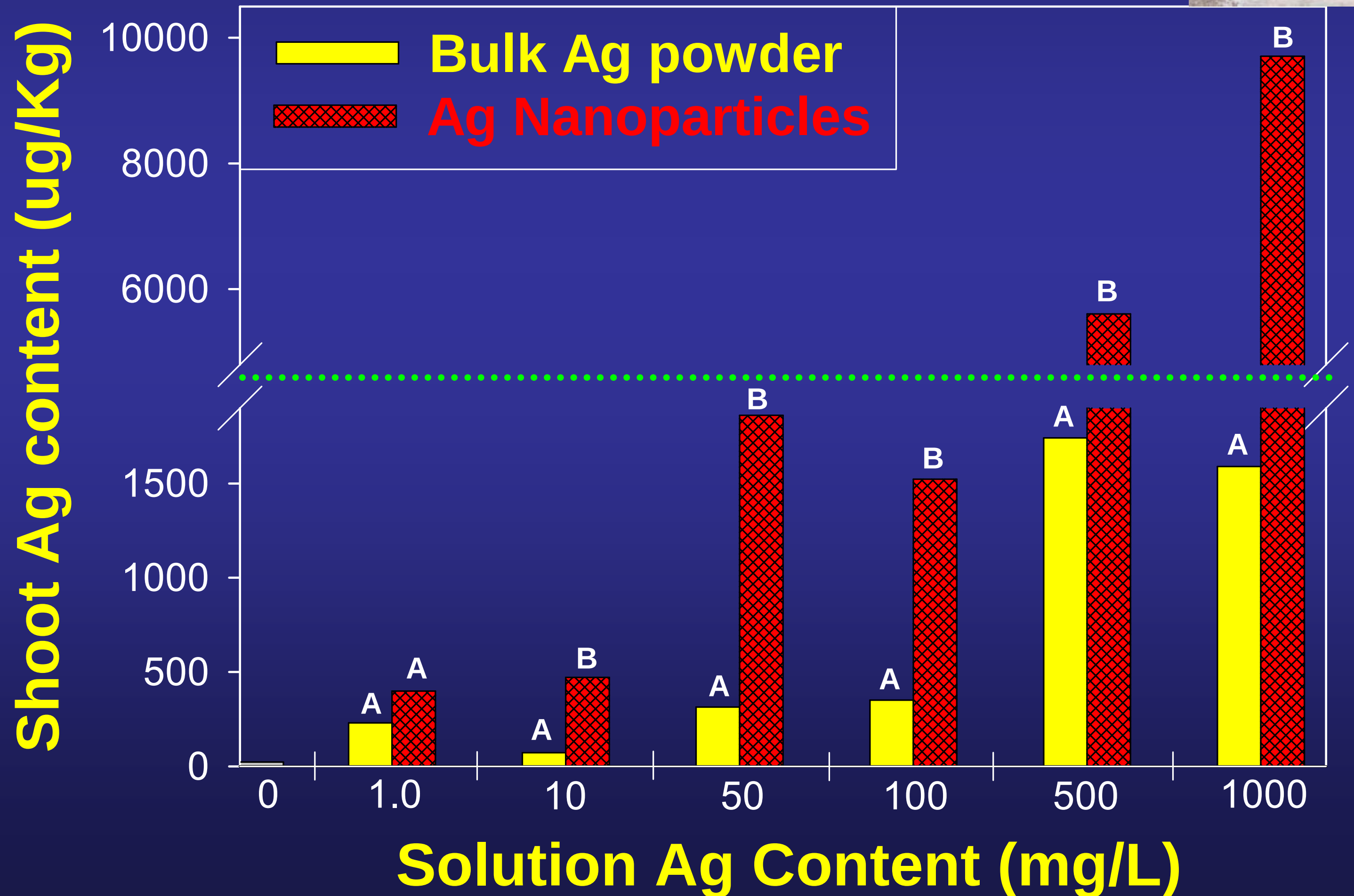
Dose-uptake study (1-1000 mg/L) assessing nanoparticle or bulk Ag on zucchini biomass





Dose-uptake study (1-1000 mg/L) assessing nanoparticle or bulk Ag on zucchini transpiration volume

Ag content of zucchini shoots grown in Ag nanoparticle or bulk solutions (1-1000mg/L)



Conclusions

- Two common EPA phytotoxicity tests fail to detect any toxicological differences between commonly encountered nanoparticles and the corresponding bulk materials
- More involved hydroponic assays reveal significantly greater toxicity from Ag, Cu, and MWCNTs as compared to the bulk materials
- Increased dissolution of ions from Ag nanoparticles only partially explains the phytotoxicity; there is something inherently toxic about the elemental Ag NP
- Uptake of MWCNTs by plants is suggested
- In a dose-uptake study, the accumulation of Ag from nanoparticle treatments was 4-fold greater than from bulk solutions

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- Craig Musante and Joe Hawthorne for Technical Assistance
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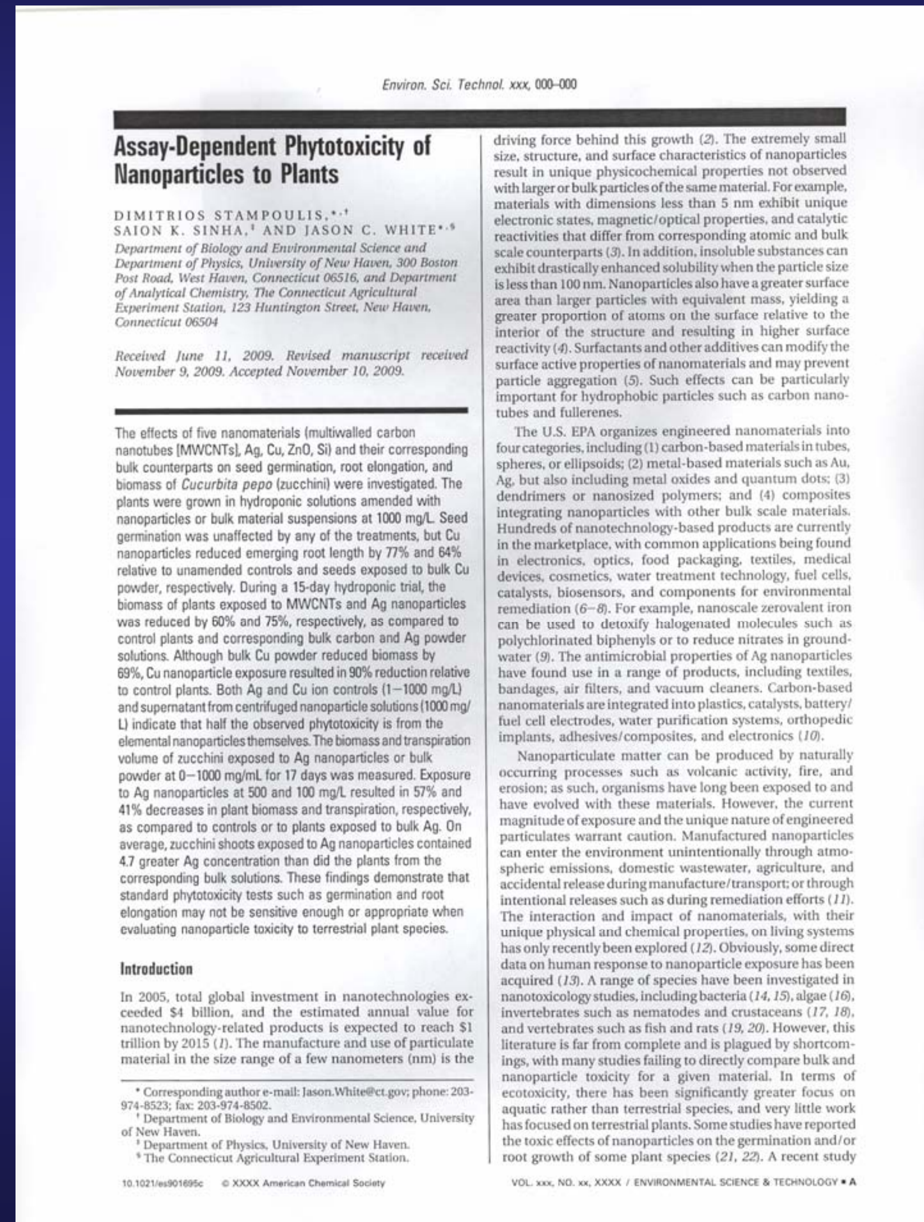

Assay-Dependent Phytotoxicity of Nanoparticles to Plants

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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