Nanomaterials and the Food Supply: Assessing the Balance Between Applications and Implications

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Presented at the Environmental Sciences: Water, Gordon Research Conference Holderness, NH June 29, 2016
What are Nanoparticles (NPs)?

- Nanoparticles (less than 100 nm) are generated *naturally* by erosion, fires, volcanoes, and marine wave action.

- **A key point** - People have been exposed to nanoparticles for as long as there have been people; in other words, “nano” isn’t inherently bad.

- Nanoparticles are also produced by human activities such as coal combustion, vehicle exhaust, and weathering rubber tires.
What are Engineered Nanomaterials?

- Our ability to construct and manipulate materials at the nano-scale has increased dramatically in the last decade.

- Why does this matter? Materials at the nano-scale behave differently than the same material at the bulk or non-nano scale.

- Have higher surface area to volume; can engineer for surface reactivity or other desired characteristics.

- Frequently, this unique behavior can be both useful and profitable.

- Nanotechnology was a $1 billion industry in 2005; will be a $3 trillion industry by 2020.

<table>
<thead>
<tr>
<th>Changes in properties</th>
<th>Bulk-scale</th>
<th>Nanoscale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si</td>
<td>Insulator</td>
<td>Conductive</td>
</tr>
<tr>
<td>Cu</td>
<td>Malleable and ductile</td>
<td>Stiff</td>
</tr>
<tr>
<td>TiO₂</td>
<td>White color</td>
<td>Colorless</td>
</tr>
<tr>
<td>Au</td>
<td>Chemically inert</td>
<td>Chemically active</td>
</tr>
</tbody>
</table>
National Nanotechnology Initiative (NNI)(http://nano.gov/)

- Started in 2000; Clinton administration
- 2016/2017 Budget Request is $1.5 Billion across 20+ Federal agencies. Applications- 93%; Implications- 7%.
- “The NNI consists of the individual and cooperative nanotechnology-related activities of Federal agencies with a range of research and regulatory roles and responsibilities.”

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Nanotechnology - Applications

- Nanomedicine
- Water treatment
- Communication\electronics
- Energy
- Agriculture\food
- Textiles
- Cosmetics

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Nanomaterials and Food Protection

- **Food Safety** - microbes and chemicals/elements
  - Antimicrobials in food packaging
  - Nano-enabled coatings for food and equipment
  - Nanosensors for pathogen detection

- **Food Defense** - microbes and chemicals/elements
  - Nanosensors for specific agents of concern (biological weapons such as *B. anthracis*, Ebola [Harvard/MIT]) and others; plant proteins such as ricin and abrin.
Nanomaterials and Agriculture

- There has been significant interest and research in using nanotechnology in agriculture
- The goals fall into several categories
  - Increase production rates and yield
  - Increase efficiency of resource utilization
  - Minimize waste production
- Specific applications include:
  - Nano-fertilizers, Nano-pesticides
  - Nano-based treatment of agricultural waste
  - Nanosensors
Nanomaterials and Agriculture

- Nano-fertilizers often contain nutrients/growth promoters encapsulated in nanoscale polymers, chelates, or emulsions.
  - Slow, targeted, efficient release becomes possible.
  - In some cases, the nanoparticle itself can stimulate growth.
- Nanosensors can be used to detect pathogens, as well as monitor local, micro, and nano-conditions in the field (temperature, water availability, humidity, nutrient status, pesticide levels...)

**The Improvement of Spinach Growth by Nano-anatase TiO₂ Treatment Is Related to Nitrogen Photoreduction**

Fan Yang · Chao Liu · Fengqing Gao · Mingyu Su · Xiao Wu · Lei Zheng · Fashui Hong · Ping Yang

**Beneficial role of carbon nanotubes on mustard plant growth: an agricultural prospect**

Anindita Mondal · Rum Basu · Sukhen Das · Papiya Nandy

**Development of Zinc Nanofertilizer to Enhance Crop Production in Pearl Millet (Pennisetum americanum)**

J. C. Tarafdar · Ramesh Raliya · Himanshu Mahawar · Indira Rathore
Nanomaterials and Agriculture

- Nano-pesticides often follow a similar model to nano-fertilizers; active pesticidal (insecticide, fungicide,...) ingredient associated with or within a nanoscale product or carrier
- Increased stability/solubility, slow release, increased uptake/translocation, and in some cases, targeted delivery (analogous to nano-based delivery in human disease research)
- Can result in lower required amounts of active ingredients

Inhibition Effects of Silver Nanoparticles against Powdery Mildews on Cucumber and Pumpkin

Kabir Lamsal¹, Sang-Woo Kim¹, Jin Hee Jung¹, Yun Seok Kim¹, Kyoung Su Kim¹ and Youn Su Lee¹

¹Division of Bio-Resources Technology, Kangwon National University, Chuncheon 200-701, Korea
Department of Agricultural Biotechnology, Center for Fungal Genetic Resources and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-724, Korea

Role of nanotechnology in agriculture with special reference to management of insect pests

Malendra Rai • Avinash Ingle

Antifungal activity of zinc oxide nanoparticles against Botrytis cinerea and Penicillium expansum

Lili He¹, Yang Liu¹, Azlin Mustapha, Mengshi Lin⁵
Nanomaterials and Agriculture

- Nanoscale based micronutrients for disease suppression (particularly root disease)
- A new research initiative at CAES
- Many micronutrients (Cu, Mn, Zn, Mg) stimulate or are part of plant defense systems.
- However, these nutrients have low availability in soil and are not readily transferred from shoot to root. What about nano versions of these nutrients?
- New USDA Grant- $480,000; March 2016-Feb. 2019
Nanoscale micronutrients for disease suppression

- Greenhouse and field trials with eggplant and tomato
- Single foliar application of NP (bulk, salt) CuO, MnO, or ZnO (100 mg/L) during seedling stage. Transplant to infested soil.
- NP CuO had greater disease suppression, higher Cu root content, and increased yield. NP CuO had no direct affect on the pathogen.
- $44 per acre for NP CuO suppressed a root pathogen of eggplant, increasing yield from $17,500/acre to $27,650/acre.

Nanoscale based micronutrients for disease suppression

- Current field trials in CT involve eggplant, watermelon and asparagus
- Single foliar applications of NP CuO, ZnO, MnO alone or in combination.
- Two separate experimental farms (soil types) being used. A range of concentrations used; salt only controls.
- Also, collaborative work in FL where field trials involve tomato growth with multiple applications during the growing season (Kocide, CuO and MgO NPs)
Implications: Nanotoxicology at CAES

- Two “simple” questions - Do NM behave differently and if so, is that difference of concern with regard to exposure and risk?

- USDA NIFA Grant 1 - Addressing Critical and Emerging Food Safety Issues - “Nanomaterial contamination of agricultural crops.”
  - Obj. 1: Determine the uptake, translocation, and toxicity of NM to crops.
  - Obj. 2: Determine the impact of environmental conditions on NM uptake, translocation, and toxicity to crops.
  - Obj. 3: Determine the potential trophic transfer of NMs.
  - Obj. 4: Quantify the facilitated uptake of pesticides through NM-chemical interactions.

- USDA NIFA Grant 2 - Nanotechnology for Ag. and Food Systems - “Nanoscale interactions between engineered nanomaterials and biochar”
Determine the trophic transfer potential of NMs

- **Experiment 1** - NP/bulk CeO$_2$ (0 or 1000 mg/Kg) added to an agricultural loam.
- Zucchini grown for 28d from seedling.
- Roots, stems, leaves, and flowers analyzed by ICP-MS.
- Leaves used to feed crickets for 14d.
- Crickets used to feed wolf spiders for 7d.
- Insect tissues/feces by ICP-MS.

Determine the trophic transfer potential of NMs: Exp. 1

- Particle size-dependent transfer from soil → plant → herbivore → carnivore observed
- NP CeO$_2$ reduced biomass of reproductive tissues by 50%
- No biomagnification; 10-100 fold decreases at each level
- Insect feces contained 10x more Ce than insect tissues

Determine the trophic transfer potential of NMs: Exp. 2

- NP/bulk La$_2$O$_3$ (0 or 500 mg/Kg) in soil; lettuce grown for 50d from seedling.
- Leaves used to feed crickets and darkling beetles for 15 d.
- Crickets used to feed mantids for 7 days

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Determine the trophic transfer potential of NMs: Exp. 2

- NP/bulk La were phytotoxic (25-30% biomass reduction)
- La accumulation and transfer were unaffected by particle size
- No biomagnification; 10-100 fold decreases at each level
- Insect feces contained 10x more La than insect tissues
Determine the trophic transfer potential of NMs: Exp. 3

- NP/bulk CeO$_2$ (1000 mg/kg) added to a TX soil; kidney bean grown for 35 d
- Leaves fed to bean beetle (larvae, pupae, adult);
- Beetles fed to spined soldier bugs
- Ce root\shoot content was unaffected by particle size
- Time-dependent Ce *increase* in the beetle; *biomagnification* in the adult.
- Time dependent *decrease* in fecal Ce content.
Determine the trophic transfer potential of NMs: Exp. 4

- Trophic transfer of NP and bulk CuO

- 500 mg/kg in soil for 0 or 60 days, lettuce, cricket, Anolis lizards.

- Soil was contaminated with weathered chlordane (3 mg/kg) and DDX (0.2 mg/kg)

- Tracked Cu, chlordane and DDX content and form (ICP-MS, μXRF, XANES, biomass, and gene expression in the plant (transcriptomics)
Determine the trophic transfer potential of NMs: Exp. 4

- Leaf Cu content unaffected by particle type or weathering
- Root Cu content affected by particle size and weathering
- Cricket and fecal Cu content largely unaffected by particle type, weathering or even Cu amendment
- Lizard Cu content (head, intestine, body, feces) unaffected by Cu amendment, type or weathering

Servin et al. In preparation

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Determine the trophic transfer potential of NMs: Exp. 4

- In NP exposed roots, Cu distribution and speciation varied with weathering status (ESRF, Grenoble France)
- Unweathered treatment had Cu hot spots in the roots; the weathered treatment had homogeneous Cu
- Cu in the weathered roots was more reduced/transformed to Cu$_2$O and Cu$_2$S forms

<table>
<thead>
<tr>
<th>Spot</th>
<th>CuO</th>
<th>Cu$_2$O</th>
<th>Cu$_2$S</th>
<th>R-factor</th>
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<tbody>
<tr>
<td>Unweathered</td>
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<tr>
<td>SR(175)</td>
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<tr>
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<tr>
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<td>AMR(263)</td>
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<td>EMR(250-)</td>
<td>0.314</td>
<td>0.238</td>
<td>0.447</td>
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</table>

<table>
<thead>
<tr>
<th>Spot</th>
<th>CuO</th>
<th>Cu$_2$O</th>
<th>Cu$_2$S</th>
<th>R-factor</th>
</tr>
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<tbody>
<tr>
<td>Weathered</td>
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<tr>
<td>A</td>
<td>0.0000</td>
<td>0.9425</td>
<td>0.0575</td>
<td>0.0009</td>
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<tr>
<td>E</td>
<td>0.0000</td>
<td>0.4599</td>
<td>0.4354</td>
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<tr>
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<tr>
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<td>0.0000</td>
<td>0.0877</td>
<td>0.8511</td>
<td>0.0019</td>
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<tr>
<td>C</td>
<td>0.0000</td>
<td>0.4647</td>
<td>0.4835</td>
<td>0.0029</td>
</tr>
</tbody>
</table>

A: aggregate sec root, E: Epidermis, SR: secondary root, MR: Main root, C: Cortex
Determine the trophic transfer potential of NMs: Exp. 4

- Soil contained weathered chlordane (3 mg/kg) and DDx (0.2 mg/kg).
- Pesticide content in roots and shoots determined by GC-MS; below is whole plant content (ng)
- Unweathered Cu type/size seems to differentially impact pesticide uptake
- Upon weathering, these differences based on type disappear
- Interesting changes between unweathered and weathered pesticide for bulk and NP Cu
NM Trophic Transfer?

- Element transfer from metal oxides in soil to biota does occur and it can be particle-size dependent
- Nanoparticles can be found in biota
- Biomagnification appears possible but perhaps atypical
- Insects often (but not always) excrete much of what they ingest
- Species and soil type seem to have significant impacts
- NP transformation processes in the soil and biota are important to fate (and effects?)
- Overall risk? Seems low?
Nanomaterial interactions with co-existing contaminants

- NMs are entering agricultural systems directly (pesticide/fertilizers) or indirectly (biosolids)
- Agricultural soils contain a number of other organic chemicals
- Interactions between NM and these co-existing contaminants may be important
  - Could bioavailability of legacy pesticides be affected? A food safety issue?
  - Could efficacy of intentional agrichemicals be affected? An economic issue?
- Multiple publications since 2012; three more underway
Nanomaterial interactions with co-existing contaminants

- Impact of C_60_ or Ag on DDE accumulation by crops in vermiculite (De La Torre Roche et al. 2012. *Environ. Sci. Technol.*; De La Torre Roche et al. 2013a. *Environ. Sci. Technol.*).

- Impact of C_60_ on weathered DDE accumulation from soil by crop and worm species (Kelsey and White, 2013. *Environ. Toxicol. Chem.*).

- Impact of C_60_ on weathered chlordane and DDE accumulation by 4 crops in soil (De La Torre Roche et al. 2013b. *Environ. Sci. Technol.*).

- Impact of functionalized/non-functionalized MWCNT on chlordane and DDE uptake by lettuce in vermiculite (Hamdi et al. 2015 *Nanotox.*).

- Impact of NP TiO_2_ on Pb accumulation by hydroponic rice (Cai et al., in review).

- Impact of functionalized/non-functionalized MWCNT on carbamazepine accumulation by collard greens (Deng et al. in prep.).

- Impact of coated and uncoated NP Ag on chlordane and DDx accumulation by earthworms in soil (Mukherjee et al. in prep.).
Nanomaterial interactions with co-existing contaminants

- Completing an experiment where zucchini was grown for 28-d in soil that contained Ag or CeO$_2$ NPs (or bulk) and imidicloprid
- Roots, shoots, flowers and pollen were analyzed for metals by ICP-MS and imidicloprid + metabolites by LC-MS/MS
- NP were accumulated at greater levels than bulk forms
- NP Ag increased pollen imidicloprid content; bulk Ce increased root and flower imidicloprid content;
Nanomaterial interactions with co-existing contaminants?

- In both model media and soil, exposure to NM/NP can influence the bioavailability of co-existing organic contaminants.
- Evident in abiotic and biotic (plants, worms) exposure assays.
- Whether availability is increased or decreased depends on NP/NM type, morphology (tubes vs fullerenes), and concentration; as well as species.
- Decreased co-contaminant availability upon NP exposure seems more common.

Impact of Ag types on chlordane accumulation by earthworms 
(Mukherjee et al.; in prep)

Impact of different TiO2-types on Pb accumulation by rice 
NP/NM Detection in Complex Matrices

- Current NP detection techniques (ICP-MS, sp-ICP-MS, FFF-ICP-MS, S/TEM-EDS, μXRF/XANES) all have significant shortcomings.
- Concerns over human exposure to and risk from NPs have increased interest in novel detection strategies.
- A group at UMass Amherst is investigating the feasibility of Surface-enhanced Raman Spectroscopy (SERS) as a method for NPs detection and quantification in complex matrices.
- An initial study published last year used ferbam (a fungicide) as an indicator molecule that binds strongly onto AgNPs.
- Detection and quantitation based on the signature SERS response of AgNPs-ferbam complex.
- A novel approach: NPs have been used to detect pesticides; we are using a pesticide to detect NPs.

SERS and NP Ag detection

- SERS is able to specifically detect and discriminate NP with ferbam (10 mg/L) as an indicator.
- The largest peak was located at 1379 cm\(^{-1}\), which can be attributed to deformation of ferbam upon Ag binding.
SERS and NP Ag quantitation

- The concentration-dependent SERS spectra of AgNPs (citrate, 60 nm) with ferbam as an indicator.
- The linear relationship between Raman intensity and AgNPs concentration.
SERS for Ag NP quantitation in commercial products

- SERS spectra for 4 Ag NPs-containing antimicrobial products confirm the effectiveness of ferbam for Ag NP binding and NP detection.

- TEM images of the four commercial products confirmed the presence of significant quantities of Ag NPs.

- Ag concentration as determined by SERS and by ICP-MS were similar for 3 of the 4 products.

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<table>
<thead>
<tr>
<th>Product</th>
<th>Average Size (nm, TEM)</th>
<th>Advertised concentration of AgNPs (mg/L)</th>
<th>Total silver concentration (mg/L)</th>
<th>Concentration of Ag by ICP-MS (mg/L)</th>
<th>SERS intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat spray</td>
<td>28.9 ± 0.8</td>
<td>30</td>
<td>29.9 ± 0.2</td>
<td>21.5 ± 0.6</td>
<td>6335</td>
</tr>
<tr>
<td>Nasal spray</td>
<td>33.2 ± 1.0</td>
<td>10</td>
<td>10.2 ± 0.1</td>
<td>6.5 ± 0.1</td>
<td>1253</td>
</tr>
<tr>
<td>Antifungal</td>
<td>15.4 ± 0.5</td>
<td>30</td>
<td>30.2 ± 0.2</td>
<td>10.0 ± 1.2</td>
<td>78</td>
</tr>
<tr>
<td>spray</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEJ</td>
<td>14.0 ± 0.4</td>
<td></td>
<td>340.7 ± 3.1</td>
<td>75.3 ± 2.1</td>
<td>141</td>
</tr>
</tbody>
</table>
Optimizing SERS

- The ferbam-based approach was a first step but sensitivity (>0.1 mg/L) is insufficient for many matrices.
- Surface modification and microextraction approach used to separate AgNPs in the matrix
  - Use a surfactant ligand to bind to the AgNPs
  - Modify the surface hydrophobicity so that the bound AgNPs can be extracted by an organic solvent
  - Produce a strong and distinct SERS signal for detection and quantification of the extracted/concentrated AgNPs

Optimizing SERS

- 4-mercaptobenzoic acid (4-MBA) modifies the surface of the coated AgNPs by displacing citrate with a thiol group.
- The 4-MBA forms acid-base pairs with tetraoctylammoniumbromide (TOAB), which significantly increases AgNPs hydrophobicity.
- 4-MBA has distinct SERS peaks at 1080 and 1590 cm\(^{-1}\), effectively serving as an AgNP probe.
- The data to the right shows that nearly all of the AgNPs are extracted from the water.

Optimizing SERS

- Detection down to 100 ng/L is possible (below, left).
- The response is linear with AgNPs concentration (below, center).
- The technique was used to detect 20 and 2 µg/g AgNPs in wheat leaves (below, right).
- Current work looking at method efficacy with different size Ag NPs and with different coatings.

Optimizing SERS

- SERS approach for Titanium dioxide (TiO\textsubscript{2}) detection
- Developing a method using flavonoid-assisted microextraction and SERS for TiO\textsubscript{2} NPs (anatase, 21 nm) detection in complex liquid matrices.
- Flavonoids bind TiO\textsubscript{2} NPs, enabling the extraction of the particles by ethyl acetate and sodium chloride.
- Using the flavonoid, myricetin (MYC), we were able to achieve detection at 0.2 mg/L TiO\textsubscript{2} NPs in water.


(A) Schematic illustration of flavonoid-assisted extraction method for TiO\textsubscript{2} NPs from water. (B) Photographs of flavonoid-based phase separation. (C) SERS spectra of MYC-adsorbed TiO\textsubscript{2} NPs from the interlayer.
Optimizing SERS

- Three flavonoids: Myricetin (MYC), Quercetin (QUC) and Luteolin (LUT).
- Raman spectra (red) of three flavonoids, and SERS spectra (blue) of flavonoid adsorbed TiO$_2$ NPs. The Raman signature of sorbed TiO$_2$ NPs at 144 cm$^{-1}$ could be clearly observed with flavonoid-binding.

- Raman characteristic peaks of TiO$_2$ NPs in the range from 100 cm$^{-1}$ -800 cm$^{-1}$. Inset: Linear fitting curve for quantification of TiO$_2$ NPs based on 144 cm$^{-1}$ Raman peak.

Conclusions

- Are NM significant emerging class of contaminants in agricultural/food systems? This is the key Pro vs Con question.
- Exposure may occur through NM-containing pesticide/fertilizers, biosolids, food packaging/processing, and as flavor/quality amendments.
- Trophic transfer/food chain contamination can occur but biomagnification seems uncommon and species-, soil-, and particle-variability seems high.
- Some NMs may significantly alter the fate and effect of co-contaminants by some biota in soil and non-soil systems.
- Again, these interactions seem to differ with species, particle size/characteristics (coating, functionalization) and exposure conditions.
- Robust and accurate NPs detection platforms are needed.
- Although the benefits of nanotechnology to food production are huge, there are some EHS warning signs.
Acknowledgements

- B. Xing, L. He, O. Parkash, UMass
- X. Ma– Texas A & M
- J. Bennett, S. Isch- CT DPH
- J. Gardea-Torresdey et al.- UTEP
- Wang et al- Ocean Univ. of China
- Marmiroli et al.- Univ. of Parma, Italy
- T. Vanek- Czech Republic
- J. Vangronsveld et al.- Hasselt Univ., Belgium
- L. Newman- SUNY ESF
- At CAES- R. De la Torre-Roche, A. Servin, A. Mukherjee, H. Hamdi (Univ. of Carthage), S. Majumdar, L. Pagano (Univ. of Parma), W. Elmer, J. Hawthorne, C. Musante
- Funding- USDA AFRI and Hatch, FDA FERN