Nanomaterials and Food/Agriculture: Assessing the Balance Between Implications and Applications



1





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Nanomaterials and Food Protection



Food Safety- microbes and chemicals/elements

- Antimicrobials in food packaging
- Nano-enabled coatings for food/equipment; EWNS & HSPH
- Nanosensors for pathogen detection







Available online at www.sciencedirect.com



Nanotechnology to the rescue: using nano-enabled approaches in microbiological food safety and quality Mary Eleftheriadou^{1,3}, Georgios Pyrgiotakis^{2,3} and Philip Demokritou²



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

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Nanomaterials and Agriculture



The goals fall into several categories; efficiency is the driver (precision ag.)

- 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



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Interaction of Nanomaterials with Plants: What Do We Need for Real Applications in Agriculture?

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Nanomaterials and Agriculture



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0

FIRST REACTIONS

- Nano-fertilizers often contain nutrients/growth promoters encapsulated in nanoscale polymers, chelates, or emulsions
 - \succ Slow, targeted, efficient release becomes possible
 - \succ In some cases, the nanoparticle itself can stimulate growth
- Nano-sensors 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...)



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IOP Publishing Vernam Academy of Science and Technology Advances in Natural Sciences. Nanoscience and Nanotechnology Adv. Nat. Sci.: Nanosci. Nanotechnol. 7 (2016) 045016 (11pp) doi:10.1088/2043-8282/7/4/045018		2017	This is an open access article published under an ACS Authorchoice <u>license</u> , which permits copying and redistribution of the article or any adaptations for non-commercial purposes.
Biofabricated zinc oxide nanoparticles coated with phycomolecules as novel micronutrient catalysts for stimulating plant		ACS central science	FIRST REACT
growth of cotton		Slow Release Nanofer	tilizers for Bumper Crops
N Priyanka ¹ and P Venkatachalam ^{1,2} 2016		Manish Chhowalla	
Agric Res 20 DOI 10.1007/s40003-014-0113-y	14	JOURNAL OF	2012
FULL-LENGTH RESEARCH ARTICLE		AGRICULTURAL AND FOOD CHEMISTRY	Article pubs.acs.org/JAFC
Development of Zinc Nanofertilizer to Enhance Crop Product in Pearl Millet (<i>Pennisetum americanum</i>)	ion	Dissolution Kinetics of Manufactured Zinc Oxi	Macronutrient Fertilizers Coated with de Nanoparticles
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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

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"Nano" Research at the CAES



Applications: Nutrition and Crop Disease Suppression

- Evaluating the use of nanoscale micronutrients to promote crop health and suppress fungal and other plant pathogens.
- Evaluating nanoscale Ag and ZnO directly on fungal pathogens.

Implications: Nanotoxicology

- Studying the fate and effects of engineered nanomaterials (NM) on plants and related biota. NM effects are often unique.
- Investigating the molecular basis of plant response; this level of understanding will be needed to ensure accurate risk assessment and safe use of nanomaterials.
- \succ Investigating trophic transfer in the food chain.
- Investigating co-contaminant interactions (NM co-exposure on the fate of pesticides, pharmaceuticals, heavy metals).







Nanoscale Nutrients and Disease

- Nanoscale based micronutrients for disease suppression (particularly root disease)
- 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?
- USDA NIFA Grant- \$480,000; 3/16-2/19 (UTEP, IFDC) IFDC)
- USDA SCBG- \$60,000; 2/17-1/19
- Center for Sustainable Nanotechnology New seed grant





I Nanopart Res. (2015) 17-92 DOT 10 10076 11051-015-2907-2 PEVIE

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A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield

Alia Servin · Wade Elmer · Arnab Mukherjee Roberto De la Torre-Roche · Helmi Hamdi · Jason C. White · Prem Bindrahan · Christian Dimkn



Nanoscale Micronutrients Suppress Disease





<u>Nutrition</u> is the first line of defense against disease. Micronutrients protect <u>roots</u> against soilborne diseases by activating enzymes to create defense products.

<u>Cu</u>: activates polyphenoloxidases



<u>Mn</u>: activates enzymes in the Shikimic acid and Phenylpropanoid pathways
Zn: activates superoxide dismutases



Micronutrient Availability?



- Increasing micronutrient levels in roots is problematic in neutral soils
- Micronutrients are not basipetally (shoot to root) translocated
- When applied to soil they frequently precipitate and become unavailable to the plant
- Limited options for preventing and treating root disease (host resistance, fumigation)





So, a chemist and a plant pathologist walk into a bar...



NP CuO (and other metal NPs?) can move basipetally whereas bulk equivalents do not.





Wang, White et al. 2012. Xylem- and phloem-based transport of CuO nanoparticles in Maize (*Zea mays* L.) *Environ. Sci. Tech.* 46:4434-4441.







- Would applying <u>nanoscale</u> micronutrients to leaves affect growth?
- Would these metals be translocated to roots?
- Could these translocated nutrients stimulate plant defense and suppress root disease (mostly fungi, nematodes)?

Nanoscale micronutrients (Cu, Zn, B, Si...)





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



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and White, 2016, Environ, Sci.: Nano. 3:1072-1079



Nanoscale based micronutrients for disease suppression



- > 2016/2017 field trials in CT involved eggplant, watermelon, asparagus.
- > Single foliar applications of NP CuO, ZnO, MnO alone or in combination.
- > Two farms/soil types used; a range of concentrations, salt only controls.
- Also, collaborative work in FL where field trials involve tomato growth with multiple applications <u>during</u> the growing season (Kocide, CuO and MgO NPs). New project with the Center for Sustainable Nanotechnology focused on better designed materials.

> A USDA SCBG- strawberries and nematodes (2017-2019).

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Foliar Application of Nanoscale Micronutrients to Watermelon



- NP CuO foliar application on watermelon seedlings suppressed *Fusarium* infection and increased plant biomass/ yield.
- Transcriptomics confirmed the upregulation of polyphenol oxidase (a Cu-activated enzyme for host defense) and Plant Resistance 1 Protein (associated with resistance) with CuO NP/infection.



This data suggests that NP CuO may activate defense

mechanisms in plants, likely via basipetal translocation of the nanoscale nutrient.

14



Nitrogen accumulation by Sorghum is Enhanced by Zn NP and Salts



Accumulation = uptake + translocation

- Zn fertilization improved overall N accumulation between 4% and 38%, dependent on NPK regime Zn application route.
- Packaging Zn as NP (slightly) mitigated inhibition of N uptake by Zn at high NPK.
- Grain translocation of N (P,K as well) at high NPK more efficient with Zn salt than with NP.

15



















Implications: Nanotoxicology at CAES



- > NM interact uniquely with crops. One "simple" question- **Does this matter**? Is this difference in behavior of concern with regard to exposure and risk? A necessary component of sustainable applications work.
- USDA NIFA Addressing Critical and Emerging Food Safety Issues-"Nanomaterial contamination of agricultural crops."
- <u>USDA NIFA</u>- Nanotechnology for Ag. and Food Systems- "Nanoscale" interactions between engineered nanomaterials and biochar."
- USDA Hatch- "Impact of particle coating and weathering on nanomaterial fate and effects on crops."
- Three main lines of inquiry
 - Mechanisms of plant response.
 - **Trophic transfer.**

17

Co-contaminant interactions.





Institute of

Experimental















1. Toxicity, Mechanisms, and Biomarkers





About 70 candidate/target genes identified in *A. thaliana* were located and validated through transcriptomic analyses in zucchini (*C. pepo*) and tomato (*S. lycopersicum*).



<u>Response: Zucchini vs Tomato</u>





<u>Comparison between the</u> tomato and zucchini:

- <u>005u</u> (heat shock protein) up regulated in all the treatments of zucchini, down regulated in all the treatments of tomato
- 152u (chloroplast electron carrier) up regulated in all the treatments of tomato, down regulated in all the treatments of zucchini







Pagano et al. 2017



- Exposure of zucchini to NP CeO₂, La₂O₃, CuO, ZnO and CdS Quantum Dots. Not only single analyte exposure but also all possible binary combinations (11 treatments).
- Physiological (mass, water content, length, pigments, cell viability) and molecular endpoints (37 genes) monitored.
- Just published in ES: Nano. Co-contaminant effects were consistently observed, at both the physiological and molecular level. Examples of additive and antagonistic effects noted, as well as potential synergism.





Mechanisms of CuO NP toxicity and transgenerational effects

- A. thaliana seeds (3 ecotypes) were soaked in CuO NPs (0, 20,50 mg/L) or BPs (50 mg/L) suspensions or in Cu²⁺ ion solution (0.15 mg/L) for 48 h.
- Ion levels determined based on measured dissolution.
- All seeds were placed in the MS-agar for a germination or aqueous solution for growth.
- Root morphology evaluated by SEM and WinRHIZO Pro 2005b.
- \succ Harvested pollen and seed viability was determined.
- Cu content determined by ICP-MS; Cu speciation (seeds) determined by X-ray absorption near-edge spectroscopy (XANES).
- Differential Display Reverse Transcription Polymerase Chain Reaction (DDRT-PCR) used to measured gene expression.



50 mg/L CuO NPs and BPs.







Transgenerational effects of CuO NP exposure

- ➤The germination of pollen (A) and seeds (B) collected from NP-exposed plants was reduced.
- The root length of seeds (C) obtained from CuO NPs-treated plants and overall seedling biomass (D) was also reduced.
- Pollen (Col-0) was grown in distilled water, 50 mg/L CuO NPs (center), and 0.15 mg/L Cu²⁺ ions. Damage to the plasma membrane is evident in the NP exposure (E).





Bav-0

Control 0.15 Cu²⁺ 20 NPs 50 NPs 50 BPs

Treatments

Ws-2

Col-0



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Wang et al. 2016 *Environ. Sci. Technol.* 50:6008-6016.



Yue et al. 2017: NP La₂O₃ and maize aquaporin gene expression

- Conducted with collaborators at Nanjing Agricultural University and the University of Massachusetts Amherst.
- Due to La₂O₃ nanoparticle (NPs) use in medical, industrial, and agricultural products, concerns over the risks of exposure have increased.
- Plants are obviously receptor of concern, but the mechanisms of La₂O₃ NPs phytotoxicity are unknown.
- The potential for growth inhibition and reduced water uptake in corn upon exposure to La₂O₃ NPs (50-500mg L⁻¹) were investigated.





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Root Damage and Water Uptake



- SEM images of morphological changes (depressions) and physical damage (cracks, shrinkage, root cap loss) in plant root tips upon NP exposure.
- Given the damage to the root system, the results suggest that La₂O₃ NPs may impact the water status of maize seedling.
- Across nearly all La₂O₃ NPs treatments, the water uptake rate was significantly reduced relative to the unexposed, bulk, and ion controls







Water Transport and AQP Expression



- > Water transport in leaf vasculature was investigated with safranin.
- NPs disrupted the water transport in plants. Upon treatment with 50-250 mg/L NPs, most leaf minor veins exhibited incomplete staining (55-76% reductions), suggesting reduced functionality.
- In roots and shoots (to a lesser extent), most AQPs genes in NPs-exposed were down regulated.
- Abscisic acid (stress-induced phytohormone) may act as a signaling molecule in response to NPs exposure, adjusting water uptake by regulating AQPs gene expression.







Metal oxide NPs reduce peanut (Arachis hypogaea L.) nutritional quality

Conducted with collaborators at China Agricultural University, Guangxi University, the Chinese Academy of Agricultural Sciences, and UMass



- Biomass, shoot height, per plant yield, and element content were determined.
- Amino acid content, fatty acid profile, and resveratrol in the peanut grain were measured.





Metal oxide NPs reduce peanut (Arachis hypogaea L.) nutritional quality



- > Exposure had no impact on plant biomass.
- NPs decreased the grain weight by 10-31% (greatest at 500 mg/kg CuO NP).
- The Cu grain content increased in a dosedependent manner; Fe₂O₃ and TiO₂ NPs did not increase the Fe or Ti content.
- TEM-EDX showed NPs of all 3 elements in the grains.
- NPs CuO altered the amino acid content as related to glycolysis, the citric acid cycle, and defense pathways.
- Elevated resveratrol content in CuO and TiO₂ NP treated grains were indicative of plant stress response





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Hawthorne et al. 2014. Environ

Experiment 1 - NP/bulk CeO₂ (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 CeO₂ reduced biomass of reproductive tissues by 50%
- > No biomagnification; 10-100 fold <u>decreases</u> at each level
- Insect feces contained 10x more Ce than insect tissues





Determine the trophic transfer potential of NMs: Exp. 2



- Trophic transfer of NP and bulk CuO
 - 500 mg/kg in soil for <u>0 or 70 days</u>, 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. 2

- Leaf Cu content unaffected by particle type or weathering
- Root Cu content affected by particle size upon 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

31

weathering

www.ct.gov/caes Servin et al. 2017. *Nanotox*. 11:98-111.









Determine the trophic transfer potential of NMs: Exp. 2



- 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

32

Cu in the weathered roots was more reduced/transformed to Cu₂O and Cu₂S forms





Components				_
Spot	CuO	Cu2O	Cu2S	R-factor
SR(175)	0.6580	0.342	0.0000	0.004
SR(178-)	0.0000	0.554	0.446	0.002
SR(192-)	0.458	0.355	0.187	0.001
ASR (208)	1	0.0000	0.0000	0.000
SR(231-)	0.635	0.430	0.0000	0.006
SR(242-)	0.229	0.353	0.417	0.004
AMR(263)	1	0.0000	0.0000	0.000
EMR(250-)	0.314	0.238	0.447	0.009

Weathered

		Components				
	Spot	CuO	Cu2O	Cu2S	R-factor	
	А	0.0000	0.9425	0.0575	0.0009	
	Е	0.0000	0.4599	0.4354	0.0009	
	SR	0.0000	0.3402	0.6239	0.0029	
	MR	0.0000	0.0877	0.8511	0.0019	
	С	0.0000	0.4647	0.4835	0.0029	
A; aggregate sec root, E; Epidermis, SR; secondary root, MR; Main root, C; Cortex						



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- 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?
- Nine publications since 2012; two more underway and one review article published.













Conclusions



- Nanotechnology has the potential to dramatically improve agriculture; to literally help feed the world.
- Because of this and because of widespread use of nanomaterials in other sectors, exposure in the food supply will be significant.
- As such, a thorough and comprehensive understanding of mechanisms of action/interaction is needed to enable accurate assessment of risk and the sustainable application of nanotechnology.
- Species- and soil-type differences, trophic transfer, co-contaminant interactions, biomagnification, rhizosphere and endophyte effects, and robust detection platforms for presence/effects are all part of the sustainable



solution.

34



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