Phytotoxicity and Ecological Safety of Engineered Nanomaterials

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Abstract

With the dramatically increasing use of nanotechnology-enabled consumer products, engineered nanomaterials (ENMs). ENMs are inevitably discharged into environment, posing unknown risks to ecosystem structure and function. Plants play important roles in soil and water resources in the environment. Although these ENMs have been widely used in medicine, agriculture, and consumer products for great economic benefit, their unique properties, may cause adverse effects on plants. Thus, toxicity and disposition of ENMs in terrestrial plants, especially agricultural crops, should be fully characterized. This mini-review summarizes the current literature on: (1) the distribution and biotransformation of ENMs; (2) ENMs induced phytotoxicity at physiological and molecular levels; (3) nutrient displacement or enhancement in ENMs treated plants; (4) trophic transfer of ENMs in terrestrial food chains.

Key words: Engineered nanomaterials (ENMs), Biotransformation, Phytotoxicity, Nanotoxicity, Nutrient displacement.

1. Introduction

Engineered nanomaterials (ENMs) have many advantages and bring a huge benefit to the fields of biomedical, agriculture and renewable energy (Nel *et al*., 2006; Mauter and Elimelech, 2008; Barreto *et al*., 2011). However, due to unique properties at the nanoscale (high surface area and greater reactivity) (Somasundaran *et al*., 2010), engineered nanomaterials (ENMs), including both carbon-based and metal-based nanomaterials, can interact uniquely with biota (microorganism, plants, and animals) in the environment (Ma *et al*., 2013; Srivastava *et al*., 2015; Van Aken, 2015). An increasing number of studies have demonstrated that ENMs can cause oxidative stress in exposed organisms by inducing excessive amounts of reactive oxygen species (ROS), production (Panda *et al*., 2011; Dimkpa *et al*., 2012a; Zhao *et al*. 2012a). A thorough characterization of ENMs distribution and biotransformation in plants may reveal important information from the aspect of nanotoxicity (Zhang *et al*., 2011a; Servin *et al*., 2012). Plants are a critical component in ecosystem structure and function, and form the basis of terrestrial food chains. Thus, evaluating the trophic transfer of ENMs can help further understand and evaluate the overall risk posed by these unique materials in the environment. This review seeks to address the impacts of ENMs on plants with regard to: (1) uptake and biotransformation of ENMs *in planta*; (2) phytotoxicity to plants; (3) nutrient displacement or enhancement; and (4) trophic transfer of ENMs in terrestrial food chains.

2. Impacts of ENMs on Plants

In plant-ENM interaction studies, root and foliar pathways are the two modes of ENMs exposure to plants. Since ENMs are used as additives in agrichemical formulations, both approaches are appropriate and simulate realistic conditions by ENMs enter the environment (Lee *et al*., 2012; Hong *et al*., 2014; Deepa *et al*., 2015). The literature shows that ENMs exposure to plants can cause both positive and negatives impacts; as such, we will review ENMs interactions in three sections: (1) ENMs uptake and biotransformation; (2) phytotoxicity of ENMs to plants; (3) nutrient displacement or enhancement in ENMs treated plants.

2.1. ENMs uptake and biotransformation

Many environmental factors will influence the aggregation, ion-release, and surface properties of ENMs, including pH, ionic strength, dissolved organic matter (DOM) presence and type, and biotic interactions (Chang *et al*., 2012). Although it can be important to characterize ENMs prior to exposure experiments, it is important to recognize that system complexities may dramatically and dynamically alter the materials from their “native” state. Post-exposure, there are multiple approaches used to assess ENM fate. The most commonly used methods to measure metal content in plant tissues are inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectroscopy (ICP-OES) (Stampoulis *et al*., 2009; De La Torre-Roche *et al*., 2012; Ma *et al*., 2013; Majumdar *et al*., 2014). Notably, neither of these methods will provide information on particle size; the techniques simply provide total metal contents. However, some ICP-MS systems can be run in single particle mode and hyphenated approaches such as Field Flow Fractionation (FFF) can be used to generate particle size specific data.

In reviewing the literature, it immediately becomes apparent that comparing metal accumulation across studies is confounded by differences in experimental conditions, exposure pathways and periods, lack of detail and artifacts of design (Petersen *et al*., 2014). Some interesting trends in ENMs uptake by plants are evident when were using bioaccumulation factor (BAF) to normalize element content data in shoot and root tissues separately (Ma *et al*., 2015b).

Substrates used for plant growth are a primary factor that determines ENMs uptake. As expected, significantly high levels of Ag nanoparticles (NPs) were noted in mung bean and sorghum grown in liquid medium are evident as compared to both plants grown in soil (Lee *et al*., 2012). Further, soil type can also determine NPs uptake in plants. For example, translocation factors (TFs) in CeO_2 NPs treated corn grown on organic matter enriched soil was found to be significantly lower than in unenriched soil (Zhao *et al*., 2012b). Additionally, NPs size is another factor that can dramatically influence NPs accumulation in terrestrial plants (Yin *et al*., 2011; Zhang *et al*., 2011b). NPs can penetrate the cell wall, of which pore size is usually in a range of 5-10 nm, although a large portion of NPs might aggregate due to pH, ionic strength, and dissolved organic matter in environment as mentioned above. Other than determination of total metal uptake using ICP-MS, synchrotron-based micro X-ray fluorescence (μ-SXRF) microscopy can be applied to investigate the oxidation of metal-based NPs in plant tissues (Servin *et al*., 2012; Zhang *et al*., 2012; Hernandez-Viezcas *et al*., 2013), which can help us further understand the role of biotransformation in the toxicity and biological fate of these materials in *planta*. Fig. 1 shows a schematic diagram of how ENMs biotransformation happens in plants. A robust literature has begun to develop on the uptake and transformation of rare earth element (REEs) oxide NPs in plants. For example, CeO_2 NPs in exposed cucumber were present as partially transformed to phosphate precipitates (Zhang *et al*., 2012). Biotransformation of other metal-based NPs such as ZnO, CuO and Ag were also reported in soybean, *Elsholtzia splendens*, and lettuce, respectively (López-Moreno *et al*., 2010; Larue *et al*., 2014; Shi *et al*., 2014). Both CuO and Ag NPs were partially complexed by binding with –SH containing compounds, including key plant defense molecules such as cysteine and glutathione (GSH) (Larue *et al*., 2014; Shi *et al*., 2014).

2.2. Phytotoxicity of ENMs to terrestrial plants

Numerous of studies have demonstrated that both carbon- and metal-based ENMs can cause oxidative stresses by inducing excessive amounts of ROS production, which subsequently results in growth inhibition, as well as damage to cell membrane integrity, chlorophyll function and to DNA (Panda *et al*., 2011; Atha *et al*., 2012; Faisal *et al*., 2013; Mirzajani *et al*., 2013). Most of metal-based NPs, including Ag, CuO, ZnO, and CeO_2 can decrease chlorophyll content and function, which subsequently compromises plant health and agricultural yield (Dimkpa *et al*., 2012a; Ma *et al*., 2013; Mirzajani *et al*., 2013; Mukherjee *et al*., 2014). Further evidence from a transcriptomic study with Ag NPs treated *Arabidopsis thaliana* demonstrated that transcription level of protochlorophyllide reductases were significantly down-regulated upon exposure and the authors suggest this as a mechanism for decreased chlorophyll content (Kaveh *et al*., 2013). Increases in lipid peroxidation is a characteristic indicator of decreased cell membrane integrity, and is directly linked to excessive amounts of ROS generation. In a range of 0-1.0 mg L^{-1} concentrations of Ag NPs treated rice root, malondialdehyde (MDA) content (an indicator for lipid peroxidation) increased in a dose-dependent fashion (Nair and Chung, 2014). Others demonstrated lipid peroxidation and loss of membrane integrity that resulted in ion leakage upon CeO_2 NPs exposure to rice and corn (Zhao *et al*., 2012a; Rico *et al*., 2013a).

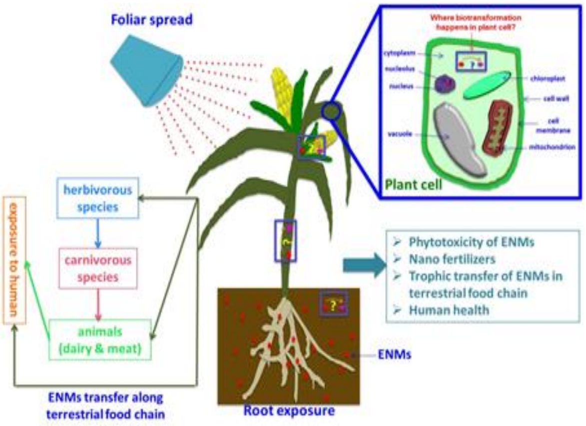

Fig. 1: Overview of ENMs biotransformation in plants, phytotoxicity and trophic transfer of ENMs within in terrestrial food chain

In response to ENMs exposure, plant defense mechanisms involved in ROS scavenging and metal detoxification can be up-regulated to counteract potential phytotoxicity. ROS scavenging antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) can convert ROS into O_2 and H_2O. However, in reviewing the literature, it is clear that antioxidant enzyme activity in ENMs treated plants also varies with plant species, exposure conditions, dose, and NPs type. For example, exposure of 500 mg L^{-1} CeO_2 NPs to the rice cultivar "Cheniere" led to significant increases of APX activity in root tissues but another rice cultivar ("Neptune") exhibited increased CAT activity at CeO_2 NPs exposures as low as 62.5 mg L^{-1} (Rico *et al*., 2013a,b).

Similarly, the up-regulation of transcription levels of APX in tobacco upon Al_2O_3 NPs exposure also suggests that inherent defense systems in plants can scavenge ENM generated ROS through the manipulation of gene expression of antioxidant enzymes (Burklew *et al*., 2012). Similarly, the relative expression of genes involved in SOD, peroxidase (POD), and glutathione S-transferase (GST) were highly induced in *Arabidopsis* when treated with 5 mg L^{-1} Ag NPs (Kaveh *et al*., 2013). The important role of the GSH metabolic pathway has been well-documented in NPs exposed plants. In NiO NPs treated tomato, GSH levels increased in a dose-dependent fashion. Further evidence has demonstrated that GSH is involved in both metal detoxification and ROS scavenging. Ma *et al*. (2015a) investigated enhanced level of GSH in γ-glutamylcysteine synthase (γ-ECS) *Crambe* could protect plants from both Ag NPs and Ag^+ ions toxicity and attained greater biomass. Another evidence for scavenging ROS induced by CeO_2 NPs in rice has been reported that ascorbate-glutathione cycle played an important role to break down excessive amounts of ROS in rice (Rico *et al*., 2013b). Induced levels of oxidized GSH (GSSG) in Ag NPs treated wheat suggested an indirect link with reduced GSH, which might be depleted during the process of ROS scavenging and metal detoxification (Dimkpa *et al*., 2012b). At molecular level, transcription level of genes involved in sulfur assimilation and GSH biosynthesis in *Arabidopsis* were highly induced upon exposures to CeO_2 and indium oxide nanoparticles (In_2O_3 NPs) (Ma *et al*., 2013). Approximate 5-folds changes of gene encoding glutathione synthase (GS) were found in 50 mg L^{-1} In_2O_3 NPs treated *Arabidopsis*, while only about 2.5-folds changes were evident in 500 mg L^{-1} CeO_2 NPs treatment.

2.3. Nutrient displacement or enhancement in ENMs treated plants and use of ENMS as growth stimulants

The presence of ENMs could interfere with nutrient acquisition and transport, subsequently causing nutritional imbalance and compromised plant health. For example, Ag NPs exposure to wild type *Crambe abyssinica* caused significant decrease in Fe accumulation in the plant tissues (Ma *et al*., 2015a). 1000 mg kg^{-1} CeO_2 NPs reduced N_2 fixation in soybean, possibly by inhibiting the growth of N_2 fixing bacteria inside of nodules (Priester *et al*., 2012); in addition, the bioavailability of phosphorus (P) was decreased in Hoagland’s solution, probably because P could precipitate with Ce^{n+} released from CeO_2 NPs (Cornelis *et al*., 2011; Schwabe *et al*., 2013). Indirect evidence of cation transporter and aquaporin down-regulation in the presence of 25 mg L^{-1} Au NPs suggests that nutrient deficiency may result from dysfunction of ion-selective channels upon NPs exposure (Taylor *et al*., 2014). More studies are needed to draw conclusions on the interactions of ENMs and nutrient ions displacement in plants and the underlying biochemical and molecular mechanisms involved.

In spite of the observed negative impacts of certain ENMs on nutrient levels in plants, other ENMs have potential as nano-enabled fertilizers and/or growth stimulants for the purposes of enhanced crop performance. Numerous examples of positive effects of ENMs on plant germination, biomass, and crop yield have been reported (Seabra *et al*., 2014; Liu and Lal, 2015; Servin *et al*., 2015). Not only could amendments of nano-scale nutrient elements promote plant growth, but also, due to their antimicrobial property, these NPs could inhibit activities of both bacterial and fungal pathogens, slowing disease course and increasing yield. For example, 500 mg L^{-1} Mg NPs could significantly increase seed weight and inhibit bacterial activity (Huang *et al*., 2005; Delfani *et al*., 2014). Similarly, low dose exposure of micronutrient Cu and Fe NPs greatly elevated the rate of photosynthesis in waterweed and soybean as compared to untreated controls (Nekrasova *et al*., 2011; Ghafariyan *et al*., 2013). In the presence of foliar application of Fe NPs, the levels of both macro- and micro-nutrients in *Spathyphyllum* were significantly increased (Rasht, 2013). Carbon-based nanomaterials (CNMs) have been shown to exert similar positive impacts on plant growth as well. One common finding across several studies was that CNMs could stimulate water uptake by activating aquaporins; this directly resulted in faster seed germination and increased plant biomass (Khodakovskaya *et al*., 2009, 2013). Although this literature seems in conflict with the observed phytotoxicity mentioned previously, the data suggests that the conditions of dose and exposure may ultimately define the balance between toxicity, no effect or growth/yield enhancement. Significant future study should be directed at this area so as to provide a scientifically sound and mechanistic basis for the use of ENMs in agriculture.

3. Environmental Implication of ENMs

3.1. ENMs in edible portion of plants

With the dramatically increasing use of ENMs, concerns over the lack of understanding of the risks posed to food safety have been raised. Evidence for the presence of CeO_2 NPs in the edible portion of soybean was reported using synchrotron based X-ray fluorescence micrscopy (Priester *et al*., 2012); although the implications of this finding are unknown, caution is clearly warranted. Long-term experiments with CeO_2 and ZnO NPs treated corn demonstrated that both NPs could reduce crop yield by 38% and 49%, respectively, and but perhaps more importantly, nutritional quality may have been compromised from altered nutrient allocation in the kernels (Zhao *et al*., 2015). Thorough investigations on long-term impacts of ENMs on both food quality and safety are just beginning. It is important that such studies be conducted under conditions of environmental relevance, including long-term, multi-generation exposure to realistic exposure concentrations with endpoints that provide mechanistic information on plant response. Only then will an accurate and meaningful evaluation of risk be possible.

3.2. Trophic transfer of ENMs along terrestrial food chain

Along with the evidence of the presence of ENMs in the edible tissues of plants, a small but increasing number of studies on trophic transfer of ENMs within terrestrial food chains, as illustrated in Fig. 1, have been published (Gardea-Torresdey *et al*., 2014). Several studies with Au NPs produced mixed results, with trophic transfer and biomagnification occurring in some scenarios but not others (Judy *et al*., 2010, 2012; Unrine *et al*., 2012). For example, Hawthorne *et al*. (2014) monitored the transfer of Ce from CeO_2 NP contaminated soil to zucchini, as well as to crickets that consumed the zucchini and spiders that consumed the crickets. The authors observed that CeO_2 transferred in a particle sizes specific fashion. Interestingly, about 10 times more Ce was found in the cricket feces than that present in the insect tissues; the implications of this finding are not known but possible ENMs detoxification/excretion mechanisms would be of great interest (Hawthorne *et al*., 2014; Koo *et al*., 2014). As noted above, the published work here is limited and there are currently no related study on how the trophic transfer of ENMs could impact human health along with food chain. There is a robust literature on human cell ENM interactions, with exposure known to induce pulmonary disease, kidney cell apoptosis, and dermal toxicity (Shvedova *et al*., 2003; Cui *et al*., 2005; Hirano *et al*., 2010). Thus, investigations into the impacts of ENMs through terrestrial food chains and their potential risks to humans and other receptors are greatly needed.

4. Conclusion

Concerns over potential negative impacts on the environment from the dramatically increasing demands for ENMs usage have been drawn more attention. Although ENMs can and have brought a huge benefit for human health and the economy, potential negative impacts caused by ENMs release into the environment should not be neglected. Current studies have illustrated that ENMs exposure can have both negative (e.g. oxidative stresses induced by ENMs) and positive (e.g. nano fertilizer in a relative low exposure range) consequences on agricultural crops. Understanding the fine and likely dynamic boundary between acceptable and unacceptable consequences of ENMs use, particularly in agriculture, poses a significant challenge. Clearly, a mechanistic understanding of key processes and interactions will be needed. In term of ENMs biotransformation in plants, techniques such as scanning/transmission electron microscopy, single particle ICP-MS, FFF-ICP-MS, and synchrotron-based techniques will be critical to this effect. For example, μ-SXRF is a powerful technique to analyze metal speciation in plant tissues, providing critical understanding on where biotransformation happens and how the transformed ENMs interact with plants at cellular and sub-cellular level. Such approaches will ultimately generate the necessary knowledge base to inform an accurate and meaningful assess of risk from ENMs exposure and use.

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