Review

Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation

Xingmao Ma a,⁎, Jane Geiser-Lee b, Yang Deng c, Andrei Kolmakov d

a Department of Civil and Environmental Engineering, Southern Illinois University Carbondale, Carbondale, IL, USA
b Department of Plant Biology, Southern Illinois University Carbondale, Carbondale, IL, USA
c Department of Earth and Environmental Studies, Montclair State University, Montclair, New Jersey, 07043, USA
d Department of Physics, Southern Illinois University Carbondale, Carbondale, IL, USA

Abstract

The rapid development and potential release of engineered nanoparticles (ENPs) have raised considerable concerns due to the unique properties of nanomaterials. An important aspect of the risk assessment of ENPs is to understand the interactions of ENPs with plants, an essential base component of all ecosystems. The impact of ENPs on plant varies, depending on the composition, concentration, size and other important physical chemical properties of ENPs and plant species. Both enhancive and inhibitive effects of ENPs on plant growth at different developmental stages have been documented. ENPs could be potentially taken up by plant roots and transported to shoots through vascular systems depending upon the composition, shape, size of ENPs and plant anatomy. Despite the insights gained through many previous studies, many questions remain concerning the fate and behavior of ENPs in plant systems such as the role of surface area or surface activity of ENPs on phytotoxicity, the potential route of entrance to plant vascular tissues and the role of plant cell walls in internalization of ENPs. This article reviewed the current knowledge on the phytotoxicity and interactions of ENPs with plants at seedling and cellular levels and discussed the information gap and some immediate research needs to further our knowledge on this topic.

© 2010 Elsevier B.V. All rights reserved.

Contents

1. Introduction ........................................................................................................ 3053
2. Engineered nanoparticles (ENPs) ..................................................................... 3054
3. Phytotoxicity of engineered nanoparticles to plant seedlings ............................. 3054
4. Phytotoxicity of engineered nanoparticles to algae and plant cells ....................... 3056
5. Uptake, translocation and accumulation (UTA) of engineered nanoparticles .......... 3057
6. Interactions of engineered nanoparticles with plant cells .................................. 3058
7. Future research needs .......................................................................................... 3059
References .............................................................................................................. 3060

1. Introduction

Nanotechnology is advancing rapidly and could soon become a trillion-dollar industry (Nel et al., 2006). As a result, release of substantial amount of engineered nanoparticles (ENPs) into the environment is inevitable. ENPs are widely accepted as materials with at least two dimensions between 1 nm and 100 nm (ASCM, 2006; SCENIHR, 2007). ENPs of these sizes fall in a transitional zone between individual molecules and the corresponding bulk materials and therefore possess unique properties distinctively different from their molecular and bulk counterparts (Taylor and Walton, 1993). Examples of the unique properties of ENPs include very large specific surface area, high surface energy, and quantum confinement. These unusual properties may result in substantially different environmental fate and behaviors than their bulk counterparts. An emerging area of research is now focused on short and medium term studies of the environmental and ecological impact of released ENPs.
Plants are an essential base component of all ecosystems and play a critical role in the fate and transport of ENPs in the environment through plant uptake and bioaccumulation (Monica and Cremonini, 2009). Even though scientific investigation on plant uptake and accumulation of ENPs is still in its rudimentary stage, some new publications have been added to the literature in the past few years yielding new advances in the area of ENP toxicology and uptake by plants. This review is intended to provide a critical assessment of the progress taking place on this topic and to shed light on future research needs.

2. Engineered nanoparticles (ENPs)

Most commonly encountered ENPs in the environment fall into one of the five following categories: carbonaceous nanoparticles, metal oxides, quantum dots, zero-valent metals and nanopolymers, with new products gradually being added to the list. Due to limited publications on the toxicological effects of dendrimers, this review will focus on the toxicological effect and interactions of the rest four categories with plants. A brief introduction of ENPs of each category will be provided in this section and detailed information on ENPs including their chemistry, environmental behavior and cytotoxicity is referred to elsewhere (Christian et al., 2008; You and Reid, 2008).

Carbonaceous nanoparticles are the most abundant ENPs and consist primarily of fullerenes and nanotubes. Fullerenes are enclosed cage-like structures comprised of twelve 5-member carbon and unspecified 6-member rings in defect-free form. Even though an icosahedrally symmetrical structure (nC60) is the most commonly encountered fullerene, both smaller fullerene such as C28 and C36 and very large spherical fullerene conformations have been identified and characterized (Ugarte, 1992; Wang et al., 2001). Carbon nanotubes (CNTs) can be defined as carbon whiskers. A carbon nanotube is a honeycomb lattice rolled on to itself, with diameter of the order of nanometers and length of up to several micrometers. An excellent review of carbonaceous nanomaterials and their applications in the environmental field is now available in literature (Mauter and Elimelech, 2008).

Metal oxide nanoparticles, represented by titanium dioxide (TiO2) and zinc oxide (ZnO), are of great technological importance in the field of heterogeneous catalysis for catalytic support of a wide variety of metals (Biener et al., 2005). Metal oxide nanoparticles also find extensive applications in sunscreen industry due to their ultraviolet blocking ability and visible transparency of nanoparticulate forms (Klaine et al., 2008). Quantum dots (QDs) are artificial “droplets” of charge that can contain anything from a single electron to several thousand electrons (Kouwenhoven and Marcus, 1998). QDs demonstrated many similar quantum phenomena as in real atoms and nuclei and therefore are frequently used as models of different atoms by simply changing its size and shape (Kouwenhoven and Marcus, 1998). QDs generally possess a reactive core which controls optical properties and an inert shell to protect the core from oxidation (Dabbousi et al., 1997).

Silver nanoparticles (AgNPs) are by far the most prevalent metallic nanoparticles in consumer products due to their antimicrobial activity (Klaine et al., 2008) while nanoscale zero-valent iron (nZVI) is the most popular metallic nanoparticles in environmental remediation applications (Zhang, 2003). Due to the high specific surface area (~24–35 m²/g) and more reactive surface sites, nZVI shows extremely high chemical reductive reactivity in the absence of oxygen and high oxidative reactivity in the presence of oxygen. The nZVIs-based technology has been used to remove diverse contaminants through reductive dechlorination or oxidation, such as chlorinated organics (Wang and Zhang, 1997; Liu et al., 2005), heavy metals (Ponder et al., 2000; Kanel et al., 2006) and other inorganics (Choe et al., 2000). Of note, bimetallic nZVI, consisting of elemental iron and another more stable metal element (e.g. Ni, Pd and Ag), have been demonstrated to have a stronger chemical activity than bare iron nanoparticles (Lee and Sedlak, 2008; He and Zhao, 2005). nZVIs produced from different methods possess somewhat different properties and caution should be taken in comparison of experimental results. Nurmi et al. (2005) characterized nZVIs prepared with two widely adopted methods, one by the reduction of goethite with heat and H2 and the other by precipitation with borohydride, and found that nano irons have different compositions and sizes and consequently result in different reactivities with different environmental contaminants. These variations complicate the determination and comparison of the toxicity, fate and impact of nZVIs.

3. Phytotoxicity of engineered nanoparticles to plant seedlings

ENPs closely interact with their surrounding environment and plants are an essential base component of all ecosystems. As a result, ENPs will inevitably interact with plants and these interactions such as uptake and accumulation in plant biomass will greatly affect their fate and transport in the environment, Fig. 1. ENPs could also adhere to plant roots and exert physical or chemical toxicity on plants. Increasing numbers of publications have emerged recently concerning the interactions of ENPs with plants (Battke et al., 2009; Lin and Xing, 2007; Lin et al., 2009). Most of these studies are focused on the potential toxicity of ENPs to plants and both positive and negative or inconsequential effects have been reported.

A recent work indicated that MWCNTs at the concentration range of 10–40 mg/L dramatically enhanced the seed germination and growth of tomato plants (Khodakovskaya et al., 2009), Fig. 2. The researchers hypothesized that the positive effect of MWCNTs arose from the capability of CNTs to penetrate seed coat and therefore

![Fig. 1. An overview and general principle of uptake, transport and accumulation of nanoparticulate matter by plants. Nanoparticulate matters in a natural environment are adsorbed by primary roots (A2) or lateral roots (A1 and then B). These nanoparticles are then transported from root (C) through stem (D and I) to leaf (E, F, G, H). Nanoparticles could also be adsorbed on the surface of roots.](image-url)
promote water uptake. Water uptake in seed germination is critical because mature seeds are relatively dry and need a substantial amount of water to initiate cellular metabolism and growth. The measured water moisture content of seeds and the detection of CNTs inside seeds supported the hypothesis; however, the specific penetration mechanisms through the coat and the enhancement of water uptake by CNTs were not reported. TiO$_2$ nanoparticles (2.5 g–40 g/kg soil) were shown to improve the growth of spinach by enhancing photosynthesis and nitrogen-fixation capability in leaves and roots respectively (Yang et al., 2007). A latest work confirmed these findings showing that TiO$_2$ could promote the energy utilization and conversion efficiency in D1/D2/cyt b559 complex (Su et al., 2009). Similarly, a mixture of SiO$_2$ and TiO$_2$ nanoparticles at low concentrations increased nitrate reductase activity in the rhizosphere of soybean and consequently expedited soybean germination and growth (Lu et al., 2002). SiO$_2$ nanoparticles also enhanced the growth of Changbai larch (Larix olgensis) and the enhancement effect increased with concentration up to 500 mg/L (Lin et al., 2004).

A few studies also indicated inconsequential effects of ENPs on plants. For instance, a study with willow tree cuttings indicated that TiO$_2$ nanoparticles have limited effects on their growth in terms of water usage and transpiration (Seeger et al., 2009). In a sand column study, aluminum nanoparticles did not demonstrate any toxic effects on kidney bean (Phaseolus vulgaris) and rye grass (Lolium perenne) at concentrations up to 17 mg/L (Doshi et al., 2008). Al$_2$O$_3$ nanoparticles up to 4000 mg/L did not have any detectable effects on root elongation and development of Arabidopsis even though slight inhibition of seed germination was detected (Lee et al., 2010).

However, most studies with ENPs indicated certain degree of phytotoxicity, especially at high concentrations. SWCNTs significantly affected root elongation of tomato, cabbage, carrot and lettuce but promoted the growth of onion and cucumber in 24 to 48 h (Canas et al., 2008). Tomato (Lycopersicon esculentum) showed the highest degree of sensitivity to SWCNTs among the six species tested. The researchers showed that functionalized CNTs demonstrated different toxic behaviors but generally were less toxic than non-functionalized CNTs. However, how CNTs were functionalized was not specified. Nevertheless, this work highlighted the importance of surface properties of CNTs in determining the phytotoxicity of CNTs. Metallic oxide nanoparticles (e.g. ZnO) were shown to be inhibitory at different developmental stages of plants such as seed germination and root elongation (Lin and Xing, 2007; Yang and Watts, 2005). In terms of metallic nanoparticles (MNPs), copper nanoparticles were shown to be toxic to two crop species, mung bean (Phaseolus radiatus) and wheat (Triticum aestivum), as demonstrated by the reduced seedling growth rate (Lee et al., 2008). Mung bean is more sensitive than wheat and the authors attributed this phenomenon to differences in root anatomy and architecture; mung bean is a dicot with one large primary root and several smaller lateral roots developing from this primary root while wheat is a monocot with numerous small roots without a primary root. Our recent lab work indicated that very low concentrations of AgNPs (<1 ppm) could be toxic to seedlings of thale cress (Arabidopsis thaliana), another dicot with root structures like mung bean. AgNPs of 20 nm to 80 nm clearly stunted the growth and their phytotoxicity is concentration and particle size dependent, Fig. 3. The root tip (cap and columella) were observed to turn light brown when primary roots were exposed to AgNPs. The brown tip was attributed to the adsorption of AgNPs either itself or in conjunction with cell wall materials or secondary metabolites produced by root tips. Exact mechanisms are yet to be elucidated. Determination of phytotoxicity of metallic nanoparticles and their oxides is complex due to the potential dissolution of metallic ions from these nanoparticles and the potential toxicity and uptake of metallic ions. A recent study on the phytotoxicity of ZnO nanoparticles to ryegrass (Lolium perenne) indicated that phytotoxicity cannot be explained by the dissolution of ZnO nanoparticles from bulk materials alone (Lin and Xing, 2008). Similarly, the phytotoxicity of ZnO nanoparticles to Arabidopsis was much stronger than solutions containing same concentration of soluble Zn (Lee et al., 2010). A separate study on the phytotoxicity of five different nanoparticles (MWCNTs, Ag, Cu, ZnO and Si) on an agricultural plant, zucchini (Cucurbita pepo), supported the conclusion that dissolution from bulk materials alone cannot account for the observed phytotoxicity of ENPs (Stampoulis et al., 2009). Even though bulk materials of Ag and Cu resulted in
reduced biomass compared to controls, plants exposed to ENPs demonstrated a higher degree of reduction, indicating that at least part of the toxicity is from elemental MNPs. Phytotoxic phenotypes in seedlings caused by ENPs include stunt growth, reduced biomass and root cap deformity. The morphology changes of rye grass roots caused by high concentrations of ZnO nanoparticles were demonstrated by Lin and Xing (2008) and are shown in Fig. 4. It can be seen that root tips shrank and epidermal and cortical cells were highly collapsed in the presence of high concentrations of ZnO.

It needs to be cautioned that the inhibition of plant growth may not derive directly from chemical phytotoxicity of nanoparticles. Instead, toxicity may result from the physical interactions between nanoparticles and plant cell transport pathways, i.e. by inhibiting apoplastic trafficking by blockage of the intercellular spaces in the cell wall or cell wall pores, or the symplastic connections between cells through blockage of the nano-sized plasmodesmata. A latest work showed that the inhibition of leaf growth and transpiration of maize seedlings (Zea mays L.) by bentonite and TiO₂ nanoparticles is primarily due to the reduction of hydraulic conductivities (Asli and Neumann, 2009). A further examination showed that diameter of maize root cell wall pores was reduced from 6.6 nm to 3.0 nm by pretreatment of nanoparticles. Another factor which needs to be considered in phytotoxicity study is the solvent effect. The duration of nanoparticles in solution is very short without stabilizers and most commercial products contain certain stabilizers. The synergistic effects of ENPs and stabilizers should be taken into consideration in phytotoxicity studies. Barrena et al. (2009) indicated that three metallic nanoparticles demonstrated low to zero toxic effects on two vegetable plants, lettuce and cucumber and the observed positive or negative effects could be primarily attributed to the presence of stabilizers. For most nanoparticles, relatively high concentrations are needed to cause observable toxicity on plants and the toxicity threshold is species dependent (Lin and Xing, 2007; Lee et al., 2008).

Several latest research papers have also indicated that particle size and specific surface area are more appropriate indicators of phytotoxicity than nominal concentrations of nanoparticles (Barrena et al., 2009). Our laboratory studies on the phytotoxicity of silver nanoparticles (AgNPs) on thale cress seedlings appear to support the hypothesis that surface area is a better indicator of phytotoxicity than nominal concentration (data not published). Yang and Watts (2005) also indicated that surface characteristics are important for phytotoxicity of nanoparticles. By coating alumina nanoparticles with phenanthrene, the authors found that phytotoxicity of alumina nanoparticles to seedlings of five different species was reduced, as indicated by root elongation. Fourier Transform Infrared Spectroscopy (FTIR) was employed to evaluate the surface characteristics and the result revealed that alumina hydroxyl radicals disappeared from the surface and the authors concluded that hydroxyl radicals contributed to phytotoxicity of alumina nanoparticles. This conclusion was proved by the reduced phytotoxicity of alumina nanoparticles after the addition of a hydroxyl radical scavenger, dimethyl sulfoxide (DMSO). Studies dedicated to investigate the effect of other physicochemical properties of ENPs on phytotoxicity such as particle shape and surface charge are not currently available.

Taken together, the apparent differences on the toxicity of nanoparticles to plants may arise from properties of nanoparticles, plant species and ages, exposure time and concentrations. Research has been conducted to highlight the importance of preparation methods because different sample preparations resulted in different size distributions and toxicological properties (Nurmi et al., 2005; Lovern and Klaper, 2006). As mentioned above, nZVI prepared with various methods demonstrate highly different properties. Other researchers who studied the toxicity of fullerene dispersions to several aquatic species reached the same conclusion that preparation methods should be considered in comparison of phytotoxicity results (Ober dorster et al., 2006; Zhu et al., 2006). An equally important parameter in the phytotoxicity study is the selection of phytotoxicity indicator. Seed germination and root elongation are two standard indicators of phytotoxicity suggested by U.S. Environmental Protection Agency, yet several research has indicated the insensitivity of seed germination for nanoparticles (Stampoulis et al., 2009). Plant biomass and chlorophyll levels have been shown to be more sensitive in several studies and more work is probably needed to standardize phytotoxicity studies of nanomaterials.

4. Phytotoxicity of engineered nanoparticles to algae and plant cells

ENPs are not only toxic to plant seedlings, but also harmful to plant cells and algae. Using AgNPs and root tip cells of onion (Allium cepa), researchers demonstrated that AgNPs could disrupt cell division process causing chromatin bridge, stickiness and cell disintegration (Kumari et al., 2010) Pseudokirchneriella subcapitata, single-celled microalgae, have been frequently used in nanotoxicity studies. The toxicity of CeO₂ nanoparticles to P. subcapitata have been observed, yet the mechanisms could not attribute to either of the mechanisms...

---

**Fig. 4.** Longitudinal sections of ryegrass primary root tips observed under light microscope, demonstrating the deformity of morphology of root tips by ZnO nanoparticles or ions. A. Controls, B. primary root tips exposed to 1000 mg/L ZnO nanoparticles and C. primary root tips exposed to 1000 mg/L Zn₃(PO₄)₂. ep: epidermis, ct: cortex and vs: vascular cylinder. Images are adapted with permission from Lin and Xing, 2008, Environ. Sci. Technol, copyright 2008 American Chemical Society.
mentioned above for plant seedlings because no evidence showed that CeO2 nanoparticles adsorbed to the cell surface nor was taken up by P. subcapitata (Hoecke et al., 2009). In this study, the authors noticed that CeO2 nanoparticles clustered around P. subcapitata cells and suggested that localized interactions of CeO2 nanoparticles with the algal cells may cause toxicity because of local aggregation resulting in local nutrient depletion and shading.

As indicated earlier, surface area and surface characteristics play an important role in the phytotoxicity of nanoparticles to seedlings. A study specifically designed to test the hypothesis that nanotoxicity is related to specific surface area instead of mass concentrations supported this statement because the toxicity threshold of suspensions of SiO2 nanoparticles with the diameters of 12.5 nm and 27.0 nm to P. subcapitata is essentially the same when nanoparticles are normalized to their surface area (Hoecke et al., 2008). In a separate study, Hoecke et al. found that the toxicity of CeO2 nanoparticles to P. subcapitata can be related to the surface area as well. Up to this point, most evidence on phytotoxicity appeared to support the hypothesis that surface area is a more important factor on phytotoxicity of nanoparticles to plants at both seedling and cellular levels. However, evidence to reject this hypothesis has also been reported in other studies using mammalian cells. For instance, Warheit et al. found that toxicity of nanoparticles is not solely determined by the surface area of nanoparticles and suggested that surface activity of nanoparticles as a dominant factor in determining the toxicity of nanoparticles (Warheit et al., 2006, 2007). A toxicity study conducted with AgNPs and nitrifying bacteria demonstrated that reactive oxygen species (ROS) may play a role in the toxicity of nanoparticles, but ROS is not the only factor controlling the toxicity of silver nanoparticles (Choi and Hu, 2008). Phytotoxicity studies on both seedling and cellular levels adding ROS in the investigation scope are needed.

In addition to the surface characteristics, the role of dissolution in the toxicity of metallic nanoparticles (both elemental and oxides) to plant cells is under intense investigation. Franklin et al. noticed that the toxicity of ZnO nanoparticles, bulk ZnO and ZnCl2 demonstrated comparative toxicity to P. subcapitata and the toxicity of nanoparticle and bulk ZnO is solely due to the dissolved Zn (Franklin et al., 2007). In a separate study of the phytotoxicity of AgNPs to algal cells, AgNPs seemed to be more toxic than Ag+ to Chlamydomonas reinhardtii, mobile single-celled algae (Navarro et al., 2008a,b). Using a strong silver ligand (cysteine) to bind silver, the authors found that toxic effects of silver ions or AgNPs can be completely eliminated, indicating that the toxicity of AgNPs was associated with the Ag+. The authors concluded that AgNPs are a source of Ag+ and the rapid accumulation of Ag+ by algal cells was the underlying mechanism of toxicity of AgNPs, which is in contrast with the conclusions drawn from seedling studies. In this study, photosynthesis efficiency was used to determine toxicity effect. A recent study concerning the uptake of SWCNTs by a commonly used cell culture, tobacco Bright Yellow (BY-2) cells showed that SWCNTs were not toxic to the cell based on their morphology, metabolic rate and membrane integrity (Liu et al., 2009). However the applied SWCNTs are considerably large and are mainly composed of CNT bundles with an average size of 500 nm.

5. Uptake, translocation and accumulation (UTA) of engineered nanoparticles

The importance of uptake and accumulation of ENPs by plants is increasingly recognized by researchers and some scientific studies have been published recently. The first report was published by Zhu et al. (2008). It unambiguously showed for the first time that iron oxide nanoparticles (Fe3O4) was taken up by pumpkin (Cucurbita maxima) roots and translocated through the plant tissues. A mass balance was conducted at the end of the experiment and the study showed that about 45.5% of fed nanoparticles were accumulated in roots and approximately 0.6% of the nanoparticles were detected in leaves. When a different plant species, lima bean (Phaseolus limensis), was tested by the same researchers however, uptake and transport of iron oxide nanoparticles were not observed. Lin et al. investigated the uptake and translocation of carbon nanomaterials by rice plants (Oryza sativa) and they found that fullerene C70 could be easily taken up by roots and transported to shoots (Lin et al., 2009). Their study also demonstrated that C70 could be potentially transported downward from leaves to roots through phloem if C70 entered into plants through plant leaves. Similar results were not observed for MWcnts by the same researchers even when the concentration of MWcnts was 800 mg/L. This discrepancy could be due to the relatively large size of MWcnts than fullerenes. With a two-photon excitation microscope, researchers demonstrated that MWcnts primarily adsorbed to root surface as individual and aggregated CNTs even though one or both ends can pierce through root cap cell walls, Fig. 5 (Wild and Jones, 2009). This unique interaction facilitates the penetration of organic contaminants into cell cytoplasm but extensive internalization of ~100 nm diameter CNTs was not observed. Other researchers who investigated the uptake of ZnO nanoparticles by ryegrass (L. perenne) noticed no upward translocation of ZnO nanoparticles from roots to shoots (Lin and Xing, 2008). ZnO nanoparticles primarily adhered to root surface and individual nanoparticles were also observed in the apoplast and protoplast spaces in root endodermis and stele.

For metallic nanoparticles, Cu nanoparticles (CuNPs) could be taken up and accumulated in the biomass of bean and wheat plants, and a responsive relationship existed between the bioaccumulated CuNPs in plant tissues and in growth media (Lee et al., 2008). The researchers also presented a linear relationship that higher concentrations of CuNPs in the growth media resulted in higher uptake and accumulation of CuNPs in plant tissues and that the aggregation state of CuNPs in plant tissues is also related to the concentrations of nanoparticles in growth media. However, this study was carried out in agar media; it is unknown how this result resembles the uptake and bioaccumulation of CuNPs in liquid medium and soil. In our laboratory studies, AgNPs colloids were used and the preliminary data indicated that AgNPs as large as 40 nm can be taken up by the roots of A. thaliana and transported to the shoots even though the majority of AgNPs are adhered to the root cap, Fig. 6. The roots were collected from seedlings fed with 680 μg/L of 40 nm AgNPs for four weeks and were imaged under a confocal/multiphoton microscope. AgNPs were observed to accumulate in an unexpected tissue, columella, in the roots. Exact reason for the accumulation of AgNPs in columella is still unknown. Due to the natural dissolution process from metallic nanoparticles and the capabilities of some plants to reduce metal ions to elemental NPs inside plant tissues (Harris and Bali, 2008), accumulated MNPs in plants could come from two sources. No attempt has been made to distinguish the sources of NPs in plant tissues in the presence of ENPs.

A recently published work showed that uptake and transport of nanocrystals from roots to leaves within a few days in moth orchid (Phalaenopsis spp.) and A. thaliana when watered with an aqueous colloidal solution of NaYF4:Yb,Er nanocrystals (Hirschmoller et al., 2009). NaYF4:Yb,Er nanocrystals are upconversion fluorescence nanocrystals which emit visible light under near infrared light. Orchid roots were soaked in the NaYF4:Yb,Er colloidal solution for different time and NaYF4:Yb,Er nanoparticles in plant tissues were visualized using a confocal laser scanning microscope. The images clearly demonstrated the route of the penetration of the nanocrystals into plant tissues, from velamen radicum (a special epidermis occurring only in aerial roots) to passage cells in about ten minutes and finally reaching vascular tissues in days (Hirschmoller et al., 2009). This work is probably the first study to show the uptake kinetics and to illustrate the potential penetration routes of nanoparticles into plant tissues. Whether this observation is true to subterranean roots is unknown. Even though progresses have been made, the investigation
of plant uptake and accumulation of nanoparticles is still in its infant stage. Many hurdles are ahead and many critical questions remain unanswered and these critical questions will be discussed in the Future research needs section.

6. Interactions of engineered nanoparticles with plant cells

ENPs have to penetrate cell walls and plasma membranes of epidermal layers in roots to enter vascular tissues (xylem) in order to

![Fig. 5](image)

**Fig. 5.** A. Unstained MWCNTs at the surface of a living root, B. piercing of epidermal cellular structure by MWCNTs. Both ends of the MWCNTs penetrated through the cell wall and the middle of MWCNTs is at the root surface, and C. 3D reconstruction of multiple xy TPEM sections showing the adsorption of MWCNTs at the root surface and piercing of MWCNTs through the epidermal cell walls of several cells. Images are adapted with permission from John and Wilds, 2009, Environ. Sci. Technol., copyright 2009 American Chemical Society.

![Fig. 6](image)

**Fig. 6.** Uptake and accumulation of 40 nm AgNPs by wall cress (*Arabidopsis thaliana*). A. Root controls grown in Hoagland solutions, B. the adsorption of AgNPs on primary and lateral root caps, and C. the uptake and accumulation of AgNPs in plant roots, small fractions of AgNPs can be detected in the vascular tissues. D. Magnified root cap indicating that AgNPs are primarily accumulated in root cap and some AgNPs are sequestered in columella. The roots were imaged with a confocal/multiphoton microscope.
be taken up and translocated through stems to leaves. Cell walls, through which water molecules and other solutes must pass to enter into roots, are a porous network of polysaccharide fiber matrices. The pore sizes of plant walls are typically in the range of 3–8 nm, which is much smaller than many tested ENPs (Carpita and Gibeaut, 1993). Navarro et al. hypothesized that cell walls typically with the thickness of about 5 to 20 nm function as natural sieves. ENP aggregates with a size smaller than the largest pore are expected to pass through and reach the plasma membrane and the larger particle aggregates will not enter into plant cells. But the authors also admitted that ENPs may induce the formation of new and large size pores which allow the internalization of large ENPs through cell walls (Navarro et al., 2008a,b). Proseus and Boyer investigated the penetration of Cu nanoparticles of various sizes into and across algal cell walls using confocal laser scanning microscopy and found that gold nanoparticles with a diameter of 10 nm or above could not penetrate through algal cell walls even under pressured conditions (Proseus and Boyer, 2005).

However, cell internalization of ENPs of different sizes and compositions has been observed for different plant species (Lin et al., 2009; Liu et al., 2009). Liu et al. demonstrated that SWCNTs treated with strong acids can be taken up by BY-2 cells. The authors further indicated that BY-2 cells can also uptake the SWCNT–fluorescein isothiocyanate and SWCNT–DNA conjugates, making SWCNTs a promising nanotransporter for intact plant cells (Liu et al., 2009). Torney et al. used protoplasts in their studies to demonstrate the internalization of mesoporous silica nanoparticles and the potential delivery of DNA and other chemicals with silica nanoparticles to plant cells (Torney et al., 2007). Other researchers showed that protoplasts from sycamore-cultured cells can effectively take up 40 nm polystyrene nanoparticles and 20 nm CdSe/ZnS QDs (Etcheberria et al., 2008). Protoplasts are plant cells after enzymatic removal of cell wall and certain surface proteins. Etcheberria et al. showed that it is likely that ENPs pass through plasma membranes of sycamore cells in a similar manner as they do through mammalian cells via endocytic pathways. The authors also demonstrated a mechanism of recognition, separation and redistribution which exists in the early stage of solute uptake: the 40 nm nano-sphere was stored in the vacuoles of sycamore cells as most other solutes but the 20 nm QDs remained in the cytoplasmatic membranous compartments after 18 h (Etcheberria et al., 2006). Whether the different sequestration location is related to the particle sizes or the composition is unknown and yet will be highly interesting to unravel. Even though protoplasts can provide invaluable information on the internalization mechanism how nanoparticles enter plant cells, it is questionable that whether protoplasts could resemble intact plant cells surrounded with cell walls. The employment of intact cells is still needed to elucidate the mechanisms of nanoparticles internalization into plant cells, especially after it was shown that intercellular transport of nanoparticles in cell walls could occur.

Once micro- and macromolecules enter plant cell walls, the molecules can be transported through plasmodesmata, the intercellular organelles of 20–50 nm in diameter (Lucas and Lee, 2004; Heinlein and Epel, 2004). Selective and non-selective pathways through plasmodesmata are found to transport regulatory proteins and RNAs in short distances (Kim, 2005). We found AgNPs of 20 nm taken by plants were mostly in the intercellular spaces could be transported inside plant cells through plasmodesmata, Fig. 7. Root samples were analyzed under a transmission electronic microscope and found that gold nanoparticles (AgNPs) in plasmodesmata of Arabidopsis thaliana. A. 20 nm AgNPs (arrows pointing to) in intercellular space, i.e. plasmodesmata. B. Control. Scale bars: 1 μm.

Fig. 7. Transmission electron micrographs showing the accumulation of silver nanoparticles (AgNPs) in plasmodesmata of Arabidopsis thaliana. A. 20 nm AgNPs (arrows pointing to) in intercellular space, i.e. plasmodesmata. B. Control. Scale bars: 1 μm.

7. Future research needs

Despite the rapid progress in the study of phytotoxicity, uptake and accumulation of ENPs in the past two years, we are still in the very early stage of this field and numerous questions with tremendous scientific or practical importance need to be investigated. In the phytotoxicity study of nanoparticles, the most urgent need is probably to build connections between the characteristics of nanoparticles (e.g. surface area, particle size, surface activity) with phytotoxicity. Equally important is the need to understand the role of plant species and the composition of nanoparticles on phytotoxicity of nanoparticles. Previous studies have clearly demonstrated the different resistivity of nanoparticles by different plant species, yet how and why different plant species demonstrate different resistance to nanoparticles remains unsettled. Does the resistance of plants correlate with the biomass of roots, or surface of roots? How does the vascular structure of plants affect the plant’s resistance to toxicity? What is the genetic response of plants and what genes are up-regulated and what genes are down-regulated in the presence of ENPs?

On the aspect of plant uptake and accumulation, little studies have been performed to assess the uptake kinetics of ENPs and to investigate how the composition, particle size and aggregation state affect the uptake kinetics and the fate and transport of nanoparticles in plant systems. Information is also lacking on how plant species and environmental factors will affect the uptake and accumulation of ENPs by plants. Xylem structure of plants could be an essential parameter because it has been recognized that xylem structures determine the speed of water transport (Guthrie, 1989). As a result, plants with different xylem structures may demonstrate different uptake kinetics of nanoparticles and studies with plants having different xylem structures will reveal tremendous information on the impact of plant species on uptake kinetics. Another important need is to understand the penetration route of NPs into vascular tissues. Solutes enter into vascular tissues either apoplastically or symplastically. Preliminary studies with AgNPs in our study appeared to indicate apoplastic pathway of entrance to plant tissues and cross membrane through plasmodesmata, yet more studies are needed to confirm this hypothetical pathway. Where ENPs are sequestered in plant tissues
and are they bioavailable to human beings and wild lives? The answer to the last question will have considerable implications for food safety because nanoparticles can be potentially accumulated in edible portions of plants and crops if they are exposed to ENPs.

From the experimental point of view, the future phytotoxicity and uptake research will benefit from the in vivo microscopy methods which allow the quantitative kinetic studies of the uptake and accumulation. Up to now, most of this kind of research relies on optical detection of the fluorescent nanoparticles (quantum dots) or on nanoparticles functionalized with fluorescent markers (Gonzalez-Melendi et al., 2008). This powerful tool however has several inherent limitations such as limited resolving power which leads to the fundamental impediments in observing the uptake, transport and accumulation dynamics on the cellular level. In addition, the vast majority of the nanoparticles relevant to phytotoxicity is not fluorescent and cannot be detected directly by this method. The common functionalization of the surface of these particles may lead to alteration of their chemical reactivity and deviate from the real world behavior.

One of the potential methods capable of addressing these challenges relies on the recent developments in environmental scanning (SEM) and transmission (TEM) electron microscopy. The development of electron transparent but gas/liquid impermeable membranes opened the possibility to image the living cells in their natural environment with unprecedented resolution (de Jonge et al., 2009). Using this approach, in vivo imaging of the individual nanoparticles and their short and long term dynamics inside the individual cells and pant tissue become feasible. Fig. 8 shows AgNPs taken up by thale cress seedlings imaged with this innovative technology by one of the authors.

Finally, the majority of studies conducted so far on phytotoxicity and uptake of ENPs by plants were carried out in hydroponic settings. Without adequate information, hydroponic studies could reveal considerable information on the interactions of plants and ENPs in water without the compounding effect of soil. Yet the complicated nature of environmental media calls for fate and transport studies of ENPs in more “nature-like” conditions. These research needs are by no means a complete list in the study of interactions of ENPs with plants, yet they probably represent some most urgent research needs in this area as the production of ENPs is expected to grow rapidly.

References


Lovern SB, Klaper R. Daphnia magna mortality when exposed to titanium dioxide and ne-quartz particles in rats: toxicity is not dependent upon particle size and surface area. Toxicol Sci 2006;93:529–32.


