

Interaction of Nanoparticles with Edible Plants and Their Possible Implications in the Food Chain

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ABSTRACT: The uptake, bioaccumulation, biotransformation, and risks of nanomaterials (NMs) for food crops are still not well understood. Very few NMs and plant species have been studied, mainly at the very early growth stages of the plants. Most of the studies, except one with multiwalled carbon nanotubes performed on the model plant *Arabidopsis thaliana* and another with ZnO nanoparticles (NPs) on ryegrass, reported the effect of NMs on seed germination or 15-day-old seedlings. Very few references describe the biotransformation of NMs in food crops, and the possible transmission of the NMs to the next generation of plants exposed to NMs is unknown. The possible biomagnification of NPs in the food chain is also unknown.

KEYWORDS: food crops, nanomaterials uptake, genotoxicity, phytotoxic effects, biotransformation

■ INTRODUCTION

Studies have shown that environmental conditions may influence plant ion concentrations in crop plants.^{1–3} Under specific growing environments, plants absorb essential and nonessential elements, which above certain concentrations may cause toxicity.⁴ In addition, toxic elements with no known function in biological systems are found to be accumulated in plant tissues, with lethal effects for nontolerant species.^{5–9} Once stored within plants, beneficial or toxic elements can be transferred from producers (plants) to consumers. For instance, selenium-laden plants can be used to supply selenium deficiencies in ruminants and other animals;^{10,11} however, this is still under scrutiny because the borderline between deficiency and toxicity is very narrow.¹²

Plants have evolved in the presence of natural nanomaterials (NMs). However, the probability of plant exposure to NMs has increased to a greater extent with the ongoing increasing production and use of engineered nanomaterials (ENMs) in a variety of instruments and goods.¹³ ENMs can reach the plants through direct application, accidental release, contaminated soil/sediments, or atmospheric fallouts. Little is known about the impact of ENMs on food crops, and their possible effects in the food chain are unknown.^{14,15} A few studies on the toxicity of ENMs have been performed on crop plants such as rape (*Brassica napus*), radish (*Raphanus sativus*), lettuce (*Lactuca sativa*), corn (*Zea mays*), and cucumber (*Cucumis sativus*), among others.^{16–18} These studies and most recent publications on the absorption, translocation, accumulation, and biotransformation of ENMs (metal based (MB) and carbon based (CB)) in edible plants are included in this review. Although MB NMs include nanoparticles (NPs, materials with at least two dimensions between 1 and 100 nm) and other NMs (materials with at least one dimension of 100 nm), only studies on NPs will be discussed.¹⁹ The reviewed papers on CB NMs include fullerenes and carbon

nanotubes (single-walled (SWCNTs) and multiwalled (MWCNTs)).²⁰

■ UPTAKE, TRANSLOCATION, AND ACCUMULATION OF NANOPARTICLES INTO EDIBLE PLANTS

The uptake of CB and MB NMs by plants is a very recent field of study. Most of the data correspond to the germination stage and cell cultures. Because the protocols for quantification of NPs within tissues are not yet well-defined, the discussion of the current literature is more oriented to the effect of the NPs on plants. Among CB NMs, the most studied materials are the fullerene C₇₀, the fullerol (C₆₀(OH)₂₀), and CNTs, whereas the most studied MB NMs are TiO₂, CeO₂, Fe₃O₄, and ZnO NPs. The uptake of other MB NMs such as Au, Ag, Cu, and Fe NPs is also discussed.

Mode of Uptake of Nanoparticles by Plants. Current literature revealed that the uptake, translocation, and accumulation of NPs depend on the species of plant and the size, type, chemical composition, functionalization, and stability of the NPs. Among the CB NMs, only the fullerene C₇₀ and fullerols were shown to be readily accumulated in plants. Conversely, most MB NPs were found to be taken up and accumulated in plants, although some conflicting data exist. The selective uptake, biotransformation, and translocation of various nanoparticles by a model plant have been schematically represented in Figure 1. As seen in Figure 1A, and discussed in the following sections, conclusive studies about the absorption and movement of NPs within living plants pertain only to fullerols, Ni(OH)₂, and Cu NPs.^{21–23} Results for the uptake of other types of NPs are shown in Figure 1B. As shown in this figure, the published data about NP uptake by plants is still not conclusive.

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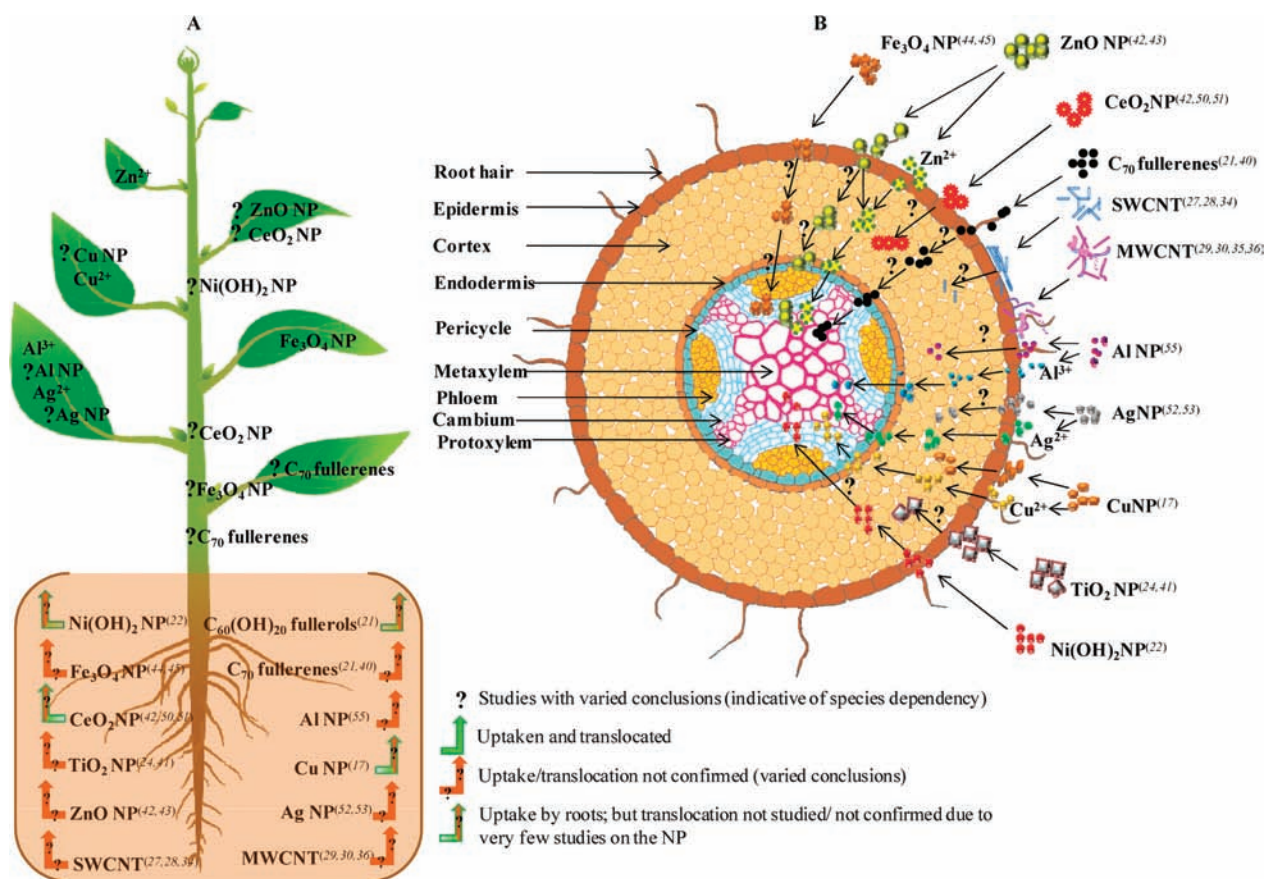


Figure 1. Uptake, translocation, and biotransformation pathway of various nanoparticles in a plant system: (A) plant showing the selective uptake and translocation of nanoparticles; (B) transverse cross section of the root absorption zone showing the differential nanoparticle interaction on exposure. The superscripts depict the reference cited.

Several avenues for the uptake of NPs by plant cells have been proposed (Figure 2). As shown in this figure, the data suggest that NPs can enter plant cells by binding to carrier proteins, through aquaporins, ion channels, or endocytosis, by creating new pores (preferably for CNTs), or by binding to organic chemicals in the environmental media. Due to increased surface area to mass ratio of the NPs as compared to the bulk metals, they are thought to deliver more reactivity with their surroundings. The NPs may form complexes with membrane transporters or root exudates (Figure 2) and subsequently be transported into the plants.^{24,25} Most of the MB NPs that have been reported as taken up by plants include elements for which ion transporters have been identified.²⁶ Once inside the cells, NPs may be transported apoplastically or symplastically. They may be transported from one cell to the other through plasmodesmata. However, the exact mechanisms of why only some plant species readily take up several NPs are still unknown and remain to be explored.

Uptake of CB NMs by Plants. With the recent developments involving CNTs in smart delivery systems of various biomolecules/genes/drugs into the cells, studies are increasingly being performed in an effort to find the uptake and transport mechanism of CNTs into the intact plant cells.

Although SWCNTs are too large to penetrate cell walls, Shen et al.²⁷ showed evidence of an endocytosis-like structure in the plasma membrane in an *Arabidopsis thaliana* leaf cell. Although not a food crop, but the model plant for studying plant biology,

the results obtained with *Arabidopsis* are deemed extremely relevant and significant to guide further studies with edible plants. Studies with cell suspensions of *Nicotiana tabacum* cv. Bright Yellow (BY-2) showed that the water-soluble SWCNTs (<500 nm in length) were found to penetrate the intact cell wall and the cell membrane through fluidic phase endocytosis.²⁸ In addition, it has been reported that MWCNTs were taken up by the seeds and root systems of developing tomato seedlings.²⁹ It was hypothesized that the MWCNTs were able to penetrate the seed coat by creating new pores, thereby enhancing water uptake. MWCNTs were also visualized initially on the root surface and ultimately piercing the epidermal and root hair cell walls and root cap of wheat seedlings.³⁰ Although the above-mentioned studies suggest potential modes of uptake of nanotubes, results are insufficient to conclude whether the nanotubes translocate from the root systems to the aerial parts of the plants. The hydrophobic properties of the MWCNTs render them capable of interacting with many organic substances.³¹ It has been suggested that the very low surface friction of CNTs facilitates the flow of organic substances into the cytoplasm.³² This property has been explored for phytoremediation purposes. Ma and Wang³³ found that C₆₀ at 15 mg L⁻¹ increased the uptake of trichloroethylene by 82% in cottonwood (*Populus deltoides*) cuttings.

Conversely, Canas et al.³⁴ found no uptake of SWCNTs and functionalized SWCNTs (F-SWCNTs) by roots of cucumber seedlings after 48 h of treatment. However, the SWCNTs were

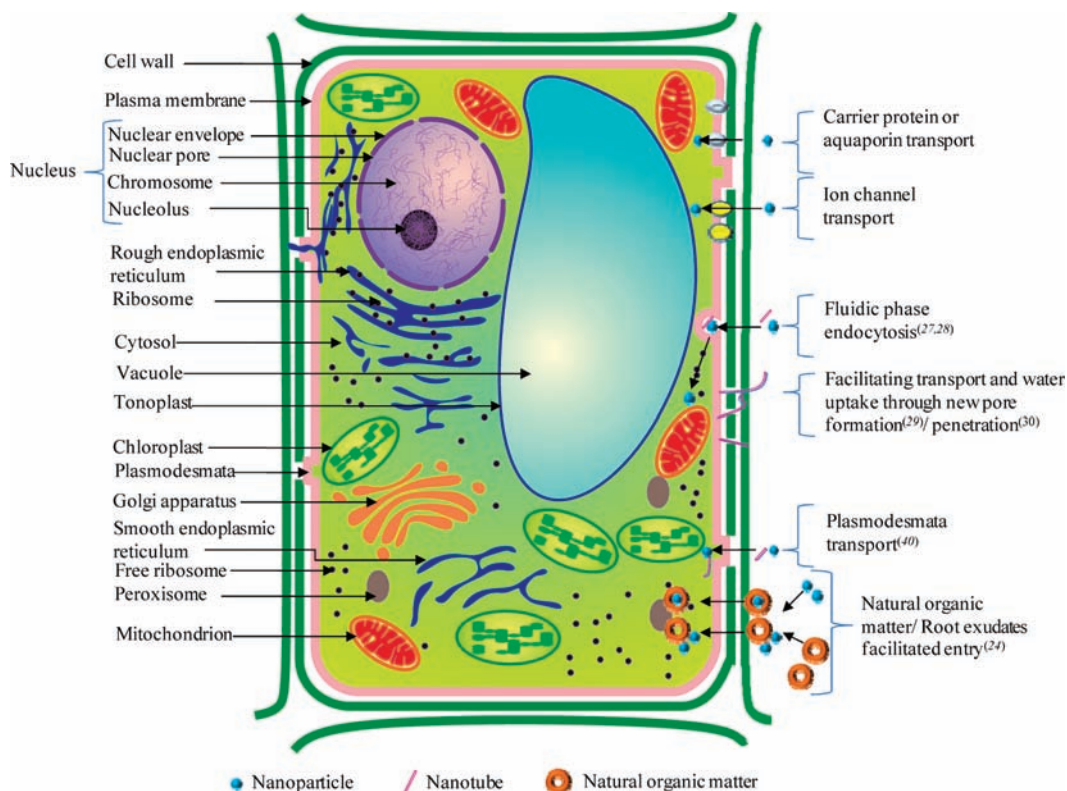


Figure 2. Probable modes of cellular uptake of the nanoparticles in a plant cell.

found adhered on the external surface of the main root and secondary roots in the form of nanotube sheets. Other studies reported that the cell walls of rice cell suspension restrict the entry of the MWCNTs into the cellular cytoplasm.³⁵ MWCNTs were reported to form black clumps tightly wrapping around and associating with the cells.³⁶ The clumps were found to increase in number and size with increase in concentration. The CNTs are hypothesized to interact with the proteins and polysaccharides on the cell wall and elicit hypersensitive responses mimicking plant pathogens due to their small size, eventually leading to cell mortality.^{35–37} This is also supported by identification of non-covalent interactions between CNTs and rice starch.³⁸ This hypersensitive response is considered to be responsible for the prevention of the entry of MWCNTs through the plant cell walls.

The uptake of the CNTs by plants also depends on its dispersion in the experimental media. In natural ecosystems the effect and uptake of NMs by the plants is expected to depend to a great extent on the chemical properties, organic content, and colloidal properties of the associated soil, sludge, or sediments. Natural organic matter (NOM) is a collection of heterogeneous organic substances from decomposed living species, which may pose as important factors affecting nanomaterial exposure to plants.²¹ The hydrophobic moieties of NOM are believed to interact with the hydrophobic carbon of NP surfaces, giving rise to a dynamic equilibrium process.³⁹ Lin et al.⁴⁰ studied the uptake, accumulation, and translocation of NOM-suspended fullerene C_{70} and MWCNT in rice plants. They reported the presence of C_{70} in the form of black aggregates that were more abundant in the seeds and roots, compared to the stems and leaves, of the rice seeds. The presence of NOM- C_{70} aggregates in leaves suggests that they followed the transmission route of water and nutrients through the xylem. In the matured plants,

NOM- C_{70} aggregates were predominantly present in or near the stem's vascular systems and leaves, whereas the roots were noted to be devoid of C_{70} , supporting the claim of robust translocation from the roots to the aerial parts of the plant. In contrast, the seeds treated with NOM-MWCNT showed insignificant uptake, with very few aggregates of nanotubes in the vascular system and none in the tissues. The individual fullerene C_{70} NPs were hypothesized to enter the plant roots through osmotic pressure, capillary forces, pores on cell walls, and intercellular plasmodesmata or via the highly regulated symplastic route. In a contrasting study, Chen et al.²¹ reported that hydrophobic fullerene C_{70} -NOM blocked the cell wall pores in *Allium cepa* cell suspensions, resulting in negligible uptake of the NPs by the cells, whereas the small size and greater hydrophilicity of the fullerenols $C_{60}(\text{OH})_{20}$ allow their permeability through *A. cepa* cell walls and eventual accumulation at the interface between the cell wall and plasma membrane. Moreover, $C_{60}(\text{OH})_{20}$ was found to accumulate between adjacent epidermal cell walls, indicating its apoplastic mode of transport in the plant tissues. The above-discussed results clearly indicate that CB NMs can be taken up by some edible plants, but more studies are needed to establish the uptake mechanisms and consequences of their accumulation in edible plants species.

Uptake of Metal Oxide NPs by Plants. *Uptake of TiO_2 NPs.* Although TiO_2 NPs are profusely used in daily life products, the study of their uptake and translocation in plants is limited, especially on food crops. Kurepa et al.²⁴ reported that an ultrasmall TiO_2 (<5 nm) complexed with Alizarin red S nanoconjugate was taken up and translocated by *A. thaliana* seedlings following tissue and cell specific distribution. They showed that roots of *A. thaliana* released mucilage that formed a pectin hydrogel capsule surrounding the root, which could either inhibit

or facilitate the entry of the TiO₂ complexed with Alizarin red S or sucrose. Other studies have shown that polysaccharides in mucilage might adsorb and inactivate toxic heavy metals in the rhizosphere or enhance accumulation depending on the plant species.²⁵ Asli and Neumann⁴¹ investigated the uptake and translocation of TiO₂ NPs (30 nm) in maize (*Z. mays*) excised roots having intact apices. The NPs were not seen to be taken up by the root cells, probably due to its large size compared to the size of the pore diameter (6.6 nm) in the root cell wall of maize.

Uptake of ZnO NPs. As in the case of other MB NPs, the uptake, translocation, and accumulation of ZnO NPs in food crops are not well understood. In addition, most of the studies have been carried out until germination stage, which provided limited information because of the incomplete plant root and vascular system development. Lopez-Moreno et al.⁴² investigated the uptake and accumulation of ZnO NPs (8 nm) by soybean (*Glycine max*) seedlings. These researchers treated the soybean seeds with ZnO NPs in the range of 500–4000 mg L⁻¹. The Zn uptake by the seedlings was significantly higher at 500 mg L⁻¹, perhaps because at this concentration the NPs have lesser aggregation. At high concentrations (1000–4000 mg L⁻¹), an increase in the probability for the formation of agglomerates is proposed. This makes passage through the cell pore walls difficult, thereby reducing uptake and accumulation as understood from the results. These researchers performed X-ray absorption spectroscopy (XAS) analysis of the ZnO NP-treated samples. The XAS results showed Zn²⁺ inside the plant tissues, but the spectrum resembled more the one of zinc acetate and nitrate than ZnO NPs. Although ZnO NPs are expected to be a source for Zn²⁺ found within tissues, the study fails to highlight whether the Zn²⁺ was contributed by the biotransformation of the ZnO NPs on/in roots. However, it may be hypothesized that root exudates ionized the ZnO NPs on the root surface, as no traces of ZnO NPs were shown by the XAS spectra. However, more elaborative studies need to be performed to confirm the biotransformation of the ZnO NPs and the associated mechanism and factors affecting the ionization. In ryegrass, scanning electron microscopy studies confirmed the adsorption and aggregation of the ZnO NPs to the root surface.⁴³ The high-magnification transmission electron microscopy (TEM) images of the ryegrass root cross sections also showed the presence of particles in the apoplast, cytoplasm, and nuclei of the endodermal cells and the vascular cylinder, presumed to be ZnO NPs. Unfortunately, X-ray absorption studies confirming the presence of the NPs were not presented.

Uptake of Iron Oxide NPs. Only studies on the edible plants pumpkin and lima beans treated with Fe₃O₄ NPs were found. Zhu et al.⁴⁴ studied the uptake of magnetite (Fe₃O₄ NP, 20 nm diameter) by pumpkin seedlings in hydroponic conditions using a vibrating sample magnetometer. It was reported that the signal for magnetic NPs was detected in roots, stems, and leaves of pumpkin plants. However, the uptake of the NPs was also seen to depend on the growth medium, because no uptake was observed when plants were grown in soil and reduced uptake was found when plants were grown on sand. This may be attributed to the adherence of the Fe₃O₄ NPs to the soil and sand grains. It seems that the uptake also depends on the species of plant because no uptake of Fe₃O₄ NPs was found to occur in treated lima bean plants (*Phaseolus limensis*). On the other hand, Wang et al.⁴⁵ did not note any uptake of 25 nm Fe₃O₄ NPs by the pumpkin plants. It has been hypothesized that it is difficult for the large size NPs to penetrate through the cell walls and transport across the plasma

membranes. The cell wall pore sizes vary from 2 to 20 nm, whereas the size of ions and water molecules is about 0.28 nm. Thus, ions and water find their way through ion channels and aquaporins, respectively.^{46–49}

Uptake of CeO₂ NPs. It was found in two recent studies that seedlings of soybean, alfalfa (*Medicago sativa*), corn, and tomato (*Lycopersicon esculentum*) accumulated Ce in tissues as the external concentration of CeO₂ NPs (7 nm) increased.^{42,50} Interestingly, at 4000 mg of CeO₂ L⁻¹, the concentrations of Ce (mg kg⁻¹ DW biomass) significantly varied between species (≈300 for corn, 462 for soybean, 4000 for tomato, and 6000 for alfalfa). This differential accumulation could be explained by specific differences in root microstructures and the physical and chemical interactions between the NPs and root exudates in the rhizosphere. This opens up an area of extensive research. X-ray absorption near-edge structure (XANES) analysis confirmed the presence of CeO₂ NPs in the root samples of all of the tested plant species. However, whether the resemblance of the spectrum of the plant roots was due to the absorption into the roots or binding of the CeO₂ nanoparticles to root hairs and exudates was not very clear. In a more recent study Birbaum et al.⁵¹ reported that CeO₂ NPs applied as aerosol or suspension on corn leaves were absorbed by the leaves, but not translocated to new leaves. It was also reported that NPs applied in the irrigation water resulted in no detectable translocation of the NPs within the plant. This could suggest that the absorption and translocation of the nanoceria is species dependent.

Uptake of Nickel Hydroxide NPs. Only one reference was found on the uptake of Ni(OH)₂ NPs. Parsons et al.²² investigated the uptake and translocation of Ni(OH)₂ NPs (8.7 nm) in mesquite (*Prosopis* sp.). The accumulation and oxidation state of the NPs were studied by X-ray absorption spectroscopy. The XANES spectra showed the presence of Ni(OH)₂ NPs in roots and shoots of plants treated with uncoated NPs, whereas plants treated with citrate-coated NPs showed Ni NPs only in roots. Results also showed that none of the treatments reduced plant size or chlorophyll production, but the study was conducted with plants at the seedling stage.

Uptake of Metallic NPs. The uptake and toxicity of NPs either may be due to their small size, surface characteristics, aggregation, and associated characteristics or may be mimicking and expressing the toxicity associated with the element. Stampoulis et al.⁵² reported on the uptake of Ag NPs in zucchini (*Cucurbita pepo*) compared to their corresponding bulk counterpart. The Ag concentration in the plant shoots was found to be on an average 4.7 times higher in the plants exposed to 10–1000 mg L⁻¹ Ag NPs than those treated with bulk Ag powder at similar concentrations. It has been suggested that the greater ion release from Ag NPs is responsible for the greater Ag concentration in the shoots. However, the form of Ag (nanoparticles, aggregates, or ionic) inside the plants was not studied. In another study, *Brassica juncea* plants treated with Ag NPs did not seem to accumulate Ag in any form.⁵³ Corredor et al.⁵⁴ reported that carbon-coated Fe NPs applied to the leaf petioles of living pumpkin plants were found only in the epidermal cells close to the application site. No NPs were traced in the cells located far from the application points or near the xylem.

Lee et al.²³ investigated the uptake and translocation of Cu NPs in mungbean (*Phaseolus radiata*) and wheat (*Triticum aestivum*) in agar growth medium. The study showed that the Cu NPs could cross the cell membrane and agglomerate in the cells. The bioaccumulation factors (amount of Cu in plant dry

weight tissue divided by the amount of Cu in the growth media) of mungbean and wheat plants exposed to 1000 mg of Cu NPs L^{-1} were 8 and 32 mg kg^{-1} , respectively. Also, a responsive relationship between the bioaccumulated NPs in plant tissues and growth media was observed. Likewise, Doshi et al.⁵⁵ investigated the uptake of Al in red kidney beans exposed to 10–10000 mg of Al NPs kg^{-1} (1–100 nm) in soil. Results showed that the Al concentration in the red kidney beans was not significantly different from that of the untreated control. Unfortunately, in both studies,^{23,55} it was not quantified how much of the Ag or Al plant content was in the form of NPs. However, the results suggested that the uptake of metallic NPs is species-specific.

There are reports on the reduction of metal ions into NPs and subsequent accumulation of the NPs in edible plants. Gardea-Torresdey et al.^{56,57} found that Au(III) and Ag(I) ions in agar solid growth media were reduced and accumulated as Au and Ag NPs inside alfalfa seedlings. Similarly, biotransformation and accumulation of Ag(I) and Pt(II) ions into Ag and Pt NPs in alfalfa and mustard seedlings were reported.^{58,59} These studies strongly suggest that Au, Ag, and Pt NPs can be produced/accumulated in alfalfa and mustard seedlings.

Storage of NPs in Plants. There are few issues arising from the observation that nanoparticles are accumulated in plants. One important question that demands attention is how and where within the plants the absorbed nanoparticles are stored. At the present time there are no specific studies addressing this gap. Available literature indicated vaguely that NPs are found in the plant's cells and tissues, although one of the above-mentioned studies with SWCNTs in *N. tabacum* plant cell suspensions found their fate in vacuoles (SWNTs–FITC) as well as cytoplasmic strands (SWCNT–DNA).²² A study on the reduction of Ag ions into Ag NPs and their accumulation in alfalfa showed that Ag NPs could accumulate on the surface of root cell organelles.⁵⁹ However, Gardea-Torresdey et al.⁵⁷ showed the presence of Ag NPs in alfalfa stems. One more issue corollary to the accumulation of NPs in edible plants is their transmission to the plant's next generation. Lin et al.⁴⁰ reported that C_{70} was detected, although much less frequently, in the leaf tissues of second-generation rice plants. If NPs are found in the second-generation plants, there is the possibility that these plants become adapted and more responsive, accumulating more of the respective NPs. Another important issue is the bioavailability of the accumulated NPs to the next trophic level, for example, in humans and ruminants. There are studies showing that NPs in algae and tobacco are transmitted to the next trophic level.^{20,60}

CELLULAR TOXICITY, GENOTOXICITY, AND TRANSMISSION OF ENGINEERED NANOMATERIALS IN EDIBLE PLANTS

Characteristics such as small size, shape, and larger surface area to mass ratio of NPs have created a new dimension in current science and technology with their varied applications in medicine, drug development, and many more applications. On the other hand, the propensity of the NPs to cross cell barriers and their interactions with intracellular structures owing to their small size and high surface reactivity contribute to potential cellular and genetic toxicity by the induction of oxidative stress.^{61,62}

Very few studies have been conducted on the genetic response rendered by the NPs on edible plants. Plants with low numbers of chromosomes have been considered to be excellent cytogenetic systems with a wide range of genetic end points, from gene

mutation to mitotic and meiotic chromosome aberrations, alterations in ploidy, sister chromatid exchanges, and DNA damage.⁶³

Tan et al.^{35,36} reported that MWCNTs reduced the cell density on cultured rice cell suspensions in a dose-dependent manner. At lower concentration of MWCNTs cell death was predominantly noted to be caused by apoptosis; however, at higher concentration cell mortality was attributed to necrosis identified by leakage of cytoplasmic content and membrane disruption. On the better side, the rice cells in the suspensions were observed to demonstrate self-defense response, when exposed to MWCNTs, by sacrificing a small population of cells that aggregates with the NPs and precipitates. This protected the remainder of the cells in culture.³⁵

The studies by Shen et al.²⁷ in rice and *Arabidopsis* protoplast cells well established that the nanosize and the concentration of the SWCNTs were responsible for potential cytotoxicity. Abundant endonucleolytic cleavage of DNA was evident in the *Arabidopsis* cells, proving the genotoxic potential of the SWCNT in plant systems. Other studies³² have shown that in *A. cepa*, the more water-soluble and small-sized fullerol $C_{60}(OH)_{20}$ produced more cell damage than the fullerene C_{70} suspended in natural organic matter (C_{70} –NOM). The cell damage was also attributed to the aggregation of the NPs, leading to blockage of the apoplastic pathway, which is a probable route of uptake of these NPs in the plant tissues. Lin et al.⁴⁰ studied the generational transmission of C_{70} –NOM in rice plants and established the presence of black aggregates of C_{70} in the leaves of the second generation of the plants treated with the fullerenes only in their first generation. Although the study at the molecular level was not included, the data highly suggest that the first-generation plants had taken up and genetically transmitted the NPs to their next generation.

Studies on the cytotoxicity of metal oxide NPs only include a recent report by Lopez-Moreno et al.⁴² with CeO_2 and ZnO NPs in soybean seedlings. The genotoxicity of both NPs was investigated by detecting new DNA bands using the random amplified polymorphic DNA (RAPD) assay. A new DNA band in the RAPD profile of soybean roots treated with ZnO NPs at 4000 mg L^{-1} was detected. According to Lopez-Moreno et al.⁴² the toxicity may rise either due to the interaction of the DNA with the Zn ions leached out from the ZnO NPs or with its direct interaction with the ZnO NPs. However, the absence of ZnO NPs in plant tissues, as shown by the XANES results, failed to conclude on the potent reason behind genotoxic response in soybean. On the other hand, the RAPD profile of soybean roots treated with CeO_2 at 2000 and 4000 mg L^{-1} showed four and three new bands, respectively. Thus, cubic CeO_2 NPs (7 nm) were shown to affect the genetic stability to a greater extent in comparison with a slightly larger sized hexagonal ZnO NPs (8 nm). Apart from the size and shape of the NPs, the differential genotoxic response may also be attributed to the fact that CeO_2 NPs were shown by the XANES spectra within the tissues and, unlike Ce, Zn is an essential element used in several biochemical processes, as established by several researchers.^{64–67}

TiO_2 NPs (~100 nm in size) were found to be genotoxic as well as cytotoxic in plant systems.⁶⁸ In their study, Ghosh et al.⁶⁸ found an initial increase in the extent of DNA damage in *A. cepa* (~3.5-fold increase at 4 mM concentration) followed by a gradual decrease until the highest selected concentration (10 mM). The decrease was explained by the precipitation of NMs at high concentrations. The genotoxic potential of TiO_2 in this plant was confirmed by comet assays and DNA laddering

Table 1. Positive or No Consequential Effects of Nanoparticles in Food Crops

nanoparticle	particle size (nm)	plant	concentration	observed toxicity	ref
zero-valent Fe		flax, red clover, white clover, meadow fescue	100, 250, 500 mg L ⁻¹	no effect on germination	82
		barley, ryegrass	100, 250 mg L ⁻¹	no effect on germination	82
Al	1–100	radish, rape, lettuce, corn, cucumber	2000 mg L ⁻¹	no effect on germination	16
		red kidney beans, ryegrass	10, 100, 1000, 10000 mg L ⁻¹	no observed toxicity	55
		radish, rape	2000 mg L ⁻¹	improved root growth	16
Ag	20	flax	20, 40, 60, 80, 100 mg L ⁻¹	no effect on the germination	82
	2	cucumber, lettuce	62, 100, 116 mg L ⁻¹	low to zero toxicity	18
Au	10	cucumber, lettuce	62, 100, 116 mg L ⁻¹	positive effect on germination index	18
Si		zucchini	1000 mg L ⁻¹	no effect on the germination	52
Cu		lettuce	0.013% (w/w)	no effect on the germination; improved shoot/root ratio	84
dodecanethiol-functionalized Au		lettuce	0.013% (w/w)	no effect on the germination; improved shoot/root ratio	84
Pd entrapped in Al(OH) ₂ matrix		lettuce	0.013–0.066% (w/w)	no effect on the germination; improved shoot/root ratio	84
3-amino-functionalized SiO ₂		lettuce	0.013–0.066% (w/w)	no effect on the germination; improved shoot/root ratio	84
CeO ₂	7	corn, alfalfa, soybean	500, 1000, 2000, 4000 mg L ⁻¹	significantly increased root and stem growth	50
	<25	wheat	100 mg L ⁻¹		30
ZnO	8	soybean	500 mg L ⁻¹	increased root growth	42
Al ₂ O ₃		radish, rape, ryegrass, lettuce, corn, cucumber	2000 mg L ⁻¹	no effect on germination	16
Fe ₃ O ₄	20	pumkin	500 mg L ⁻¹	no toxic effect	44
	7	cucumber, lettuce	62, 100, 116 mg L ⁻¹	low to zero toxicity	18
TiO ₂	<100	wheat	100 mg L ⁻¹		30
nanoanatase (TiO ₂)	4–6	spinach	0.25%	enhanced rca mRNA expressions (51%), protein levels (42%), activity of Rubisco activase, Rubisco carboxylation, the rate of photosynthetic carbon reaction, single plant dry weight, chlorophyll content	91
	5	spinach	0.25%	improved spinach growth related to N ₂ fixation by TiO ₂	92
	5	spinach	0.25%	improved light absorbance, transformation from light energy to electron energy, and active chemical energy, and promoted carbon dioxide assimilation	93

Table 1. Continued

nanoparticle	particle size (nm)	plant	concentration	observed toxicity	ref
rutile (TiO ₂)		spinach (naturally aged)	0.25–4%	increased germination and germination and vigor indices, plant dry weight, chlorophyll formation, ribulosebisphosphate carboxylase/oxygenase activity, photosynthetic rate	86
		spinach	0.25–4%	promoted photosynthesis, the rate of evolution of oxygen in the chloroplasts was accelerated	87
Ni(OH) ₂	8.7	mesquite	2 mg L ⁻¹	no effect	22
mixture of SiO ₂ /TiO ₂		soybean		increased germination and shoot growth; increased nitrate reductase activity; increased absorption and utilization of water/fertilizer; enhanced antioxidant system	85
mixture of Au/Cu		lettuce	0.013% (w/w)	no effect on the germination; improved shoot/root ratio	84
multiwalled carbon nanotube		tomato	10–40 mg L ⁻¹	significant increase in germination rate, fresh biomass, and length of stem significantly enhanced moisture content inside tomato seeds	29
		radish, rape, ryegrass, lettuce, corn, cucumber	2000 mg L ⁻¹	no effect on germination	16
		ryegrass	2000 mg L ⁻¹	increased root length	16
		zucchini		no effect on the germination	52
	internal dimension: 110–170	wheat	100 mg L ⁻¹	no significant effect on root or shoot growth	30
single-walled carbon nanotube	8	onion, cucumber	104, 315, 1750 mg L ⁻¹	significantly increased root length	34
	8	cabbage, carrot, lettuce	104, 315, 1750 mg L ⁻¹	no effect	34
functionalized single-walled carbon nanotube	8	cabbage, carrot, tomato, onion, lettuce	9, 56, 315, 1750 mg L ⁻¹	no effect	34

technique. The presence of chromosomal aberrations and interphase micronuclei in *A. cepa* plants validated the occurrence of cellular fragmentation in the previous cell cycle. The genotoxic and cytotoxic effects observed were well correlated with the results of the generation of superoxide radicals resulting in lipid peroxidation in the *A. cepa* cells.

The literature on the cytotoxicity of metallic NPs on edible plants includes only Ag and Fe NPs. In *A. cepa* root meristem cells, Ag NPs have been reported to possess mitodepressive, mitoclastic, and clastogenic properties.^{63,69} A dose-dependent decrease has been noted in the frequency of mitotic index in the Ag NP treated *A. cepa* cells from 60.30% (control) to 27.62% (100 mg of Ag NPs L⁻¹).⁶³ Babu et al.⁶⁹ noted dose- as well as duration-dependent decreases in the mitotic index in response to Ag NPs in *A. cepa* root meristems. This mitodepressive and cytotoxic response may be attributed to the inhibition of DNA

synthesis at S-phase of the cell cycle.⁷⁰ Kumari et al.,⁶³ on treating the *A. cepa* cells with various concentrations of Ag NPs, noted different kinds of chromosomal aberrations such as stickiness, chromosomal breaks, gaps, disturbed metaphase, and cell wall disintegration at different doses. In a similar in vivo cytogenetic assay carried out by Babu et al.,⁶⁹ various types of chromosomal and mitotic abnormalities such as fragments, C-metaphase, sticky metaphase, laggard chromosomes, anaphasic bridge, and disturbed anaphase were identified in the treated meristem cells. The interaction of Ag NPs with the tubulin SH group may be responsible for the ineffective mitotic spindle function.⁷⁰ As explained by Kumari et al.,⁶³ the stickiness in the metaphase and anaphase stages may be attributed to degradation or depolymerization of chromosomal DNA or by intermingling of interchromosomal chromatin fibers, which leads to sub-chromatid connections between chromosomes.^{71–73} The

Table 2. Negative Effects of Nanoparticles on Different Food Crops

nanoparticle	particle size (nm)	food crop	concentration (mg/L)	growth media	observed toxicity	ref	
zero-valent Fe		flax, barley, ryegrass	2000, 5000	aqueous suspension	completely inhibited germination	82	
		barley	300	sandy soil	reduced germination	82	
		flax, barley, ryegrass	>1500	sandy and clay soil	no germination	82	
Ag (colloid)	0.6–2	ryegrass	10	aqueous suspension (0.1% v/v Tween 20)	reduced germination (20%)	82	
		ryegrass	20	aqueous suspension (0.1% v/v Tween 20)	reduced germination (50%)	82	
		flax, ryegrass	10	aqueous suspension (0.1% v/v Tween 20)	reduced shoot length	82	
		barley, ryegrass, flax	20	aqueous suspension (0.1% v/v Tween 20)	reduced shoot length	82	
Ag	5	barley	10	aqueous suspension (0.1% v/v Tween 20)	reduced germination	82	
		flax, barley	10	aqueous suspension (0.1% v/v Tween 20)	reduced shoot length	82	
Ag	20	barley	10	aqueous suspension (0.1% v/v Tween 20)	reduced germination	82	
		barley	10	aqueous suspension (0.1% v/v Tween 20)	reduced shoot length	82	
		barley, ryegrass	20	aqueous suspension (0.1% v/v Tween 20)	reduced shoot length	82	
Ag	100	zucchini	100, 500, 1000	25% Hoagland solution	reduced transpiration (41–79%)	52	
	100	zucchini	500, 1000	25% Hoagland solution	reduced biomass (57–71%)	52	
Ag	<100	onion	100	aqueous suspension	decreased mitosis; disturbed metaphase; sticky chromosome; cell wall disintegration and breaks	63	
Cu		mungbean	<200	agar culture media	reduced seedling growth	23	
		mungbean	800	agar culture media	reduced shoot growth	23	
		wheat	<200	agar culture media	reduced root and seedling growth	23	
		50	zucchini	1000	25% Hoagland solution	reduced biomass (90%)	52
		50	zucchini	1000	aqueous suspension	reduced root growth	52
Si	10	zucchini	1000	aqueous suspension (0.2% sodium dodecyl sulfate)	completely inhibited germination	52	
Al		ryegrass	2000	aqueous suspension	decreased root length	16	
		ryegrass	2000	aqueous suspension	reduced germination	16	
		corn, lettuce	2000	aqueous suspension	reduced root length	16	
Zn		radish, rape, ryegrass, lettuce, corn, cucumber	2000	aqueous suspension	highly reduced root growth	16	
ZnO	9–37 (mean: 19 ± 7)	ryegrass	1000	1 strength Hoagland solution	reduced biomass, shrank root tips, epidermis and rootcap were broken, highly vacuolated and collapsed cortical cells	43	

Table 2. Continued

nanoparticle	particle size (nm)	food crop	concentration (mg/L)	growth media	observed toxicity	ref
		corn	2000	aqueous suspension	reduced germination	16
		radish, rape, ryegrass, lettuce, corn, cucumber	2000	aqueous suspension	highly reduced root growth	16
	5	zucchini	1000	25% Hoagland solution	reduced biomass (78–90%)	52
	8	soybean	2000, 4000	aqueous suspension	decreased root growth	50
CeO ₂	7	alfalfa	1000, 2000	aqueous suspension	slightly reduced shoot growth	50
		tomato	2000	aqueous suspension	significantly reduced shoot growth (30%)	50
		cucumber	2000	aqueous suspension	reduced shoot growth (20%)	50
		maize	500, 1000, 2000	aqueous suspension	significantly reduced shoot growth (30%)	50
		alfalfa	500	aqueous suspension	significantly reduced the biomass	50
		maize	500–2000	aqueous suspension	reduced germination	50
		maize	4000	aqueous suspension	reduced root growth	50
		tomato, cucumber	2000	aqueous suspension	reduced germination	50
		tomato	1000–2000	aqueous suspension	reduced root growth	50
		alfalfa	2000–4000	aqueous suspension	reduced root growth	50
		soybean	2000	aqueous suspension	reduced germination	50
Al ₂ O ₃	13	maize, cucumber, carrots, cabbage	2000	aqueous suspension	reduced root growth	83
		corn	2000	aqueous suspension	reduced root length	16
single-walled carbon nanotube	1.19 (major), 18, 722 nm	rice	400	half-strength Murashige and Skoog basal medium	delayed flowering, decreased yield	40
	8	tomato	104, 315, 1750	suspension (35:65 w/w CNT:poly-3- aminobenzoic acid)	most sensitive in root reduction	34
functionalized carbon nanotube	8	lettuce	104, 315, 1750	suspension (35:65 w/w CNT:poly-3- aminobenzoic acid)	reduced root length at longer exposure	34
multiwalled carbon nanotube		zucchini	1000	25% Hoagland solution	reduced biomass (38%)	52
		lettuce	2000	aqueous suspension	reduced root length	16
	diameter range: 10–30	rice	20, 40, 80	Murashige and Skoog basal medium	chromatin condensed inside the cytoplasm and caused cell death, plasma membrane detachment from cell wall and cell shrinkage	36
TiO ₂ /inorganic bentonite clay	30/1–60	maize	300, 1000	0.1 strength Hoagland solution	inhibited hydraulic conductivity, leaf growth, and transpiration	41

induction of chromosomal breaks and micronuclei by Ag NPs indicates the clastogenic potential of the xenobiotic, which may lead to a loss of genetic material.⁷⁴ Thus, these may be regarded as end points of irreversible genotoxicity on the chromosomes. Although the genotoxicity of the Ag NP is well established, the causative mechanism has not been reflected in this study. Moreover, no studies have been performed to clarify if the toxicity is caused by the Ag NPs themselves or the Ag(I) ions released from the NPs in the external/biological media.

The water-based magnetic NPs (50–300 $\mu\text{L/L}$) coated with perchloric acid have been reported to decrease the nucleic acid level in the cells of corn, revealing an inhibitory effect on biosynthesis.⁷⁵ In a related study, treatment with magnetite NPs coated with β -cyclodextrin ($\text{C}_{42}\text{H}_{70}\text{O}_{35}$) as stabilizer resulted in increased total prophase, metaphase, anaphase, and telophase at high volume fractions of the fluid, coupled with a linear increase in mitotic index with increasing volume fraction of magnetite NPs.⁷⁵ The coatings on the magnetic NPs prevent close approach of the nanomagnetic cores, reducing

aggregation via van der Waals or magnetic attractions. Răcuciu and Creangă⁷⁶ hypothesized that the ferrophase might have penetrated the nuclear membrane, and the extranuclear DNA from the chloroplasts is the one of the most probable targets of magnetic fluids. The magnetic NPs were also reported to induce chromosomal aberrations and perturbation of the proliferative capacity.

It is not clear from the above-mentioned studies whether the genotoxicity in plants is caused by the NPs themselves or their biotransformation within the plants. Therefore, more research needs to be focused on the differences in toxicity of MB NPs with their respective bulk counterparts, the increased concentration of the element if it is an essential micronutrient, and the effect of the ions produced inside or outside the organism exposed to the NPs. Moreover, the factors associated with the varied toxicological responses of different plant species to different NPs have not, yet, been well explored.

■ FACTORS AFFECTING THE TOXICITY OF NANOPARTICLES IN EDIBLE PLANTS

The toxicity of NPs in edible plants has been discussed in several reviews.^{77–80} Studies revealed that not all plants treated with NPs manifested toxicity effects; in fact, more studies revealed positive or no consequential effects in plants (Table 1). However, caution must be observed in making conclusions about the effects of particular NPs. Physiological and visual toxicological effects in plants might not be a sensitive indicator of toxicity. Studies at proteomic, genomic, and metabolic levels are needed.

Currently, there is no definite mechanism on the toxicity of NPs in plants. Many researchers believe that the observed toxicity of NPs in plants is based on plant–NP physical interactions. The presence of NPs on the root surface could alter the surface chemistry of the root such that it affects how the roots interact with their environment.³⁴ Studies revealed that plant development is negatively affected because NPs clog the root openings and both hydraulic conductivity and nutrient uptake in roots are inhibited.⁴¹

Studies on NPs in food crops revealed that various factors influence toxicity and that, at present, no general trend could be elucidated. On the basis of several studies, the following are the principal factors that influenced toxicity in agricultural food crops: concentration of NPs, particle size and specific surface area, physicochemical properties of NPs, plant species, plant age/life cycle stage, growth media, NP stability, and dilution agent.⁸¹

The size of seeds could render more sensitivity to NP exposure.^{34,82} This is because a large-seeded species (e.g., cucumber) has a lower surface to volume ratio than a small-seeded species (e.g., tomato). SWNTs showed higher toxicity effects in smaller-seeded species (lettuce, onion, and tomato) than in large-seeded species (cucumber).^{34,80} However, a clear effect of the size of seeds on the toxicity of NPs in plants cannot be confirmed at this time.^{16,23,50}

Lee et al.²³ reported that mungbean was more sensitive than wheat to Cu NPs toxicity, probably due to differences in root anatomy because xylem structures determine the speed of water transport and different xylem structures may demonstrate different uptake kinetics of NPs.⁷⁸ Mungbean is a dicot with one large primary root and several smaller lateral roots, whereas wheat is a monocot with numerous small roots without a primary

root. However, generalization on whether the toxicity is based on dicot or monocot classification cannot be made.^{16,50,82}

Solvent was reported to affect the toxicity of NPs. In the study conducted by Barrena et al.,¹⁸ it was observed that the effect of nanoparticles–solvents was sometimes more significant than that of the NPs themselves. Three MB NPs (Au, Ag, and Fe₃O₄) demonstrated low to zero toxic effects on lettuce and cucumber, and effects could be primarily due to the presence of stabilizers. The very small size of NPs is believed to cause higher toxicity in plants. This was generally observed in many studies. Various sizes of silver NPs were tested, and results showed that Ag_{colloid} (0.6–2 nm) had greater toxicity in flax, barley, and ryegrass than Ag NPs of 5 and 20 nm.⁸² The particle surface characteristic was also an important factor in nanoparticle toxicity.⁸³

The concentration of NPs is another major factor affecting toxicity in food crops. On the basis of the macroscopic standard phytotoxicity tests (germination/root elongation or vigor test), it is possible to conclude that food crops tested required high concentrations of NPs (1000–4000 mg L⁻¹) before toxic effects began to manifest (Table 2). Zero-valent Fe NPs completely inhibited germination of ryegrass, flax, and barley at very high concentrations (2000 and 5000 mg L⁻¹). Likewise, ZnO NPs at 1000 mg L⁻¹ caused the death of almost all living cells at the root tip of ryegrass.⁴³ Cu NPs reduced root and seedling growth of wheat only at a relatively higher concentration (<200 mg L⁻¹). These observations raise important considerations in future toxicity studies. First, high concentrations of NPs may not be realistic because they are not commonly found in the natural environment. Second, if high concentrations are required for NPs to exhibit toxicity at hydroponic setup, a much higher concentration might be needed to cause toxicity in the natural environment such as the soil. In fact, toxicity studies conducted in different soil media required higher amount of NPs to induce toxicity in plants.^{55,82,84} Finally, most plants showed visible signs of recuperation from NP toxicity, indicating that toxicity was temporary.

The above-mentioned studies showed that NP phytotoxicity studies are at the very beginning. Studies must be conducted for a longer duration to investigate if plants that initially exhibited toxicity will recover after some time. Toxicity studies covering the whole life cycle of a plant similar to that conducted by Lin et al.³⁷ are much desired to determine the long-term effect of nanoparticle toxicity. It may also be recommended to conduct toxicity studies in soil media because NPs are likely to react with the constituents of environmental matrices that will enhance, lessen, or modify the toxic effect of NPs. Also, because germination and root and shoot growth assays appeared to be poor indicators for phytotoxicity studies of NPs, toxicity indicators based on biological markers, plant defense mechanism, changes in plant integrity at cellular or genetic levels tested periodically during the plant's life cycle would be more appropriate. Ma et al.⁷⁸ pointed out that one of the most urgent needs in plant–NP interaction studies is to determine what the genetic response of plants is and what genes are up-regulated/down-regulated in plants exposed to NPs. Furthermore, elucidation of mechanisms of toxicity and genotoxicity remains an unexplored field of study.

■ BIOTRANSFORMATION OF NANOPARTICLES IN EDIBLE PLANTS

Very few references were found on the biotransformation of NPs in plants. The differential biotransformation of ZnO and

CeO₂ NPs in soybean was investigated by Lopez-Moreno et al.⁴² CeO₂ NPs were taken up by soybean and did not undergo biotransformation. In the case of ZnO, results revealed that Zn was found inside the soybean plant only at Zn²⁺ oxidation state. Because no ZnO NPs were detected inside the plant and the Zn²⁺ ions released from ZnO NPs in the hydroponic solution were too low (8–25 mg L⁻¹ for the concentrations of 500–4000 mg L⁻¹) to cause a spike in Zn concentration inside the plant, it was hypothesized that ZnO NPs became transformed on the root surface. Recent studies in our laboratory on the biotransformation of ZnO NPs in mesquite also indicated that the NPs were transformed on/in the root surface. An interesting XAS study by Parsons et al.²² showed that mesquite plants treated with Ni(OH)₂ NPs had Ni(OH)₂ in the roots but the XAS spectra showed Ni²⁺ in the shoots and leaves, demonstrating the biotransformation of the Ni(OH)₂ NPs by mesquite. Stampoulis et al.⁵² reported that Ag was found at a greater concentration in zucchini grown in Ag NPs than in those cultivated in Ag bulk solution. Similarly, a study showed an increase in Al concentration in ryegrass treated with Al NPs⁵⁵ and Cu concentration in mungbean and wheat treated with Cu NPs.²³ However, no test was done to determine whether the Ag, Al, or Cu in the plant existed as NPs or ions; thus, no definite conclusion could be made on the basis of the reported studies.

In the case of other nanoparticles (C₇₀, SWCNTs, MWCNTs, Fe₃O₄, and TiO₂ NPs), biotransformation was not observed.^{37,40,44,52} These studies revealed that NPs can undergo biotransformation or just be accumulated in plants. There are limited studies in this field, and more questions on the mechanism of biotransformation, specific storage sites within the plant, transmission of NPs to the plant's next generation, long-term effects on the genetic integrity of the plant, and transfer of NPs in the food chain, among others, are still in need of research. Beyond that, it remains to be elucidated if those entities experiencing/not experiencing biotransformation have an adverse or beneficial effect on animal and human health.

■ EFFECTS OF THE NANOPARTICLES IN EDIBLE PLANT SPECIES

Carbon NMs. When discharged into environmental matrices, CNTs could be stabilized by NOM by averting the hydrophobicity,³⁹ with unknown consequences on the ecosystem.

According to the study of Lin and Xing,¹⁶ MWCNTs were reported to have no significant effect on seed germination of rape, radish, lettuce, corn, and cucumber at 2000 mg L⁻¹ after 5 days of treatment. Similarly, no phytotoxic symptoms or increased physiological responses were reported in the study of Wild and Jones³⁰ on living wheat roots. In tomato seeds, MWCNTs at 10–40 mg L⁻¹ accelerated seed germination and increased germination percentage rate and vegetative mass with respect to control (no CNTs).²⁹ This may be attributed to the increased water uptake induced by the CNTs as previously explained. On the other hand, SWCNTs and poly-3-aminobenzenesulfonic acid functionalized SWCNTs adversely affected root elongation in tomato. The result was explained by the high concentration of CNTs observed around the base of the apical meristem (where root elongation occurs) of tomato roots.³⁴ Lin et al.³⁷ reported that the C₇₀ and MWCNT (400 mg L⁻¹) suspended in NOM inhibited rice plant reproduction by delaying flowering by at least 1 month. Interestingly, it was also found that exposure to the MWCNT induced a self-defense and hypersensitive response in

the rice cells.^{35,36} Lin et al.³⁷ suggested that the presence of metallic impurities (residual metals used as catalysts for the synthesis of CNTs) may also be a factor contributing to the toxicity of MWCNTs.

Metal Oxide NPs. Studies on the toxicity of metal oxide NPs on crop plants are limited. So far, only Fe₃O₄, CeO₂, SiO₂, TiO₂, and ZnO have been studied in a few plant species. Furthermore, very few papers include plant life cycle studies. Accumulation of TiO₂ NPs in maize root cell walls was accompanied by a significant reduction in the cell pore diameter.⁴¹ This was shown to reduce the hydraulic conductivity in the primary roots, thereby leading to reduced transpiration and leaf growth. This is attributed to the physical interaction between the colloidal particles and a physical inhibition of the apoplastic flow through the cell walls, rather than toxic effects. In soybean, a mixture of SiO₂ and TiO₂ NPs increased the nitrate reductase activity, enhancing the uptake of water and fertilizer, stimulating the antioxidant system.⁸⁵ In spinach, TiO₂ NPs were reported to increase chlorophyll formation, photosynthesis, and plant dry weight, among others.^{86,87}

It seems that ZnO NPs do not affect seed germination in soybean even at the very high concentration of 4000 mg L⁻¹.⁴² In addition, experimental data have shown that soybean root elongation was promoted at 500 mg of ZnO NPs L⁻¹ but reduced at higher concentrations.⁴² This could be attributed to an excess of Zn ions released by the NPs or to an interaction between the NPs and the root surface. The interaction of ZnO NPs with plants could be influenced by the species of plants, as reported by Lin and Xing.⁴³ Besides the effect on seedling growth, ZnO NPs have been associated with cortical cells highly vacuolated and collapsed along with the shrinking and partial death of the vascular cells.⁴³

In contrast to ZnO NPs, the nanoceria were found to reduce seed germination in alfalfa, soybean, tomato, and cucumber at high concentration (4000 mg L⁻¹).⁵⁰ However, corn germination was significantly reduced even at the minimum concentration of 500 mg L⁻¹. Corn was seen to be more sensitive to ZnO and CeO₂ NPs, demonstrating toxicity symptoms even at concentrations that were not found to significantly affect other food and forage crops.^{16,50} Ce is generally precipitated as cerium oxide in cell walls and intercellular spaces of epidermal and cortical walls, and not in the growing zones. Thus, the authors hypothesized that the oxidative stress in the growing zone is reduced, promoting root growth. On the other hand, CeO₂ was found to inhibit root growth significantly in tomato and alfalfa seedlings at higher concentrations. This can be correlated with the very high amount of Ce found in the tissues of these plants (4000 mg of Ce kg⁻¹ DW for tomato and 6000 mg of Ce kg⁻¹ DW for alfalfa), which is not comparable to those of cucumber and soybean (≈400 and 462 mg of Ce kg⁻¹ DW). This may be responsible for inducing negative effects on root growth in tomato and alfalfa.

The toxicity of NPs depends upon the particle surface characteristics. For instance, alumina NPs were shown to inhibit root growth in corn, cucumber, soybean, cabbage, and carrot.⁸³ However, phenanthrene mitigated (changed the surface characteristics of the alumina NPs) the effect on cucumber root growth inhibition caused by uncapped alumina NPs.⁸⁸

The iron oxide NPs have been found to increase soybean pod and leaf dry weight.⁸⁹ Also, iron oxide NPs have been reported as facilitators for iron and photosynthate transfer to the leaves of peanut.⁹⁰ In the case of pumpkin, iron oxide NPs increased root elongation,⁴⁵ which was attributed to the Fe dissolution.

Although there was positive/no significant negative effect on pumpkin plants, the Fe₃O₄ NPs were found to induce oxidative stress and higher antioxidative enzyme activity than the bulk Fe₃O₄ particles.⁴⁵ The Fe₃O₄ NPs adsorbed on the root surface or absorbed by the roots were thought to disturb the metabolic activities in roots, leading to local instability of the cell wall and/or membrane, eventually producing oxidative stress.

Metallic NPs. References on the effect of metallic NPs are scarce. According to Stampoulis et al.,⁵² Si NPs at 1000 mg L⁻¹, in the presence of sodium dodecyl sulfate (SDS, surfactant to suspend the NPs in the solution), completely inhibited the germination of zucchini seeds (*C. pepo*), whereas Si NPs in the absence of SDS resulted in 80% germination. Reduction in germination was also noted in plants treated with MWCNT and Ag NPs, after excluding the additive effect on the inhibition of the SDS itself. As described in Tables 1 and 2, the effects of NPs thus depend upon the plant species, types of NPs, concentration, size, aggregation, functionalization, and experimental conditions including temperature and time, and method of exposure (seeds/seedlings/cell suspensions).^{90–93}

This literature review has confirmed that knowledge on plant toxicity of ENMs is at the foundation stage. Practically, there are no conclusive studies on the nanotoxicity; however, with the limited pieces of information, the new field of nanoecotoxicology has emerged to address the effects of ENMs on the living components of ecosystems.⁹⁴

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