Mohammad Faisal
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Phytotoxicity of Nanoparticles

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Editors

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ISBN 978-3-319-76707-9 ISBN 978-3-319-76708-6 (eBook)
https://doi.org/10.1007/978-3-319-76708-6

Library of Congress Control Number: 2018945882

Printed on acid-free paper

This Springer imprint is published by the registered company Springer International Publishing AG part of Springer Nature.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

In recent years, nanotechnology has exhibited exponential growth in various sectors to accomplish market commodities with higher prospective applications. The small size particles (nanomaterials) are rapidly being used in manufacturing of products of our daily life such as biosensors, cosmetics, food packaging, imaging, medicines, drug delivery, and aerospace engineering, etc., and these products are coming in the global market approximately at the rate of 3–4 per week. In spite of manifold benefits of the power of nanomaterials, there are open questions about how the small size materials affect the environment and human health, while very few reports are available on the hazards of nanoparticles. Compared to the bulk counterpart, the small size and large specific surface area of nanoparticles endow them with high chemical reactivity and intrinsic toxicity. Such unique physicochemical properties of nanoparticles draw global attention of scientists and environmental watchdogs to study potential risks and adverse effects of nanomaterials in the environment. Nanoparticle toxicity has pronounced effects and consequences not only for plants but also for the ecosystem in which the plants form an integral component. Plants growing in nanomaterial-polluted sites exhibit altered metabolism, growth reduction, lower biomass production, and nanoparticle accumulation, and these functions are of serious human health concern. Edible plants with excessive amounts of accumulated toxic nanoparticles are harmful not only to humans but also to the animals when used as animal feed. Nanoparticles adhere to plant roots and exert physical or chemical toxicity and subsequently cell death in plants. On the other hand, plants developed various defense mechanisms to counteract nanoparticle-induced toxicity. Only detailed study of these processes and mechanisms would allow researcher and student to understand the complex plant–nano interactions. However, there are several unresolved issues and challenges regarding the interaction and biological effects of nanoparticles. Therefore, the book was aimed to provide relevant state-of-the-art findings on nanoparticle toxicity, its uptake, translocation, and mechanism of interactions with plants at the cellular and molecular level. Being involved in this area we comprehend that information on the nanoparticle toxicity and their mechanism of interaction with plants is still obscure, and there is no single book available on this aspect.

The intended volume comprised several chapters on relevant topics contributed by experts working in the field of nanophytotoxicity so as to make available a

comprehensive treatise designed to provide an in-depth analysis of the subject in question? The book is a compilation of 18 chapters having relevant text, tables, and illustration describing the experimental work on nanomaterial-induced toxicity in plants and current trends reported and some general conclusions also drawn by the contributors. All the chapters have been organized in a way to provide crisp information on phytotoxicity of different types of nanoparticles. Special attention has been given to explore the uptake and mechanism of nanoparticle-induced toxicity and cell death in plants.

The book has been designed to serve as reference for scientist, researchers, and students in the fields of nanotoxicology, environmental toxicology, phytotoxicology, plant biology, plant physiology, plant biochemistry and plant molecular biology and who have interest in nanomaterial toxicity.

We are extremely thankful to all the contributors who wholeheartedly welcomed our invitation and agreed to contribute chapters to embellish toxicological information on nanoparticles (NPs), thus helping in this endeavor.

Riyadh, Saudi Arabia
March 2018

Mohammad Faisal
Quaiser Saquib
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Acknowledgments

We the editors are thankful and acknowledge the generous support established from contributors who spared precious time in scripting the chapters for this edited volume and also genuinely thank the publisher for their cooperation in making this book a reality. We thank the reviewers for their comments and suggestions, which have been very helpful for improving the manuscript. We express our heartfelt thanks to Mr. Eslam M. Abdel-Salam and Ahmad A. Qahtan for their assistance and generous help in editing of the manuscript. We express our special thanks to the King Saud University for all the support.

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Nanoparticle Uptake by Plants: Beneficial or Detrimental?

1

Ivan Pacheco and Cristina Buzea

1.1 Introduction

Nanoparticles can be defined as very small particles with size in the nanometer range. They can be as small as 1 nm and as large as hundreds of nm.

Due to their small size, nanoparticles can be internalized by plants, animals, and humans. Further, they can enter cells and organelles and affect cellular processes. Nanoparticles with selected compositions had shown some beneficial effects in selected plants, and, as a result, some scientists are promoting their use in agriculture. However, nanoparticles are phytotoxic for many other plants. In addition, nanoparticles are toxic to humans and animals, being associated to a multitude of diseases, ranging from respiratory and cardiovascular to neurological diseases. As a result of their toxicity, it is necessary to environmentally monitor man-made nanoparticles and to pass regulations and laws regarding the use and safe handling of nanoparticles.

What makes nanoparticles different from larger particles of the same material are surface and quantum effects (Buzea and Pacheco 2017). A material in nanoform exhibits different physical, chemical, and mechanical properties than the material in bulk form. Decreasing the size of a nanoparticle, the ratio between the atoms on its surface compared to those in its interior increases, leading to a smooth scaling of its physical and chemical properties. As a result, nanoparticles will have higher surface/volume ratio, increased chemical reactivity, and reduced melting point. Due to the

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_1

small size of a nanoparticle, its electrons become confined and will have a quantized energy spectrum, resulting in quantum size effects. An example of quantum size effect is the appearance of magnetic moments. For example, there are nanoparticles of materials nonmagnetic in bulk that, when in nanoform, develop magnetic moments. Among these are gold, platinum, and palladium.

Nanoparticles can have various sizes, morphologies, and crystallinities, as illustrated in Fig. 1.1. They can have a short aspect ratio, with spherical or cubic morphologies, or a long aspect ratio, in the form of tubes or long whiskers (Soto et al. 2005; Murr and Soto 2004; Rui et al. 2015; Qiu et al. 2010).

Nanotoxicology is a branch of toxicology that studies the toxicity of nanoparticles in humans and animals. It encompasses in vitro studies performed on animal and human cell lines, in vivo experiments on animals and humans, epidemiological data related to particle pollution, and occupational exposure studies of workers involved in handling nanoparticles (welding, mining, etc.).

Nanoparticles are being increasingly used in applications, including agriculture. However, many types of nanoparticles are proved to be toxic, despite the fact that the same material in bulk form is harmless. It is impossible to predict the toxicity degree of a nanoparticle type without experimental data. As most of the nanotoxicity studies are published in very specialized journals, the dissemination of information on nanoparticle toxicity is not readily available for the scientists that are starting to use these nanoparticles in applications, including agrichemicals.

The researchers working in their application in agriculture, being unaware of nanoparticle toxicity, are likely to suffer health effects in the coming years due to incorrect handling and inadvertently exposure to nanoparticles. Due to their small size, nanoparticles can easily become airborne and be inhaled and ingested and enter in contact with the skin. Secondly, the use of agricultural nanoparticles poses a risk for the population and ecosystem.

Having remembered asbestos and the severe health effects due to its use in construction, we would like to prevent a similar situation from happening. However, nanoparticles use in agriculture might pose a higher environmental and toxic threat than asbestos. Asbestos use was limited mainly to the construction industry, being confined to buildings, and is now relatively easy to remove. Nanoparticles used in agriculture will not be confined to a specific place; they will enter the atmosphere and become respirable particles, pollute the water, and lead to devastating consequences for humans and other life species.

This chapter will focus on evaluating the beneficial and detrimental effects of nanoparticles on plants together with their toxicity in humans and animals. Weighting the pros and cons will allow the reader to form an idea whether or not nanoparticles should be used in agriculture. We show research regarding nanoparticle uptake and accumulation in plants, together with phytotoxicity studies. We also show selected beneficial effects in some plants. Following are subchapters dedicated to toxic effects of nanoparticles in humans and animals together with comparative toxicity for various compositions. After reading this chapter, the reader should be informed on the pros and cons of using nanoparticles in agriculture and the environmental risks and toxicity that they will pose for life.

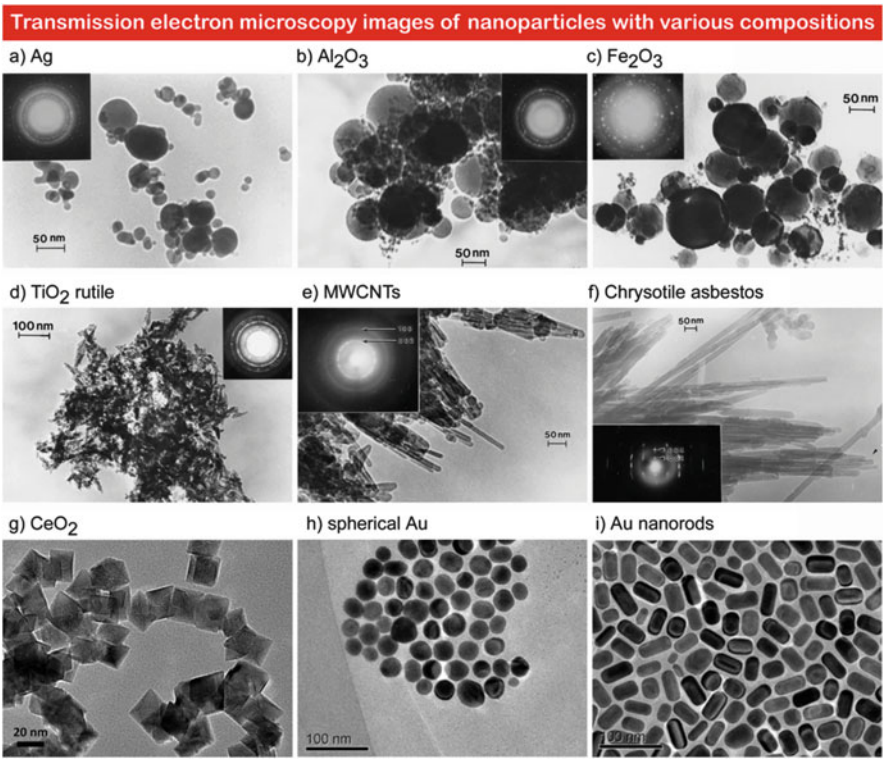

Fig. 1.1 Transmission electron microscopy images of nanoparticles of (**a**) Ag, (**b**) Al_2O_3, (**c**) Fe_2O_3, (**d**) TiO_2 rutile, (**e**) MWCNTs, and (**f**) chrysotile asbestos. Inserts are showing selected area electron diffraction (SAED) patterns that indicate the degree of crystallinity of nanoparticles. Notice the similarity between the morphology of MWCNTs and asbestos. Images (**a**–**d**) are reprinted from Soto K. F. et al. 2005. Comparative in vitro cytotoxicity assessment of some manufactured nanoparticulate materials characterized by transmission electron microscopy. Journal of Nanoparticle Research, 7, 145–169, with permission from Springer (Soto et al. 2005). Images (**e**–**f**) are reproduced from Murr L. E. & Soto K. F. 2004. TEM comparison of chrysotile (asbestos) nanotubes and carbon nanotubes. Journal of Materials Science, 39, 4941–4947. Copyright 2004 Kluwer Academic Publishers. With permission of Springer (Murr and Soto 2004). (**g**) CeO_2 nanoparticles. Reprinted from Environmental Pollution, vol. 198, Rui Y. et al., Transformation of ceria nanoparticles in cucumber plants is influenced by phosphate, pp. 8–14, Copyright (2015), with permission from Elsevier (Rui et al. 2015). (**h**) Gold nanospheres and (**i**) gold nanorods; images (**h**–**i**) adapted from Biomaterials, Vol 31, issue 30, Qiu Y. et al, Surface chemistry and aspect ratio mediated cellular uptake of Au nanorods, Pages 7606–7619, Copyright (2010), with permission from Elsevier (Qiu et al. 2010)

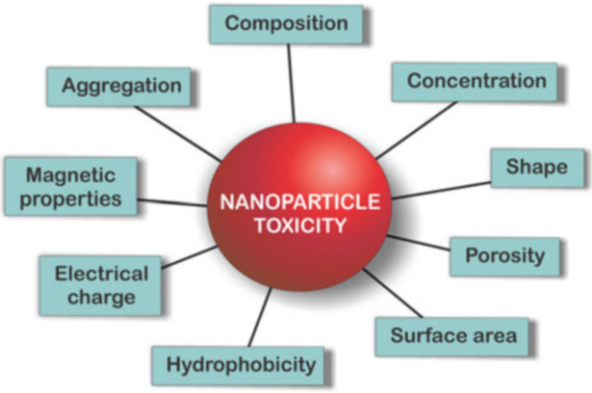

Fig. 1.2 Nanoparticle toxicity is determined by its physicochemical and morphological characteristics

1.2 Nanoparticle Physicochemical Properties

Nanoparticle interaction with their environment and uptake and toxicity in plants, humans, and animals depend on their size, aggregation, composition, concentration, shape, porosity, surface area, hydrophobicity, electrical charge, and magnetic properties, as illustrated schematically in Fig. 1.2.

It is important to note that nanoparticles suffer chemical transformation in the soil, within the plants, and within organisms in general. They are able to undergo various transformations, for example, acquiring a protein corona or changing their oxidation state, depending on their environment conditions. These transformations dictate ultimately their uptake, translocation, and toxicity. Even nanoparticles that may be considered stable are still able to change chemically, and their beneficial properties might become detrimental. For example, under hydroponic conditions Ce (IV)O_2 in cucumber plants is reduced to Ce(III) (Rui et al. 2015). CeO_2 nanoparticles in hydroponic cucumber plants treated with phosphate suffer chemical transformation, being located outside the epidermis, while in phosphate free plants, they were observed only in the intercellular spaces and vacuole of root (Rui et al. 2015).

1.3 Nanoparticles in Agriculture

1.3.1 Pesticides and Fertilizers

The topic of nanoparticle applications in agriculture emerged around the year 2000 (Gogos et al. 2012). Nanoparticles used in agriculture can be solid (such as metal and their oxides) or nonsolids (such as lipid or polymer) (Gogos et al. 2012). They are used for plant crop protection and for soil/water remediation (Fig. 1.3). Nanoparticles in plant protection are used as fungicides, herbicides, and insecticides, as depicted in Fig. 1.4. Nanoparticles can be the active ingredient or an additive that

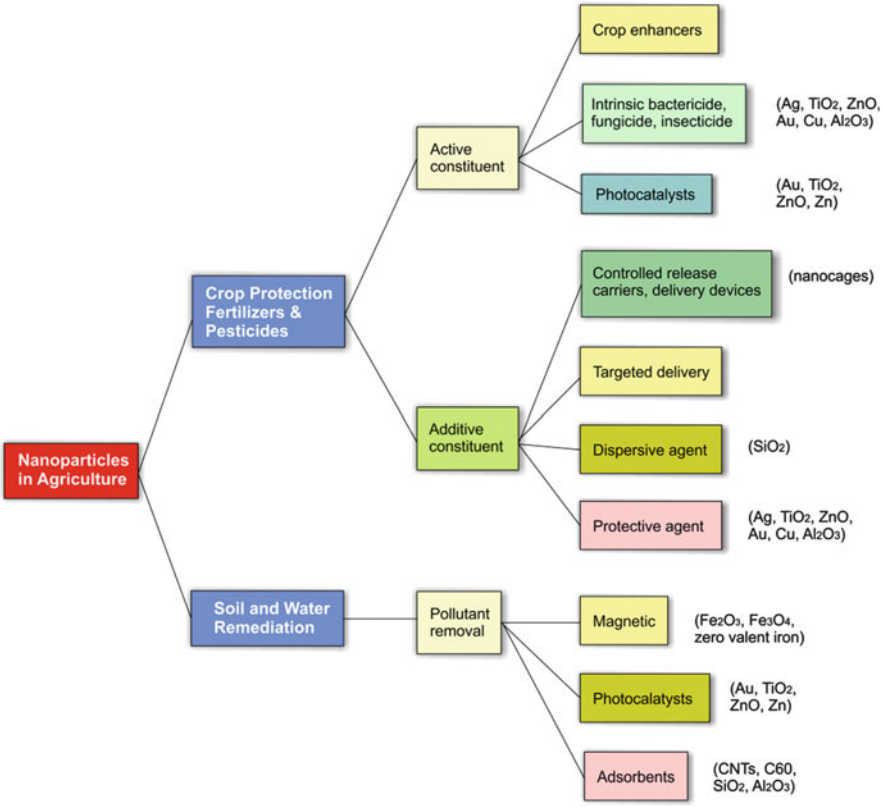

Fig. 1.3 Schematics for the applications of nanoparticles in agriculture for plant protection and soil and water remediation

can act for the controlled release of the main ingredient, as dispersing agent, targeted delivery agent, protective agent, or photocatalyst.

Figure 1.3 shows a schematic of nanoparticle function used in agriculture together with examples of nanoparticle compositions. Figure 1.4 shows comparative results of nanoparticles used in agriculture. Nanoparticles can act as active constituents and additives: they can serve as delivery devices that targeting specific tissues, nanopesticides (small particles of pesticides), and nanocages filled with pesticides act as controlled release devices. Nanoparticles themselves can have pesticidal properties when in nanoform, such as Ag, Au, TiO_2, Cu, and ZnO, several of these having photocatalytic properties. They can be pesticide additives that serve for enhancing the solubility of active ingredients. Some nanoparticles can also be used for soil and water remediation (Aragay et al. 2012). Due to their high surface area, adsorption capacity, and electromagnetic properties, nanoparticles are prospected for the adsorption of organic and inorganic pollutants from soil and water (Gupta and Saleh 2013). Among them are metal-containing particles, CNTs,

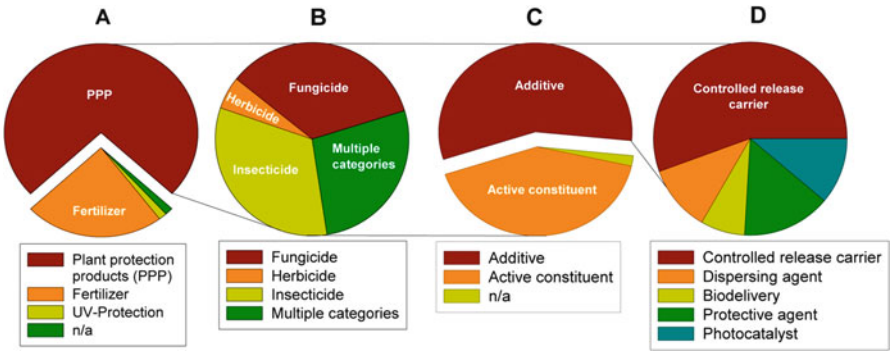

Fig. 1.4 Comparative results of nanoparticles used in agriculture. (**a**) Applications of nanoparticles in agriculture. (**b**) Types of plant protection products containing nanoparticles, (**c**) the function of nanoparticles within these products, (**d**) the role of the additive nanoparticles in plant protection products. Reprinted with permission from Gogos A. et al., Nanomaterials in Plant Protection and Fertilization: Current State, Foreseen Applications, and Research Priorities. Journal of Agricultural and Food Chemistry, vol. 60, pp. 9781–9792. Copyright (2012) American Chemical Society (Gogos et al. 2012)

C_{60}, and zeolites. Magnetic nanoparticles, such as iron oxide (Fe_3O_4) and zerovalent iron, are unique agents for water treatment (Xu et al. 2012; Deng et al. 2014). Magnetic nanoparticles are used for selected pollutant removal. Heavy metal pollutants are adsorbed by nanoparticles of Fe_2O_3, Fe_3O_4, SiO_2, and Al_2O_3 (Bakshi et al. 2015).

Some nanoparticles are found to be beneficial for plant protection and growth of selected plants, as discussed in Sect. 1.6. Unfortunately, the same types of nanoparticles are shown to be toxic to animals, humans, and some plants, such as carbon nanotubes (CNTs), Ag, titanium dioxide (TiO_2), silica (SiO_2), and alumina (Al_2O_3), as seen in Sect. 1.7.

The use of nanoparticles in agriculture should be limited by legislation. Very concerning is the increasing number of patents being filed related to nano-agrichemicals. The buildup of nanoparticles in plants, soil, water, and the environment, their trophic transfer, will detrimentally and irreversibly affect the health of humans and animals as well as plants. Many nanoparticles are shown to enter edible plants, and once they are in the food chain, they are likely to cause adverse health effects. It is imperative that regulatory agencies address and control the utilization of nanoparticles in agriculture (Kookana et al. 2014).

The reader interested in finding out more details about nanoparticles used in agriculture as agrichemicals, crop enhancers, crop protection, and soil and water remediation, can research the following reviews: Iavicoli et al. (2017), Khot et al. (2012), Liu and Lal (2015), Servin et al. (2015), Deng et al. (2014), Aragay et al. (2012), Gogos et al. (2012), Kah and Hofmann (2014), Ruttkay-Nedecky et al. (2017), and Wang et al. (2016).

1.3.2 Nanoparticle Soil Interaction and Accumulation

The use of nanoparticles in agriculture results in their accumulation in soil and the environment in general as well as trophic transfer. We must specify that when speaking about soil and nanoparticles, we are referring to man-made nanoparticles. Within the soil there are a multitude of natural nano- and microparticles, some of them having beneficial properties for the soil fertility. For example, clay nanoparticles may prevent leakage of nutrients in the groundwater by forming electrostatic bonds with them (Bernhardt et al. 2010).

Several types of nanoparticles are known for their antibacterial properties; hence their availability in soil is likely to affect soil bacteria, which are essential for their role in various ecosystems (Dinesh et al. 2012). The negative effects on endophytic bacteria symbionts are of special concern (Deng et al. 2014). Nanoparticles in soil will modify their properties in a dynamic manner, affecting their aggregation, dispersibility, dimensions, surface area, charge, and chemistry, which will affect their transport and availability.

Nanoparticle interaction with the soil and their bactericidal properties depends on the soil properties (Bakshi et al. 2015; Layet et al. 2017; Schlich and Hund-Rinke 2015). For example, silver nanoparticle toxicity against ammonia-oxidizing bacteria decreases for soils with higher clay content and larger pH. As a result, the toxicity of nanoparticles on plants may be affected by the soil type (Josko and Oleszczuk 2013).

The existence of nanoparticles with bactericidal properties in soil is likely to affect plants. It was found that the exposure of legumes to some nanoparticles severely lowers nitrogen fixation due to their bactericidal effects. Soybean plants exposed to ceria nanoparticles have a reduced nitrogen fixation correlated with almost absent bacteroids in its nodules (Priester et al. 2012).

1.4 Nanoparticle Uptake in Plants

1.4.1 Nanoparticle Uptake Routes

The interaction of nanoparticle with plants is a relatively new field of study. Nanoparticle uptake is plant specific. While the topic of uptake and transport of nanoparticles within plants is still not entirely understood, there is a consensus that it depends on the type of nanoparticle, their physicochemical properties, plant species, and the plant substrate—soil, hydroponics, or culture medium (Arruda et al. 2015; Aslani et al. 2014; Bakshi et al. 2015; Bernhardt et al. 2010; Chichiricco and Poma 2015; Deng et al. 2014; Dietz and Herth 2011; Ma et al. 2015; Miralles et al. 2012a, b; Navarro et al. 2008; Rico et al. 2011; Yadav et al. 2014; Schwab et al. 2015; Zuverza-Mena et al. 2017; Reddy et al. 2016).

It is known already that some nanoparticles translocate within the plants by forming complexes with membrane transporter proteins or root exudates (Yadav et al. 2014). Nanoparticle properties, such as size, porosity, hydrophobicity, and

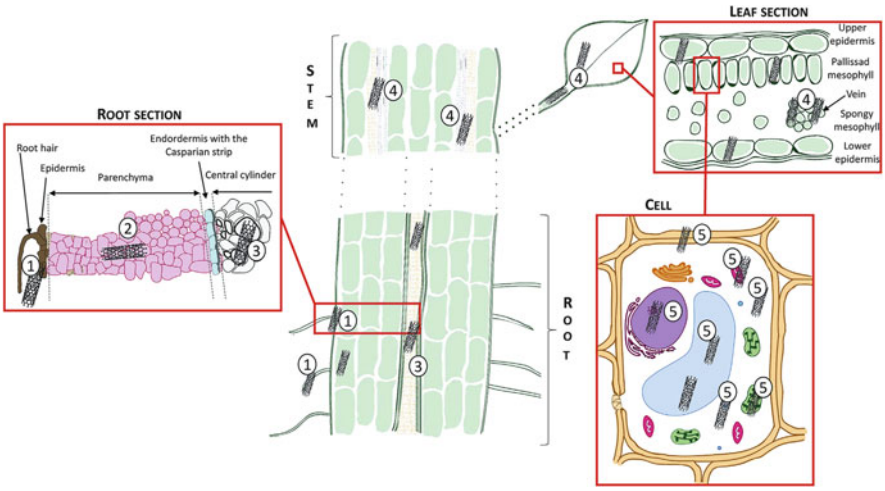

Fig. 1.5 Schematics of the uptake and translocation of CNTs in plants. Image not at scale. Within the cell blue represents vacuole; green, chloroplasts; purple, nucleus; orange, smooth endoplasmic reticulum; blue, plasmode. *1*. The uptake of CNTs by plant roots can occur via osmotic pressures, capillary forces, pores on cell walls, intercellular plasmodesmata, or through direct penetration. *2*. Endocytosis allows CNTs to cross both cell wall and cell membrane. *3*. CNTs may use the vascular system together with water and nutrients and can translocate to the upper parts of the plants. *4*. CNTs may reach the upper part of plants. Their preferential location in leaves is the xylem. *5*. Inside the cells CNTs can be found in cytoplasm, cell wall, cell membrane, chloroplast, mitochondria, and plasmodes. Reprinted from Carbon, vol. 123, Line C. et al., Carbon nanotubes: Impacts and behaviour in the terrestrial ecosystem—A review, pp. 767–785. Copyright (2017), with permission from Elsevier (Line et al. 2017)

surface, are modulating the interaction of nanoparticles with plants. A schematic of nanoparticle uptake in plants is shown in Fig. 1.5 (Line et al. 2017).

Roots can uptake small nanoparticles through pores (with size around 5–20 nm) within the root epidermal cell walls—called the apoplastic route (Deng et al. 2014). Particles larger than the pore size will be stopped. Small nanoparticles that cross the cell walls may be subjected to osmotic pressure and capillary forces and diffuse through the apoplast and reach the endodermis (Lin et al. 2009; Deng et al. 2014).

Another route of nanoparticle uptake in plants is the symplastic pathway via the inner side of the plasma membrane. The cell wall is a porous matrix of polysaccharide fibers that can be crossed by nanoparticles that bind to protein carriers, via aquaporins, ion channels, and endocytosis, or by piercing the cell membrane and creating new pores (Tripathi et al. 2017; Rico et al. 2011; Wild and Jones 2009). Nanoparticles can migrate to neighboring cells through plasmodesmata (20–50 nm diameter channels) (Deng et al. 2014).

Another way of entry of nanoparticle in plants is via foliar through stomatal pores (Larue et al. 2014a, b; Hong et al. 2014). From leaves nanoparticles can translocate to other parts of the plants, including roots (Hong et al. 2014). Examples of plants that internalize nanoparticles through leaves are rapeseed, wheat, beans, corn,

lettuce, and cucumber (Chichiricco and Poma 2015). Nanoparticles ranging from a few nanometers up to several hundred nanometers and with different compositions can be internalized through leaves, such as ceria, titania, iron oxide, zinc oxide, and silver (Chichiricco and Poma 2015).

Within the cells nanoparticles are shown to interact with cell organelles, and depending on their physicochemical properties, many produce oxidative stress, genotoxicity, and metabolic changes (Deng et al. 2014).

1.4.2 Nanoparticle Composition-Dependent Uptake in Plants

In the following, we will focus mostly on the nanoparticle uptake on crops due to their immediate trophic transfer to humans and animals. Many crops exposed to various nanoparticles have been shown to internalize them (Deng et al. 2014). Once inside, they translocate to various plant tissues: stems, leaves, petioles, flowers, and fruits (Deng et al. 2014). While there are some reports on beneficial effects on selected plants, there is an overwhelming evidence of adverse effects of nanoparticles on many crops.

Below are examples of studies showing the uptake of nanoparticles with various compositions in various edible plants:

- Au—tomato plants (Dan et al. 2015), tobacco (Judy et al. 2011; Sabo-Attwood et al. 2012), *Arabidopsis thaliana* (Avellan et al. 2017; Taylor et al. 2014), barley (Feichtmeier et al. 2015), rice, radish, pumpkin (Zhu et al. 2012)
- Ag—*Arabidopsis thaliana* (Geisler-Lee et al. 2013; Kaveh et al. 2013; Nair and Chung 2014), tomato (Antisari et al. 2015), wheat (Dimkpa et al. 2013b), lettuce (Larue et al. 2014a), mung bean and sorghum (Lee et al. 2012), rice (Mirzajani et al. 2013; Thuesombat et al. 2014), broad bean (Patlolla et al. 2012), corn, cabbage (Pokhrel and Dubey 2013), review (Cox et al. 2016)
- CeO_2—alfalfa, corn (Lopez-Moreno et al. 2010b; Wang et al. 2013b), cucumber (Zhang et al. 2011; Lopez-Moreno et al. 2010b; Rui et al. 2015; Hong et al. 2014), tomato (Antisari et al. 2015; Lopez-Moreno et al. 2010b; Wang et al. 2013b), soybean (Lopez-Moreno et al. 2010a), barley (Rico et al. 2015), lettuce (Gui et al. 2015; Zhang et al. 2015), wheat (Rico et al. 2014)
- MWCNTs—wheat (Miralles et al. 2012b; Larue et al. 2012b), rapeseed (Larue et al. 2012b), tomato (Khodakovskaya et al. 2013), red spinach (*Amaranthus tricolor* L), (Begum and Fugetsu 2012), lettuce, rice, cucumber (Begum et al. 2014), onion (Ghosh et al. 2015), alfalfa (Miralles et al. 2012b), corn (Yan et al. 2013), review (Line et al. 2017)
- TiO_2—corn (Asli and Neumann 2009), wheat (Du et al. 2011; Larue et al. 2012a, c), rapeseed (Larue et al. 2012a, c), lettuce (Larue et al. 2014b), *Arabidopsis thaliana* (Kurepa et al. 2010, Wang et al. 2011b), cucumber (Servin et al. 2012, 2013), tomato (Antisari et al. 2015), onion (Pakrashi et al. 2014; Ghosh et al. 2010), review (Cox et al. 2016; Jacob et al. 2013), tobacco (Ghosh et al. 2010)

- C60 or C70—*Arabidopsis thaliana* (Landa et al. 2012; Liu et al. 2010), bitter melon (Kole et al. 2013), rice (Lin et al. 2009), onion (Chen et al. 2010), review (Husen and Siddiqi 2014)
- Zn and ZnO—*Arabidopsis thaliana* (Landa et al. 2012), soybean (Lopez-Moreno et al. 2010a), radish, rape, lettuce, corn, cabbage (Pokhrel and Dubey 2013; Lin and Xing 2007), cucumber (Lin and Xing 2007), wheat (Dimkpa et al. 2013a; Du et al. 2011), cress (Josko and Oleszczuk 2013), onion (Kumari et al. 2011), garlic (Shaymurat et al. 2012)
- Carbon-Fe—pea, sunflower, tomato, wheat (Cifuentes et al. 2010)
- Fe_3O_4—pumpkin (Zhu et al. 2008), soybean (Ghafariyan et al. 2013), tomato (Antisari et al. 2015)
- Al_2O_3 or Al—onion, cress (Asztemborska et al. 2015), corn (Lin and Xing 2007; Asztemborska et al. 2015), review (Singh et al. 2017b)
- Co—tomato (Antisari et al. 2015), onion (Ghodake et al. 2011)
- Ni—tomato (Antisari et al. 2015; Faisal et al. 2013)
- SnO_2—tomato (Antisari et al. 2015)
- CuO_2—radish (Atha et al. 2012), wheat (Dimkpa et al. 2013a), rice (Shaw and Hossain 2013), review (Anjum et al. 2015)
- CdSe quantum dots—rice (Nair et al. 2011)
- Rare-earth La_2O_3, Gd_2O_3, Yb_2O_3—rape, radish, wheat, lettuce, cabbage, tomato, cucumber (Ma et al. 2010)

The accumulation of nanoparticles in plants is not yet entirely understood; however several trends are emerging (Deng et al. 2014). Nanoparticle uptake in plants is species specific and depends on the nanoparticle composition and their size. For example, tobacco uptakes Au nanoparticles, while wheat does not (Judy et al. 2012). One must emphasize that future research might show a different picture of nanoparticle uptake, as various researchers uses nanoparticles with different sizes, surface charge and functionalization, crystallinity, etc.

Nanoparticle uptake and toxicity in plants is composition specific. For example, the exposure of tomato plants to nanoparticles with various compositions (CeO_2, Fe_3O_4, SnO_2, TiO_2, Ag, Co, and Ni) has different effects on root growth, accumulation site, and fruit yield (Antisari et al. 2015). Longer roots are achieved after exposure to iron oxide nanoparticles, while the opposite effect is obtained by using tin oxide. While most metal nanoparticles accumulate in roots, silver and cobalt nanoparticles were found in below- and aboveground plant organs. Tomato fruits had higher amount of silver nanoparticles compared to other compositions (Antisari et al. 2015).

The uptake of nanoparticle by plants is a function of exposure condition, nanoparticle physicochemical properties, and plant species. Similar to the process in humans, the uptake and translocation of nanoparticles within plants can be very swift. The time of translocation from roots to shoots of carbon-coated magnetic nanoparticles in sunflower, tomato, pea, and wheat is less than 24 h (Cifuentes et al. 2010).

1.4.3 Nanoparticle Size-Dependent Plant Uptake

Particle size is one of the most important factors that determine the uptake of nanoparticles in plants. Smaller nanoparticles are internalized by plants, while larger ones are not (Zhu et al. 2008; Wang et al. 2011a). For example, in the case of TiO_2 nanoparticles with sizes between 14 and 655 nm, only the smallest ones are able to translocate through the entire wheat plant (Larue et al. 2012a). The ones smaller than 140 nm pass through wheat root epidermis, while those smaller than 36 nm can transfer through root parenchyma and translocate from root to shoot (Larue et al. 2012a). Another example is the uptake of ceria nanoparticles in cucumber (Zhang et al. 2011). Nanoparticles with sizes of 7 and 25 nm are both absorbed by cucumber roots and translocate to leaves; however a larger number of smaller nanoparticles are absorbed compared to larger ones (Zhang et al. 2011).

1.4.4 Nanoparticle Crystalline Structure-Dependent Plant Uptake

Nanoparticles with the same composition but different crystalline structure can suffer a different uptake and translocation in plants. For example, titanium dioxide nanoparticles in anatase and rutile crystalline form are differentially translocated in cucumber plants (Servin et al. 2012). The anatase nanoparticles remained mainly in the roots, while the rutile nanoparticles translocated and accumulated mostly in the aerial tissue of cucumber.

1.4.5 Nanoparticle Charge-Dependent Plant Uptake

Studies show that the uptake of nanoparticles in plants is a function of nanoparticle surface charge or functionalization. Nanoparticles can be neutral; have a positive charge, in which case are called cationic; or have a negative charge—being called anionic. There seems to be a different behavior in the uptake of nanoparticles according to their charge by woody plants compared to herbaceous plants.

Woody Plants A recent study on the uptake of CdSe/CdZnS quantum dots coated with cationic polyethylenimine (PEI) or poly(ethylene glycol) of anionic poly (acrylic acid) (PAA-EG) in poplar trees shows that both types of nanoparticles are internalized after 2-day exposure (Wang et al. 2014). Cationic quantum dot absorption is tenfolds faster than anionic nanoparticles, most likely due to electrostatic forces between positively charged quantum dots and the negatively charged root cell wall (Wang et al. 2014). Slower absorption of anionic quantum dots might be a result of the repulsive electrostatic forces between the negatively charged root surface and the negatively charged nanoparticles.

Herbaceous Plants Interestingly, the uptake of cationic and anionic nanoparticles in herbaceous plants differs from the one in woody plants (Koelmel et al. 2013; Zhu et al. 2012).

Rice under hydroponic conditions uptakes and bioaccumulates 2 nm gold nanoparticles. Their distribution is a function of the nanoparticle surface charge (Koelmel et al. 2013). The accumulation in roots follows the order AuNP (+) > AuNP(0) > AuNP(−), where "+," "0," and "−" denoted positive, zero, and negative electrical charged nanoparticles, respectively. In contrast, the rice shoots showed a reverse order of nanoparticle charge uptake compared to the roots, having a preferential uptake of anionic nanoparticles.

Similar results were obtained in a study on the uptake of (6−10 nm) gold nanoparticles with different surface charge under hydroponic conditions in rice, radish, pumpkin, and perennial ryegrass (Zhu et al. 2012). Nanoparticle uptake is surface charge and plant specific. Cationic nanoparticles translocate mainly in plant roots, while anionic nanoparticles suffer uptake mainly in plant shoots. A larger number of nanoparticles are found in radish and ryegrass roots than rice and pumpkin roots. Nanoparticles accumulate in rice shoots in larger amounts compared to none in radish and pumpkin shoots (Zhu et al. 2012).

Cerium oxide nanoparticles (4 nm in size) also have a preferential uptake and tissue localization in wheat according to their surface charge (Spielman-Sun et al. 2017). Positively charged CeO_2 adhere to wheat roots the strongest, while negatively charged and neutral nanoparticles have higher concentrations in leaves compared to plants exposed to cationic CeO_2.

Therefore, the trend for herbaceous plants is to absorb positively charged nanoparticles in roots, while the shoots, stems, and leaves uptake mainly negatively charged nanoparticles.

1.5 Detrimental Effect of Nanoparticles in Plants

1.5.1 Composition and Plant-Specific Phytotoxicity

The interaction between plants and nanoparticles may range from subtle to notable changes in plant morphology, physiology, biochemistry, and genetics (Deng et al. 2014). Plant morphology changes include germination index (germination time and rate), root elongation, shoot and root biomass, root tip morphology, etc. (Deng et al. 2014).

Many studies indicate a detrimental effect of nanoparticles in many plant species, while a minority is trying to promote the use of nanoparticles for selected beneficial effects in a few plants. It is important to note that while some plants will have beneficial effects as a result of exposure to a type of nanoparticle, other plants are negatively affected by the same nanoparticles.

Many types of nanoparticles are phytotoxic, inhibiting plant growth and physiological, biochemical, and genetic traits (Tripathi et al. 2017; Brar et al. 2010; Deng et al. 2014). Table 1.1 shows examples of edible plants adversely affected by

Table 1.1 Detrimental effects of nanoparticles on selected crops

	Au	Ag	CNT	C_{60}	TiO_2	CeO_2	ZnO	CuO_2	Fe_3O_4
Alfalfa (*Medicago sativa*)					D	D	D	D	
Arabidopsis thaliana	D	D	D			D	D	*D*	D
Barley (*Hordeum vulgare*)	D	D				D		D	D
Corn (*Zea mays*)		D		D	D	D	D	D	
Cress (*Lepidium sativum*)					D		D		D
Cucumber (*Cucumis sativus*)		D	D		D	D	D	D	D
Lettuce (*Lactuca sativa*)		D	D		D	D	D	D	D
Onion (*Allium cepa*)		D	D	D	D		D	D	
Pumpkin (*Cucurbita*)									D
Radish (*Raphanus raphanistrum*)		D			D	D	D	D	D
Red spinach (*Amaranthus tricolor*)			D					D	D
Rice (*Oryza sativa*)	D	D	D	D	D	D	D	D	
Soybean (*Glycine max*)	D		D	D		D	D	D	D
Tomato (*Lycopersicon esculentum*)	D	D	D		D	D	D		D
Wheat (*Triticum aestivum*)		D	D		D	D	D	D	D

D—found detrimental in at least one of the growth inhibition, physiological and biochemical traits, and toxicity at genetic level

nanoparticles with several compositions that are promoted or already being used as agrichemicals (Au, Ag, CNT, C_{60}, CeO_2, ZnO, CuO_2, Fe_3O_4). Here "D" refers to detrimental.

Table 1.2 shows examples of plant-specific detrimental effects of nanoparticles as a result of plant exposure to nanoparticles with several compositions. These range from adverse effects in their physiological, biochemical, and genetic traits. Noble metal nanoparticles, such as Au, induce necrosis in tobacco plants (Sabo-Attwood et al. 2012). Exposure to Ag nanoparticles leads to retarded germination in rice and corn (Thuesombat et al. 2014; Pokhrel and Dubey 2013) and reduction in mitotic index and fragmented chromosomes in onion (Kumari et al. 2009). Carbon-based nanoparticles (CNTs, C_{60}) lead to cellular toxicity in rice, spinach, and onion (Shen et al. 2010; Begum and Fugetsu 2012; Chen et al. 2010), reduction in biomass for zucchini (Stampoulis et al. 2009), and delayed flowering together with decreased yield (Lin et al. 2009). Exposure to TiO_2 nanoparticle results in damaged chloroplast and reduced photosynthetic rate in spinach (Lei et al. 2008), stress in cucumber

Table 1.2 Examples of detrimental effects as a result of plant exposure to different nanoparticles

NPC	Size (nm)	Plant	Effect	References
Au	3	Tobacco	Necrosis	Sabo-Attwood et al. (2012)
Ag	20	Rice	Seed germination	Thuesombat et al. (2014)
	11	Corn	Retarded germination	Pokhrel and Dubey (2013)
	<100	Onion	Fragmented chromosomes, reduction in mitotic index	Kumari et al. (2009)
CNT	1–2	Arabidopsis	Cell death	Shen et al. (2010)
		Rice	Delayed flowering, decreased yield	Lin et al. (2009)
		Rice	DNA damage, cell viability	Shen et al. (2010)
		Zucchini	60% reduction in biomass	Stampoulis et al. (2009)
		Spinach	Cell damage	Begum and Fugetsu (2012)
C_{60}		Onion cells	Necrosis	Chen et al. (2010)
TiO_2	27	Cucumber	Stress	Servin et al. (2013)
	30	Corn	Inhibited leaf growth	Asli and Neumann (2009)
		Corn	DNA damage	Castiglione et al. (2011)
	5	Spinach	Damaged chloroplast, reduced photosynthetic rate	Lei et al. (2008)
	100	Onion	DNA damage	Ghosh et al. (2010)
CeO_2	7	Soybean	Genotoxicity	Lopez-Moreno et al. (2010a)
	8	Cucumber	Stress	Hong et al. (2014)
	8	Rice	Stress	Rico et al. (2013)
	8	Wheat	Nutrition	Rico et al. (2014)
	10	Cucumber	Nutrition	Zhao et al. (2014)
	10–30	Tomato	Detrimental effects on second-generation plants	Wang et al. (2013b)
ZnO	20	Corn	Plant growth	Lin and Xing (2007)
	<100	Onion	Genotoxicity	Kumari et al. (2011)
	8	Soybean	Plant growth	Lopez-Moreno et al. (2010a)
	<50	Soybean	Seed formation	Yoon et al. (2014)
	4	Garlic	Genotoxicity	Shaymurat et al. (2012)
	10	Green peas	Chlorophyll/stress	Mukherjee et al. (2014)
	100	Rice	Root length/formation	Boonyanitipong et al. (2011)
	30,50	Chinese cabbage	Root and shoot formation	Xiang et al. (2015)
	<50	Buckwheat	Genotoxicity	Lee et al. (2013)

(continued)

Table 1.2 (continued)

NPC	Size (nm)	Plant	Effect	References
CuO, Cu	<50	Rice	Stress	Shaw and Hossain (2013)
		Zucchini	77% reduced root length 90% reduced biomass	Stampoulis et al. (2009)
	<100	Radish	Decreased root growth, DNA damage	Atha et al. (2012)
	<50	Buckwheat	Genotoxicity	Lee et al. (2013)
Ni	23,34	Tomato	Stress, mitochondria, cell damage	Faisal et al. (2013)

NPC nanoparticle composition

(Servin et al. 2013), inhibited leaf growth, and DNA damage in corn (Asli and Neumann 2009; Castiglione et al. 2011). CeO_2 nanoparticle adversely affects the nutrition and genetics of soybean, cucumber, rice, and wheat (Lopez-Moreno et al. 2010a; Hong et al. 2014; Rico et al. 2013, 2014; Zhao et al. 2014). ZnO is genotoxic to onion, garlic, and buckwheat (Kumari et al. 2011; Shaymurat et al. 2012; Lee et al. 2013); affects the seed formation in soybean (Yoon et al. 2014); inhibits plant growth in corn, soybean, rice, and cabbage (Lin and Xing 2007; Lopez-Moreno et al. 2010a; Boonyanitipong et al. 2011; Xiang et al. 2015); and affects chlorophyll in green peas (Mukherjee et al. 2014). CuO is genotoxic to radish and buckwheat (Atha et al. 2012; Lee et al. 2013), produces stress in rice (Shaw and Hossain 2013), and severely reduces root length (77%) and biomass (90%) in zucchini (Stampoulis et al. 2009). Nickel nanoparticles induce stress and damage of mitochondria and cells in tomato (Faisal et al. 2013).

A type of nanoparticle can sometimes have both beneficial and detrimental effects on the same plant. For example, barley exposed to CeO_2 nanoparticles (500 mg/kg) led to a more than 300% increase in shoot biomass; however it formed no grain (Rico et al. 2015).

In the following subchapters, we will elaborate on the adverse effects of nanoparticles on plant physiological, biochemical, and genetic traits.

1.5.2 Plant Growth Inhibition

Phytotoxicity related to growth inhibition manifests in reduced biomass; decreased germination and leaf growth; reduced root elongation, root biomass, root tip morphology, and shoot growth; delayed flowering; and decreased yield among others (Tripathi et al. 2017). The adverse biochemical traits involve the generation of reactive oxygen species, lipid peroxidation, decreased rate of transpiration, disturbed mitosis, breakdown of cell wall, reduction in chlorophyll content, and reduced photosynthesis (Tripathi et al. 2017). Toxicity at genetic level involves reduction in mitotic index, sticky and fragmented chromosomes, chromosome aberrations, alteration of genes, damaged DNA structure, and decreased cell viability (Tripathi et al. 2017). Examples of toxic effects of nanoparticle on plants are given in Table 1.2.

Some adverse effects of nanoparticles on plant growth are easily assessed by measuring the germination index, the elongation of roots and shoots, root biomass, root tip morphology, total biomass, and flowering (Deng et al. 2014).

For plants exposed to nanoparticles from soil or hydroponically, an important indicator of toxicity is the shoot and root biomass. While studies use different exposure times and doses, the general conclusion is that phytotoxicity is plant and nanoparticle specific. This toxicity can be due to the release and subsequent accumulation of ions in plant tissue and/or nanoparticle uptake and translocation (Deng et al. 2014). Nanoparticles with various compositions have an adverse effect on seedling roots and shoot elongation, mainly due to the adsorption of nanoparticles into the roots. Among phytotoxic materials to roots and shoots are gold, silver, zinc oxide, copper oxide, alumina, and carbon nanotubes (Begum and Fugetsu 2012; Begum et al. 2012; Burklew et al. 2012; Feichtmeier et al. 2015; Deng et al. 2014; Dimkpa et al. 2013b; Ghodake et al. 2011).

Figure 1.6 shows photographs of plants detrimentally affected by exposure to nanoparticles. Figure 1.6a–h illustrates the trend of decreased shoot and root length in a concentration-dependent manner in tomato and cauliflower exposed to CuO nanoparticles (Singh et al. 2017a), wheat exposed to Ag nanoparticles (Dimkpa et al. 2013b), barley seedlings exposed to Au nanoparticles (Feichtmeier et al. 2015), red spinach exposed to MWCNTs (Begum and Fugetsu 2012), rice exposed to MWCNTs (Begum et al. 2012), and rice exposed to CuO nanoparticles (Shaw and Hossain 2013). Figure 1.6i–j shows various aberrant features observed in onion after exposure to titanium dioxide nanoparticles, such as chromosome break and nuclear blebbing (Pakrashi et al. 2014).

It is important to note that nanophytotoxicity is material and species specific. This can be seen in a study comparing the toxic effects of several rare-earth oxide nanoparticles (CeO_2, La_2O_3, Gd_2O_3, Yb_2O_3) on several crops (cabbage, cucumber, lettuce, radish, rape, tomato, wheat) (Ma et al. 2010). For example, only the root elongation of lettuce is affected by CeO_2, while all remaining (La_2O_3, Gd_2O_3, Yb_2O_3) nanoparticles lead to a large reduction in root elongations for all studied plants.

Silver nanoparticles are known for their antibacterial, antifungal activity and are consequently used extensively as agrichemicals. As a result, the existence of Ag nanoparticles in soil can have an effect upon soil microbiota (such as nitrogen-fixing bacteria) that will in turn affect the physicochemical characteristics of soil and plants (Anjum et al. 2013). Silver nanoparticles can be internalized and accumulate in edible plants and consequently enter the food chain. Some plants exposed to silver nanoparticles show reduced germination, biomass, transpiration, shoot and root length, and cytotoxicity involving modifications in gene expression, oxidative stress, decreased mitosis, chromosomal abnormalities, and cell death (Anjum et al. 2013; Arruda et al. 2015; Thuesombat et al. 2014; Pokhrel and Dubey 2013; Kumari et al. 2009). Silver nanoparticles have a concentration-dependent growth inhibition effect upon mung bean and sorghum (Lee et al. 2012).

MWCNTs are the type of nanoparticle that shows the entire array of effects on plants, ranging from beneficial to detrimental. They are promoted for their use in

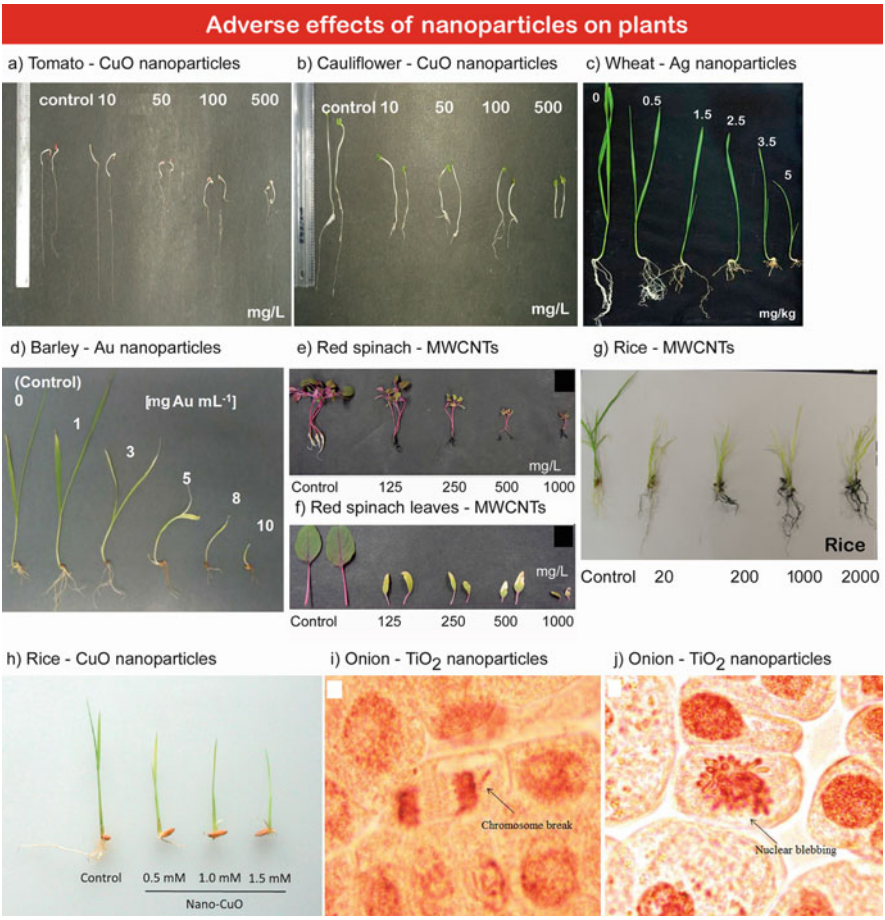

Fig. 1.6 Photographs showing detrimental effects following the exposure to different concentrations of various nanoparticles of selected crops. (**a**) Tomato exposed to CuO nanoparticles, (**b**) cauliflower exposed to CuO nanoparticles. Images (**a**–**b**) are reprinted from Singh A. et al., Effect of biologically synthesized copper oxide nanoparticles on metabolism and antioxidant activity to the crop plants *Solanum lycopersicum* and *Brassica oleracea* var. botrytis. Journal of Biotechnology, 262, 11–27, Copyright (2017), with permission from Elsevier (Singh et al. 2017a). (**c**) Wheat exposed to Ag nanoparticles. Reprinted with permission from Dimkpa C. O. et al, Silver Nanoparticles Disrupt Wheat (*Triticum aestivum* L.) Growth in a Sand Matrix. Environmental Science & Technology, 47, 1082–1090. Copyright (2013) American Chemical Society (Dimkpa et al. 2013b). (**d**) Barley seedlings exposed to Au nanoparticles. Reprinted from Feichtmeier, N. S. et al, Uptake, effects, and regeneration of barley plants exposed to gold nanoparticles. Environmental Science and Pollution Research, vol. 22, 2015, pp. 8549–8558, with permission from Springer (Feichtmeier et al. 2015). (**e**–**f**) Red spinach exposed to MWCNTs. Images (**e**–**f**) reprinted from Begum P and Fugetsu B, 2012, Phytotoxicity of multi-walled carbon nanotubes on red spinach (*Amaranthus tricolor* L) and the role of ascorbic acid as an antioxidant, Journal of Hazardous Materials, 243, 212–222, Copyright (2012), with permission from Elsevier (Begum and Fugetsu 2012). (**g**) Rice exposed to MWCNTs. Image reprinted from Begum P. et al., Applied Surface Science, vol. 262, Phytotoxicity of multi-walled carbon nanotubes assessed by selected plant species in the seedling stage, pp. 120–124, Copyright (2012), with permission from Elsevier (Begum et al. 2012). (**h**) Rice exposed to CuO nanoparticles. Reprinted from Shaw A. K. &

aiding germination of some seeds (Khodakovskaya et al. 2011). CNTs are phytotoxic to red spinach, lettuce, and cucumber, showing decreased roots and shoot lengths, while no negative effects were observed for chili and soybeans (Begum et al. 2014). They accumulate in onion plants and are cytotoxic and genotoxic, altering cellular morphology, affecting membrane integrity and mitochondrial function, resulting in DNA damage and chromosome aberration (Ghosh et al. 2015).

1.5.3 Nutrient Depletion in Nanoparticle-Contaminated Plants

The intake of plants and fruits is important for the mineral and nutrients they contain. Plant exposure to nanoparticles results in modified content of nutrients, fruit flavor, antioxidant content, and growth performance (Antisari et al. 2015; Deng et al. 2014; Petersen et al. 2014; Rico et al. 2011; Zhao et al. 2014).

Therefore, the use of nanoparticles as agrichemicals raises some serious concerns.

Cerium oxide nanoparticles affect the amounts of nutrients for important crops, such as rice, corn, soybean, tomato, and cucumber (Antisari et al. 2015; Peralta-Videa et al. 2014; Rico et al. 2013; Zhao et al. 2014, 2015). Rice exposed to ceria nanoparticles yields grains with compromised nutritional value, showing smaller amounts of iron, glutelin, lauric and valeric acids, starch, and some antioxidants (Rico et al. 2013). Cucumber fruits of plants exposed to nanoceria have an altered Mo micronutrient, sugar, and phenolic content in addition to protein fractionation (Zhao et al. 2014). Corn exposed to nanoceria has 38% reduced yield and less calcium translocation from the cob to the kernels compared to control (Zhao et al. 2015).

Zinc oxide nanoparticles have a profound effect on corn plants, accumulating in corncobs, alter its nutrient contents, and reduce photosynthesis and relative chlorophyll content, resulting in a reduced yield by 49% (Zhao et al. 2015).

The fruits of tomato plants exposed to CeO_2, Fe_3O_4, SnO_2, TiO_2, Ag, Co, and Ni nanoparticles exhibit a depletion of Mg, P, and S (Antisari et al. 2015).

1.5.4 Nanoparticle-Induced Genotoxicity

Due to their small size, nanoparticles are able to enter cells and elicit a genetic response from plants. Nanoparticles with many compositions (CuO, Ag, ZnO, CeO_2, TiO_2, carbon nanotubes, etc.) induce genotoxicity in various plants (radish, onion,

Fig. 1.6 (continued) Hossain Z. 2013. Impact of nano-CuO stress on rice (*Oryza sativa* L.) seedlings. Chemosphere, 93, 906–91, Copyright (2013), with permission from Elsevier (Shaw and Hossain 2013). (**i**, **j**) Various aberrant features observed in *Allium cepa* after exposure to titanium dioxide nanoparticles: (**i**) chromosome break, (**j**) nuclear blebbing. Images (**i**–**j**) reprinted from Pakrashi S. et al., 2014. In Vivo Genotoxicity Assessment of Titanium Dioxide Nanoparticles by *Allium cepa* Root Tip Assay at High Exposure Concentrations. Plos One, 9, 12 (Pakrashi et al. 2014)

soybean, buckwheat, fava beans, ryegrass, tobacco, etc.) (Atha et al. 2012; Chichiricco and Poma 2015; Ghosh et al. 2015; Kumari et al. 2009, 2011; Lee et al. 2013; Lopez-Moreno et al. 2010a; Pakrashi et al. 2014; Patlolla et al. 2012; Shaymurat et al. 2012; Burklew et al. 2012). The plants with inhibited roots displayed errors in cell division, chromosomal abnormalities, microRNA deregulation, and DNA damage.

1.5.5 Nanoparticle Transgenerational Effects in Plants

As shown previously, nanoparticles can accumulate in plants within various tissues, such as leafs, roots, fruits, and seeds. Nanoparticle uptake in seeds has been shown to cause transgenerational effects in some plants (Lin et al. 2009; Wang et al. 2013b). These studies raise the questions if other nanoparticles might cause long-term multigenerational effects in plants.

Nanoparticles can be transmitted to plant progenies through seeds, in the absence of external nanoparticle exposure. For example, C_{70} can be found in second-generation rice plants (Lin et al. 2009). Seeds harvested from plants exposed to C_{70} were planted in a media free of C_{70} nanoparticles. The germinated rice plants (second-generation plants) were found to contain C_{70} black aggregates near the stem's vascular system and in leaf tissue.

Some authors found that ceria nanoparticles might have a beneficial effect on some plants. Exposure to ceria nanoparticles had a minor beneficial effect on first-generation seedlings and, however, had a detrimental effect on the growth of second-generation plants (Wang et al. 2013b). Second-generation tomato plants grown from seeds harvested from parent plants exposed to ceria nanoparticles were weaker and had a lower biomass, lower water transpiration, and a higher reactive oxygen species amount (Fig. 1.7).

1.6 Beneficial Effects of Nanoparticles in Plants

Nanoparticles can influence plant phenotype, some plants will be negatively affected, others will show beneficial effects, while others will show no response.

Some nanoparticles are used for their beneficial effects as crop enhancers or/and inhibiting plant pathogens. Several reviews report positive effects of some nanoparticles as crop enhancers in selected plant species, such as enhanced seed germination, crop yield, improved photosynthesis, increased resistance against stress, and suppressed plant disease (Rico et al. 2011; Du et al. 2017; Siddiqi and Husen 2017; Rizwan et al. 2017; Ruttkay-Nedecky et al. 2017; Gardea-Torresdey et al. 2014; Zuverza-Mena et al. 2017; Wang et al. 2016; Khan et al. 2017; Arruda et al. 2015). The composition of nanoparticles that show beneficial effects includes Au, Pd, Cu, Si, CeO_2, TiO_2, Fe_2O_3, MWCNTs, and fullerol $C_{60}(OH)_{20}$ (Arruda et al. 2015; Chichiricco and Poma 2015; Siddiqui and Al-Whaibi 2014; Zheng et al. 2005).

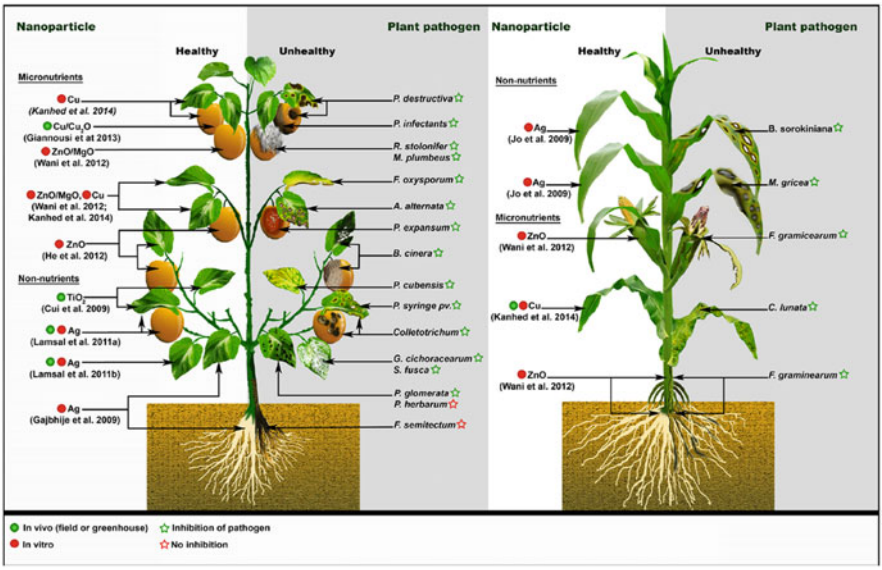

Fig. 1.7 Effect of nanoparticle used as micronutrients (left) and non-nutrients (right) on crop disease. Reproduced from Journal of Nanoparticle Research, A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield, Servin A. et al, vol. 17, 2015. Copyright (2015) with permission of Springer (Servin et al. 2015)

While some authors chose to focus mainly on the positive aspects of nanoparticle in plants, promoting their use as plant growth enhancers (Servin et al. 2015; Khodakovskaya et al. 2012, 2013; Lahiani et al. 2013; Lyu et al. 2017), it is difficult to predict what is the effect of nanoparticle progressive accumulation in soil. Here we must mention hormesis, which describes a beneficial effect for a low-dose agent, while a higher dose is toxic or inhibits growth.

Nanoparticles with selected compositions have been shown to inhibit plant pathogens as a result of their antimicrobial properties (Servin et al. 2015). Examples of pathogen inhibitor nanoparticles are Ag (Lamsal et al. 2011b; Gajbhiye et al. 2009), Cu (Giannousi et al. 2013; Kanhed et al. 2014), ZnO (Wani and Shah 2012; He et al. 2011), MgO (Wani and Shah 2012), and TiO_2 (Cui et al. 2009).

MWCNTs Several authors describe the positive effects of MWCNTs related to increased germination of tomato, barley, soybean, and corn and increased growth of tobacco cells (Khodakovskaya et al. 2009; 2011, 2012, 2013; Lahiani et al. 2013; Alexandru et al. 2012).

MWCNT exposure leads to enhanced germination and growth of tomato seedlings (Khodakovskaya et al. 2009, 2011) and enhanced flowering and fruit yield for tomato plants (Khodakovskaya et al. 2013). Raman spectroscopy detected MWCNT nanoparticles in the fruits of tomato plants (Khodakovskaya et al. 2011), which raises questions about the safety of their consumption. The presence of these

nanoparticles in the edible parts of plants together with the increased evidence of their toxicity in humans should not justify their use for increased flowering and fruit yield.

MWCNTs penetrate tomato seeds and increase their germination and growth rates compared to control (Khodakovskaya et al. 2009). The exposure of tomato plants to MWCNTs results in significant changes in total gene expression in leaves and roots (Khodakovskaya et al. 2011). The enhanced germination and growth of tomato plants are due to the upregulation of the stress-related genes, such as those induced by pathogens and water-channel LeAqp2 gene (Khodakovskaya et al. 2011). There are a large number of altered genes with various functions in the leaves of first-generation plants exposed to MWCNTs: 29 genes involved in cellular responses, 39 genes in stress response, 14 genes in transport, 13 genes in signal transduction, and 25 genes in metabolic processes. Similarly, there are a large number of genes in the roots: 19 genes involved in stress responses, 9 genes in cellular processes, 6 genes in transport, and 22 genes related to catabolic, metabolic, and biosynthetic processes.

The biochemical mechanism of MWCNT hormesis in plants is not yet understood. Cell culture experiments indicated that small concentrations (5 μg/ml) of MWCNTs lead to an augmented growth of tobacco cell, while higher concentrations (100–500 μg/ml) showed the opposite effect (inhibited cell growth) (Khodakovskaya et al. 2012).

Some authors studied the effects of CNTs on crops (such as corn, barley, soybean) and observed that they lead to accelerated seed germination also concluding that they have no negative effect on the biological endpoints showing the development of plants grown from the exposed seeds (Lahiani et al. 2013).

One must emphasize that while beneficial effects of nanoparticles were reported for first-generation plants, the nanoparticles accumulated in the seeds might be detrimental to second-generation plants (Wang et al. 2013b).

One must remind that the beneficial effects of carbon nanotubes are plant species specific. While CNTs might be beneficial for tomato plants, they inhibit the growth of red spinach, lettuce, cucumber, and rice in a dose-dependent manner (Begum and Fugetsu 2012; Begum et al. 2012, 2014).

In addition, taking into account their similarities to asbestos, CNTs are believed to have comparable toxicity to humans (Stella 2011).

C60 Fullerenes The use of C60-based nanoparticles in the growth of bitter melon was reported to increase biomass, water content, and fruit yield (Kole et al. 2013). The authors show that C60 nanoparticles biodistribute to petioles, leaves, flowers, and fruits. Again, the accumulation of nanoparticles in the edible part poses risks for human exposure to this nanoparticle.

Au, Pd, Si, and Cu Nanoparticles Low concentration of nanoparticles made of Au and Pd and higher concentrations of Si and Cu nanoparticles were found to increase the shoot ratio for lettuce after 15 days of incubation (Shah and Belozerova 2009). Nanoparticles with these compositions are shown to be toxic to humans and animals.

Ag Silver nanoparticles are used in agriculture for their bactericidal effects. Silver nanoparticles in small concentrations (30 μg/ml) accelerated root growth in rice plants while just doubling the concentration inhibited root growth (Mirzajani et al. 2013). Silver nanoparticles were shown to enter cell wall, damaged cells and produce reactive oxygen species.

TiO_2 Titania nanoparticles had some beneficial effects on seeds, especially those with low germination (Zheng et al. 2005; Feizi et al. 2013). Some authors believe that the induction of reactive oxygen species by nanoparticles results in subsequent enhancement of stress resistance and facilitation of seed penetration of water and oxygen (Khot et al. 2012).

Spinach exposed to titania nanoparticles shows increased plant dry weight, chlorophyll, and photosynthetic rate (Zheng et al. 2005).

Another example of hormesis is titanium dioxide in fennel plants. Titania nanoparticles were found to increase fennel seed germination, while larger particles lowered by 50% the shoot biomass compared to the control (Feizi et al. 2013). This fact is important as small nanoparticles can aggregate into larger particles, which can in turn become phytotoxic to the same plant species.

It seems that increased root length is an adaptation process of roots clogged with nanoparticles (Asli and Neumann 2009). Titania nanoparticles are shown to accumulate in roots and block pores in hydroponic maize treated with nanoparticles. As a result, the plants have lower water supply, leaf growth, and transpiration rate (Asli and Neumann 2009). In order to survive, the maize plant adapts by forming a larger root system.

Rare Earth Rare-earth additives are used in fertilizers for their promotion of larger yields, longer rots, darker green foliage, and better fruit color (Yuan et al. 2001).

1.7 Nanoparticle Toxicity in Humans and Animals

Unfortunately, many of the nanoparticles that have some agricultural benefits, including those shown to be internalized by crops, have varying degrees of toxicity in humans and laboratory animals. A multitude of reviews discuss the topic of nanoparticle toxicity in humans and animals (Buzea et al. 2007; Ema et al. 2016, 2017; Sohaebuddin et al. 2010; Kendall and Holgate 2012; He et al. 2017; Shah et al. 2015).

While the discipline of nanotoxicology is a fairly new, some older epidemiological studies give a plethora of information on environmental nanoparticle (particulate matter) toxicity on humans. What makes nanoparticles toxic is their size and, as a result, their ability to enter organisms; enter circulatory system; translocate to organs, such as the liver, spleen, kidneys, brain, and heart; enter cells; and go further into organelles (Buzea et al. 2007). They are able to be internalized, depending on their entry and size, within several minutes to several hours following exposure (see Fig. 1.8d) (Nemmar et al. 2002). Once inside cells and organelles, they produce

cytotoxicity and genotoxicity. They are associated to inflammation and various diseases, including cancer. In the following we will give examples of such adverse effects of nanoparticles on humans. While our examples are not comprehensive, we expect to be compelling and inform the scientists planning to use nanoparticles in agriculture of the potential adverse effects on humans, including themselves.

1.7.1 Nanoparticle Physicochemical Characteristic-Dependent Toxicity

Nanoparticle toxicity depends on their physical and chemical properties, such as size, aggregation, composition, concentration, shape, porosity, surface area, hydrophobicity, electrical charge, and magnetic properties (Buzea et al. 2007; Podila and Brown 2013; Silva et al. 2014, 2015; Hanley et al. 2009; Chithrani et al. 2006; Naqvi et al. 2010; Schlinkert et al. 2015; Sharma et al. 2014; Li et al. 2015b; Teske and Detweiler 2015). A schematic illustrating this idea is given in Fig. 1.2.

There is no universal law for determining the toxicity of nanoparticles, which cannot be extrapolated from the behavior of the bulk material. Each type of nanoparticles has to be tested in order to assess its toxicity. Nanoparticles of the same material can have different toxicities for different sizes, surface functionalization, or surface charge. Nanoparticles with the same size but made of different materials will also have different toxicities.

Usually nanoparticles with smaller size have higher toxicity than larger ones (Buzea et al. 2007).

Nanoparticles with the same composition but different crystalline form can exhibit different properties and toxicity, such as titanium dioxide in rutile and anatase forms. Rutile titania 200 nm in size induced oxidative DNA damage and other cytotoxic effects in human bronchial epithelial cell, while anatase titania did not (Gurr et al. 2005).

Some nanoparticles are hydrophobic, while others are hydrophilic (Garcia-Ivars et al. 2015). This property can be modulated by coating of nanoparticles of various substances (Podila and Brown 2013). For example, coating with polyethylene glycol (PEG) renders nanoparticles highly hydrophilic (Kettler et al. 2014).

Nanoparticles can have positive, negative, or neutral charge. Nanoparticle surface charge is very important in deciding how a nanoparticle interacts with biological systems (Gatoo et al. 2014; Salatin et al. 2015). Positively charged nanoparticles are attracted to the negatively charged cell membrane and have a higher cellular uptake versus negatively charged or neutral nanoparticles (Kettler et al. 2014). Nanoparticle toxicity depends on whether or not nanoparticles are internalized within cells. For example, cationic gold nanoparticles are toxic, while anionic nanoparticles are nontoxic (Goodman et al. 2004). Studies also show that nanoparticles with large surface charge, either negative or positive, show an increased receptor-mediated endocytic uptake of nanoparticle compared to neutral nanoparticles (Kettler et al. 2014).

1.7.2 Nanoparticle Internalization and Biodistribution

1.7.2.1 Inhalation, Ingestion, and Dermal Exposure

Due to their minute size, nanoparticles can be inhaled and ingested or penetrate through the skin. From the respiratory and gastrointestinal systems, they can rapidly enter blood and lymphatic system (Landsiedel et al. 2012).

Numerous studies indicate that inhaled nanoparticles accumulate in lungs, and some nanoparticles, depending on their size and other physicochemical properties, can reach the alveoli, translocate to organs, and become systemic. They can be found in the circulatory system and lymphatic system and in the brain, heart, thyroid, liver, spleen, colon, bones, and kidney (Anderson et al. 2015; Bakand et al. 2012; Bruinink et al. 2015; Buzea et al. 2007; Davidson et al. 2015; Fischer and Chan 2007; Geiser and Kreyling 2010; Gosens et al. 2014, 2015; Khlebtsov and Dykman 2011, Johnston et al. 2010; Lin et al. 2015; Landsiedel et al. 2012; Nakane 2012; Theodorou et al. 2014).

From lungs, nanoparticles can go further to the circulatory system. Nanoparticles with various compositions have been collected from the blood of patients with various diseases (Gatti and Montanari 2006). In the circulatory system, they interact with plasma and form a protein corona that will determine their toxicity and translocation. Next, nanoparticles move to and accumulate in various organs and tissues: the liver, spleen, pancreas, heart, kidneys, brain, lymph nodes, bone marrow, etc. (Landsiedel et al. 2012; Sonavane et al. 2008). The smaller the nanoparticles, the greater their accumulation in tissues (Sonavane et al. 2008).

Ingested nanoparticles that enter the gastrointestinal tract are partly excreted in feces, and some are absorbed and become systemically available (Hillyer and Albrecht 2001).

The site of accumulation in the body depends on the composition and surface functionalization of the nanoparticles. Metallic nanoparticles usually localize in the liver, spleen, and lymph node (Lin et al. 2015; Johnston et al. 2010).

Nanoparticles are able to cross the placental barrier, reach fetus, and have adverse effects on pregnancy and fetuses, as shown by in vivo and ex vivo studies on animals (Kulvietis et al. 2011; Wick et al. 2010; Semmler-Behnke et al. 2014; Snyder et al. 2015; Melnik et al. 2013; Yamashita et al. 2011).

1.7.2.2 Nanoparticle Persistence and Disease

Nanoparticles can persist in the body for longer than 6 months (Lin et al. 2015). Long-term residence of nanoparticles in the body will produce tissue injuries and inflammation which is the precursor to cancer and other diseases. The residence of metallic nanoparticles within a tissue favors tumorigenesis (Sighinolfi et al. 2016). Indeed, recent studies indicate that nanoparticles accumulate in tissue of patients with various diseases, such as deep-vein thrombosis, pulmonary embolism, colon cancer, prostate cancer, stroke, asthma, emphysema, lung cancer, Crohn's disease, ulcerative colitis, liver necrosis, renal failure, and Hodgkin's lymphoma (Gatti 2004; Gatti and Montanari 2006; Gatti and Rivasi 2002; Roncati et al. 2015a, b; Ballestri et al. 2001; Iannitti et al. 2010).

Figure 1.8a–c shows nanoparticles persistent in the lungs that are likely to be the cause of the disease observed in the respective subjects. Figure 1.8a shows images of MWCNTs inside lung cells and in the bronchoalveolar lavage fluids of asthmatic children living in Paris (Kolosnjaj-Tabi et al. 2015). The carbon nanotubes found inside the lungs of asthmatic children are very similar in morphology and shape to those from Fig. 1.8b, which shows MWCNTs from vehicle exhausts and from pollution dust collected near a busy traffic intersection in Paris. Figure 1.8c shows black carbon deposits inside the lung of a patient diagnosed with emphysema, which are likely to be the cause of his emphysema. Figure 1.8d illustrates very fast internalization of inhaled ^{99m}Tc-labeled carbon nanoparticles in a human volunteer, nanoparticles being detected within 5–60 min of exposure (Nemmar et al. 2002).

In some of the following paragraphs, we will discuss in more detail various diseases associated to nanoparticle exposure.

1.7.2.3 Nanoparticle Size-Dependent Accumulation

Experiments on mice with orally ingested gold nanoparticles having size between 4 and 28 nm show translocation to the blood, brain, lung, heart, kidney, spleen, liver, small intestine, and stomach, while nanoparticles with larger size (58 nm) were not detected in most studied tissue (Hillyer and Albrecht 2001).

Inhaled nanoparticles smaller than 50–100 nm can travel to and accumulate in the brain, through olfactory nerves and blood-brain barrier (Buzea et al. 2007; Lin et al. 2015; Sonavane et al. 2008).

The maximum size of nanoparticles that can enter and be cleared from the body is between 200 and 250 nm (Bruinink et al. 2015). If nanoparticles existent in tissues aggregate in complexes with larger size, their clearance becomes less likely. Consequently, long-term exposure to small amounts of nanoparticles can lead to adverse health effects due to their accumulation without clearance.

Experiments on human volunteers show fast internalization and long-term persistence of gold nanoparticles in humans (Miller et al. 2017). Within 15 min following inhalation, gold nanoparticles are detected in the blood of human volunteers. They can persist 3 months following inhalation exposure. Smaller nanoparticles (5 nm diameter) are more persistent than the larger ones (30 nm).

1.7.2.4 Nanoparticle Corona

When in contact with organic matter, nanoparticles will interact dynamically with biomolecules via electrostatic and van der Waals forces (Kumar et al. 2014). This interaction will result in acquiring a corona formed of biomolecules, which will determine their subsequent interaction with cells and tissue/organ accumulation (Grillo et al. 2015; Khlebtsov and Dykman 2011). This corona will dictate the degree of nanoparticle toxicity in addition to the intrinsic properties of the nanoparticle (Foroozandeh and Aziz 2015). There are cases when nanoparticle corona might be more important than the intrinsic physical properties of a nanoparticle in deciding its toxicity (Walkey et al. 2014). In general, nanoparticle physicochemical properties determine to some extent the composition of its corona together with the composition of the biological environment (Kreyling et al. 2014). It is believed that the

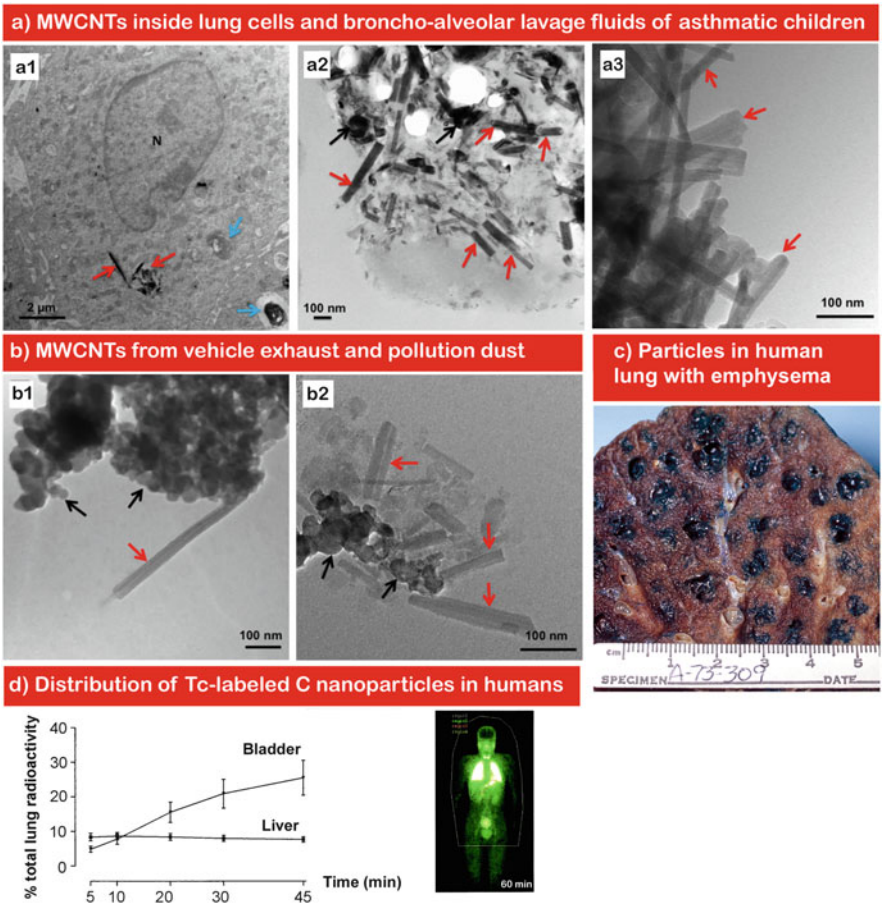

Fig. 1.8 High-resolution TEM images showing MWCNTs inside lung cells (**a1**) and in the bronchoalveolar lavage fluids (**a2**–**a3**) of asthmatic children together with MWCNTs from vehicle exhausts (**b1**) and pollution dust collected near a busy traffic intersection in Paris (**b2**). N indicates nucleus; blue arrows, lamellar bodies; black arrows, nanospheres; red arrows, MWCNTs. Image (**a2**) is a magnified view of (**a1**). Image (**b2**) is a magnified view of (**b1**). Reprinted from Kolosnja-Tabi J. et al., Ebiomedicine, vol. 2, Anthropogenic Carbon Nanotubes Found in the Airways of Parisian Children, pp. 1697–1704, Copyright (2015), with permission from Elsevier (Kolosnjaj-Tabi et al. 2015). (**c**) Pathology of lung with centrilobular emphysema with multiple cavities heavily lined by black carbon deposits. Courtesy of Dr. Edwin P. Ewing, Jr., http://phil.cdc.gov/phil_images/20040517/4/865_lores.jpg. (**d**) (left) Radioactivity on the bladder and liver compared to lungs versus time of exposure to ^{99m}Tc-labeled carbon nanoparticles in humans. (Right) Radioactivity in the body of a human volunteer after 1 h of exposure to ^{99m}Tc-labeled carbon nanoparticles. Image reproduced from Nemmar A. et al., Passage of inhaled particles into the blood circulation in humans. Circulation, vol. 105, pp. 411–414. Copyright (2002) American Heart Association, Inc. with permission of Wolters Kluwer Health, Inc. (Nemmar et al. 2002)

existence of a corona, with its overall negative charge, will diminish nanoparticle toxicity due to a reduced interaction with the negatively charged cell wall (Docter et al. 2015). On the other side, nanoparticles with a positive charge (without a corona), being electrostatically attracted to the negatively charged cell membrane, will have an increased cellular uptake.

1.7.2.5 Nanoparticle Uptake by Cells

Depending on their physicochemical properties, nanoparticles can be internalized by cells and locate within various organelles, cytoplasm, mitochondria, nucleus, lysosomes, endoplasmic reticulum, etc. (Singh et al. 2010; Huk et al. 2015; Sathuluri et al. 2011). The cellular uptake of nanoparticles is also cell specific and depends on the experimental conditions (Kettler et al. 2014). Cell internalization of nanoparticles usually results in cytotoxicity. Inside the cells, nanoparticles have been observed to affect cellular processes; produce reactive oxygen species, DNA damage, and epigenetic changes; and even cause cell death (Gatoo et al. 2014; Karlsson et al. 2008; Stoccoro et al. 2013). Nanoparticles can be genotoxic simply by their direct interaction with the genetic material or due to generating reacting oxygen species (Tortiglione 2014).

1.7.3 Nanoparticle Association to Respiratory Diseases

Epidemiological studies indicate that exposure to particulate pollution is associated with different respiratory diseases, such as chronic obstructive pulmonary disease (COPD), respiratory infections, lung cancer, and asthma (Mannucci et al. 2015; Rückerl et al. 2011). For each 10 $\mu g/m^3$ increase in particulate pollution, there is a 2.5% increase in hospital admissions of patients with COPD (Mannucci et al. 2015). Children exposed to air pollution have more respiratory tract infections and asthma episodes. Figure 1.8 shows nanoparticles accumulated in the lung of subjects with asthma and emphysema.

Nanoparticles with composition of iron, manganese, and chromium were found in the lungs of welders suffering from various respiratory diseases (Andujar et al. 2014). Welders that are exposed to welding fumes for a long time have higher incidence of high blood pressure (Li et al. 2015a; Xu et al. 2017).

1.7.4 Nanoparticle Association to Cardiovascular Diseases

In vivo, in vitro, and epidemiological studies show that exposure to nanoparticles of various compositions is associated to cardiovascular diseases (Franklin et al. 2015; Yu et al. 2016; Savi et al. 2014; Cosselman et al. 2015; Rückerl et al. 2011). Epidemiological studies show a relationship between particulate pollution and a gamut of cardiovascular diseases. Among them are blood clot formation, pulmonary embolism, increased blood pressure, atherosclerosis, arrhythmia, ischemic heart disease, myocardial infarction, and heart failure; stroke and stroke mortality correlate

to particulate pollution in a dose-dependent manner (Mannucci et al. 2015; Cosselman et al. 2015; Shah et al. 2015; Yu et al. 2016).

The composition of nanoparticles that are associated to adverse cardiovascular effects includes, but is not limited to, titanium dioxide, silver, silicon, silica, carbon black, carbon nanotubes, zinc oxide, etc. (Many of these nanoparticles are promoted for their use in agriculture due to some benefits on selected plants.)

Nanoparticles of metal oxide produce clotting irrespective of their charge (Steuer et al. 2014). Studies show that environmental nanoparticles are associated with an increased risk of thrombotic complications that lead to an increased and worse prognosis of cardiovascular events (Ilinskaya and Dobrovolskaia 2013).

Nanoparticles are found in biopsies and various human specimens of diseased tissue or collected from the blood of patients with various diseases (Gatti 2004; Gatti and Montanari 2006; Gatti and Rivasi 2002; Bitounis et al. 2016; Rinaldo et al. 2015; Ballestri et al. 2001). Nanoparticles tend to accumulate at the site of vascular lesions (Miller et al. 2017).

Figure 1.9a shows microscopy images of nanoparticles found in blood clots collected from diseased patients by using a vena cava filter (Gatti and Montanari 2006). With the help of environmental scanning electron microscopy (ESEM) and energy-dispersive spectroscopy (EDS), nanoparticles and their composition are identified in thrombi and fibrotic tissue taken from patients at risk of developing deep-vein thrombosis or to prevent pulmonary embolisms in potentially relapsing patients (Gatti et al. 2005; Gatti and Montanari 2006). The composition of micro- and nanoparticles collected from the tissue of the patients had various compositions, such as Fe, Cr, Cu, W, and Al. It is likely that the particles within the thrombi are the sole cause for thrombosis (Gatti et al. 2005).

Nanoparticles from automobile exhaust pollution lead to adverse cardiovascular effects which can occur as fast as within a few hours in the case of acute exposure, or within a few years for chronic exposure (Donaldson et al. 2013). Living near highway is associated with increased risk of high blood pressure (Chung et al. 2015).

Each 10 $\mu g/m^3$ increased in particulate pollution is associated with a 21% increase in fatal and nonfatal coronary artery disease according to an epidemiological study in more than 65,000 postmenopausal US women (Mannucci et al. 2015). An increase in the levels of particulate matter of 10 $\mu g/m^3$ results in a 35–85% increase in nonfatal and fatal strokes for population suffering a long-term exposure to particulate pollution (Mannucci et al. 2015).

Particulate pollution is also associated to higher mortality due to cardiovascular and respiratory events (Cohen et al. 2017; Chen et al. 2012; Brook 2008). The segment of population exposed to particulate pollution has a lower life expectancy, the life span being reduced by several months to several years (Brook 2008).

High concentration of particulate pollution is associated with increased hospital admission, stroke, acute myocardial infarction, and mortality (Shah et al. 2015). Even small increases in particulate pollution were associated with a large (19%) increase for developing cerebrovascular disease (ischemic and hemorrhagic) (Shah et al. 2015).

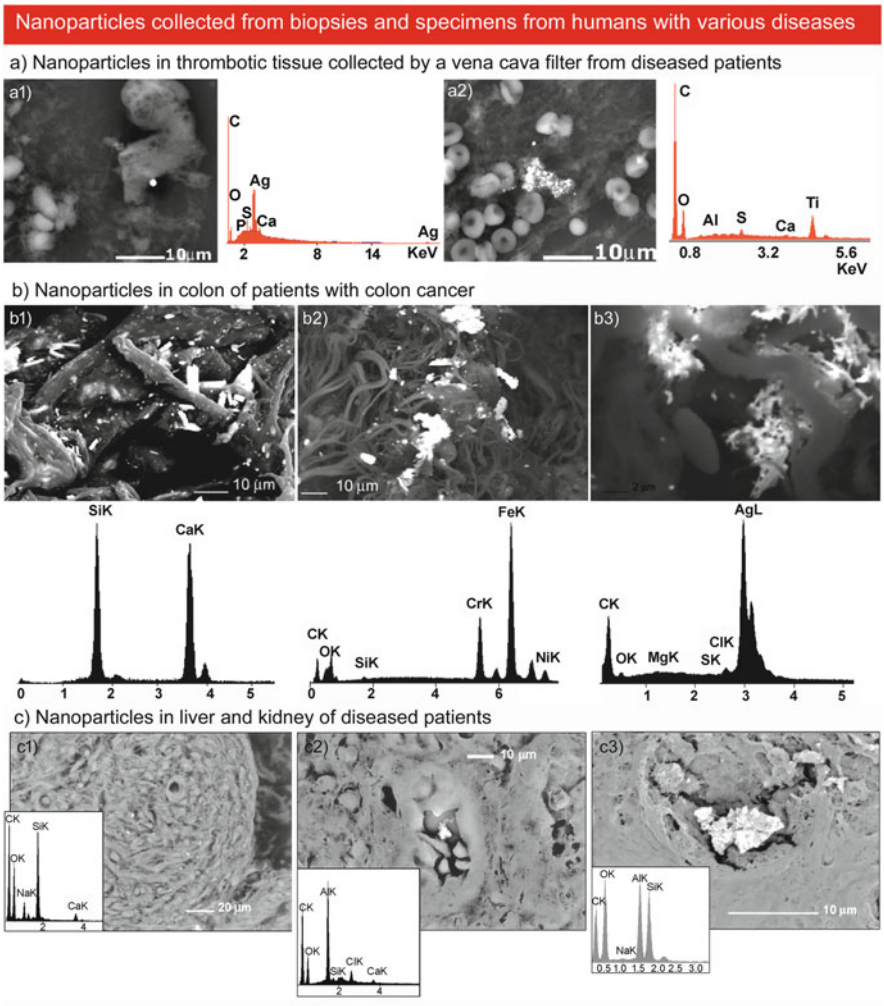

Fig. 1.9 Scanning electron microscope (SEM) images of nanoparticles observed in the blood, colon, liver, and kidney biopsies of patients with various diseases together with their EDS spectra indicating their compositions. (**A**) Nanoparticles in thrombotic tissues collected from diseased patients by using a vena cava filter; (a1) SEM image shows thrombus containing nanoparticle with the composition of Ag, S, and O; (a2) SEM image of red blood cells with a cluster of nanoparticles having the composition: Ti, O, and. Images (a1) and (a2) are reprinted from Gatti A. M. & Montanari S., Journal of Biomedical Materials Research Part B-Applied Biomaterials 77B (2006) 307, with permission from John Wiley & Sons, Inc. (Gatti and Montanari 2006). (**B**) SEM images showing nanoparticles with different compositions in the colon of patients with various diseases. Upper side shows the electron microscopy image and bottom the EDS spectrum indicating the composition of the nanoparticles; (b1) nanoparticles of calcium and silicon in the colon of a patient with adenocarcinoma; (b2) nanoparticles of stainless steel (Fe, Cr, Ni) in the colon of a patient with adenocarcinoma; (b3) nanoparticles of Ag in the colon of a patient with colon cancer. Images (b1), (b2), and (b3) are reprinted from Biomaterials, vol. 25, Gatti A. M., Biocompatibility of micro- and nano-particles in the colon. Part II., pp. 385–392, Copyright (2004), with permission from Elsevier (Gatti 2004). (**C**) SEM images of the liver and kidneys from diseased patients together with inset showing the EDS spectrum indicating the composition of nanoparticles;

1.7.5 Nanoparticles in the Central Nervous System

Increasing evidence show that nanoparticles can reach and accumulate in the brain and are associated to neurotoxicity (Cupaioli et al. 2014; Buzea et al. 2007; Song et al. 2015; Hillyer and Albrecht 2001; Wang et al. 2017; Heusinkveld et al. 2016; Maher et al. 2016). In vitro, in vivo, and epidemiological studies relate nanoparticle exposure to neuro-inflammation and neurodegenerative disease, nanoparticles being involved in oxidative stress, inflammation, and impaired activity of cellular organelles. It is believed that the accumulation of nanoparticles in the brain may accelerate the appearance of neurodegenerative diseases (Wang et al. 2017).

Animal Experiments In vivo experiments show that titania nanoparticle-exposed rodents suffer impaired ability of recognition, spatial memory, and learning (Song et al. 2015).

Occupational Exposure Manganese nanoparticles generated during welding and mining operations are associated with increased risk of neurological diseases in miners and welders (Buzea et al. 2007). For example, some welders develop Parkinson's disease in their mid-1940s, while the general population is affected by this disease at around 60 years of age.

Environmental Nanoparticle Pollution Environmental nanoparticles are linked to neurodegenerative disease, such as Alzheimer's disease, Parkinson's disease, and dementia (Calderon-Garciduenas et al. 2016a, b; Gonzalez-Maciel et al. 2017; Chin-Chan et al. 2015).

A recent study showed that pollution nanoparticles are able to translocate to the brain of adults and children living in a polluted environment, can enter cells and organelles, and produce neurotoxicity and cellular damage (Gonzalez-Maciel et al. 2017). Spherical incomplete combustion nanoparticles (29 nm size) have been observed in the neurons, endothelium, nasal, and olfactory epithelium of Mexico City residents that suffered accidental deaths. They were found at sites with abnormal mitochondria, vascular damage in the prefrontal white matter, and other disease pathologies. Samples from control residents living in less polluted environment had intact mitochondria usually with no nanoparticles, compared to those from Mexico City residents that had numerous abnormal mitochondria containing nanoparticles (Gonzalez-Maciel et al. 2017).

Children and adults residing in polluted environments show neuropathological traits of neurodegenerative disease, such as amyloid-beta diffuse plaques and tau

Fig. 1.9 (continued) (c1) liver section with giant-cell granuloma showing nanoparticles with composition of Si, Na, Al, Mg, Ca, O, and C; (c2) kidney granuloma with ceramic nanoparticles; (c3) kidney granuloma with an alumina particle. Images (c1), (c2), and (c3) are reprinted from Biomaterials, vol. 23, Gatti A. M. & Rivasi F., Biocompatibility of micro- and nanoparticles. Part I: in liver and kidney, pp. 2381–2387, Copyright (2002), with permission from Elsevier (Gatti and Rivasi 2002)

hyper-phosphorylation with pre-tangle disease (Calderon-Garciduenas et al. 2016a). Nanoparticles are present inside cells and organelles with abnormal pathologies of adults and children exposed to high concentrations of pollutant nanoparticles (residents of Mexico City) (Calderon-Garciduenas et al. 2016b; Gonzalez-Maciel et al. 2017).

A population-based cohort study of all Ontario adults between 2001 and 2012 found that living closer than 300 m from heavy traffic was associated with a higher incidence of dementia as opposed to those living further away than 300 m (Chen et al. 2017). In addition, dementia involved predominantly urban residents versus rural residents, with urban environments being known to have a higher concentration of particulate matter pollutants than rural ones.

Proof of nanoparticle internalization by human brain tissue is shown in Figs. 1.10a–b). Figure 1.10a shows nanoparticles of iron oxide, silica, and titania inside human cerebral endothelial cells (HCEC) (Kenzaoui et al. 2012).

Figure 1.10b shows nanoparticles in the prefrontal white matter of children living in Mexico City that were exposed to high concentrations of particulate pollution. Nanoparticles were internalized inside (b1) a red blood cell (RBC), (b2) mitochondria, (b3) and a degenerating myelinated axon (Calderon-Garciduenas et al. 2016b).

1.7.6 Nanoparticle Toxicity Following Maternal Exposure

Nanoparticles with different compositions and sizes can be transmitted from mother to offspring through the placental barrier or through milk (Muoth et al. 2016; Ema et al. 2016, 2017; Kulvietis et al. 2011; Melnik et al. 2013; Semmler-Behnke et al. 2014; Snyder et al. 2015; Wick et al. 2010; Yamashita et al. 2011). Rats exposed to titanium dioxide nanoparticle during gestation result in negative cardiovascular effects in progenies that last into adulthood (Hathaway et al. 2017). The potential mechanism of impaired functionality of the heart is believed to be related to mitochondrial dysfunction.

1.7.7 Nanoparticles in the Liver, Kidneys, and Other Organs

Systemically available nanoparticles larger than 6 nm locate mainly in the liver and spleen, and other reticular connective tissues (Lu and Gu 2017). Spleen localization is likely for nanoparticles with a diameter of 200–500 nm, comparable to the inter-endothelial slit. Liver fenestration size of ~100 nm allows accumulation of nanoparticles with smaller and comparable sizes, while the urinary system will eliminate those smaller than ~6 nm (Lu and Gu 2017; Landsiedel et al. 2012).

The long-term retention of nanoparticles in organs can be cytotoxic (Wang et al. 2013a). For example, 20 nm gold nanoparticles accumulate in the liver of rats and change the expression of gene expression involved in detoxification, lipid metabolism, and the cell cycle (Kermanizadeh et al. 2014). Silver nanoparticles with sizes of

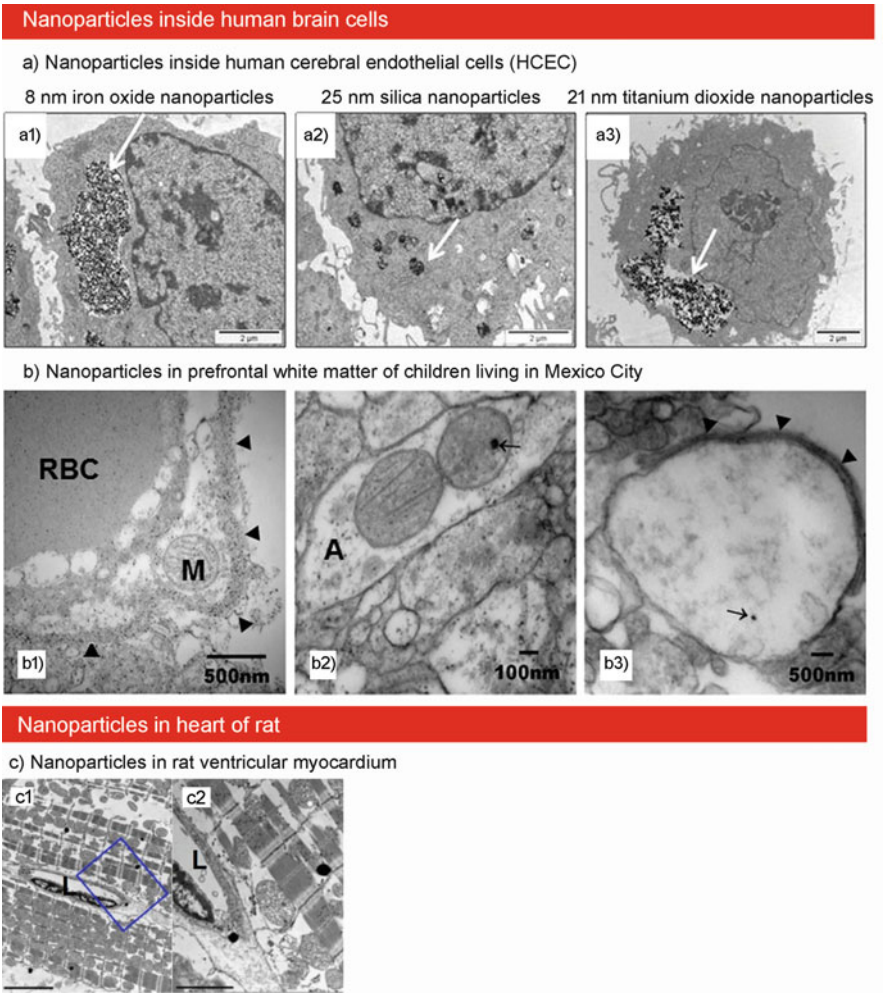

Fig. 1.10 Nanoparticles inside human brain cells. (**A**) Uptake of nanoparticles with various compositions by human cerebral endothelial cells (HCEC); (a1) 8 nm iron oxide, (a2) 25 nm silica, and (a3) 21 nm titanium dioxide nanoparticles. Reproduced from reference (Kenzaoui et al. 2012), courtesy of Portland Press. (**B**) Nanoparticles in the prefrontal white matter of children living in Mexico City and exposed to high concentrations of nanoparticulate pollution. (b1) Nanoparticles in a red blood cell (RBC), an endothelial cell mitochondria (M), and the basement membrane (arrowheads); (b2) one 30 nm diameter nanoparticle inside a mitochondria without cristae in a poorly preserved unmyelinated (short arrow); (b3) a nanoparticle (short arrow) inside a degenerating myelinated axon with remnants of myelin (arrowheads). Reprinted from Environmental Research, vol. 146, Calderon-Garciduenas L. et al., Prefrontal white matter pathology in air pollution exposed Mexico City young urbanites and their potential impact on neurovascular unit dysfunction and the development of Alzheimer's disease, pp. 404–417, Copyright (2016), with permission from Elsevier (Calderon-Garciduenas et al. 2016b). (**c**) Titanium dioxide nanoparticles in the rat ventricular myocardium after tracheal instillation. Nanoparticles are located in longitudinally oriented cardiomyocytes and in the wall of a vascular structure. Reprinted from (Savi et al. 2014) courtesy of Particle and Fibre Toxicology

20 nm can be found in the cytoplasm and nuclei of liver cells and upregulate pro-inflammatory genes (Kermanizadeh et al. 2014). Nanoparticles of copper and zinc oxide produce renal damage in mice (Wang et al. 2013a).

Nanoparticles of different materials have been found in the liver and kidneys of humans with different diseases (Gatti and Rivasi 2002). Some examples are illustrated in Fig. 1.9b–c where one can see nanoparticles in diseased tissue of the liver and kidneys (Gatti and Rivasi 2002).

Nanoparticles can be found in heart tissue as well. Titanium dioxide nanoparticles are internalized by heart cells following tracheal instillation of nanoparticles in rats (Savi et al. 2014). Figure 1.10c shows nanoparticles in the rat ventricular myocardium located in longitudinally oriented cardiomyocytes and in the wall of a vascular structure.

Nanoparticle exposure is also associated to diabetes, exacerbation of allergic diseases, and immune system disruption (Mannucci et al. 2015; Ilinskaya and Dobrovolskaia 2014).

A recent meta-analysis study found a positive association between particulate pollution and type 2 diabetes mellitus (He et al. 2017). In individuals suffering long-term exposure to particulate matter, for each 10 $\mu g/m^3$ increase in particulate concentration PM2.5, the risk of type 2 diabetes mellitus increases by 25% (He et al. 2017).

Nanoparticles with different compositions (Co, ZnO, TiO_2) are toxic to immune cells or immune system (Bregoli et al. 2009; Hanley et al. 2009; Andersson-Willman et al. 2012; Moon et al. 2011). When the immune system is deregulated, it can trigger the onset of cancer and autoimmune diseases (Ilinskaya and Dobrovolskaia 2014).

1.7.8 Toxicity of Nanoparticles with Various Compositions

In the following we will present information on the toxicity of nanoparticles with different compositions in humans and animals. Many of these nanoparticles are suggested to be beneficial agrichemicals.

1.7.8.1 Toxicity of Gold Nanoparticles

One might think that, just because bulk gold is chemically inert and biocompatible, gold nanoparticles should react very little and be biocompatible. Indeed, some gold nanoparticles can be chemically inert and have little or no toxicity depending on their size and surface functionalization. However, there is experimental evidence that indicates gold nanoparticles are toxic, the toxicity degree depending on their size and functionalization.

Gold Nanoparticle Magnetism The toxicity of gold nanoparticles might be related to the fact that when sufficiently small, gold in nanoform develops magnetic moments. Several materials at the nanoscale develop magnetic moments, while their bulk counterparts do not show magnetism (Pacheco and Buzea 2018). Gold in bulk form is diamagnetic—an external magnetic field will have a very weak effect

on it; after the removal of the field, the material will not show a magnetic moment. Decreasing the size of gold particles below 3 nm, they become ferromagnetic (Hori et al. 1999, 2004; Yamamoto et al. 2004; Maitra et al. 2011; Greget et al. 2012; Nealon et al. 2012). A similar phenomenon happens to platinum (Sakamoto et al. 2011) and palladium (Hori et al. 1999), these materials becoming magnetic in nanoform. Hence, gold nanoparticles smaller than 3 nm become magnetic, and their aggregation and chemical reactivity will differ from that of larger nanoparticles.

The magnetic behavior of nanoparticles can be modified by coating with various ligands (Krishna et al. 2014; Crespo et al. 2013). Gold nanoparticles of selected sizes can be made diamagnetic, paramagnetic, or ferromagnetic, just by changing surface ligand types (Krishna et al. 2014). In addition, gold surface functionalization can modify their electrical charge rendering them positively or negatively charged (Gerber et al. 2013; Cheng et al. 2013). The electromagnetic behavior of nanoparticles will influence their translocation and accumulation and hence their toxicity.

Accumulation Experiments demonstrated that intravenously administered gold nanoparticles accumulate in the liver, spleen, kidney, lung, brain, heart, thymus, skin, and testis (Johnston et al. 2010). For example, 8.5% of radiolabeled (1.4 nm size) gold nanoparticles are found in secondary targets, including the blood and liver of rats after 24 h of exposure (Semmler-Behnke et al. 2008). Gold nanoparticles administered via intravenous injection in pregnant rodents were detected in fetal tissue as soon as 2 h after exposure (Lin et al. 2015).

Mice injected intraperitoneally with gold nanoparticles with sizes 8–37 nm showed fatigue, loss of appetite, fur color changes, weight loss, camel-like back, and crooked spine; most of the mice died within 21 days (Chen et al. 2009). Gold nanoparticles with small size translocate from maternal blood into fetus as showed by experiments on rats (Semmler-Behnke et al. 2014).

Cytotoxicity The cytotoxicity to gold nanoparticles is cell specific and depends on their surface coating (Cheng et al. 2013; Schlinkert et al. 2015). Gold nanoparticles were found to be internalized by cells and, as a function of their surface functionalization, may locate in endosomes/lysosomes, mitochondria (Cheng et al. 2013), vacuoles (Khlebtsov and Dykman 2011), and nuclei (Ojea-Jimenez et al. 2012). In vivo cytotoxicity studies showed the presence of gold nanoparticles inside liver Kupffer cells and spleen macrophages accompanied by acute inflammation and apoptosis in the liver (Cho et al. 2009). Analysis of liver and spleen samples of rats exposed to gold nanoparticle via injection indicate gene expression changes in genes pertaining to detoxification, lipid metabolism, the cell cycle, defense response, and circadian rhythm (Balasubramanian et al. 2010).

Many experimental results suggest that in small enough concentrations, gold nanoparticles with sizes down to 3–5 nm are not toxic; however, smaller nanoparticles may be cytotoxic due to their irreversible binding to biomolecules such as DNA (Khlebtsov and Dykman 2011). This fact could be explained by the newly acquired magnetic behavior of very small gold nanoparticles (Pacheco and

Buzea 2018). Some in vitro genotoxicity studies indicate that gold nanoparticles may induce DNA strand break and chromosomal damage via oxidative stress in some mammalian cells (Li et al. 2011; Hadrup et al. 2015).

Charge-Dependent Cytotoxicity Cytotoxicity studies of gold with different surface properties on Cos-1 cells (kidney fibroblasts from the African monkey) indicate that cationic nanoparticles are several times more toxic than anionic ones (Khlebtsov and Dykman 2011). Study on other four cell lines (HeLa, Sk-Mel-28, L929, J774A1) shows that 1.4 nm nanoparticles cause cell necrosis 12 h after exposure, while 1.2 nm gold nanoparticles caused apoptosis (Khlebtsov and Dykman 2011).

1.7.8.2 Toxicity of Silver Nanoparticles

Silver nanoparticles are promoted as agrichemicals due to their broad-spectrum antimicrobial properties (Cox et al. 2016; Anjum et al. 2013; Jo et al. 2009; Lamsal et al. 2011a). However, silver in nanoform is found to be toxic to humans and animals.

Due to its antibacterial properties, silver is used in wound dressing (Marx and Barillo 2014). Sometimes overexposure of humans to silver from drugs or wound dressing leads to a condition called argyria, where the skin has a blue-gray discoloration, being accompanied by liver toxicity (Hadrup and Lam 2014; Christensen et al. 2010).

Accumulation Experiments on mice show that following silver nanoparticle inhalation, ingestion, and injection, they accumulate in the blood, stomach, liver, spleen, kidney, lung, brain, heart, and testis (Johnston et al. 2010; Gaillet and Rouanet 2015). Inhaled silver nanoparticles have a long residence time in the lung, about a third being still found in the lungs 2 months after exposure, as indicated by in vivo studies in rats (Anderson et al. 2015). Studies on animals showed that radioactive isotope-labeled silver nanoparticles with size of 35 nm can be passed through the placenta to fetuses and can be passed from mother to infants from breast milk (Melnik et al. 2013).

Toxicity Animal studies show that silver is toxic to animals resulting in immune system effects, enlarged heart, weight loss, pulmonary toxicity, changes in liver enzymes and blood biochemistry, and liver damage (Ahlberg et al. 2014; Gaillet and Rouanet 2015; Hadrup and Lam 2014; Kim et al. 2008). Silver nanoparticles accumulated in immune system organs are accompanied by damage in the liver, kidneys, thymus, and spleen, and possible genotoxicity due to chromosomal breakage (Wen et al. 2017). Experiments also indicated that silver nanoparticles might have a toxic effect on myocardial electrophysiology and induce lethal bradyarrhythmias in mice (Lin et al. 2017). They produce cardiac dysfunction and genotoxicity in chicken, heart malformation in fish, and thrombus formation in rats (Yu et al. 2016). A low concentration of silver nanoparticles with size of 45 nm produces vasoconstriction in isolated rat aortic rings, while a high concentration stimulates vasodilation (Rosas-Hernandez et al. 2009).

Cytotoxicity Silver nanoparticles enter cells and may localize in endosomes/lysosomes, mitochondria, and nuclei (Cheng et al. 2013). Once inside the cells and organelles, they interfere with their functions. Smaller Ag nanoparticles seem to be more toxic than larger nanoparticles (Avalos et al. 2014; Carlson et al. 2008). Experimental evidence indicate that human and animal exposure to silver nanoparticles is associated to oxidative stress, apoptosis, genotoxic effects, chromosome aberration, and DNA breaks (Ahlberg et al. 2014; Cheng et al. 2013; Hackenberg et al. 2011; Kim and Ryu 2013; Liu et al. 2015).

1.7.8.3 Toxicity of Titanium Dioxide Nanoparticles

Titanium dioxide nanoparticles are found to have some beneficial effect on selected plants (Zheng et al. 2005; Feizi et al. 2013). They are already widely used as additives in food and other consumer products; however there are studies that indicate they might be toxic (Song et al. 2015; Coccini et al. 2015; Buzea et al. 2007).

Accumulation Animal experiments in vivo demonstrate that 3 nm titania nanoparticles enter blood, cross the blood-brain barrier, and accumulate in the brain (Li et al. 2010; Moon et al. 2011). Fluorescently labeled titanium dioxide nanoparticles with size of 35 nm intravenously injected into pregnant mice crossed and distributed into the placenta, fetal liver, and brain (Yamashita et al. 2011).

Toxicity Animal models show that regardless of their way of internalization (instillation, inhalation, injection, or ingestion), titanium dioxide nanoparticles are toxic (Tortiglione 2014). Toxicity includes immune system effects, reduced sperm production, neurobehavior alteration, abnormal fetal brain development, smaller fetuses, fetal deformities, and mortality. Titanium dioxide nanoparticles promote arrhythmia in rats as a result of a direct interaction with cardiac tissue (Savi et al. 2014). Just one single dose of nanoparticles results in higher cardiac conduction velocity and tissue excitability, increasing the probability of arrhythmias. Titania nanoparticles accumulate in heart tissue of rodents and are associated with cardiac damage and dysfunction, myocarditis, vascular dysfunction, arrhythmia, and inflammatory responses (Yu et al. 2016).

Cytotoxicity Titanium dioxide nanoparticles are cytotoxic and/or genotoxic in various human cell lines (Karlsson et al. 2008; Coccini et al. 2015; Gurr et al. 2005; Yu et al. 2016).

1.7.8.4 Toxicity of Zinc Oxide Nanoparticles

Zinc oxide nanoparticles are known for their antibacterial properties (Saliani et al. 2015; Seil and Webster 2012). Despite being widely used in cosmetics, recent studies show that ZnO are toxic (Roy et al. 2015; Vandebriel and De Jong 2012). They lead to DNA and mitochondrial damage, decreased cell viability, chromosome aberration, and oxidative lesions and upregulate genes controlling apoptosis (Roy et al. 2015; Karlsson et al. 2008). Inhalation of Zn nanoparticles in rats results in

their translocation to the liver and was found to produce liver and lung tissue damage (Vandebriel and De Jong 2012). Zn nanoparticles were also found to produce cardiac inflammation, DNA damage, and apoptosis in mice and rats (Yu et al. 2016).

1.7.8.5 Toxicity of Copper Oxide Nanoparticles

Experiments on human and animal cell lines demonstrate that copper oxide nanoparticles are cytotoxic, neurotoxic, and genotoxic and generate oxidative stress in many cell types (Karlsson et al. 2008; Magaye et al. 2012). Copper nanoparticles degrade DNA via generation of oxygen species. In vivo experiments on mice indicate that copper oxide nanoparticles translocate to the liver, kidneys, and spleen and produce inflammation in these organs. Copper smelter workers exposed to copper dusts or fumes have an increased risk of cancer (Magaye et al. 2012).

1.7.8.6 Toxicity of Cerium Oxide Nanoparticles

Cerium oxide nanoparticles are special in the sense that can have either a beneficial or a toxic effect on mammalian cells depending on cerium oxidation state and oxygen vacancies. The preparation method seems to have an essential role in dictating their toxicity (Gagnon and Fromm 2015; Kumar et al. 2014). Synthesis at lower temperature usually results in reduced toxicity ceria, with a higher number of oxygen vacancies and hence a more beneficial antioxidant effect (Gagnon and Fromm 2015). In addition to synthesis, the storage conditions, aging, and oxidation state influence ceria nanoparticle properties (Kumar et al. 2014). Cerium oxide nanoparticles can be cytotoxic, most likely as a result of reactive oxygen species, and lead to apoptosis in different cell lines (Gagnon and Fromm 2015; Mittal and Pandey 2014). Some CeO_2 nanoparticles with size smaller than 20 nm can have beneficial properties: protection of cells against irradiation, inhibition of oxidative stress, and prevention of inflammation (Gagnon and Fromm 2015; Niu et al. 2011).

1.7.8.7 Iron Oxide, Cobalt, and Nickel Nanoparticle Toxicity

Nanoparticles made of Fe, Ni, and Co are ferromagnetic. Magnetic nanoparticles are chemically unstable over long periods of time, oxidizing easily in air (Rao et al. 2015). Magnetism makes nanoparticles more prone to aggregate. As learned from their applications for in vivo imaging, there is always a concern of their aggregation in organs that may lead to inflammation and immunological responses (Markides et al. 2012). Intravenously administered ultrasmall superparamagnetic iron oxide nanoparticles (USPION) promote the formation of blood clots, cardiac oxidative stress, and DNA damage in mice (Nemmar et al. 2016). Injected magnetite nanoparticles with size of 10 nm were found to accumulate in the liver and spleen of rats (Ruiz et al. 2016).

Cobalt exhibits extreme toxicity in large amounts, and cobalt long-term exposure is associated to health effects related to the thyroid gland, lungs, skin, and immune system (Simonsen et al. 2012). Cobalt and nickel compounds are carcinogenic; their inhalation and dermal exposure lead to skin allergies, lung fibrosis, and cancer (Magaye et al. 2012). Nickel nanoparticles elicit severe cytotoxicity producing oxidative stress, genotoxicity, and cell death (Magaye et al. 2012).

1.7.8.8 Toxicity of Silicon and Silica Nanoparticles

Silicon and silicon dioxide (silica) nanoparticles have been found to produce cardiovascular disorders: cerebrovascular toxicity, coagulation disorders, and endothelium dysfunction in rodents (Yu et al. 2016). They are cytotoxic and genotoxic to a range of human cell types, such as human umbilical vein endothelial cells, epithelial cells, microvascular endothelial cells, platelets, and aortic vessel cells (Yu et al. 2016).

1.7.8.9 Toxicity of Carbon-Based Nanoparticles

Carbon-based nanoparticles include carbon black, fullerenes C60 and C70, single-walled carbon nanotubes, SWCNTs, and multiple-walled carbon nanotubes MWCNTs. Sometimes SWCNTs and MWCNTs are referred to as simply "carbon nanotubes," CNTs.

Carbon black particles are a by-product of automobile exhaust and were found to be toxic to humans and animals. Carbon black nanoparticles were detected in the urine of 289 children at an average of 98.2×10^5 particles/ml (Saenen et al. 2017). Children living close to a major road (<160 m) had a higher amount of urinary particles (Saenen et al. 2017). Carbon black and CNTs were found to produce cardiac and endothelial dysfunction, vasorelaxation, thrombus formation, placenta vessel damage, and thrombus formation in rodents (Yu et al. 2016). They produce cytotoxicity and genotoxicity in a wide range of cell types, such as human umbilical vein endothelial cells, blood cells, smooth muscle cells, human dermal microvascular endothelial cell, and human aortic endothelial cells, to name a few (Yu et al. 2016).

A review of CNT toxicity and exposure concludes that CNTs lead to oxidative stress and inflammation, resulting to fibrosis and granulomas with possible genotoxic and carcinogenic effects (Aschberger et al. 2010). Figure 1.11a shows the persistence of MWCNT in mice lungs. These nanoparticles can be found in lungs and diaphragm of mice 17 months following inhalation exposure and promote lung adenocarcinoma (Sargent et al. 2014). Due to their morphological similarities, MWCNTs are suspected to be as toxic as asbestos (Stella 2011). A comparison between CNTs and asbestos can be seen in Fig. 1.1e–f (Murr and Soto 2004).

MWCNTs are shown to be teratogenic in mice, resulting in deformity of limbs and fusion of vertebrae and ribs (Fujitani et al. 2012; Ema et al. 2016). Figure 1.11b shows fetal external and skeletal malformations in mice administered with MWCNTs. The mice show deformity of the forelimbs, short or absent tail, and fusion of the vertebrae and ribs (Ema et al. 2016).

Consequently, the use of CNTs in agriculture is not justified taking into account their adverse effects on humans and animals, as well as environment overload.

1.7.9 Comparative Toxicity of Nanoparticles with Various Compositions

Comparative toxicity studies indicate that some nanoparticles are more toxic than others. For example, metallic nanoparticles are inherently toxic due to their

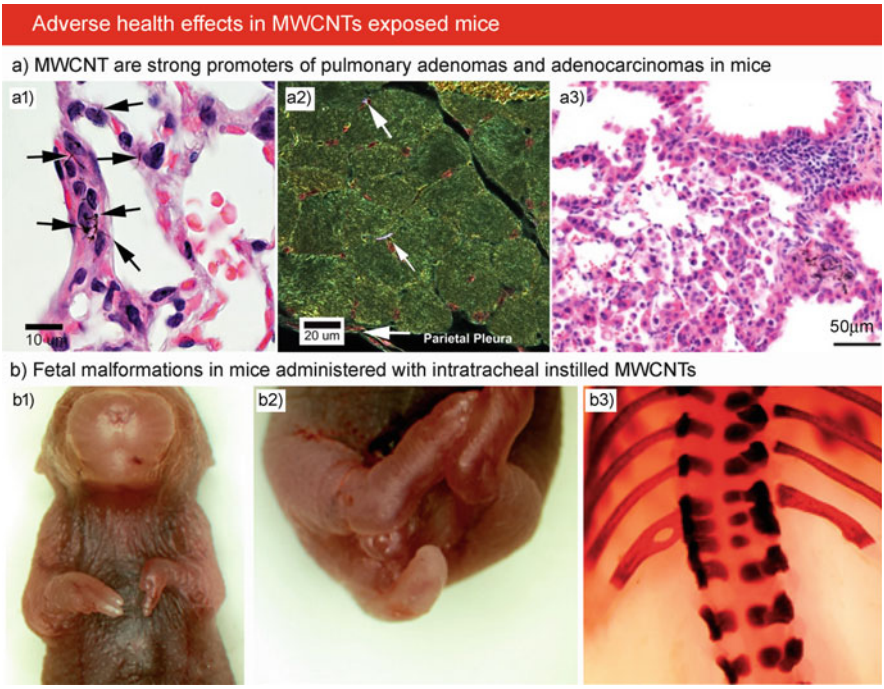

Fig. 1.11 Examples of adverse health effects in mice exposed to MWCNTs. (**A**) Light and enhanced dark-field imaging of MWCNT in lungs and diaphragm of mice exposed with MWCNTs 17 months following inhalation exposure. (a1) MWCNTs (black fibers) can be observed in the alveolar interstitium; (a2) enhanced dark-field imaging shows MWCNT (upper two arrows) in the diaphragm. MWCNTs are bright white; nuclei, brown-to-orange; muscle cells, green; and red blood cells, yellow. The lower arrow indicated the parietal pleural border; (a3) image showing a focal adenomatous hyperplasia in a mouse following the inhalation of MWCNT. Images (a1–a3) are reproduced from Sargent L. M. et al. 2014, Promotion of lung adenocarcinoma following inhalation exposure to multi-walled carbon nanotubes. Particle and Fibre Toxicology, 11, 18 (Sargent et al. 2014). (**B**) Fetal external and skeletal malformations in mice administered with intratracheal instilled MWCNTs at 4 mg/kg on GD 9. Malformations include (b1) reduction deformity of the forelimbs, (b2) short or absent tail, and (b3) fusion of the vertebrae and ribs. (Courtesy of Dr. T. Fujitani, Tokyo Metropolitan Institute of Public Health, Japan, Fujitani et al. (2012). Reproduced from Reproductive and developmental toxicity of carbon-based nanomaterials: A literature review, Ema M. et al., Nanotoxicology vol. 10 (2016) 391–412, reprinted by permission of the publisher (Taylor & Francis Ltd, http://www.tandfonline.com) (Ema et al. 2016)

composition, such as metal dust and welding fumes (cobalt, nickel, cadmium, zinc, manganese) (Buzea et al. 2007). Even though some materials are not considered toxic in bulk, in nanoparticle form they cause cancer in animals, as in the case of titanium dioxide (Borm et al. 2004). The toxicity of nanoparticles is also cell specific, as can be seen below.

Table 1.3 illustrates comparative toxicity of nanoparticles with various compositions on murine macrophage cells (Soto et al. 2005). The scale for the

Table 1.3 Nanoparticle comparative cytotoxicity

Material	Mean aggregate size (μm)	Particle size and (mean particle size)	RCI at 5 μg/ml	RCI at 10 μg/ml
Silver Ag	1	3–100 nm (30 nm)	1.5	0.8
Silver Ag	0.4	5–65 nm (30 nm)	1.8	0.1
Alumina Al_2O_3	0.7	4–115 nm (50 nm)	0.7	0.4
Iron oxide Fe_2O_3	0.7	5–140 nm (50 nm)	0.9	0.1
Zirconia ZrO_2	0.7	7–120 nm (20 nm)	0.7	0.6
Titanium dioxide TiO_2	0.8	5–100 nm (40 nm)	0.4	0.2
Chrysotile asbestos $Mg_3Si_2O_5(OH)_4$	0.5–15	15–40 nm diameter (20 nm); 15 μm length	1.0	1.0
Carbon black powder	0.5	2–50 nm (20 nm)	0.8	0.6
Single-wall carbon nanotubes SWCNTs	10	10–100 nm diameter	1.1	0.9
Multiple-walled carbon nanotubes MWCNTs	2	5–30 nm diameter (15 nm), 20 nm–1 μm length	0.9	0.8

Reprinted from Soto K. F. et al. 2005. Comparative in vitro cytotoxicity assessment of some manufactured nanoparticulate materials characterized by transmission electron microscopy. Journal of Nanoparticle Research, 7, 145–169, with permission from Springer (Soto et al. 2005)
RCI relative cytotoxicity index

comparison of nanoparticles toxicities is based on value "1" for asbestos, a known carcinogen. Nanoparticles with values smaller than "1" are less toxic than asbestos. At a concentration of 5 μg/ml, silver nanoparticles and SWCNTs are more toxic than asbestos. They are followed in decreasing toxicity order by MWCNTs and iron oxide, carbon black, zirconia and alumina, and titanium dioxide. At a concentration of 10 μg/ml, the materials show the following toxicity in decreasing order: SWCNTs, MWCNTs and silver, carbon black and zirconia, alumina, and titanium dioxide. It is interesting to note that the aggregation strongly modulates the cytotoxicity of silver.

In Fig. 1.12 is illustrated the comparative cytotoxicity of CuO, TiO_2, ZnO, $CuZnFe_2O_4$, Fe_2O_3, Fe_3O_4, C, and CNT nanoparticles in human lung epithelial cell line A549 (Karlsson et al. 2008). This study reveals that CuO nanoparticles show the highest level of toxicity, followed by ZnO, carbon nanotubes, $CuZnFe_2O_4$, and Fe_2O_3. Titania and Fe_3O_4 did not decrease cell viability; however they produced oxidative lesions.

The discrepancy between results on comparative cytotoxicity from different studies is likely due to the fact that the nanoparticles used in various experiments have different sizes, aggregations, and synthesis methods, which will result in different reactivity and interaction with cells and subcellular structures (Staszek et al. 2015). In addition, cytotoxicity is cell dependent, as we will see in the following.

Table 1.4 shows cellular responses of two types of cells (ht bronchial epithelial cells and RAW macrophages) subjected to TiO_2, SiO_2, and MWCNTs, having

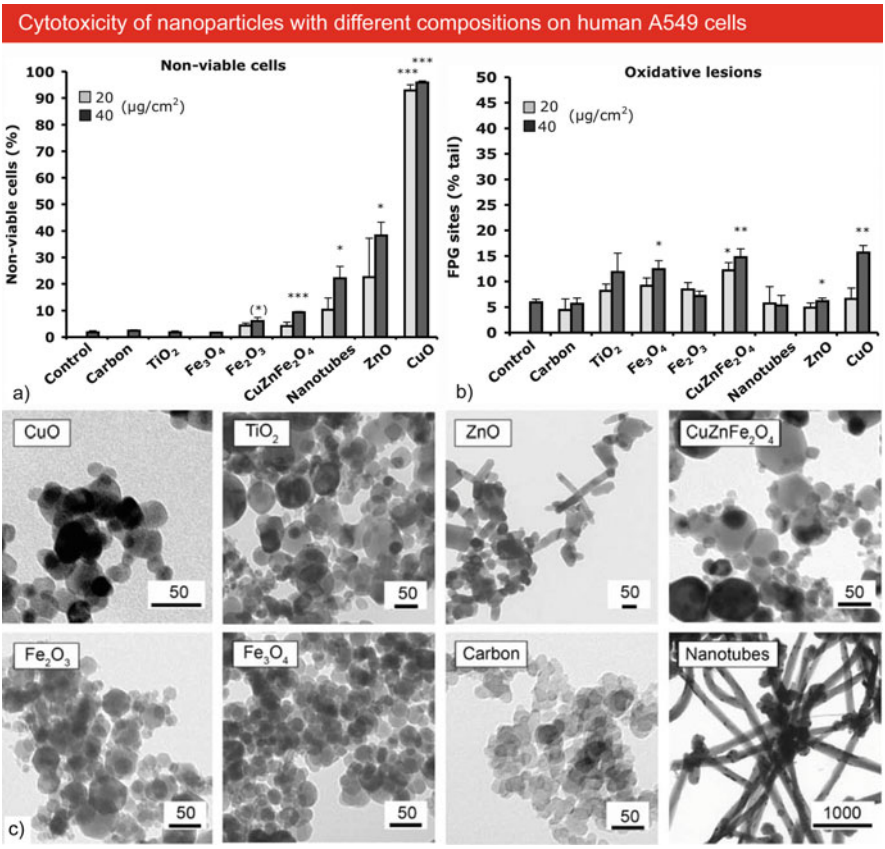

Fig. 1.12 (**a**) Cytotoxicity of various nanoparticles in cultures of human A549 cells (human adenocarcinomic alveolar basal epithelial cells) after exposure to 20 and 40 μg/cm^2 of nanoparticles. (**b**) Oxidative DNA lesions. (**c**) Transmission electron microscopy images of nanoparticles used in the experiment: CuO, TiO_2, ZnO, $CuZnFe_2O_4$, Fe_2O_3, Fe_3O_4, C, and CNTs. Reprinted with permission from Karlsson H. L. et al. 2008. Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. Chem Res Toxicol, 21, 1726–32. Copyright (2008) American Chemical Society 45 (Karlsson et al. 2008)

different sizes (Sohaebuddin et al. 2010). The response to nanoparticles is cell specific. For example, titania nanoparticles are more cytotoxic to macrophages than the ht bronchial epithelial cells, showing a higher apoptosis rate. In general, macrophages are more prone to cytotoxicity than fibroblasts and epithelial cells as a response to nanoparticle exposure.

Other cytotoxicity studies on pulmonary cell lines show that Cu and Zn nanoparticles have the highest toxicity, followed by TiO_2, Al_2O_3, CeO_2, Ag, Ni, and ZrO_2 (Lanone et al. 2009).

Table 1.4 Cellular responses of two types of cells (ht bronchial epithelial cells and RAW macrophages) subjected to several nanomaterials (TiO_2, SiO_2, and MWCNTs with different sizes in nm)

Cell responses	hT bronchial epithelial cells					RAW macrophages				
			MWCNT					MWCNT		
	TiO_2	SiO_2	<8	20–30	>50	TiO_2	SiO_2	<8	20–30	>50
Cytotoxicity	+	++	++	+	–	+++	+++	++	++	+++
ROS	–	+	+	–	–	+	+	+	–	+
LMD	–	–	–	–	+	–	–	–	–	+
MMP	–	+	++	–	–	–	+	+	–	+
Caspase	–	++	++ +	–	–	–	–	–	–	–
Apoptosis	–	+++	++	–	–	+++	+++	+	+	++
Necrosis	–	–	–	–	–	–	–	–	–	–

ROS reactive oxygen species, *LMD* lysosomal membrane destabilization, *MMP* mitochondrial membrane potential. The symbols "+" and "−" denote toxicity or its absence. "+" means a significant increase in the intracellular event, while "−" means that the material did not induce a toxic effect. "++" and "+++" signify twice and three times the response. Reproduced from (Sohaebuddin et al. 2010) under a Created-Commons CC-By license

1.8 Beneficial or Detrimental Effect of Nanoparticles?

Some nanoparticles are promoted as agrichemical for selected beneficial effects on some plants. While they show benefits for some plants, they are phytotoxic to others. If used in agriculture, nanoparticles will become ubiquitous in the soil, atmosphere, and water and will be available for uptake in other plant species for which they are phytotoxic. Last but not least, most nanoparticles are shown to be associated to many diseases in humans and animals. The environmental overload with agricultural nanoparticles together with the consumption of plants containing nanoparticles will pose a serious health risk to humans and animals. For example, silver, which is increasingly used in agriculture, can be more cytotoxic than asbestos. Another example is carbon in the form of nanotubes. Carbon nanotubes have morphologies very similar to asbestos and show similar toxicities in animal cells.

Here below we give the most important arguments explaining why nanoparticles should not be used in agriculture:

- Most studies of beneficial effects of nanoparticles were carried out on first-generation plants. The benefits shown in first-generation plants might not be present in second-generation plants, as in the case of ceria nanoparticles on tomato plants.
- The use of agrichemical nanoparticles as plant growth regulators will result in their environmental accumulation in soil and water. Due to their small size, it is very likely they will become airborne.

- Nanoparticle benefits are found to be plant species specific. Some nanoparticles show benefits for one plant, while they are phytotoxic for another plant type. Agrichemical nanoparticles cannot be confined to a specific location, becoming airborne and bioavailable for other plants that might suffer phytotoxic effects as a result of exposure.
- The accumulation of nanoparticles in soil will make a higher concentration of nanoparticles available to plants for uptake. In many cases a higher concentration of nanoparticles has been shown to have detrimental effects compared to the lower concentrations that might show beneficial effects.
- Edible plants have been shown to uptake and accumulate nanoparticles. The possible toxic effect on humans from nanoparticles within edible plants poses serious concerns.
- The use of nanoparticles can adversely affect soil microbiota, creating an imbalance in the bacterial diversity.
- Last but not least, most nanoparticles are shown to have toxic effects on humans and animals, leading to a plethora of diseases, ranging from respiratory, cardiovascular, and neurodegenerative diseases to various cancers. Agricultural nanoparticles can become available to humans via ingestion of edible plants that contain nanoparticles, via environmental exposure with nano-agrichemicals from atmosphere, soil, and water.

We should ask ourselves: are the beneficial effects of nanoparticles in some plants enough to warrant their use in agriculture with possible catastrophic consequences for humans, animals, and other plants?

Figure 1.13 shows a schematic of adverse effects of nanoparticles in humans, animals, and plants. There is overwhelming evidence demonstrating that nanoparticles with different composition are associated with health effects in humans and animals, such as arteriosclerosis, high blood pressure, blood clots, stroke, arrhythmia, heart disease, heart attack, respiratory diseases, neurodegenerative diseases, reproductive system diseases, and various cancers. Nanoparticle toxicity in plants relate to growth inhibition, physiological and biochemical traits, and toxicity at genetic level.

It is likely that many authors working in the agricultural field are not aware of the current knowledge on nanoparticle toxicity to humans and animals and are conducting experiments expected to improve plant yield and promote their growth. We hope that this chapter has shed some light on the most stringent problems related to nanoparticles use in agriculture and will help scientists decide whether or not to pursue research in different avenues of nano-agrichemicals.

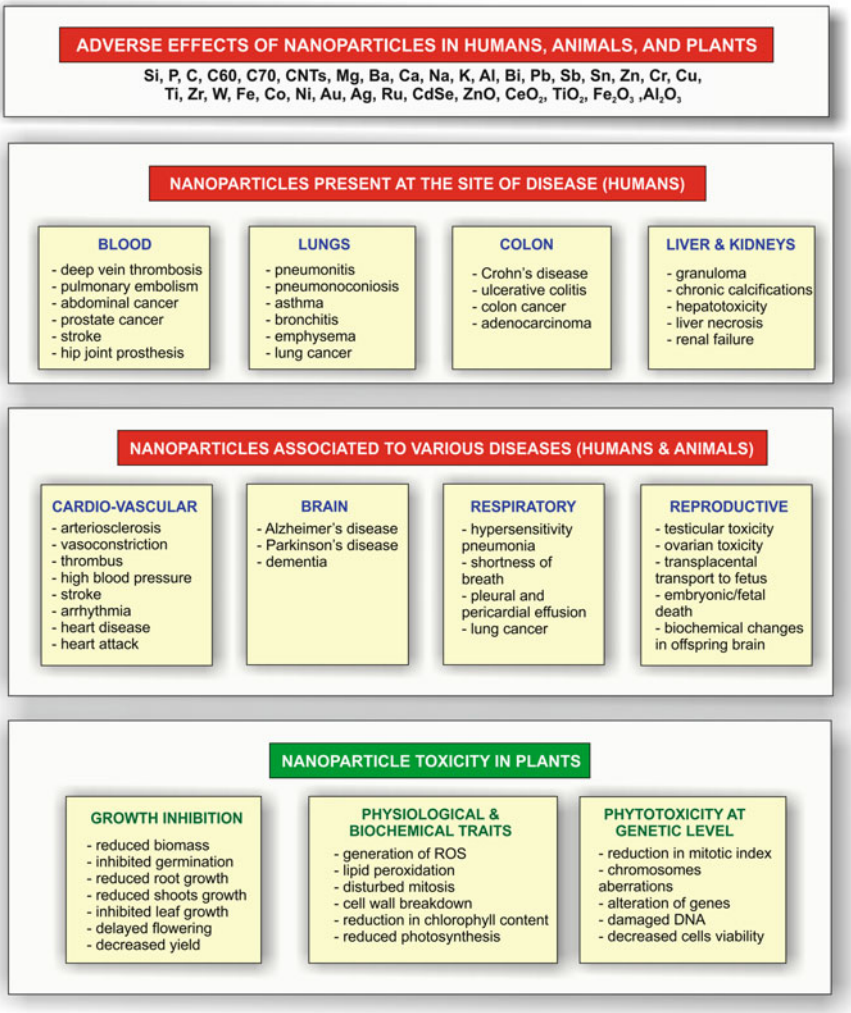

Fig. 1.13 Schematics of adverse effects of nanoparticles in humans, animals, and plants. Nanoparticles with different compositions shown in the upper part were found to localize in humans at the site of various diseases. Several health effects in humans and animals are associated to exposure to nanoparticles. Nanoparticle toxicity in plants relate to growth inhibition, physiological and biochemical traits, and toxicity at genetic level

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2 Interplay Between Engineered Nanomaterials (ENMs) and Edible Plants: A Current Perspective

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2.1 Introduction

Engineered nanomaterials (ENMs), emerging from the dynamically developing nanotechnology industry, are being used at a greater pace in photonic crystals, coatings, personal care products, food, bioremediation, paints, material science, and catalysis (Fig. 2.1) (Falcaro et al. 2016; Zuverza-Mena et al. 2016; Du et al. 2016). The ENMs also have multiple applications in health-care industry and energy production (Safari and Zarnegar 2014; Vilela Neto 2014). Nanotechnology has brought about a new industrial revolution through its wide range of end uses. For instance, it has been reported that about 3000 tons of TiO_2-NPs are manufactured in a different array of products each year, and >50% of the total production is used in personal care products (Weir et al. 2012). Super paramagnetic iron oxide nanoparticles (Fe_3O_4 SPION), due to their magnetic properties, are usually used as a drug carrier, in medical devices such as MRI, and treatment of many diseases (Siddiqi et al. 2016). Considering the human exposure and environmental implications, personal care products represent one of the most important applications of ENMs (Keller et al. 2014). The number of nanotechnology products available in the marketplace is likely

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_2

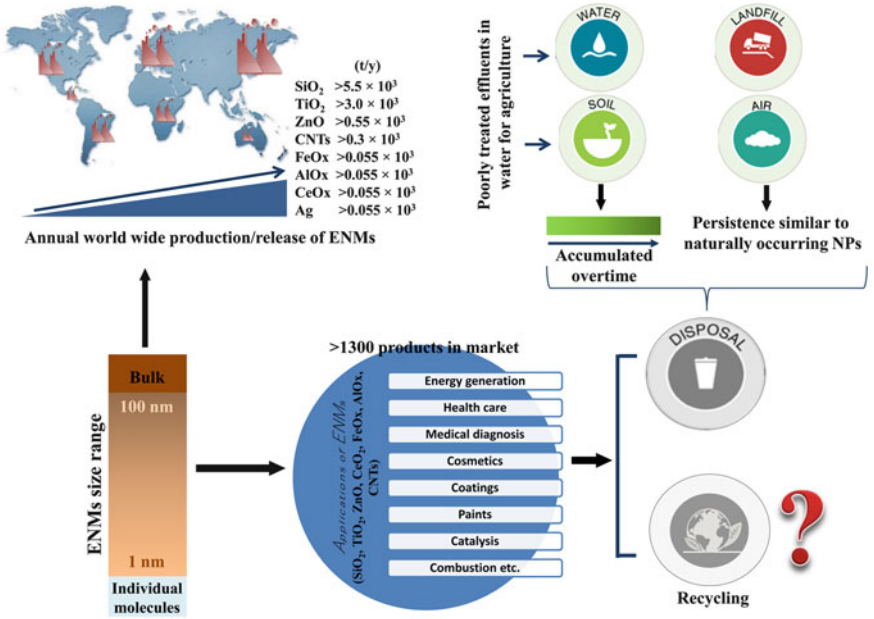

Fig. 2.1 Schematic representation of worldwide production and application of ENMs and their disposal into the environment

to upsurge by many folds over the next coming years (Aslani et al. 2014). It has been estimated that the growth of global nanotechnology industry will reach US$75.8 billion by 2020 (Global Nanotechnology Market Outlook 2022). Besides their use in multiple consumer products, the potential risks associated with ENMs in the environment and through the different trophic levels of the food chain to humans still necessitates further investigations. The smaller size and enhanced surface reactivity due to a greater surface area to volume ratio of ENMs may pose key toxicological consequences in human cells (Krug and Wick 2011). As an integral part of the ecosystem and the most significant in terms of food production for humans, interactions of plants with ENMs are certain. Since the production, application, recycling, and accidental spill of ENMs can result in the unintentional or intentional release of ENMs in the soil, water, and atmosphere. The release can commence from waste incineration plants, discharges from wastewater treatment plants, and landfills. All these processes are expected to receive ENMs from nanotechnology-enabled products (Nowack et al. 2012). At every stage of ENMs life cycle, it is imperative to study the outcome of plant-ENM interactions and evaluate their possible consequences (Smirnova et al. 2011). The ENM accumulation and their entry into the plant system, most likely, alter the magnitude and nature of free radical scavenging potential, antioxidant defense system, and microRNA expression that is responsible for regulating physiology, morphology, and metabolism in plants (Siddiqi and Husen 2016). The effects of ENMs may vary with varying concentration, size, and

shape (Siddiqi and Husen 2016). The overall impact of such an exposure on edible crop plants has not been extensively and scientifically explored. The results of the plant-ENM interaction are not always detrimental to plants. In some cases, the uptake and accumulation of ENMs in plant system may enhance shoot length and inhibit root growth and their proliferation (Lin and Xing 2007; Atha et al. 2012). Realizing the current status of ENMs production and importance of safe and quality cultivation of edible plants for human population, this chapter is aimed to understand the behavior of ENMs particularly the metal-oxide nanoparticles (MONPs) and carbon nanotubes (CNTs) on plant surfaces, various modes of entry of ENMs into plant system, translocation, assessment of their impacts on plant growth, and their transfer to next trophic level.

2.2 Engineered Nanomaterials (ENMs)

The ENMs are generally considered as materials having at least two dimensions between 1 and 100 nm (Ma et al. 2010). Typically, ENMs at this scale fall in a nano-transitional zone between individual molecules (<1 nm) and the corresponding bulk materials (>100) which therefore enables them with some characteristic features peculiarly different from their molecular and bulk equivalents (Taylor and Walton 1993) (Fig. 2.1). These inherent features of ENMs include larger surface area, quantum confinement, and higher surface energy that substantially distinguish them from their bulk counterparts in terms of their behavior and fate in different environments (Ma et al. 2010). The ENMs which are found in different compartments of environment fall into one of the following groups: zero-valent metals (Diao and Yao 2009), metal-oxide nanoparticles (Lang et al. 2011; Rizzello and Pompa 2014), carbonaceous nanomaterials (Baughman et al. 2002), lipids (Yang and Ma 2010), quantum dots, and nanopolymers (Ljubimova and Holler 2012). Also, ENMs with different topographical properties such as nanofibers, nanowires, nanorods, nanoplates, and nanosheets are also being manufactured for various applications. Wahab et al. (2016) reported the self-designed synthesis of ZnO structures such as nanoplates, nanosheets, nanorods, and nanoflowers for targeted cancer nanotechnology. The array of MONPs includes both individual (Al_2O_3, TiO_2, CeO_2, CuO, ZnO, CrO_2, Bi_2O_3, and MoO_3) and binary oxides ($LiCoO_2$, InSnO, and $BaTiO_2$) (Lang et al. 2011). The MONPs have widespread industrial applications. For example, owing to visible transparency and ultraviolet-blocking property, nano-ZnO and nano-TiO_2 are widely being used in bottle coatings, sunscreen, and cosmetics (Chekin et al. 2013). The production of nano-TiO_2 and nano-ZnO for use in various skin care products reached up to 3000 and 550 tons/year (Bagheri et al. 2013). Furthermore, CeO_2 has found a key utilization as a combustion catalyst in diesel fuels to augment the emission quality, as well as in gas sensor, oxygen pumps, metallurgical ceramic, and solar cells applications (Chekin et al. 2012). Another principal class of ENMs is carbonaceous nanomaterials (CNMs). The action of carbonaceous nanomaterials and MONPs is the direct reflection of different environmental conditions (Qi and Hegmann 2008). The application of CNMs are as follows:

fuel cell electrodes, in the catalyst, plastics, orthopedic implants, battery, water purification system, super capacitors, aircraft, adhesive, conductive coatings, composites, sensor electronics, and automotive industries (Klaine et al. 2008). CNMs have the property to group themselves with other rods and tubes as high aspect ratio NMs which are similar to asbestos (Zhu et al. 2002). CNMs are very hydrophilic in nature and tend to aggregate and precipitate in an aqueous medium (Lam et al. 2004). These inherent properties limit their stability in aqueous suspensions. The surface functionalization of CNMs such as non-covalent modification, self-assembly, the attachment of polyethylene glycol, and conjugation of phospholipids, lysophosphatidylcholine and lysophosphtidylcholide, increase their stability, specifically in an aqueous medium (Wu et al. 2006; Hou et al. 2009).

2.3 Production and Release of ENMs into the Environment

The industrial production and application of ENMs are ever increasing (Fig. 2.1). By 2012, the worldwide production or utilization amounts of ENMs such as nano-TiO_2, nano-SiO_2, nano-ZnO, nano-FeOx, nano-AlOx, nano-CeOx, fullerenes, and quantum dots have been assessed in terms of tons/year as 3000, 5500, 550, 55, 55, 55, and 0.6, respectively, with >800 products in the market (Piccinno et al. 2012). By 2011, the annual commercial production of CNMs was valued to exceed 1000 tons. By 2014, the number of nanotechnology products with widespread of potential applications in the marketplace was reached up to >1300 tons (Keller et al. 2014). This commercial annual production of ENMs is very likely to increase in forthcoming years. Most significant application of ENMs is in personal care products which currently have substantial environmental implications (Keller et al. 2013; Keller and Lazareva 2014). By 2014, from the use of personal care products in the United States, nano-TiO_2, with $0.87–1.0 \times 10^3$ metric tons/year, and nano-ZnO, with $1.8–2.1 \times 10^3$ metric tons/year, represent 94% of ENMs discharged into the landfills and environment (Fig. 2.1). Among them 36–43% of ENMs from personal care products were estimated to be discharged in landfills, 0.7–0.8% to air, 28–32% to water bodies, and 24–36% released to soil system. Nano-ZnO and nano-TiO_2 as ultraviolet-blocking agents in sunscreen represent around 81–82% of total discharge, followed by facial moisturizer (7.5%) and foundation (5.7%) (Keller et al. 2014). In particular, nano-TiO_2, owing to its heavy demand in industries and regular consumption in everyday life, such as cosmetics, pharmaceuticals, food additives, and paints are discharged into the local environment (Frazier et al. 2014).

The natural concentrations of ENMs in soils were expected to be low in terms of their biological relevance. Inadvertent release of engineered nanoparticles (ENPs) as a result of industrial production and use will augment their accumulation over time in different environmental compartments analogous to naturally occurring nanoparticles (Gottschalk et al. 2009). Intentional application of ENMs in agrichemicals and for soil remediation to increase crop safety may account for the major entry routes of ENMs in the soil system. In agrichemicals, ENMs are incorporated into nano-devices for the specific delivery of agrichemicals to target tissues. ENMs have been researched for

their use as fungicides (Arruda et al. 2015; Saharan et al. 2015), insecticides (Wibowo et al. 2014), and nano-fertilizers (Raliya et al. 2014, 2016a). Other applications include the use of ENMs as additives in pesticides to enhance the solubility of essential ingredients or to protect against premature degradation of active ingredients (Kah et al. 2013; Kumari and Yadav 2014). It is the need of the hour to realize the overall impact of ENMs when released intentionally or accidentally in an open agriculture system. The ENMs owing to their larger surface area to volume ratio and enhanced surface reactivity which is absent in their bulk counterparts might be toxic to plants (Lang et al. 2011; Rizzello and Pompa 2014). The root system of edible crop plants is in direct contact to ENMs released in soils or comes with the poorly treated effluents in water used for crop irrigation. Plant leaves and stems, on the other hand, are in contact with ENMs persisting in the atmosphere like other naturally occurring nanoparticles (Handy et al. 2008; Gottschalk et al. 2009). The accidental transport of ENMs from other environmental compartments into soil system is likely to happen. For instance, the combustion of commercial diesel fuel added with nano-CeO_2 as combustion catalyst emits nano-CeO_2 into the atmosphere. It has been reported that the treatment of drinking water can only eliminate 3%–8%, 2%–20%, and 48%–99% of nano-TiO_2, nano-Ag, and nano-ZnO, respectively, and the rest of the metal content has been detected in treated water (Chalew et al. 2013).

2.4 Exposure Conditions of Plants to ENMs

Plants are a principal component of all ecosystems and provide a very large surface area for ENMs contact. Furthermore, plants play a key role in the bioaccumulation, fate, and transport of ENMs in the environment through various routes (Dietz and Herth 2011). ENMs can opt one of the following routes: atmosphere, water, and soil, for their distribution and direct interaction with terrestrial plants. The ENMs designed for potential applications in agriculture and biotechnology and sometimes for uptake by plants are also subject to their bioaccumulation and transport via the food chain (Fig. 2.2). Once in the atmosphere, the ENMs can deposit or aggregate on the surface of plant leaves or shoots and from there can penetrate plant tissues through stomatal or other openings/wounds (Navarro et al. 2008; Eichert et al. 2008). Within the soil system, the waterborne ENMs intentionally applied for remediation processes could also have interactions with plant system (Mauter and Elimelech 2008). Some of the ENMs used for the treatment of wastewater sludge (Lee et al. 2010) or by leaching from enriched farms or landfills into groundwater end up into the soil (Doshi et al. 2008) (Fig. 2.1). To assess the overall impact of ENMs on edible crops, the long-term growth period of plants in soils contaminated with ENMs must be taken into account. Conversely, some environmentally less relevant approaches such as hydroponic or other nutrient media have been used under short-term exposure conditions which therefore are necessary for thorough evaluation of mechanistic aspects of ENMs toxicity and accumulation. For instance, in several studies hydroponic seedling growth experiments in nutrient media such as Hoagland's solution amended with varying concentrations of different ENMs for >7

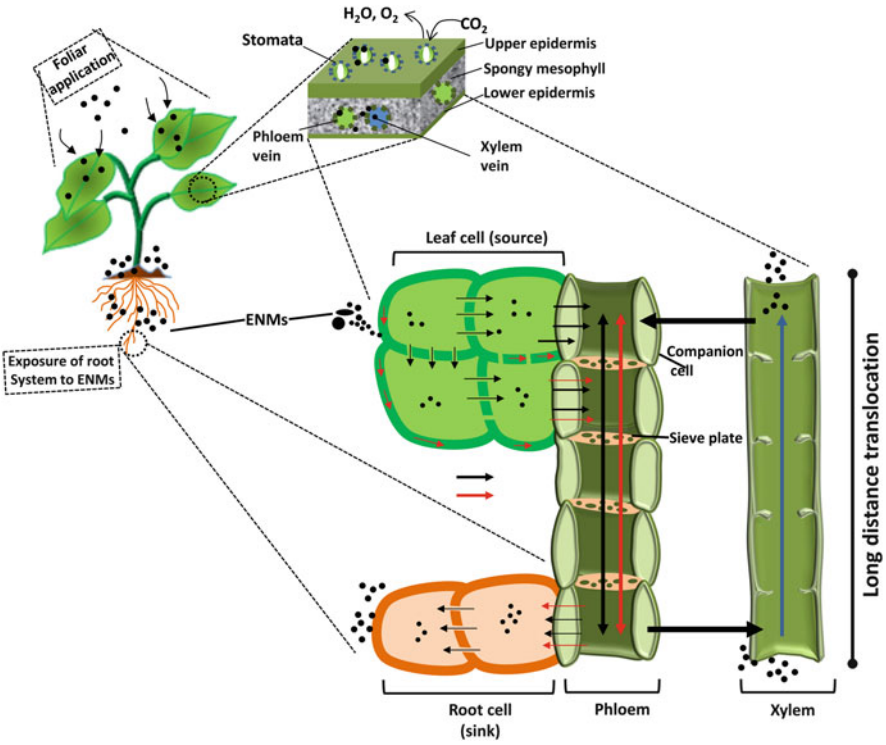

Fig. 2.2 Major sites of ENMs entry into plant system and their translocation to different parts of plant. Red arrows indicate the apoplastic movement, while black arrows indicated symplastic movement of ENMs

days were performed (Stampoulis et al. 2009; Castiglione et al. 2011; Wang et al. 2012b). Semisolid agar media and hydroponic systems are much simpler providing uniform distribution of applied ENMs and thus maximum and immediate contact with plant roots. Murashige and Skoog (MS) medium is one of the examples of semisolid nutrient media which may be supplemented with ENMs prior to solidification at the temperature suitable for applied ENMs (Yang et al. 2012; Miralles et al. 2012b; Yan et al. 2013). Although the soilless media for toxicity assessment of ENMs on crop plants is desirable from the perspective of control measurements of different susceptible parameters but from a realistic viewpoint, a soil-based approach is more relevant and practical since the soil system has its own buffering capacity to alter the characteristic activity of a particular material. Besides soil, other porous materials like sand or sand mixed with soil have also been amended with ENMs powder or suspension that could modify the stability and/or availability of ENMs to the biotic components of plant ecosystem (Du et al. 2011; Dimkpa et al. 2012; Priester et al. 2012; Khodakovskaya et al. 2013). Thus, it is understood that without the proper disposal or recycling of increasing production volumes of ENMs in the environment will increase the consequential exposure of plants to ENMs and their

accumulation kinetics in plants and through them to other trophic levels of the ecosystem (Miralles et al. 2012a).

2.5 Mechanisms of ENMs Uptake

Major edible crops that have been assessed for ENMs uptake, accumulation, and toxicity include *Allium cepa* (onion), *Triticum aestivum* (wheat), *Zea mays* (corn), *Cucumis sativus* (cucumber), *Lycopersicon esculentum* (tomato), *Cucurbita* spp. (zucchini/pumpkin), *Glycine max* (soybean), *Nicotiana tabacum* (tobacco), *Lactuca sativa* (lettuce), and *Oryza sativa* (rice). Among these, *Cucumis sativus* and *Cucurbita* spp. are species of choice to study the ENMs uptake and translocation owing to their significant water uptake capacity and bigger size vascular bundles. The persistence of ENMs in the environment of these major food crops obviously raises serious concerns due to their direct consumption by humans. The available literature suggests that ENMs uptake, accumulation, translocation, and impacts on plant growth depend upon the plant species, growth phase of the plant, size, shape, surface functionalization, chemical composition, and stability of the ENM under test conditions (Nair et al. 2010; Rico et al. 2011). The studies revealed that ENMs can enter plant system through various routes, such as passive diffusion, ion channels, aquaporins, binding to carrier proteins, endocytosis, and new pore formation (Khodakovskaya et al. 2009), or by associating with organic matter (Kurepa et al. 2010) present in local media. The interaction with root exudates may also influence the subsequent transport of ENMs (Watanabe et al. 2008; Kurepa et al. 2010).

2.6 Foliar Uptake

Engineered nanoparticles (ENPs), similar to other naturally occurring nanoparticles in the atmosphere, are in contact with aerial avenues of plants such as stomata on leaf surfaces, hydathodes on leaf tip, and leaf trichomes (Raliya et al. 2015) (Fig. 2.2). Trichomes are uni- or multicellular appendages of epidermis present on most surfaces of a plant (Schwab et al. 2015), although trichomes due to their permeability may also take up NPs (Navarro et al. 2008). To study the impact of ENMs and their uptake and movement from aerial parts to belowground parts of plants, foliar applications have been proven successful (Fig. 2.2). For instance, studies have suggested that nanoparticles following foliar application either by direct application or by an aerosol-mediated spray can directly penetrate into plant system owing to their small size by means of gas uptake (Wang et al. 2013a). In plant system, cuticle is the primary barrier for nanoparticle uptake (Wang et al. 2013a), which preferably repels charged nanoparticles (Schwab et al. 2015). The foliar application encourages the uptake of NPs by stomatal openings which are typically ~100 nm in diameter in plant leaves (Schwab et al. 2015). Stomata are abundant at lower leaf surface and therefore provide an enhanced permeability for polar materials (Schreiber 2005). The pore size of leaves in the presence of stomata was determined in three dicot

plants by comparing the uptake rates of C^{13} and N^{15} and was found >100 nm (Eichert and Goldbach 2008). On the contrary of ENPs passage, the stomata may be clogged by the NPs or their larger micro-aggregates (Hussain et al. 2013). This blocking may result in the reduction of water transpiration which in turn elevates leaf temperature leading to growth reduction and rate of photosynthesis (Hirano et al. 1995). In a study, an aerosol-mediated spray of ZnO NPs and TiO_2 on tomato plant in the concentration range from 0 to 1000 mg kg^{-1} was applied after 14 days of growth. After 14 days of NPs application, enhancement in plant height was observed for both the NPs up to 250 mg kg^{-1}. On the other hand, TiO_2 NPs was found to be significantly toxic to root growth of tomato plant at all concentrations except for 1000 mg kg^{-1} (Raliya et al. 2015). Additional to stomata, hydathodes present on leaf tip also assist in NP uptake or excretion (Hong et al. 2014). Hydathodes are small cavities which lack covering of a cuticle and permit guttation of surplus water across the leaves of plants such as *Brassica* (Huang 1986). The transport of NPs occurs particularly after a guttation period when the water present on leaf tip is imbibed back into the leaf (Huang 1986). Some studies have reported the uptake or excretion of NPs near hydathodes, for instance, the presence radioactive cerium in insoluble nano-$^{141}CeO_2$ was observed at leaf tips and at the end of vascular system in cucumber plant (Zhang et al. 2011). Schaller et al. (2013) reported excretion of nano-silica in salt form near leaf tip.

2.7 Uptake by Roots

Roots are the other major avenue for ENMs to enter the plant system (Fig. 2.2). The thinner and more permeable cuticle and cell wall of root hairs as compared to those of normal cells (Galway 2006) help the nanoparticle uptake by roots. Many studies have suggested the uptake and accumulation of various ENMs such as metal, metal oxides, quantum dots (QDs), and CNTs by root system of plants (Lin and Xing 2008; Navarro et al. 2012; Ahmed et al. 2017; Faisal et al. 2013). The presence of ENMs in environmental media frequently retards growth of root hairs in plants (Aubert et al. 2012; Wang et al. 2011c). Although a few nanoparticles are taken up by plant roots, the rest of the fraction gets adsorbed on root hairs and is thought to deliver toxic effects through physical association (Navarro et al. 2012; Miralles et al. 2012b). It is believed that NPs are transported in plants along with the transpiratory water (Zhai et al. 2014), and the rate of water uptake correlates the uptake of NPs (Rico et al. 2013b). Moreover, the smaller pore size of roots limits the uptake of larger individual particles or aggregates. For example, the average diameter of pores of primary roots of *Zea mays* is 6.6 nm that selectively allowed the CeO_2 nanoparticles having a smaller diameter than 6.6 nm to penetrate root tissues and to be transported from root to shoots (Zhao et al. 2012a). In contrast to this, CeO_2 NPs >7 nm diameter have been shown to be taken up by four edible crops, namely, corn, cucumber, alfalfa, and tomato (Lopez-Moreno et al. 2010). Moreover, CeO_2 NPs with the diameter between 7 and 25 nm were observed to translocate to shoots of cucumber plant which is suggestive of some other mechanisms of root uptake of

NPs. Some previous studies have also advocated that NPs with much bigger sizes are able to penetrate across the epidermal cells, flow into the cortex, and even the vascular tissues (Aubert et al. 2012). The root uptake of carbonaceous nanomaterials (CNMs) is slightly different that they have some sort of interaction with suspended organic matter and are influenced by homo- or heterogeneity of media. Association of multi-walled carbon nanotubes MWCNTs and fullerene (C_{70}) with natural organic material enhances the hydrophilicity of CNMs. Single-walled carbon nanotubes (SWCNTs) with <500 nm length and labeled with fluorescein isothiocyanate (FITC) have been demonstrated to penetrate the cell wall of plants by the process of endocytosis (Samaj et al. 2004; Liu et al. 2009b). Similarly, tomato roots have shown the absorption of MWCNTs (Khodakovskaya et al. 2009).

2.8 Role of Plant Cell Walls and Membranes in Uptake

The entry of ENMs in plants exclusively varies among different plant species. Furthermore, the exposure pathways, metabolic processes of plants such as secretion of root exudates, pH, and microbial population of soil also influence the uptake of ENMs (Raliya et al. 2015). NP uptake across the cell wall was mostly dependent on the size of the particles and pores of the cell wall (Asli and Neumann 2009; Glenn et al. 2012; Judy et al. 2012) (Fig. 2.3). The thickness of plant cell walls varies from 100 nm to several micrometers (Campbell et al. 2008). The major components of plant cell walls are cellulose, hemicellulose, and pectin. The pore size of the cell walls in plants is fairly constant and works as a selective size barrier for NPs.

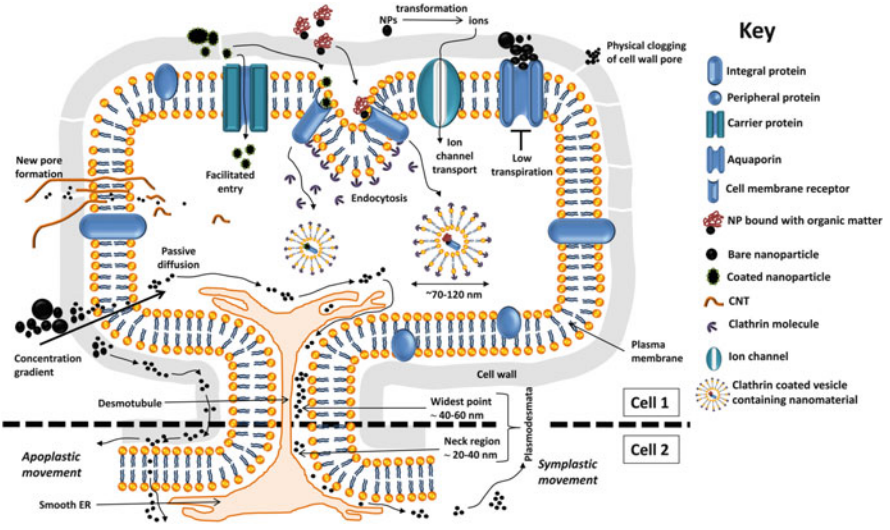

Fig. 2.3 Conceptual illustration of ENMs uptake inside the plant cell and their apoplastic and symplastic movement

Electron microscopic (EM) observations and recent advances in pectin research have fairly revealed the flexible porosity of cell walls owing to the degree of structural heterogeneity of cell walls and dynamic nature of pectins (Willats et al. 2001; Albersheim 2011). Pore diameter of cell wall as observed by EM analysis was found to be <10 nm, with an infrequent maximum of 20 nm, and 30–40 nm with a largest diameter of 60 nm after the pectins. Extraction of hemicellulose unrevealed the spaces of ~100 nm (McCann et al. 1990). In a recent study, NPs ~50 nm in diameter were found translocating across the cell wall and internalized in the cytoplasm (Lee et al. 2008; Fig. 2.3). Some other studies have shown that NPs between 5 and 20 nm are able to cross the cell wall (Ma et al. 2010; Navarro et al. 2008). Also, the NPs with ~20 nm diameter were observed in cell walls (Zhang et al. 2012a; Geisler-Lee et al. 2013). Recently, the abundance of ZnO NPs between 4 and 8 nm has been reported in treated root cells of *A. cepa* (Ahmed et al. 2017). ZnO NPs >40 nm have been demonstrated in root cell, and it was assumed that the NPs may enhance the permeability of root cell wall by creating new holes of variable sizes (Lin and Xing 2008). This behavior of ZnO NPs has challenged the common assumption that only NPs with ≤20 nm are able to cross the cell walls (Ma et al. 2010). Raliya et al. (2016a) have also confirmed the uptake and translocation of Au-NPs with ≤20 nm in diameter in watermelon plant. Additionally, small size nanoparticles owing to their higher surface reactivity encourage new pore formation and are supposed to enhance hydromineral flow and nutrient uptake (Castiglione et al. 2011). Another study on the role of cell wall in NP uptake by Kim et al. (2014) revealed that nano-zero-valent iron (nZVI) enhances root elongation by 150−200% as compared to control by encouraging OH radical-induced loosening of cell wall. The oxidation capacity of nZVI leads to enhanced hydrogen peroxide, which results in OH radical—stimulated cell wall loosening in *A. thaliana* roots. Moreover, the asymmetrical distribution of tensional strength developed from the OH radical stimulated loosening of cell wall enhanced endocytosis (a mechanism of NP uptake). The penetration of CNTs, on the other hand, is limited by their diameter as well as the length. Serag et al. (2012a) showed that, if the size of SWCNTs is too large, it may penetrate the cell wall but cannot acquire complete entry in cell and therefore may remain immobilized. The chemically trimmed SWCNTs have been demonstrated to pass through both the cell wall and the cell membrane of *Catharanthus* and *N. tabacum* (Liu et al. 2009b; Serag et al. 2011a, 2012a).

Following the penetration, ENMs as small substances seem to move across the plasma membrane by various mechanisms (Fig. 2.3). The plasma membrane is a lipid bilayer composed of phospholipids containing hydrophilic heads and hydrophobic tails. This polar nature of plasma membrane acts as a selective barrier for channeling of substances across the membrane. Depending upon the surface properties of NPs, the passage across the membrane appears to be regulated by passive diffusion or by facilitated entry of NPs (Fig. 2.3). However, the channeling of NPs across the lipid bilayer depends on several factors such as size, charge, hydrophobicity, composition, shape of NPs, membrane fluidity, lipid composition, and embedded ligands, or molecular species are also central in passage of NPs across the membrane (Mehrian and Lima 2016). Selective impermeability of plasma

membrane renders the diffusion of large and polar substances including ions, water molecules, and NPs (Schwab et al. 2015). The uptake of ENMs through plasma membrane can be categorized as endocytosis-dependent and endocytosis-independent. "Endocytosis" as pathway in which a lipid bilayer coated vesicle is generated to internalize extracellular particles including nanoparticles (Fig. 2.3). Endocytosis allows cells to communicate, to transfer nutrients, to import signaling receptors, and to mediate an immune response toward exotic species. The process starts with invagination of lipid bilayer around the NPs and ends up with the dissociation of formed vesicle containing NPs by some cellular processes (Mehrian and Lima 2016). The endocytosis is of two types: receptor-dependent or receptor-independent (Schwab et al. 2015). In receptor-dependent endocytosis, NPs bind to a molecular configuration of cell membrane such as membrane lipids, proteins, or carbohydrate moieties which as a result induce vesicle formation and be internalized inside the cell (Mehrian and Lima 2016). For the uptake of positively charged nanoparticles, receptor-mediated and clathrin-dependent endocytosis are reported (Samaj 2012). Clathrin like coat proteins are used to generate small vesicles 70–120 nm in diameter (Samaj 2012) in order to transport molecules inside the cell, and this size of vesicles may, therefore, limit NPs bigger than this size to be incorporated into the vesicle (Fig. 2.3). Clathrin protein forms a triskelion shape composed of three light chains and heavy chains. The interactions of triskelia form a polyhedral lattice that borders the vesicle (Pearse 1976). For CNT uptake through membrane, a nonspecific receptor-independent mechanism has been discussed (Liu et al. 2009b). In this study, oxidized SWCNTs, $<$500 nm in length, were found able to cross plasma membrane in *Nicotiana tabacum*. The uptake of SWCNTs has been found to be drastically reduced upon treatment with inhibitors of receptor-independent fluid phase endocytosis (FPE). The smaller MWCNTs $<$100 nm in length can skip endosomal processing and get direct entry in protoplasts of *Catharanthus roseus* (Serag et al. 2011b). In contrast to this, the penetration of cell wall and membrane by large MWCNTs has been mechanically proven in wheat roots (Fig. 2.3). This interaction caused limited penetration since the MWCNTs cannot be completely internalized (Wild and Jones 2009). For uptake of opposite charge Au NPs in protoplasts, FPE is also found significant (Onelli et al. 2008). Positively charged Au NPs as compared to negatively charged Au NPs have been observed to be internalized to a greater extent by the process of nonspecific FPE (Onelli et al. 2008).

In some recent studies, the change in the genetic expression of cell membrane-embedded water channel proteins, aquaporins, occurred upon exposure to NPs. Aquaporin channels are sometimes found downregulated (Lu et al. 2010; Taylor et al. 2014) or upregulated (Khodakovskaya et al. 2012) which is indicative of NP uptake through aquaporins (Rico et al. 2011). Aquaporins serve as nonselective channels for uptake of water and small nonionic solutes of typically $<$1 nm in diameter (Zangi and Filella 2012) and help the plant to switch between apoplastic and symplastic transport of nutrients (Schwab et al. 2015). Considering this limitation and according to sufficient scientific data, the uptake of NPs of $>$1 nm in diameter through aquaporins or other similar channels is questionable (Hu et al. 2010; Schaller

et al. 2013). Though, it may be concluded that the change of aquaporin expression is a response to the reduced water flow in plant system as a result of clogging of apoplast by (Fig. 2.3) (Asli and Neumann 2009; Lu et al. 2010).

2.9 Bioaccumulation and Subcellular Distribution of ENMs in Plants

After uptake into plant system, ENMs either travel to different organs of plants, e.g., stem, leaves, and fruits, or get accumulated in different cellular compartments, e.g., cell walls, vacuoles, cytoplasm, stellar system, plastids, nucleus, and lipid envelope of subcellular organelles, etc. The available literature suggests accumulation of ENMs at various sites in plants. It has been shown that under hydroponic conditions, soybean (a major crop containing protein) plant accumulates metal and metal-oxide nanoparticles (Priester et al. 2012). Accumulation of nano-CeO_2 has also been observed in root tissues of wheat, rice, and barley without affecting seed germination and root elongation (Rico et al. 2015). Zhao et al. (2012a) have reported the presence of cerium in roots of corn plant, and the accumulated cerium corresponds to nano-CeO_2. Confocal microscopy of root tissues confirmed the presence of nano-CeO_2 in the cell wall of root cortex cells. Moreover, nano-CeO_2 may carry out molecular modification in plants (Rico et al. 2013a, b). Zinc as a result of nano-ZnO exposure of wheat and soybean also found accumulated in the phloem of wheat and grains of soybean (Riesen and Feller 2005). ZnO NPs labeled with fluorescein isothiocyanate (FITC) have been detected and accumulated in stele of corn roots only and are not transported to aerial parts of corn plant (Zhao et al. 2012b). Copper nanoparticles also get accumulated 10- to 20-fold greater compared to threshold level (10 mg kg^{-1}). Higher accumulation of CuO nanoparticles in root tissues results in root growth inhibition (Lei et al. 2011; Adhikari et al. 2012). Wheat under stress of anatase and rutile phase of nano-TiO_2 has been studied till the germination and development (Larue et al. 2012), and it has been observed that anatase form of nano-TiO_2 having a diameter $<$140 nm gets accumulated in roots. Particles with $<$36 nm diameter are able to translocate from roots to leaves. Furthermore, the aggressive physical clogging of pores of root cell wall by colloidal TiO_2 NPs brings about a significant reduction in hydraulic conductivity of roots. This blockage of root pores further interrupts the uptake of TiO_2 NPs via apoplastic route (Rico et al. 2011). In a study, Fe_3O_4 NPs have not been found in leaves and stem of tomato plant, but significantly large amount of Fe has been detected in tomato fruits and roots (Vittori et al. 2015). This suggests that after uptake, nanoparticles undergo extensive processing in cellular environment which alters their native forms. In a recent study by Iannone et al. (2016), TEM analysis of wheat roots grown hydroponically with citrate-coated Fe_3O_4 NPs showed the localization of Fe_3O_4 NPs in cell wall of epidermal cells. However, relatively large amounts of Fe_3O_4 NPs (2.01–8.07 mg g^{-1}) were detected in root tissue, but no paramagnetic signal has been noticed in leaves and stem of plant which advocated that Fe_3O_4 NPs get accumulated only in root tissues and are not able to traverse to other parts of plant through vascular bundle. Vascular bundle plays a key role in transport of ENMs to various organs of plant. It seems that

once ENMs are in vascular tissues, their upward movement to shoots through xylem is very quick. Nanoparticles are also able to cross phloem tissues and get accumulated in fruits. For instance, a study on tomato-CeO_2 NPs interaction revealed that the transportation of CeO_2 NPs is not limited to shoots only, but they were also accumulated in fruits. These findings are in approval that NPs with certain size limit can cross phloem channels and travel along the phloem because phloem is the only vascular bundle to enter fruit tissues (Wang et al. 2012b). Similarly, fullerene C_{70} has also been reported to travel along with phloem channel (Lin et al. 2009). Ultrastructure analysis of plant tissues and fluorescence-based detection has also been proven to be successful while considering the fate of carbonaceous nanomaterials in plants. Transmission electron microscopy has revealed the presence of CNTs in different parts of plant (Elena et al. 2012). The fluorescence study of *N. tabacum* plant cell suspensions treated with fluorescently labeled SWCNTs (SWCNTs-FITC) exhibited the presence of SWNTs-FITC in vacuoles as well as in cytoplasmic strands (Siddiqi and Husen 2016). A pioneer study on plant nano-bionics approach to augment photosynthesis in *A. thaliana* leaves by Giraldo et al. (2014) showed that SWNTs can passively transport through the lipid membrane and become irreversibly localized in lipid envelope of extracted chloroplasts (Giraldo et al. 2014). Based on the length of CNTs, their deposition sites in plants may vary. High-resolution TEM (HR-TEM) studies showed that MWCNTs >200 nm get deposited in subcellular organelles, while MWCNTs with length 30–100 nm can be seen in the nucleus, vacuoles, and plastids (Serag et al. 2011b, 2012b). The adsorption followed by penetration and bioaccumulation of MWCNTs in plant tissues is associated with altered physiological parameters. The adsorption of MWCNTs on root surface has been documented by many studies (Ma et al. 2010; Khodakovskaya et al. 2009; Lin et al. 2009). Still there is sufficient literature that supports the penetration of tissues by MWCNTs and their ability to be transported. MWCNTs from industrial material "Taunit" are found in root and leaf tissues inferring their penetration and accumulation in roots (Smirnova et al. 2011). It has been shown that isolated single MWCNT-FITC is more opportunistic to enter different subcellular organelles, and accumulation of this complex increased the chance to be present in different cellular compartments. Owing to direct penetration, the occurrence of MWCNT-FTIC complex is much frequent in cytoplasm, although single MWCNT-FITC has been detected inside many organelles or cellular compartments of plant cell such as cell plastids, vacuole, and the nucleus (Serag et al. 2011b).

2.10 ENM Interactions with Secretions of Plant

In different environments, ENMs are subject to transformation by plant exudates. Plants have strong influence on the structure of soil and entry of NPs into plant system (Siddiqi and Husen 2017). Plants have been reported to secrete considerable amount of inorganic ions and biomolecules including low molecular organics such as organic acids, amino acids, aldehydes, and phenols and high molecular substances such as polysaccharide and fatty acids. These root exudates forms a micro nutritional environment around the root called "rhizosphere" (Bais et al. 2006). ENMs in direct

contact with these exudates are easily aggregated and adsorbed on the surface of root (Zhang et al. 2011, 2012a; Ma et al. 2013b). They may undergo extensive physico-chemical transformation due to specific or random interactions with plant exudates and humic acid (Rico et al. 2011). Plant exudates mediated transformation can change the bioaccumulation and fate of ENMs or vice versa. For instance, the biotransformation of nano-ZnO has been studied by many researchers (Du et al. 2011; Priester et al. 2012; Dimkpa et al. 2013; Wang et al. 2013b). None of these studies showed the presence of nano-ZnO internalized in plant cells as analyzed by synchrotron-based techniques (XANES). Nevertheless, zinc existed as transformed Zn^{2+} species such as zinc citrate in soybean (Hernandez-Viezcas et al. 2013). A Large amount of mucilage, a hydrated polysaccharide, is secreted by root tips and hairs on the root surface (Campbell 1990). This mucilage, in turn, can contribute to the adsorption of nano-ZnO on to the root surface followed by their transformation. In an earlier study, *Lolium perenne* has been reported to secrete a large amount of phenolic acids, proteins, sugars, and amino acids (Hodge et al. 1998). Among these organics, particularly the macromolecules are responsible for the stabilization of nano-ZnO in rhizosphere (Lin and Xing 2008). Although in soil system, plants roots are the key component to bringing about transformation of nanomaterials. But on the other hand, microflora of soil especially the fungal and bacterial population also play role in transformation of nanomaterials by producing or secreting enzymes such as phytases and phosphatases (Tarafdar and Claassen 1988). Since Zn is a cofactor of phytase and phosphatase, therefore it helps in mobilization of native P (Nelson et al. 2008). In a recent study, exposure of plants to nano-ZnO enhanced the activity of acid and alkaline phosphatases and phytase in soil. ZnO-NPs of 23 nm in diameter as compared to bulk ZnO significantly enhanced the activity of phytase (108%), acid phosphatase (98.07%), alkaline phosphatase (93.02%), and dehydrogenase (84.21%) enzymes (Raliya et al. 2016a). The role of other microbial products in ENM transformation such as proteins, carbohydrates, organic acids, and other by-products secreted in rhizosphere is yet to be explored. Before entering in plant system, agglomeration of NPs also influences their uptake. Also, plant species exposed to nano-CeO_2 and nano-TiO_2 have shown that none of the nanomaterials cause severe toxicity during germination and initial growth stage (Andersen et al. 2016). It is assumed from these results that the macromolecules secreted by roots cause aggregation of NPs around root tips. Furthermore, in alkaline soil, metals such as copper and iron are precipitated as their hydroxides and thus are not available for plant uptake (Dimkpa et al. 2015). The organic acids produced by plant roots bring the soil pH down and as a result, Cu is dissolved from nano-CuO and is accumulated or transported to various organs of plant (Shi et al. 2011).

2.11 Biotransformation of ENMs

The ENMs in environmental settings necessarily have some interactions with biotic and abiotic factors and experience physicochemical modifications such as dissolution, random coating by natural organics, and redox reactions (Lowry et al. 2012). Also, the

phosphorylation, sulfidation, and molecular modification play a role in biotransformation of ENMs (Lowry et al. 2012). Due to different physicochemical reactions, metal and metal-oxide NPs such as Ag, CuO, and ZnO may release metal ions and get chemically transformed by interacting with inorganic and organic substances of environment and with other living entities (Levard et al. 2012; Dimkpa et al. 2013). The interactions of ENMs with inorganic ions, biological macromolecules, and natural organic matters may result in the altered aggregation and change in surface chemistry of ENMs (Zhang et al. 2009). In soil-plant system, some of the ENMs containing metal elements of variable valence shell can undergo redox reactions and subsequent transformation by interacting with reducing and oxidizing agents of plants (Wang et al. 2012a; Zhang et al. 2012a). The biotransformation of ENMs may either enhance or reduce toxicity of subsequent ENMs. Nano-CuO is reduced to Cu_2O and Cu_2S in *Zea mays* (Wang et al. 2012a). Likewise, comparable transformation and toxicity of nano-forms of Yb_2O_3 and La_2O_3 in cucumber have been reported (Ma et al. 2011; Zhang et al. 2012b). Organic acids secreted by roots of cucumber enhanced the solubility Yb_2O_3 and La_2O_3. The presence of phosphate salts increases the likelihood of biotransformation of oxides into phosphates. Yb_2O_3 and La_2O_3 are present as their phosphates in cucumber roots. Furthermore, the biotransformation of $Ni(OH)_2$ to Ni^{2+} species is reported in leaves and shoots (Parsons et al. 2010). Some metal-based nanoparticles could be oxidized or reduced dependent on the availability of biochemicals in certain parts of plant. For instance, CuO NPs are reduced to Cu_2O and Cu_2S in maize. Thus the toxicity of NPs may be altered depending upon the reduced or oxidized form of elements. On the other hand, many studies have reported the phytotoxicity of nano-ZnO through dissolution in Zn^{2+} species. Nano-ZnO has the property of substantial dissolution from 1 mg L^{-1} to several thousand mg L^{-1} in water depending upon its size and the pH of nutrient solution (Franklin et al. 2007). Lin and Xing (2008) demonstrated that a soluble amount of zinc from nano-ZnO amended nutrient solution to be <8 mg L^{-1}. This amount is much lower than the toxic level of zinc ions to *Lolium perenne* (Lin and Xing 2008). The phytotoxicity is also dependent on pH of the medium. Watson et al. (2015) studied inhibition of root growth *T. aestivum* by nano-ZnO under acidic soil condition. Interestingly, the phytotoxicity under alkaline soil is alleviated regardless of the doubled absorption of nano-ZnO. Soluble zinc in acidic soil has been reported to be 200-fold greater and 10-fold higher in shoots than those in alkaline soil. In the same way, Zn^{2+} ions released from nano-ZnO are also detected in plant. Zn^{2+} ions released from nano-ZnO in nutrient solution are too low (8–25 mg L^{-1} Zn^{2+} for 500–4000 mg L^{-1} nano-ZnO). Therefore, it has been assumed that nano-ZnO underwent biotransformation at the surface of roots. The Cu (II) may be partially transformed to Cu (I) by citrate in roots of cucumber and bean (Dimkpa et al. 2015). Cu (I) is highly unstable and get oxidized in presence of air and water and air. Following the adsorption of Fe_2O_3 NPs on root hairs, root tips, and middle zone of plant, they may be mineralized (biotransformed) due to phytochemicals present in plant system (Shankramma et al. 2016). Many earlier studies on C_{70}, SWCNTs, MWCNTs, Fe_3O_4, and TiO_2 NPs have suggested that NPs without any transformation are just accumulated in plants (Rico et al. 2011). For instance, μ-XANES analysis of plant tissues showed that untransformed TiO_2

nanoparticles are able to translocate from the roots to leaf trichomes in cucumber plant and are also observed in fruits (Servin et al. 2012, 2013).

2.12 Translocation of ENMs in Plant Tissues

The ENMs once accumulated in plant tissues are biotransformed by phytocompounds or translocated to various organs of plants. ENMs applied to roots may travel through aerial parts of plant via xylem or applied to leaf surfaces may be translocated to belowground parts of plant via phloem (Fig. 2.2). It is suggested that the transport of nanoparticles depends on size, charge, and surface chemistry of NPs, internal environment, and growth phase of plant. There are two ways of nanoparticles translocation: (1) apoplastic and (2) symplastic. In apoplastic pathway, NPs travel through cell wall pores, intercellular spaces, longitudinal channels of cell wall, and xylem (Sattelmacher and Horst 2007; Geisler-Lee et al. 2013). In symplastic pathway, NPs have to cross the plasma membrane and then travel through plasmodesmata (Figs. 2.2 and 2.3) or sieve plates of phloem (Zangi and Filella 2012). The apoplastic passage is known as a nonselective pathway of least resistance (Sattelmacher and Horst 2007). It is accepted generally that many nutrients water and non-essential metal complexes prefer the apoplastic route for translocation (Sattelmacher and Horst 2007). To gain access to the vascular cylinder, the ENMs traverse the protoplast of endodermal cells due to the blockage of any apoplastic transport by Casparian strip between endodermal cells (Lin and Xing 2008). Plasmodesmata which regulate the cell to cell transfer of any component are microscopic channels in plant cells (Lin and Xing 2008) and are the only connections between cytoplasm of two adjacent cells (Roberts and Oparka 2003). Plasmodesmata may be linear or branched channels containing desmotubule which is lined by the cell wall and connects endoplasmic reticulum of two cells. The desmotubule of plasmodesmata and plasma membrane are linked by proteins making further microchannel divisions of 3–4 nm in diameter in cytoplasmic sleeve (space between desmotubule and cell membrane) (Roberts and Oparka 2003; Lucas and Jung-Youn 2004). Earlier, plasmodesmata were described to be cylindrical in shape with ~40 nm in diameter (Tilney et al. 1991). For the size bigger than this, the nanomaterials upon interaction may possibly increase the pore size of root cell walls by creating new holes of variable sizes (Lin and Xing 2008). Later on, the diameter of plasmodesmata in mature cells was observed to ~20–40 nm in neck zone of cytoplasmic sleeve, and ~50–60 nm at broadest point (Lin et al. 2009). The translocation of nanomaterials through plasmodesmata has been suggested based on their radial transport in plants (Corredor et al. 2009), root to shoot movement (Lin et al. 2009), accumulation of metal in various parts of plant (Nekrasova et al. 2011), presence of electron dense particulate matter in vascular tissues (Huang et al. 2011), and their size (Ghafariyan et al. 2013). Many other studies have also proposed the presence of nanoparticles in xylem (Corredor et al. 2009; Wang et al. 2011b).

There are several studies that emphasize the translocation of nanoparticles either via symplast or apoplast. For instance, CeO_2 NPs when applied on leaves of

Cucumis sativus, ~3% of the total was found in roots of the plant which is an indicator of leaf-root translocation most probably through phloem (Hong et al. 2014). Moreover, 73–81% of the total NPs adsorbed remains to the outer surface of leaf. Plant leaves are the places where transport of solutes occurs via xylem and then are transported back into symplast. In leaf tissues, NPs were observed both in symplast and apoplast (Ma et al. 2011; Larue et al. 2014). Hydroponically grown *T. aestivum* and *Cucurbita maxima* have shown to translocate CeO_2 NPs (17–100 nm) at 100 mg L^{-1} without exhibiting inhibition of root growth in the presence and absence of gum arabic and fulvic acid (Schwabe et al. 2013). Also, the foliar application revealed the uptake and translocation of NPs from stomatal openings to plant tissues such as adjacent cells, vascular bundle, and then to roots (Wang et al. 2013a; Larue et al. 2014). The solubility of NPs, in particular, has a profound effect on translocation of NPs. Notably, the highest solubility of MgO NPs and lowest solubility of TiO_2 NPs remarkably affected their translocation from leaf to root tissues, 1.49–5.45% and 11.5–26.14% for TiO_2 and MgO, respectively (Wang et al. 2013a). Similarly, the insoluble TiO_2 NPs translocated from leaves to vascular tissues and roots (Larue et al. 2014). Under hydroponic conditions soybean accumulate metal ions as well as the metal nanoparticles such as CeO_2, and Zn/ZnO (Lopez-Moreno et al. 2010). Aggregation of Fe_3O_4 NPs occurred in cucurbits followed by translocation in stem and roots (Zhu et al. 2008). In another study, significant amount of ZnO NPs translocated into beans and leaves while CeO_2 NPs bioaccumulated in root nodules where they led to consequential reduction in nitrogen fixation (Priester et al. 2012). Engineered iron oxide NPs on hydroponically grown *Cucurbita maxima* exhibited that different quantities of NPs were bioaccumulated and translocated through the root, leaves, and stem of plant (Zhu et al. 2008). In a study with bitter melon (*Momordica charantia*), Kole et al. (2013) confirmed the uptake, accumulation, and translocation of fullerol in every part of the plant through bright field microscopy and FTIR spectroscopy. It was also demonstrated that with increasing concentration the hydrodynamic size of fullerol increases owing to extensive formation of hydrogen bonds.

2.13 Impacts of ENMs on Plants: Negative Impacts

The uncontrolled and ever-increasing release of ENMs into the environment is evidently an additional stress on the plants and other organisms thriving in that environment. The threshold amount of nanomaterials in plants as well as in their surroundings is considerably low than the intentionally or unintentionally released amount. However, the interactions between plants and ENMs and the impact of ENMs on plants do occur (Fig. 2.4). It has been suggested that ENMs equally affects the plant growth and soil fertility, and hence, the controlled amount of ENMs be discharged into the environment (Rico et al. 2011). Many studies have documented the negative impacts of ENMs uptake on edible crop plants (Table 2.1). In this context, Mushtaq (2011) have reported inhibition of seed germination and root elongation by Fe_3O_4 NPs in *Cucumis sativus* over a broad range of concentration

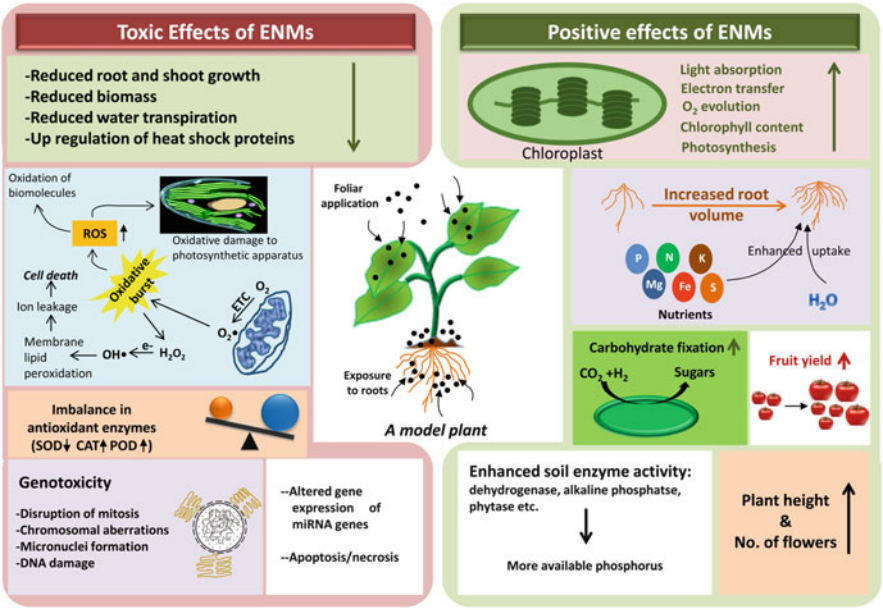

Fig. 2.4 Graphical representation of toxic and growth-promoting effects of ENMs on plant

(0.5–5.0 mg mL^{-1}) after 6 days. ZnO NPs also reduce the seed germination and cause tissue damage under hydroponic conditions (Lin and Xing 2007, 2008). In *Brassica oleracea* and *Zea mays*, seed germination and root growth measurements exhibited that the NPs are more toxic than free metal ions (Pokhrel and Dubey 2013). Germination index has also been reported to decrease by Fe_3O_4 NPs at 0.5, 2.5 and 5.0 mg mL^{-1} consistency (Zhu et al. 2008). Root growth of bean has been suppressed but shoot growth can be enhanced by CuO NPs (Lei et al. 2011; Adhikari et al. 2012). Moreover, the NPs attached to root surface of *Zea mays* inhibited water transpiration through leaves (Asli and Neumann 2009). Likewise, pristine CNTs have been found to cause toxic impacts in plants. For instance, SWCNTs have been reported to produce toxicity in *Oryza sativa* and *A. thaliana* which led to death of ~25% of protoplast within 6 h (Shen et al. 2010). The biomass of *Cucurbita pepo* plant gets reduced in presence of MWCNTs (Stampoulis et al. 2009).

2.14 Membrane Lipid Peroxidation and Generation of Oxidative Stress

Reactive oxygen species (ROS) are highly reactive chemical species such as hydroxyl radicals [•OH], peroxides $[O–O]^{2-}$, superoxides $[O_2]^-$, and singlet oxygen $[^1O_2]$ (Hayyan et al. 2016) represent total oxidative stress of the cell. The generation of ROS has been proven to damage plasma membrane via membrane lipid

Table 2.1 Toxic response of some ENMs on edible crop plants

Nanomaterial	Size (nm)	Food crop	Concentrations used	Toxicity	References
CuO	<50	*Brassica juncea*	0, 20, 50, 100, 200, 400, 500 mg L^{-1}	Reduced shoot and root growth	Nair and Chung (2015)
	10–50	*Vigna radiata*	0, 20, 50, 100, 200, 500 mg L^{-1}	Reduced biomass and root length at all concentrations, reduced chlorophyll content at >100 mg L^{-1}, enhanced H_2O_2 and lipid peroxidation, increased ROS production, altered gene expression	Nair et al. (2014)
	<50	*Triticum aestivum*	500 mg kg^{-1}	Inhibition of root and shoot growth, generation of oxidative stress possibly due to Cu released from CuO NPs	Dimkpa et al. (2012)
	<50	*Cucurbita Pepo*	0, 100, 500 mg L^{-1}	Reduced growth and transpiration (60–70%)	Musante and White (2012)
	<100	*Raphanus sativus*	10, 100, 500, 1000 mg L^{-1}	Growth inhibition and DNA damage	Atha et al. (2012)
	20–40	*Zea mays*	2–100 mg mL^{-1}	Reduced biomass and root elongation	Wang et al. (2012a)
ZnO	10	Soybean	0–500 mg kg^{-1}	Reduced Fe, Mg, and K	Peralta-Videa et al. (2014)
	<50	Soybean	500 mg kg^{-1}	Reduced roots and shoots	Yoon et al. (2014)
	<10	*C. pepo*	1000 mg L^{-1}	Reduced biomass (78–90%)	Stampoulis et al. (2009)
	90	*Z. mays*	800 mg kg^{-1}	Reduced growth and inhibition of AM fungi	Wang et al. (2016)
	10	*M. sativa*	250, 500, 750 mg kg^{-1}	Reduced root biomass (80%)	Bandyopadhyay et al. (2015)
	–	*T. aestivum*	25 ppm	Decreased biomass	Du et al. (2011)

(continued)

Table 2.1 (continued)

Nanomaterial	Size (nm)	Food crop	Concentrations used	Toxicity	References
TiO_2	<100	*T. aestivum*	~91 mg kg^{-1}	Reduced biomass	Du et al. (2011)
	20–100	*T. aestivum*	90 ppm	Reduced biomass	Shah and Belozerova (2009)
Fe_3O_4	6	Lettuce, radish, cucumber, spinach, tomato, leek, peppers	0.67 mg mL^{-1}	Reduced germination	García et al. (2011)
	25	Ryegrass, pumpkin	30, 100 and 500 mg L^{-1}	Blockage of aquaporins, oxidative stress	Wang et al. (2011a)
Al_2O_3	13	Maize, cucumber, carrots, cabbage	2000 mg L^{-1}	Reduced root growth	Yang and Watts (2005)
	–	Corn	2000 mg L^{-1}	Reduced root length	Lin and Xing (2007)
C_{60} fullerenes	1450–1900	Corn	500 mg kg^{-1}	Reduced biomass	Torre-Roche et al. (2013)
MWCNT	–	Zucchini	1000 mg L^{-1}	Reduced biomass	Stampoulis et al. (2009)
	10–30	Rice	20, 40, 80 mg L^{-1}	Plasma membrane detachment, condensation of chromatin	Tan et al. (2009)
SWCNT	8	Tomato	104, 315, 1750 mg L^{-1}	Most sensitive in root reduction	Cañas et al. (2008)

peroxidation leading to disruption of the cellular metabolism and ion leakage ultimately leading to cell death. Due to extremely short half-life, the ROS tend to be very difficult to evaluate directly. Instead of ROS, the other products of damage caused by oxidative stress, such as thiobarbituric acid reactive species (TBARS), can be measured (Pryor 1991). Assay of TBARS measure the malondialdehyde (MDA) content in a cell (Trevisan et al. 2001). The generation of ROS also causes damage to biomolecules and photosynthetic apparatus (Miller et al. 2010; Das and Roychoudhury 2014) (Fig. 2.4). Lipid peroxidation as a result of ROS generation is an indicator of cell membrane integrity (Mittler et al. 2004; Husen 2010). The antioxidant enzymes, nonenzymatic components, and low molecular weight antioxidants provide defense to plants against the oxidative damage by metal-oxide nanoparticles (Wang et al. 2011a; Zhao et al. 2012b). However, in a study TBARS were not detected in *Oryza sativa* plant treated with nano-CeO_2 at the concentration range of 0–500 mg L^{-1}. Nevertheless, ion leakage was recorded at higher concentrations (Rico et al. 2013b). Similarly, SPION have been proven to be cytotoxic to many terrestrial plants via generation of ROS (Wang et al. 2011a). In case of ZnO NPs, ROS production was detected in exposed roots (Nel et al. 2006). The higher risks of nano-form of ZnO with respect to the Zn^{2+} ions have been reported toward hydroponically grown *A. cepa* which may be attributed to higher production of ROS (Kumari et al. 2011). Likewise, ZnO-NPs were also found to trigger TBARS and ROS-mediated mitochondrial swelling and chromosomal abnormalities in hydroponically grown *A. cepa* after 12 h exposure (Ahmed et al. 2017). *Cucurbita maxima* and *Lolium perenne* seedlings treated with Fe_3O_4 NPs revealed 210 and 248% increase in lipid peroxidation in roots, compared to untreated controls (Wang et al. 2011a). Fe_3O_4 NPs were also reported to increase the levels of TBARS and to block aquaporin channels leading to reduce rate of respiration in roots (Wang et al. 2011a). Similarly, MWCNTs induced the production and accumulation of ROS in rice cells resulting in plant cell death (Tan et al. 2009).

2.15 Imbalance in Antioxidant Enzymes (AOEs) Activities

The defense system of plants comprising antioxidative enzymes such as catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and peroxidase (POD), etc. and low molecular weight antioxidants such as glutathione, ascorbate, carotenoids, proline, phenolics, α-tocopherols and α-carotenoids, etc. protect the cellular system from adverse effects of ROS (Das and Roychoudhury 2014; Getnet et al. 2015; Ozyigit et al. 2016). These components reduce the extent of oxidative damage or mitigate toxicity due to exposure to nanoparticles (Wang et al. 2011a; Zhao et al. 2012a). For instance, SOD and CAT together convert $[O–O]^{2-}$ and H_2O_2 to O_2 and H_2O and additionally reduce •OH radical, while POD acts as scavenger of ROS. It has been reported that ZnO NPs at 500–1500 μg L^{-1} increase the content of nonenzymatic antioxidants and antioxidative activities in *Brassica nigra* plant (Zafar et al. 2016). Additionally, the toxicity of ZnO NPs may also depend on the extent of soluble Zn in solution (Franklin et al. 2007). At 400 mg kg^{-1}, CeO_2 NPs cause a 39-fold higher catalase activity in shoots, as compared to control, but result in

30-fold decline at greater concentration of 800 mg kg^{-1}. Likewise, the APX activity has also found to be reduced at the concentration of 800 mg kg^{-1} with simultaneous drop in H_2O_2 level. It suggests that antioxidant enzyme activity at higher doses of CeO_2 NPs may decrease steadily. The TBARS generation and increased CAT and SOD activity in roots and shoots of pumpkin and ryegrass suggested Fe_3O_4 NPs mediated oxidative stress as compared to Fe_3O_4 bulk (Wang et al. 2011a). Although the Fe_3O_4 NPs were not able to travel from roots to shoots, the oxidative stress-mediated toxicity of Fe_3O_4 NPs might result from physical clogging of root pores inhibiting required water uptake (Ma et al. 2013a, b; Martínez-Fernández et al. 2015). Carbon-based nanomaterials have also been observed for AOEs in plants. For instance, it has been demonstrated that MWCNTs deposited at root surface may penetrate the epidermal cell wall which as a result cause damage and elevate peroxidase enzyme activity (Elena et al. 2012). The activities of AOEs may be increased (Kim et al. 2011) or decreased (Mukherjee et al. 2014) in NP-exposed plants. Thus it is widely accepted that the type of response and magnitude of damage is largely dependent on plant species, plant organ to be examined, and exposure time and strength of NPs treatment (Bandyopadhyay et al. 2015). For example, Amooaghaie et al. (2016) studied the effect of two zinc species, namely, nano-Zn and nano-ZnO, on tomato and wheat plant. Exposure to 100 mgL^{-1} nano-Zn and nano-ZnO slightly enhances the activity of CAT, POD, and APX in both treated species. Exposure to 200 mg L^{-1} nano-Zn and nano-ZnO sharply increased SOD activity in wheat leaves. While in tomato, 200 mg L^{-1} nano-Zn and nano-ZnO slightly strengthened the SOD and CAT activity.

2.16 Genotoxicity and DNA Damage

Plant cells containing a low number of chromosomes are considered as an excellent model for genotoxicity testing with a broad range of toxicological end points such as chromosomal aberrations in mitosis/meiosis, sister chromatid exchanges, alterations in ploidy, gene mutations, and DNA damage (Rico et al. 2011). Although, the genotoxicity of NPs to plants has been poorly understood with limited known examples. *A. cepa* bioassay for genotoxicity assessment has been chosen in many studies. Numerous chromosomal abnormalities, such as chromosome stickiness, chromosome bridges, breakages and laggings, and micronuclei formation by Al_2O_3, Ag, ZnO, bismuth (III) oxide, TiO_2, copper, zinc nanoparticles, and MWCNTs, have been reported in *A. cepa* root cells (Ahmed et al. 2017). Genotoxicity of TiO_2 NPs in *A. cepa* has also been demonstrated by DNA laddering technique and comet assay. Micronuclei formation and chromosomal abnormalities corroborated the manifestation of cellular fragmentation in preceding cell cycle (Rico et al. 2011). Besides mito-depressive effects of ZnO-NPs, alterations in mitochondrial membrane potential and oxidative stress were also studied along with ZnO bulk and Zn^{2+}-treated roots (Ahmed et al. 2017). Xi et al. (2004) reported that TiO_2 NPs cause oxidation of purine nucleotides in DNA which is a major causative factor for DNA damage. DNA damage in some grassland (*Lolium rigidum* and *Lolium perenne*) and agriculturally important plant (*Raphanus sativus*) has been

reported by Atha et al. (2012). Measurement of DNA-based lesions has been performed using GC/MS with isotope dilution to detect the levels of three different oxidatively altered bases (8-OH-Gua, FapyAde, and FapyGua) in DNA extract of each plant. Substantial accumulation of oxidatively altered, mutagenic DNA lesions (2,6-diamino-4-hydroxy-5-formamidopyrimidine; 7,8-dihydro-8-oxoguanine; 4,6-diamino-5-formamidopyrimidine) and inhibition of plant growth were observed (Atha et al. 2012). Disturbed and disoriented chromosomes at metaphase and anaphase in *Vicia faba* root of germinated seeds after 72 and 120 h have been reported as a result of Ag NPs exposure (Abou-Zeid and Moustafa 2014). Significant accumulation of mutagenic DNA lesions in germinated seeds of radish has been due to CuO NPs (Atha et al. 2012). In a study, RAPD profiles of hydroponically cultivated *Cucurbita pepo* under TiO_2 NPs stress revealed differences in band intensity and appearance or loss of bands (Moreno-Olivas et al. 2014).

2.17 Altered Gene Expression

Gene expressions have continuously been studied in different varieties of *A. thaliana*, such as Columbia-0 (García-Sánchez et al. 2015), and wild type, cv. Columbia (Landa et al. 2012). In the root tissue of Columbia-0 variety of *A. thaliana* exposed to TiO_2 NPs and MWCNTs for 7 days, gene expression analysis was performed using DNA microarrays. The NPs exposure suppressed transcriptional responses to microbial pathogens which in turn increased bacterial colonization. Also, the inhibition of transcription of phosphate starvation response and root hair development were observed. Likewise, in another study also based on microarray analysis, the root exposed to nano-TiO_2, nano-ZnO, and fullerene soot for 7 days showed that both biotic (defense to pathogens and wounding) and abiotic (salt, oxidative, and water deprivation) stress-responsive genes were upregulated, while biogenesis-associated genes and cell organization were downregulated upon nano-ZnO exposure (Landa et al. 2012).

Analysis of microRNA (miRNA) gene expression has been performed in *Nicotiana tabacum* (Burklew et al. 2012) and *Arabidopsis thaliana* (Nair and Chung 2014). The microRNAs (miRNA), as small noncoding RNA molecules (~22 nucleotides) (Ambros 2004; Bartel 2004), are a class of endogenous posttranscriptional gene regulators and are known to alter gene expression by either degrading mRNAs or inhibiting mRNA translation into polypeptides. miRNAs have been reported to mediate responses to abiotic stresses such as salinity and drought in plants by modifying genetic expression. Since carbon nanotubes can stimulate growth, gene, and protein expression of aquaporin in tobacco cells (Khodakovskaya et al. 2012), it may also trigger the reproductive genes in similar other plants. It has been reported that miRNA genes are upregulated and played an important role in the ability of *N. tabacum* to survive under Al_2O_3 NPs stress. *N. tabacum* plants (as a model organism) are exposed to 0–1% Al_2O_3 NPs. As the concentration of Al_2O_3 NPs increases, the average leaf count, biomass, and root length are substantially decreased. Earlier studies on miRNA genes, such as miR156, miR157, miR159,

miR162, miR167, miR169, miR172, miR395, miR396, miR397, miR398, and miR399 with identified functions in plants to mitigate the stresses, revealed that nine miRNA genes (miR159, miR162, miR167, miR169, miR395, miR396, miR397, miR398, and miR399) were notably upregulated with increasing concentration of Al_2O_3 NPs (Burklew et al. 2012). Similarly, in another study with *N. tabacum* plant, the miR395 and miR399 gene exhibited a dramatic change of 285-fold and 143-fold, respectively, at low concentrations (0.1 and 1%) of TiO_2 NPs, which suggests that TiO_2 NPs negatively impact growth and development of *N. tabacum* (Frazier et al. 2014).

2.18 Induction of Programmed Cell Death

ENMs are believed to cause cytotoxicity of plant cells through pathways of apoptosis (programmed cell death). Very few studies have focused on ENMs mediated systematic cell death of plant cells via apoptotic pathways. In this context, Shen et al. (2010) reported that SWCNTs induce chromatin condensation and ROS accumulation as compared to control treatments. A dose-dependent survival of cells has been observed with 25 μg mL^{-1} SWCNTs, primarily due to SWCNTs mediated apoptotic cell death (Shen et al. 2010). It has also been reported that MWCNTs are capable of reducing the density of rice cell suspensions in a dose-related fashion (Tan et al. 2009). This cell death at lower doses of MWCNTs has been noted to be induced by apoptosis. However, at higher concentrations reduction in cell viability has been attributed to necrosis as identified by membrane disruption and leakage of cytoplasmic content. On the other hand, a self-defense response in rice cell suspension was also observed by precipitation of small population of cells with NPs. Thus the rest of the cell population was indirectly safeguarded from the risk of cell death (Rico et al. 2011). Likewise, the NiO NPs have been examined to stimulate apoptosis in tomato root cells (Faisal et al. 2013). The comet assay revealed a noteworthy increase in the number of necrotic (24.0%) and apoptotic (21.8%) cells at 2.0 mg mL^{-1} of NiO NPs compared to untreated control groups. Flow cytometric studies also show 65.7% of apoptotic/necrotic cells and > twofold higher caspase-3-like protease activity at 2.0 mg mL^{-1} of NiO NPs. The apoptotic effects via mitochondrial-dependent intrinsic pathway have been attributed to dissolution of Ni^{2+} ions from NiO NPs (Faisal et al. 2013). Faisal et al. (2016) also reported apoptosis-mediated toxicity in eggplant (*Solanum melongena*) by Co_3O_4 NPs. Comet assay revealed about 2.4-fold higher level of DNA damage compared to unexposed control group, and flow cytometric-based cell cycle analysis revealed 73.2% apoptotic cells at 1 mg mL^{-1} of Co_3O_4 NPs (Faisal et al. 2016).

2.19 Impacts of ENMs on Plants: Positive Impacts

Besides producing negative impacts on growth and development of edible crops, ENMs also have some growth-promoting effects on plants which may differ with plant species, NPs type, and exposure conditions (Table 2.2). The growth enhancement by ENMs may bring changes in the field of agriculture to fulfill the ever-increasing nutritional requirements for human population. For instance, carbon-based nanomaterials (CNMs) have been utilized to increase crop production. The CNMs are known to be useful for enhanced root development, seed germination, and photosynthesis. As another example, the bitter melon (*Momordica charantia*) seeds grown in medium supplemented with fullerene increased the production by 112–128%, while at higher concentrations of fullerene, the growth-enhancing effects diminish (Kole et al. 2013). Other nutrients such as charantin (20%), lycopene (82%), cucurbitacin-B (74%), and insulin (91%) were also observed to increase with respect to untreated control (Kole et al. 2013). Better results have been obtained with oxidized MWCNTs at 2.3 μg mL^{-1}, but at 46 μg mL^{-1}, the inhibition mustard seed germination occurred (Mondal et al. 2011). If ENMs are able to increase the biomass and fruit count without producing toxicity, they may be combined with the applications of biofertilizers and may produce more beneficial effects. It is important to mention that the calcium content in root and stem increased from 25.6 to 69.8% in all cases of nanoparticle-treated tomato plant (Vittori Antisari et al. 2015). Foliar application of nano-ZnO to tomato plant also resulted in positive outcomes in terms of increased chlorophyll, biomass production, and total soluble leaf protein (Raliya and Tarafdar 2013; Raliya et al. 2015). Besides, the soil amended with TiO_2 NPs exhibited an increase in the chlorophyll content, catalase, nitrate reductase, and peroxidase activities in many plant species (Feizi et al. 2012).

2.20 Improvement of Root and Shoot Growth

Many of ENMs particularly metal-oxide NPs in soilless media as well as in soil environment have shown promoting effects to shoots and roots of edible plants (Table 2.2 and Fig. 2.4). In a study, CeO_2 NPs at 1–10 mg mL^{-1} slightly improved stem elongation, and a substantial increase in the total weight of fruits has been observed at 10 mg L^{-1} (Wang et al. 2012b). In barley, higher concentrations of CeO_2 NPs (500 mg kg^{-1}) brought about a rapid shoot development with a 331% increase in biomass. However, at the abovementioned concentration, the grain production halts severely. On the contrary, soil amended with low concentrations of CeO_2 NPs (125 and 250 mg kg^{-1}) encourages grain production, while large amount of Ce gets accumulated in grains and leaves (Rico et al. 2015). In an early report, mixed effects of CeO_2 NPs on the root growth of four edible plants, e.g., *C. sativus*, *Z. mays*, *M. sativa*, and *L. esculentum*, have been observed, while the shoot elongation has been stimulated in all four plant species at almost all concentrations (Lopez-Moreno et al. 2010). In case of iron oxide NPs (IONPs), an increase in dry weight of soybean pod and leaf has been detected. Also, IONPs have been reported as facilitators for

Table 2.2 Positive response of some ENMs on edible crop plants

Nanomaterial	Size (nm)	Food crop	Concentrations used	Growth-promoting effect	References
ZnO	1.2–6.8	Cluster bean	10 mg L^{-1}	Increased biomass (27.1%), shoot/root length, root area, chlorophyll content, and total soluble leaf protein	Raliya and Tarafdar (2013)
		Mung bean	20 ppm (foliar spray)	Increased biomass	Dhoke et al. (2013)
Activated carbon-based TiO_2	30–50	Tomato	0–500 mg L^{-1}	Improved germination, reduced germination time	Singh et al. (2016)
TiO_2	–	Soybean	0, 0.01, 0.03, 0.05%	Increased height (0.05%) and dry weight	Rezaei et al. (2015)
	4–6	Spinach	0.25%	Improved growth, increased glutamine synthetase, glutamate dehydrogenase, and glutamic pyruvic transaminase activity	Yang et al. (2006)
	27 ± 4	Cucumber	250, 500, 750 mg kg^{-1}	Enhanced CAT activity in leaves, enhanced P and K availability in fruit	Servin et al. (2013)
	30	*Z. mays*	300–1000 mg L^{-1}	Inhibition of root hydraulic conductivity	Ghodake et al. (2011)
		T. aestivum	1000 mg L^{-1}	Chlorophyll content	Mahmoodzadeh et al. (2013)
		Lycopersicon esculentum	0.05–0.2 g L^{-1}	Net photosynthetic rate, conductance to H_2O, and transpiration rate, regulation PS-II	Qi et al. (2013)
Nano-anatase TiO_2		*Spinacia oleracea*	0.25% (foliar spray)	Rubisco activase (rca) mRNA expressions	Ma et al. (2008)
Fe_3O_4	20	Pumpkin	500 mg L^{-1}	No toxic effect, translocation to stem and leaves	Zhu et al. (2008)
	7	Cucumber, lettuce	62, 100, 116 mg L^{-1}	Low to zero toxicity on germination	Barrena et al. (2009)
		Glycine max	0.5–0.75 g L^{-1}	Quality and yield	Sheykhbaglou et al. (2010)

Functionalized SWCNT	8	Cabbage, carrot, lettuce, onion, tomato	9, 56, 315, 1750 mg L^{-1}	No effect	Cañas et al. (2008)
MWCNT		Tomato	10–40 mg L^{-1}	Germination rate, fresh biomass, and length of stem significantly increased moisture content inside tomato seeds	Khodakovskaya et al. (2009)
		Ryegrass	2000 mg L^{-1}	Increased root length	Lin and Xing (2007)
		Lycopersicon esculentum	40 μg mL^{-1}	Uptake of nutrients (K, Ca, Fe, Mn, and Zn)	Tiwari et al. (2014)
SWCNT	8	Cucumber, onion	104, 315, 1750 mg L^{-1}	Increased root length	Cañas et al. (2008)
SiO_2	4–10	*Oryza sativa*	5 mM	Higher shoot biomass and grain weight	Liu et al. (2009a)
CuO		*Triticum aestivum*	500 mg kg^{-1} (sand culture)	Biomass	Dimkpa et al. (2012)

iron and assisted in photosynthate transfer of iron to the leaves of peanut. While in pumpkin, IONPs enhanced root elongation which is ascribed to iron dissolution (Rico et al. 2011). IONPs substantially promoted the growth of tomato plant but caused a reduction in green biomass (Siddiqi and Husen 2017). The TiO_2 NPs at 500 mg L^{-1} significantly increased the root growth of *C. sativus*, but the concentrations from 500 to 4000 mg L^{-1} cease the root to grow further (Servin et al. 2012). The assessment of organic nitrogen in the roots of plant shows that 51.1% nitrogen (N) has been found in roots as compared to control which is an indication that TiO_2 NPs mediate root growth promotion by stimulating N accumulation.

2.21 Enhanced Photosynthetic Rate

The nano-forms of TiO_2 have been studied in detail in terms of enhanced photosynthesis. TiO_2 NPs produces a positive impact on growth of spinach by increasing the activity of rubisco activase enzymes and improving light absorbance or decreasing the oxidative stress to chloroplast caused by ultraviolet radiations (Yang et al. 2007; Lei et al. 2008). The TiO_2 NPs have three crystalline structures, namely, rutile, anatase, and brookite. Among these three forms, anatase exhibits the highest catalytic activity (Yin et al. 2005). The anatase form is known to promote chlorophyll and carotene synthesis in *C. sativus*. The TiO_2 NPs promote chloroplast activity and Hill reaction by increasing light absorption in chlorophyll-a molecules, oxygen evolution, and electron transfer rate in spinach leaves (Su et al. 2007; Wang et al. 2008). The rutile form of TiO_2 NPs can prevent the damage of chloroplast membrane free radicals and ROS (Hong et al. 2005). Earlier studies showed that TiO_2 NPs could enhance energy conversion efficiency for photosynthesis (Wang et al. 2011b). In a recent research, both soluble protein and chlorophyll content in ZnO NP-treated plants increased up to 25% and 34.5%, respectively, with respect to untreated control (Raliya et al. 2016b). An aerosol-foliar spray of increasing concentrations of TiO_2 NPs up to 500 mg kg^{-1} exhibited chlorophyll content increasing from 62.67 to 227.42%. In comparison to this, TiO_2 NPs amended in soil causes a maximum increase in chlorophyll content of 216.29% at the concentration of 750 mg kg^{-1} (Raliya et al. 2015). Also, the SWCNTs penetrated into and accumulated in lipid envelope of extracted plant chloroplasts carried out over three times higher photosynthetic rate as compared to control and increased electron transport rates through a mechanism consistent with amplified photoabsorption (Giraldo et al. 2014).

2.22 Increased Absorption of Water and Fertilizer

The positive effects of ENMs to plants have also been characterized as allowing the plants to take more available form of nutrients from applied fertilizers or water from soil. For instance, nano-ZnO has been reported to significantly increase available form of P, soil microbial population, and root volume in mung bean rhizosphere. This could be

verified from increased dehydrogenase activity which is an indicator of microbial activity and uptake of P by plants from soil. The microbes thriving in soil assist in sustaining the soil health and structure for regular biogeochemical cycling of nutrients (Raliya et al. 2016b). Since nano-ZnO can be transformed to Zn^{2+} ions after uptake in plant cell, it may be used to control the activity of carbonic anhydrase for fixation of CO_2 to carbohydrates in plants. Zinc is also a cofactor of several enzymes including catalase and superoxide dismutase and thus helps to prevent oxidative damage in plant cells (Raliya et al. 2016b). SiO_2 in combination with TiO_2 NPs also increases nitrate reductase activity which serves as a central point in reduction of nitrate (NO_3^-) to nitrite (NO_2^-) and intensified absorption capacity of plant enhancing the increased uptake of fertilizer and water (Rico et al. 2011). The foliar application of ZnO NPs positively affected the growth of tomato plant and hence unleashed a possibility for potential use of ZnO NPs as a future nano-fertilizer. Similarly, foliar spray of ZnO NPs at 20 mg mL^{-1} in pot-grown plants also resulted in improved biomass production (Sekhon 2014). ZnO-, CeO-, and carbon-based nanoparticles have been reported to significantly increase the yield of tomato, wheat, and bitter melon (Raliya et al. 2015).

2.23 Transmission to Next Trophic Level

Once the ENMs are accumulated in edible parts of plant, they may be transferred to consumer via food chain. It is assumed that solubility of ENMs is a driving force for their transport. They may be transported by capillary movement to locations where the channel is broader than their diameter. The carbon-based NMs are known to be genetically transmitted to progeny (Rico et al. 2011; Lin et al. 2009). The second generation of rice plant showed the accumulation of fullerene at different stages and different organs of plant. Microbial cells can also accumulate fullerene that via eating mechanism of worms increases the likelihood to be assimilated into the food chain (Warheit et al. 2004). Two pioneer studies explained the trophic transfer of CeO_2 NPs in terrestrial food chains in detail (Hawthorne et al. 2014; Majumdar et al. 2016).

2.24 Conclusion

ENMs may toxify/detoxify the plant system or may pose no effect on growth of plant. An engineered nanomaterial should be tested for longer duration on different growth phases of plant in real environments because after sometime plants show signs of restoration of ENM-mediated toxicity. On the basis of documented studies, following are the fundamental factors that influence the effect of ENMs on agriculturally valuable crops: concentration, surface charge, specific surface area, crystal structure, size, and physicochemical properties of ENM, plant species, growth stage, anatomy of tissues, root exudates and their effect on ENMs, growth media, pH of soil, and duration of exposure. Results reported in the literature from different laboratories are diverse. Hence, thorough characterization of ENMs before testing

on plants must be taken into account for better understanding the behavior of ENMs in plants. Higher concentrations for ENMs toxicity testing must be avoided because they are not realistic in natural environments such as the soil. Understanding of toxicity mechanism, accumulation, biomagnification, and correlation with physico-chemical properties of ENMs is still limited. Also, some positive effects of ENMs on plant growth suggest the low potential risk of ENMs release into the environment. Systematic genomics, transcriptomics, and metabolomics studies are warranted to unleash the genetic points for promotion or deterioration of agro-economic characters.

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Penetration and Accumulation of Carbon-Based Nanoparticles in Plants

3

Olga Zaytseva and Günter Neumann

3.1 Introduction

Nanotechnology is a research which investigates manipulation of matter at an atomic and molecular level (Fig. 3.1). Several existing definitions of nanoparticles often vary as they reflect their specific application (Horikoshi and Serpone 2003). In general, nanoparticles can be defined as particles with the size between 1 and 100 nm (10^{-9}–10^{-7} m) at least in one dimension (Fig. 3.1). In this range properties of materials become size-dependent, and therefore nanomaterials acquire features which often differ from those of the same material in bulk form. Therefore, the key characteristics describing nanoparticles are not solely their small size but also specific features determining their unique physical and chemical properties.

Although nanotechnology has been rapidly developed only in the recent past, nanoparticles of natural origin were always present in the environment in neglectable quantities. Thus, carbon-based nanomaterials, such as carbon nanotubes, fullerenes and other ultrafine carbon particles have been detected in samples of several natural materials such as coal-petroleum mix (Velasco-Santos 2003), in mineral shungite (Buseck et al. 1992; Parthasarathy et al. 1998; Misra et al. 2007) as well as in objects of cosmic origin (Becker et al. 1994). It has been shown that carbon-based nanomaterials can be accidentally synthesized during combustion of fuels such as coal, firewood, diesel, gasoline and propane (Tiwari et al. 2016) and can be detected in the exhausts of diesel- and gasoline-fuelled vehicles and even can be released during cooking with a regular domestic gas stove (Wagner et al. 2010). Beside existence of natural and accidental formation of nanoparticles, preparation of

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_3

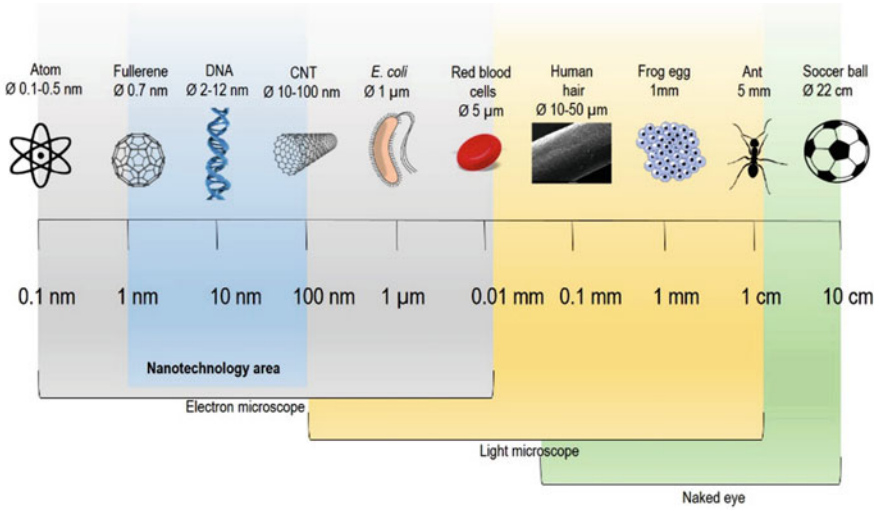

Fig. 3.1 Dimensional scale of various biological and physical objects (nanotechnology area is between 1 and 100 nm)

artificial nanoparticles and their intentional use are well known from the ancient times. For example, mixtures of gold and silver nanoparticles with various diameters (under 100 nm) were used for changing optical properties and colouring pieces of glass already before the fourth century AD (Horikoshi and Serpone 2003).

In the second part of the twentieth century, advanced development of analytical methods and tools, such as electron microscope, allowed to accomplish a significant progress in investigation and understanding of unique physicochemical characteristics of nanoparticles. This was followed by a rapid development of a wide range of nanomaterials application for industry, medicine and science, which in turn stimulated development of methods for their industrial synthesis. Thus, the overwhelming majority of nanoparticles nowadays are engineered.

Currently, carbon nanomaterials (nanotubes, fullerenes and graphene) belong to the ten most produced nanomaterials for industrial applications (Keller et al. 2013). Nowadays there are available various methods for synthesis of carbon-based nanomaterials with required properties; however those techniques still need to be further improved. According to marketing forecasts, production of carbon-based nanomaterials will grow in the near future, and it is expected to reach 20,000 tons by 2022 (Grand View Research Inc. 2015).

To the key segments of carbon nanomaterials applications belong electronics and transportation, where they can be used for mechanical reinforcement of polymer composites and functionalized textiles (Yengejeh et al. 2017) and for synthesis of multifunctional coating materials for automotive and aerospace industry (Luinge 2011; Chinnappan et al. 2016), as well as they can be utilized as conductors and semiconductors in electronic devices (De Volder et al. 2013; Saito et al. 2017), for energy storage (Sun et al. 2017) and others. Also carbon nanomaterials find their

application in consumer goods such as sporting equipment, cosmetics, health care, goods for home and garden and others (Hansen et al. 2016). Moreover, the specific fields of carbon nanomaterials utilization include also environmental and agricultural applications (Zaytseva and Neumann 2016a).

Intensive development of novel applications and rapid growth of nanomaterial production raise concerns regarding potential nanomaterials release into the environment. To date, there is no reliable information about current nanomaterial concentrations in different environmental compartments (soil, water, air); however the probabilistic computer modelling shows that the amount of nanomaterials discharged into the environment assuming worst-case scenario comprised 3200 metric tons in 2010 (Keller et al. 2013). According to Keller et al. (2013), the main part of the released CNTs ends up in landfills and soils, and in 2010 it comprised 2700 (84% of the total amount) and 500 (15% of the total amount) metric tons correspondingly. Due to potential applications of nanoparticles in agriculture (Zaytseva and Neumann 2016a) as well as soil application of sewage sludge with potential CNT contaminations, concentration of CNTs in sludge-treated agricultural soils in the European Union will increase faster and will grow by 990 ng kg^{-1} every year, while in natural and urban soils, CNT concentration is predicted to grow only by 5.1 ng kg^{-1} per year (Sun et al. 2014). Therefore there is a particular concern regarding potential contamination of agricultural crops.

The anxiety regarding nanomaterials release into the environment stimulated an active research in nanotoxicology. There are numerous of studies investigating the effects on carbon-based nanomaterials on various living organisms including terrestrial and aquatic plants. Those studies reported positive (Khodakovskaya et al. 2009; Nair et al. 2012) but also adverse effects including acute toxicity (Zaytseva and Neumann 2016b; Begum and Fugetsu 2012) of nanomaterials. However, there are studies which report absence of any influence (Lin and Xing 2007). In several studies the possibility of carbon nanomaterials to penetrate into plants and to accumulate in their tissues was investigated. There were reported evidences of carbon-based nanomaterial accumulation in plant tissues. These findings created concerns about possible translocation of carbon nanomaterials into food chain and finally consumption by humans.

In this chapter we will summarize the evidences of carbon-based nanomaterials penetration in plants, their translocation within the plant organism and their potential pathways into a food chain.

3.2 Uptake of Carbon-Based Nanomaterials into Plants

To date, a significant progress in investigation of the impact of nanomaterials on plant physiology and development has been made. Additionally numerous studies have focused on nanoparticles uptake, translocation and accumulation in plants. This feature of nanoparticles was studied in different plant systems including cell culture systems in vitro but also in vivo using various species of aquatic and terrestrial plants. These studies have clearly shown penetration of nanoparticles into the plant

tissues and even cell walls perforation and cell internalization (Liu et al. 2009). In addition, several studies reported translocation of nanoparticles from one organ to another within a whole plant (Zhai et al. 2015). Table 3.1 summarized evidences of uptake and translocation of different carbon-based nanoparticles within various plant systems.

3.2.1 Carbon Nanotubes in Plant Tissues

Carbon nanotubes (CNTs) comprise cylindrical structures of one or more layers of carbon atoms in sp^3 hybridization state. Based on the number of carbon layers, CNTs are divided into two major groups: (1) single-walled carbon nanotubes (SWCNTs), with only one layer of carbon atoms, and (2) multi-walled carbon nanotubes (MWCNTs), with two or more layers of carbon atoms. For phytotoxicity studies it is recommended to use a bulk carbonaceous material as a control treatment. It has been reported that activated carbon often used as negative control does not show penetration into plant tissues (Khodakovskaya et al. 2011). This fact suggests that specific physicochemical features of CNTs might be a key factor which ensure their ability to penetrate and to be translocated within plant tissues. To those features belong dimensional properties of CNTs (outer and inner diameter and length), degree of agglomeration and surface functionalization. The diameter of SWCNTs varies between 1 and 3 nm, while the MWCNTs have greater diameter which varies between 5 and 40 nm. The length of CNTs is also an important physical parameter which can affect the ability of CNTs to penetrate plant cell walls. For example, in the study of Lin et al. (2009), carbon nanotubes with the length up to 2 mm failed to penetrate into the root cells and were detected mainly on the outer root surface. Chemical functionalization, or in other words attachment of various functional groups onto external walls of CNTs, can change their physicochemical properties and therefore mode of interaction with plant cells. For example, attachment of carboxylic groups (–COOH) to the CNT surface can improve their "solubility" (Lin and Xing 2007; Lee et al. 2011) and thus ensure well-dispersed and stable suspension of nanotubes which often have a tendency to agglomerate in aqueous solutions. Furthermore, changing surface charge can affect distribution of CNTs in plant tissues. Thus, Zhai et al. (2015) have shown preferential accumulation of positively charged carbon nanotubes (MWCNT–NH_2) on the surface of negatively charged membranes, while negatively charged nanotubes (MWCNT–COOH) were detected in the xylem due to repulsion from the membranes.

In studies investigating nanomaterial uptake by plants, the duration of plant exposure to nanomaterials varies greatly from several hours to several weeks. However, a few studies have shown that uptake of nanoparticles can occur quite rapidly. For example, carbon nanotubes were detected in germinating soybean seeds already after 36 h of exposure (Zaytseva et al. 2017) and in maize seedlings after 72 h (Yan et al. 2013). In case of fullerols application, 48 h of seed treatment was enough to find these nanoparticles 3 months later in all organs of developed plants (Kole et al. 2013).

Table 3.1 Evidences of carbon-based nanomaterials penetration into plant tissues and methods of their detection

Type of NM	Size of NM	Plant species	Organ	Translocation	Method of detection	References
wsSWCNTs	Length <500 nm	*Nicotiana tobacum*	Cell culture	–	Confocal microscopy, fluorescence, fluorescence microscopy	Liu et al. (2009)
SWCNTs	na	*Arabidopsis thaliana*	Culture of mesophyll cells	–	Optical microscopy and transmission electron microscopy, fluorescence microscopy	Yuan et al. (2010)
MWCNTs	OD: Ø 10–35 nm Length: 6 μm	Tomato	Roots, leaves, fruits	+	Raman, photothermal and photoacoustic spectroscopy	Khodakovskaya et al. (2011)
MWCNTs	OD: 40–70 nm ID: 5–40 nm Length: 0.5–2 mm	Rice	Roots, stems	+	SEM, TEM	Lin et al. (2009)
MWCNTs	Length 0.05–2 μm	Maize and soybean	roots, stems, and leaves	+	TEM	Zhai et al. (2015)
MWCNTs	OD: 20–70 nm ID: 5–10 nm Length of >2 μm	Soybean	Germinating seeds	–	Light microscopy	Zaytseva et al. (2017)
SWCNTs	OD 1–2 nm Length ≈ 30 μm	Maize	Roots	–	TEM	Yan et al. (2013)
MWCNTs	na	Tomato	Germinating seeds	–	Raman spectroscopy	Khodakovskaya et al. (2009)
MWCNTs	na	Tomato	Leaves and roots of 25-day-old seedlings	+	TEM	Khodakovskaya et al. (2009)
MWCNTs	OD: 25 nm ID: 10 nm Length—few μm	Tomato	Flowers	+	Raman spectroscopy	Khodakovskaya et al. (2013)
MWCNTs	na	Maize, barley, soybean	Germinating seeds	–	Raman spectroscopy and TEM	Lahiani et al. (2013)

(continued)

Table 3.1 (continued)

Type of NM	Size of NM	Plant species	Organ	Translocation	Method of detection	References
SWCNTs	D: 1–2 nm Length 5–30 µm	*Arabidopsis thaliana* and rice	Cell suspension (leaf protoplasts)	–	TEM	Tan et al. (2009)
MWCNTs	Length 50–500 nm	*Catharanthus roseus*	Cell suspension (leaf protoplast)	–	TEM and confocal imaging	Serag et al. (2011)
MWCNTs	na	Onion	Roots	–	SEM	Ghosh et al. (2015)
MWCNTs	D: 11 nm Length >1 µm	Red spinach	Root and leaves	+	Raman spectroscopy	Begum and Fugetsu (2012)
MWCNTs	OD: 41.2 nm	Wheat and rapeseed	Roots, leaves	+	Radioimaging, TEM, raman spectroscopy	Larue (2012)
MWCNTs	OD: 13 nm ID: 4 nm Length > 1 µm	Lettuce	Roots	–	TEM	Ikhtiari et al. (2013)
Oxidized MWCNTs	Length 50–630 nm	Wheat	Roots	–	Transmission electron microscopy	Wang et al. (2012)
C70		Rice	Roots, leaves, seeds	+	TEM, Raman spectroscopy	Lin et al. (2009)
Fullerols $C_{60}(OH)_{20}$	–	Bitter melon	Roots, stems, petioles, leaves, flowers and fruits	+	Bright-field imaging, Fourier transform infrared spectroscopy	Kole et al. (2013)
Graphene oxide sheets	D 5 µm, thickness 0.8–1 nm	Chlorella	Algal cells	–	Confocal fluorescent microscopy and TEM	Hu et al. (2014b)

ws water soluble, *SWCNTs* single-walled carbon nanotubes, *MWCNTs* multi-walled carbon nanotubes, *TEM* transmission electron microscopy, *SEM* scanning electron microscopy, *GO* graphene oxide, *D* diameter, *OD* outer diameter, *ID* inner diameter

One of the most important structural features of plant cells is their rigid cell wall, made of fibres and cellulose. The cell wall has many important functions including cell protection from internalization of foreign objects including pathogens and viruses. Cell wall has numerous pores with average diameter varying between plant species from 2 to 5 nm (Berestovsky et al. 2001; Carpita et al. 1979). Thus, penetration through cell wall pores of foreign object with bigger size (including majority of carbon nanotubes) might be restricted. However, there were studies showing penetration of SWCNTs and MWCNTs into individual plant cells. In the experiments with cell culture of tobacco, Liu et al. (2009) have demonstrated penetration of large SWCNTs with the length ~500 nm mediated through endocytosis. The same mechanism was also suggested for penetration of SWCNTs into the cell culture of *Arabidopsis* (Yuan et al. 2010). Similar observations were reported in study of Shen et al. (2010), who have demonstrated formation of endocytosis-like structure in the *Arabidopsis* protoplast treated with SWCNTs. Alternative way of cell internalization was reported by Serag et al. (2011) for MWCNTs. They have demonstrated endosome-escaping uptake mode of MWCNTs (<100 nm) by plant protoplasts. Despite the above-mentioned evidences, the exact mechanism of the cell wall penetration and internalization of nanotubes still remains unclear and deserves further investigation.

Effects of CNTs on tomato plants were thoroughly investigated by Khodakovskaya et al. (2009), and they have demonstrated penetration of carbon nanoparticles into plant tissues. The earliest report from this working group has shown stimulation of tomato germination by amendment of the germination medium by MWCNTs (Khodakovskaya et al. 2009). With the help of Raman spectroscopy, they were able to detect nanotubes inside the germinating seeds which suggest that CNTs are able to penetrate relatively hard seed coat. The authors hypothesized that the penetration of nanoparticles was mediated through mechanical perforation of the seed surface due to outstanding mechanical properties of CNTs. An alternative explanation to the observed phenomenon was gating of water channels present in the seed coat. Mechanical disturbance of the tomato seed coat integrity by CNTs have been confirmed later in the study of Ratnikova et al. (2015). Using bright-field microscopy, the authors have shown an almost complete removal of the seed coat from tomato seeds exposed to aqueous solution of MWCNTs and treated with ultrasonication only for 60 min. However, nanotubes failed to penetrate further into the embryo due to protective function of the seed coat's semipermeable layer. Penetration of MWCNTs through seed coat was also reported for other agronomic crops such as maize, barley and soybean (Lahiani et al. 2013). Carbon nanotubes were detected inside of germinating seeds regardless of the method of nanotube application: deposition on the seed surface or seeds soaking in medium amended with nanotubes. Penetration of MWCNTs through the seed coat of soybean has been confirmed in the study (Zaytseva et al. 2017): after a short-term (36 h) seed exposure to MWCNTs, their agglomerates were detected in radicles of germinating seeds. These findings suggest that carbon nanotubes can readily cause mechanical damages to seed coats and make their way towards the embryo; however, due to variability of seed coat textures and semipermeable layers between various plant species, the results of those experiments are inconsistent.

The most probable scenario of plant exposure to nanomaterials in the environment is through growing substrate, e.g. soil; therefore the majority of studies focus on plant root treatment with nanoparticles. Such exposures were often associated with sorption of nanomaterials on the external root surface due to high affinity to epidermis. It has been reported for various plant species, including onion (Ghosh et al. 2015), rice (Lin et al. 2009), maize, soybean (Zhai et al. 2015) and lettuce (Ikhtiari et al. 2013). As a consequence of root exposure to CNTs, there were reported structural and morphological deformations of exposed roots including disturbance of root caps, reduction of root hairs (Begum et al. 2011; Begum and Fugetsu 2012; Ikhtiari et al. 2013) as well as various abnormalities in cellular organization (Ghosh et al. 2015). Tight adhesion of nanotubes in the root surface causing morphological disturbances can finally lead to the root blockage which can significantly affect plant development, e.g. to delay flowering and seed setting (Lin et al. 2009).

Several studies have reported that nanoparticles can penetrate through organs exposed to nanomaterials, e.g. roots, and can be further translocated and be detected in other plant parts which did not contact with nanomaterials: stems, leaves and even flowers and fruits. Thus, translocation of MWCNTs from root to leaves was shown in red spinach (Begum and Fugetsu 2012), tomato (Khodakovskaya et al. 2011, 2013), wheat and rapeseed (Larue et al. 2012).

Khodakovskaya et al. (2011) have demonstrated uptake of MWCNTs via roots of tomato seedlings and their distribution into different tissues including leaves and fruits. Because the nanotube treatment was applied only into growing substrate (soil amended with MWCNTs), it suggests that the nanomaterials were absorbed by roots and subsequently distributed within the plant most probably through the vascular system. However, the majority of detected in leaf tissues nanoparticles were accumulated outside of the vascular system, which demonstrates that nanoparticles can also be released from the vascular system. In more detailed investigation of CNT accumulation, the authors have demonstrated presence of CNTs in generative tissues of flowers (Khodakovskaya et al. 2013). For the analysis the authors for the first time used a promising method of carbonaceous nanomaterials detection representing a combination of photothermal and photoacoustic spectroscopy (Khodakovskaya et al. 2011). The high sensitivity of the above-mentioned technique allowed authors to recommend this method for detection of nanomaterials in plant samples grown in the areas with presumable soil contamination by nanomaterials.

Many authors suggest that the transport of nanotubes within plant organism occurs most probably together with water via vascular system. Thus, Zhai et al. (2015) have detected MWCNTs in xylem and phloem of maize roots, while Smirnova et al. (2011) have shown accumulation of MWCNTs in vascular tissues of *Onobrychis*. In wheat and rapeseed (Larue et al. 2012), CNTs were translocated from root to leaves and finally were accumulated in leaf tip. The authors suggest that transport of nanoparticles was most probably due to capillarity. A transpiration was also suggested to force CNTs transport from roots to upper organs of mustard (Chen et al. 2015).

Several studies have shown that penetration of CNTs into plants can affect plant tissues on genetic level. For instance, Khodakovskaya et al. (2011) have reported upregulation of stress-related genes (Les.564.1.S1, heat shock protein 90, and Les.49.1.S1, TDR3 protein) including those usually activated upon pathogen attacks (Les.3648.1.S1, subtilisin-like endoprotease; Les.3048.1.S1, DB163 *Meloidogyne*-induced giant cell protein; LesAffx.64585.1.S1, threonine deaminase). This is a very interesting finding, because the dimensional similarity between many microbial pathogens and nanoparticles suggests that plants might recognize interaction with nanomaterials as a pathogen attack. Similar conclusions were drawn by Tan et al. (2009). The authors have found a formation of reactive oxygen species (ROS) in suspension of rice cells in response to MWCNTs treatment. The authors suggest that upon nanoparticle penetration into the plant tissues, plants can trigger oxidative burst as it happens during hypersensitive response. A cascade of oxidative stress in response to a short-term seed treatment with MWCNTs was also demonstrated in soybean (Zaytseva et al. 2017). The agglomerates of nanotubes were detected inside the radicles of germinating seeds and were associated with decreased tissue viability and activity of superoxide dismutase (SOD) enzyme.

In the articles discussed above, the accumulation of nanomaterials in plant tissues was detected using various analytical techniques including microscopy (light, transmission and scanning electron microscopy), Raman spectroscopy and combination of various other methods. Unfortunately, those techniques are not suitable to provide quantitative data. The advantages and disadvantages of different detection methods of carbonaceous nanomaterials in plant tissues were discussed in details in review (Zaytseva and Neumann 2016a). Only a few studies reported estimation of absolute amounts of nanotubes accumulated by plants. Thus, using 14C-radiolabeled MWCNTs, Larue et al. (2012) reported that uptake on nanotubes by 15-day-old plantlets of wheat and rapeseed roots exposed to nanotubes during 1 week was less than 0.005 ‰ of the applied MWCNT dose. This nanomaterial uptake was not associated with morphological or physiological plant parameters, and therefore the authors claim that there is not a great risk of environmental contamination and that nanotubes transfer to the food web can be very low.

3.2.2 Fullerene and Their Derivatives in Plant Tissues

Fullerenes are a class of carbon allotropes characterized by spherical shape which can contain different number of carbon atoms. The most common natural and synthesized fullerenes contain 60 (C_{60}) or 70 (C_{70}) carbon atoms; however fullerenes with lower (e.g. C_{20}, C_{26}, C_{28}, C_{32}, C_{50}) and higher number (e.g. C_{72}, C_{76}, C_{84} and even C_{100}) of carbon atoms were reported as well (Karthik et al. 2014; Voytekhovsky and Stepenshchikov 2003). The size of fullerenes is significantly smaller than the size of carbon nanotubes. It has been reported that the diameter of fullerenes can vary from 0.5 nm (for C_{36}) to 1.2 nm (for C_{176}) (Goel et al. 2004). Similar to carbon nanotubes, the surface of fullerenes can be functionalized which results in changing their physicochemical properties. The most important and often

produced derivatives of fullerenes are called polyhydroxy fullerenes, fullerols or fullerenols ($C_{60}(OH)_x$, $x = 18-36$) (Borišev et al. 2016). These nanoparticles can be obtained by attachment of hydroxy groups (–OH) to the surface of fullerene. After this modification fullerols maintain unique properties of original fullerenes but additionally acquire solubility in water and therefore are often used in toxicology studies. The production volumes of fullerenes and their derivatives are lower than those of carbon nanotubes, and therefore fullerenes were not considered as a major class of contaminants for the environment. According to the modelling studies, a yearly increase of fullerenes concentration in natural and urban soils in the European Union will comprise 0.1 ng kg^{-1}, which is 50 times less than yearly increase for CNTs (Sun et al. 2014). The latter might be a reason why only a few studies focused on fullerene toxicity and their penetration in plant systems.

The effects of fullerenes and their derivatives were studied on several agricultural crops such as rice (Lin et al. 2009), sugar beet (Borišev et al. 2016) and on medicinal plant bitter melon (Kole et al. 2013).

In these studies, there were reported evidences of fullerenes and their derivatives uptake into plant organism, their translocation from one organ to another (Kole et al. 2013) and even cases of nanoparticles transmittance from one generation to another through seeds (Lin et al. 2009). Accordingly, interesting results were presented in the study of Kole et al. (2013). The authors have shown that fullerols ($C_{60}(OH)_{20}$) which were adsorbed during short-term treatment (only 48 h) in germinating seed retained in plant organism and after termination of the treatment nanoparticles were transported and distributed between all organs of plants developed from treated seeds, including vegetative and generative organs (roots, stems, petioles, leaves, flowers and fruits). The authors speculate that the translocation of nanoparticles from seeds to plant might occur (1) due to the concentration gradient of nanoparticles in plant organism and (2) hydrophobic interaction between nanoparticles and waxy layers between plant cells (Kole et al. 2013).

Translocation and accumulation of fullerenes (C_{70}) in generative organs (seeds) was also shown in rice in a study of Lin et al. (2009). The nanomaterial was added into germination media for rice seedlings during 2 weeks after seed germination. Thereafter plants were grown in soil without any nanoparticle amendment. Later black aggregations were detected in stems and leaves using bright-field microscopy, and the presence of fullerenes in those samples was confirmed with the help of Raman and infrared spectroscopy. It was suggested that translocation of fullerenes within the plant occurs through xylem as the nanoparticles were accumulated in vascular system. Since there were no fullerenes in the roots of mature plants, it was suggested that all nanoparticles were transported to the above-ground parts. From the seeds collected in this experiment, second generation of rice plants was cultivated and subjected on nanoparticles content in tissues. Similar black accumulations of fullerenes were detected in their leaves.

Beside root exposure to fullerenes and their derivatives, there was also evaluated an alternative way of nanoparticles internalization through the leaf surface. Borišev et al. (2016) applied an aqueous solution containing fullerols onto leaves of sugar beet. According to authors, after penetration through leaf cuticle into the leaf tissues,

fullerols can serve as supplementary water sources due to their strong water-binding properties. Alleviation of drought stress in plants treated with fullerols was shown by a lower proline level as well as oxidative stress markers similar as it was detected in control plants receiving sufficient irrigation. Similar results were obtained for the roots of fullerol-treated plants. These results suggest possible mobility of fullerols within plant tissues and their possible translocation downwards from treated leaves to untreated roots. However these conclusions are indirect, and therefore an identification of nanoparticles in plant tissues is needed in order to confirm this hypothesis.

3.2.3 Graphene and Their Derivatives in Plant Tissues

Graphene is one of the newest carbon-based nanomaterials which represent planar sheets made of one-atom-thick layer of carbon in sp^2-hybridization state. For the first time, this material was exfoliated from ordinary graphite in 2004 (Novoselov et al. 2004). Due to its extremely strong electrical conductivity, it has a great potential in such industrial applications as electronics (Choi et al. 2010; Jang et al. 2016). Graphene can be also functionalized similarly to nanotubes and fullerenes. Its chemical derivative—graphene oxide (GO)—can be obtained by treating graphite with strong oxidizers such as sulfuric acid (H_2SO_4), sodium nitrate ($NaNO_3$) and potassium permanganate ($KMnO_4$) (Kovtyukhova et al. 1999; Marcano et al. 2010). Graphene nanostructures have also attracted a research interest from a toxicological point of view; however the majority of toxicological studies were focused on microorganisms and animal cell culture (for review see Zhang et al. 2016). To date, there is only a few studies which have documented penetration of graphene and their derivatives into plant cells and tissues.

A few-layer graphene structure with the size of 100–120 nm and thickness of 2–5 nm added into germination medium did not affect early development of tomato seedlings (Khodakovskaya et al. 2011). According to authors the few-layer graphene material was not able to penetrate through the seed coat and root tissues, although the applied particles were in the nano-sized range. Contradictory results were presented in the study of Zhang et al. (2015) where the authors reported penetration of graphene sheets with the size in the micrometre range through husks into germinating tomato seeds. Due to damages in seed coats, water uptake by seeds was significantly improved what eventually resulted in increased germination rate and greater stem and root length as compared to the control treatment. The authors have detected graphene sheets inside the cell walls and vacuoles in the cells of root tips of young tomato seedlings. Interestingly the authors have emphasized that graphene sheets detected inside the cells were wrinkled, while the original materials contained only flat sheets. This suggests that nanomaterials can undergo physical deformation in plant tissues. Damages of plant tissues by graphene oxide sheets (diameter 1–5 mm, thickness 0.8–1 nm) due to their sharp edges were reported also for wheat (Hu et al. 2014a). Using Raman spectroscopy, the authors confirmed internalization of graphene oxide inside the cells which was associated with damages to cellular structures: cell wall, plasma membrane and membrane of chloroplasts

thylakoids. In more details cell internalization by graphene oxide sheets was investigated using unicellular green algae *Chlorella vulgaris* as a test object (Hu et al. 2014b). By the mean of confocal fluorescent microscopy and TEM, the authors confirmed nanoparticle penetration inside the algal cells. They also have documented irregular depositions of GO sheets between the cell wall and the plasma membrane and destruction of the chloroplast structure. GO sheets were adhered to the external surface of algal cells. Although the exact mechanism of such algal cells enveloping by GO sheets is unknown, nitrogen-containing functional groups might play an important role in this process.

3.3 Implications for Food Chains

As volumes of carbon nanomaterials synthesis increase over time, it raises a risk of environmental contamination. Exposure to nanoparticles can affect plant development, for example, cause damages to root tissues, blockage of nutrient uptake, reduction of biomass accumulation and other disorders (Begum et al. 2011; Ghosh et al. 2015; Lin et al. 2009). As was reported in studies discussed above, carbon-based nanomaterials are able to penetrate plant tissues and accumulate in various plant organs such as in roots and stems but also in edible parts of important agricultural crops such as in leaves, fruits and grains. Thus, carbon nanotubes were found in tomatoes (Khodakovskaya et al. 2011), while accumulation of fullerenes has been detected in rice grains (Lin et al. 2009) and in fruits of bitter melon (Kole et al. 2013). Higher plants are an essential component of all ecosystems and often are the first-step organisms in a food chain. From an ecotoxicological perspective, it is very important to investigate a risk of nanomaterials transfer from plants to the next members of the food chain. It has been already shown that plants can uptake and accumulate environmental pollutants such as heavy metals (Wenzel and Jockwer 1999) and organic pollutants (Simonich and Hites 1995). Subsequently, accumulated in plants toxic compounds can enter food chain if those plants are used as a food source. Thus organisms in higher levels of the food chain including human beings can be exposed to toxic compounds, which can cause various health disorders, poisoning and serious illnesses (for review see Peralta-Videa et al. 2009). It has been already reported that nanoparticles can flow through the food chain from plants to herbivores, accumulate in their bodies and cause metabolic disorders similar as it was shown for other environmental toxicants. Thus, Cedervall et al. (2012) have shown that polystyrene nanoparticles added for 24 h into growth media were accumulated in algae (*Scenedesmus* sp.) and then through zooplankton (*Daphnia magna*) were transferred to fish Crucian carp (*Carassius carassius*). Nanoparticles accumulation in fish tissues was confirmed using fluorescence and bright-field imaging. The accumulation of nanoparticles in tissues affected feeding behaviour of fish, fat metabolism and weight loss. Transfer of nanoparticles and their biomagnification was studied also in terrestrial food chains. Judy et al. (2011) added gold nanoparticles with a different size (5, 10, 15 nm) into a hydroponic medium and used it for cultivation of tobacco plants which they feeded later to agricultural pest

tobacco hornworm (*Manduca sexta*). Using X-ray fluorescence and laser ablation inductively coupled mass spectrometry, the authors have confirmed presence of gold nanoparticles in both organisms. Notably, the effect of biomagnification was reported as a concentration of nanoparticles in the worms' tissues was an order of magnitude more concentrated than in the plants. As to our knowledge, only a few studies were investigating health effects of consumption of plants contaminated with carbon-based nanomaterials. For example, Parks et al. (2013) tested a possibility of nanotubes to be bioaccumulated and translocated from plant material to animal organism. The authors fed SWCNTs-amended algae to estuarine amphipod *Leptocheirus plumulosus* during 28 days. The sediment in the experimental system was also amended with nanotubes. The concentration of SWCNTs in the tissues of animals exposed to nanoparticles-amended food source was 0.50 μg g^{-1}. However, the concentration of nanomaterial on the surface of amphipods was five times higher (5.38 μg g^{-1}) which suggests that much more nanotubes were accumulated on the surface of *Leptocheirus plumulosus*. Although the authors did not detect any toxic effects in *Leptocheirus plumulosus*, the issue is still deserving further investigation whether the concentration of SWCNTs can be biomagnified in the next trophic level. The obtained results indicate that consumption of nanomaterials-contaminated food represent a much higher risk for human health than consumption of contaminated drinking water or exposure to airborne nanoparticles. Therefore, the nanoparticles transfer in more complicated food chains and possible health effects represent a knowledge gap which need to be investigated in the near future.

3.4 Summary

Carbon-based nanomaterials have a great structural variability which affects their possibility to penetrate and to be accumulated in plant tissues. Thus, for nanotubes it was shown that their size (diameter and length) and correct orientation towards plant cell or surface of plant organ are important prerequisites for their penetration into plants. One of the most often suggested mechanism of carbon nanotubes internalization was mechanical piercing of plant tissues, including hard seed coats. Penetration of graphene was also often associated with mechanical damages of plant tissues due to sharp edges of graphene sheets. Carbon nanomaterials can be transported within plant organisms most probably via vascular system due to capillary effects, transpiration and difference in nanoparticles concentration. Once leaves are reached, nanoparticles can be released from vascular system. Additionally, nanoparticles uptake via leaves and their transport downwards from leaves to roots might also be possible. Carbon-based nanoparticles were shown to be accumulated in edible plant organs and also were detected in plants of second generation, which raises concerns regarding their entering into food chain and finally human exposure.

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Phytotoxicity of Rare Earth Nanomaterials

4

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4.1 Introduction

Rare earth elements (REEs), including lanthanides, scandium, and yttrium, are an essential part of many high-tech devices. Rare earth nanomaterials (RENMs) generally exhibit unique magnetic, catalytic, and optic properties and have been widely used in various fields. For example, nano-CeO_2 is the main active ingredient of three-way catalyst, which is used in automobile exhaust treatment. It can change CO to CO_2 by generation-elimination of surface oxygen vacancies and the redox reactions between oxygen-containing substances (Gorte 2010). The synthesized CeO_2 nano-cube can catalyze the oxidation of toluene to benzaldehyde (Lv et al. 2010), while the CeO_2 nano-plate can catalyze the reduction reaction of nitromethane with high selectivity at room temperature (Zhang et al. 2012c). RE-doped upconversion nanomaterials (UCNMs) can convert low-frequency stimulated luminescence into high-frequency emission light by multiphoton mechanism. The deposit of nano-La_2O_3 on the nickel surface can improve the high-temperature

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_4

resistance and oxidation resistance of nickel material and prevent the short-circuit diffusion of nickel ion, which can improve the growth mechanism and mechanical properties of oxide layer and prolong the service life at high temperature (Sokolov et al. 2013). With their increasing applications, RENMs could be inevitably released into the environment, raising more concerns about their potential hazard to environment as well as human beings' health (Peng et al. 2012; Ma et al. 2013). As part of the first trophic level in the food chain, plants may serve as the primary target and a pathway for the transporting of RENMs. Therefore, the interactions between RENMs and plants are of particular concern. The interactions between plants and ENMs can shed light on the environmental consequences of these materials. RENMs can exert positive or negative physiological effects on plants depending on physicochemical properties of RENMs, dosages, plant species, and other conditions. For example, nano-La_2O_3 is highly toxic to almost all plants, but nano-CeO_2 is only toxic to lettuce (Ma et al. 2015a). At low concentrations, some RENMS even stimulate the growth of plants (Yin et al. 2015). These complex effects of RENMs on plants have attracted the attention of scientists. However, most of the available studies have focused mainly on toxic symptoms of plants, and relatively few studies examined the mechanisms of phytotoxicity, uptake, translocation, and biotransformation of RENMs in plants. Therefore, it is necessary to have a systematic review of the published researches in this field. Till now, the phytotoxicity of RENMs based on Y, La, Ce, and Yb have been studied. Accordingly, this review will emphasize on the above materials.

4.2 Toxicological Effects of RENMs on Plants

Phytotoxicity assays are generally performed during germination or seedling growth stage. At the former stage, germination rate and root elongation are usually measured; and during the latter stage, length and dry weight of root/shoot are frequently used to assess exposure effects. In addition, enzyme activities, gene expression, and uptake of nutrient elements have also been examined to reveal the effects of RENMs on plants (Ghosh et al. 2010; López-Moreno et al. 2010; Khodakovskaya et al. 2011, 2012; Wang et al. 2011).

4.2.1 Effects of RENMs on Root Elongation and Biomass

Root elongation and biomass are direct and visible signs that reflect the phytotoxicity of RENMs to plants. RENMs containing different REEs have different effects on plant growth. Most reports showed that La-, Y-, Gd-, and Yb-based RENMs can reduce the biomass of plants. For instance, Ma et al. (2010) investigated the phytotoxicity of nano-CeO_2, nano-La_2O_3, nano-Gd_2O_3, and nano-Yb_2O_3 on seven higher plant species (radish, rape, tomato, lettuce, wheat, cabbage, and cucumber) and found that RENMs did not affect the germination of these plant species. At 2000 mg L^{-1}, nano-La_2O_3, -Gd_2O_3, and -Yb_2O_3 severely inhibited root elongation

in all tested species, while nano-CeO_2 showed no effects at the same concentration on the majority of the test species. Lettuce was the most sensitive species, with significant reduction in root length following exposure to all of the four RENMs. Further, Zhang et al. (2015a) found that nano-CeO_2 had species-specific phytotoxicity on *Lactuca* plants. Cui et al. (2014) reported that the toxicity of nano-CeO_2 to asparagus lettuce in agar media was higher than in aqueous suspensions at the same exposure concentration. Recent studies have shown that the phytotoxicity of nano-CeO_2 to *Lactuca* plants was influenced by the phosphates in the culture media (Wang et al. 2017; Zhang et al. 2017a). The same kind of RENM may have different effects on different plants species. López-Moreno et al. reported that the root growth in maize and cucumber seedlings was significantly promoted by nano-CeO_2, whereas the same treatments resulted in a negative effect on root development in alfalfa and tomato (Lópezmoreno et al. 2010). In addition, the phytotoxicity of RENMs was related to the exposure concentrations. Yin et al. (2015) demonstrate the $NaYF_4$ NPs could promote the root and stem elongation of soybean plant at low concentration (<10 μg mL^{-1}), while inhibit the growth when the concentration exceeded 50 μg mL^{-1}.

It is very important to demonstrate the different toxicity between the nanomaterials and their corresponding bulk or ion counterparts. Zhang et al. (2012a) compared the phytotoxicity of nano-Yb_2O_3, bulk Yb_2O_3, and dissolved $YbCl_3{\cdot}6H_2O$ to hydroponic cucumber seedlings. Both bulk and nano-Yb_2O_3 inhibited growth, with the nano-Yb_2O_3 particles having the more severe effect and producing higher Yb concentration in the plants. $YbCl_3{\cdot}6H_2O$ was the most harmful. They speculated that the observed phytotoxicity was resulted from the dissolved RE^{3+} ions. More Yb was found in the aerial parts of plants exposed to nano-Yb_2O_3 than bulk ones and thus higher toxicity. Zhang et al. (2015b) evaluated how different forms of cerium (bulk cerium oxide, cerium oxide nanoparticles, and the cerium ion) affected the growth of radish (*Raphanus sativus* L.). They found that the Ce^{3+} ions had a negative effect on radish growth at 10 mg $CeCl_3{\cdot}L^{-1}$, whereas bulk-CeO_2 enhanced plant biomass at the same concentration. Treatment with 10 mg L^{-1} nano-CeO_2 had no significant effect on radish growth.

4.2.2 Enzyme Activity

Enzyme is a macromolecule substance with biological catalysis function and participates in most physiological processes of plants. Studying the activity of enzymes in plants is important for understanding the phytotoxicity of RENMs. It has been reported that RENMs can modify the antioxidant enzymatic system of plants, although no visible signs of toxicity on seedling growth was observed (Du et al. 2015). Some studies have shown that the antioxidant defense system in plants is activated by nano-CeO_2. For example, Rico et al. (2013) described the differences in antioxidant defense mechanisms between two rice seedlings (cultivar Cheniere and Neptune) following CeO_2 NP exposure. The toxic effects of nano-CeO_2 on plants were thought to be due to the production of reactive oxygen species

(ROS) caused by the redox cycle between Ce^{3+} and Ce^{4+} on the NP surface. On the other hand, scavenge ROS can protect plants from oxidative damage (Hong et al. 2014). Therefore, it is necessary to analyze the activity of ROS scavengers, such as dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), etc. Ma et al. (2016) indicated that activities of superoxide dismutase (SOD), CAT, APX, and POD of plant were significantly elevated upon exposure to CeO_2 NPs. Hong et al. (2014) showed that even foliar applied $nCeO_2$ can modify the antioxidant enzymatic activity including CAT, APX, and DHAR in both shoot and root. These results indicate that oxidative stress may be involved in the phytotoxicity of $nCeO_2$.

Generally, chemical composition of RENMs determined the effect on enzyme activity of plants. Some reports suggested that the change of enzyme activity resulted from the release of metal ions from nanomaterials. Luca Pagano et al. (2016) compared effects among nano-CuO, nano-La_2O_3, and nano-CeO_2 on several crop plants and found that the toxicity of nano-La_2O_3 was greater than that of nano-CeO_2. This was likely caused by the high dissolution level of nano-La_2O_3 and La^{3+} ions release. The low dissolution rate of CeO_2 NPs correlates with the decreased level of translocation to the leaves and with the low impact on plant growth and enzyme activity. Furthermore, enzyme activity of plants may depend on the surface modification of RENMs. Barrios et al. (2015) found the citric acid-coated nano-CeO_2 increased catalase (CAT) activity of tomato plants at 500 mg kg^{-1} compared to uncoated nano-CeO_2 and bulk-CeO_2. On the other hand, both coated and uncoated NPs showed similar reducing effects on APOX, except at 62.5 mg kg^{-1}, at which coated NPs did not affect APOX. Exposure concentration of RENMs can also influence the enzyme activity of plants. Numerous studies have demonstrated dose-dependent phytotoxic effects of RENMs in plants. Zhang et al. (2017a) grew romaine lettuce exposed to nano-CeO_2. They found that nano-CeO_2 can alter antioxidant enzymatic activities and malondialdehyde levels in the plants at concentrations higher than 100 mg kg^{-1}. Rico et al. (2013) demonstrated that glutathione reductase (GR) activity was increased in the roots of rice cultivar (Neptune) upon exposure to 62.5 and 500 mg L^{-1} of CeO_2 NPs, whereas the decreases in the GR activity was evident in both rice roots and shoots at the other two concentrations of CeO_2 NPs.

4.2.3 Nutrient Content

Nutrient elements, such as Ca, Fe, P, Mg, and K, relate to the growth and metabolism of plants. Whether RENMs can affect nutrient uptake by plants is one of the keys to study the phytotoxicity of RENMs. Measuring the micro- and macroelements in plants is the main method to analyze the effect of RENMs in the present research.

Ma et al. (2016) found that CeO_2 and In_2O_3 NPs can disrupt the uptake of elemental nutrients, with the significant elevation of Ca and decrease of P and Fe accumulation in *Arabidopsis* root tissues. Then the author analyzed the reasons for this phenomenon. First, the reason for the elevation of Ca in *Arabidopsis* roots might be that NP-induced ROS trigger Ca^{2+} ion channels and thus increase the

concentration of Ca. Second, the decrease of P uptake in 1000 mg L^{-1} CeO_2-NP-treated *Arabidopsis* root indicated that CeO_2 NPs could bind P and lower nutrient bioavailability. Third, CeO_2 NPs can cause more decreases in Fe content than In_2O_3 NPs, the most likely reason for which was that upregulation of iron-regulated transporter (IRT) in the In_2O_3 NP treatment was to compensate for Fe deficiency. However, these conclusions did not agree with those drawn by Barrios et al. (2015), who found neither bare nano-CeO_2 nor citric acid-coated nano-CeO_2 affected the homeostasis of nutrient elements in the roots, stems, and leaves. In contrast, CeAc at 62.5 and 125 mg kg^{-1} increased B (81%) and Fe (174%) in roots, while at 250 and 500 mg kg^{-1}, increased Ca in the stems (84% and 86%, respectively). On the other hand, bulk-CeO_2 at 62.5 mg kg^{-1} increased Zn (152%) but reduced P (80%) in stems. The reason may be that the surface structure of the nano-CeO_2 used in the experiment is different. Ge et al. (2014) demonstrated that nano-CeO_2 at high concentrations shut down the nitrogen fixation system in soybean, which posed risks to the agriculture of leguminous crops. But at low concentrations, it had no significant effect on soybean. Hong et al. (2015) studied the influence of foliar applied NPs on fruit quality and showed that nano-CeO_2 (50 mg L^{-1}) and bulk-CeO_2 (200 mg L^{-1}) could significantly reduce fruit firmness. In addition, nano-CeO_2 and bulk-CeO_2 (200 mg L^{-1}) reduced fruit Zn by 25%, which indicated that differences in particle size are less significant through the foliar exposure than root-based exposure.

4.2.4 Gene Expression

Genes store all of the basic structures and information of living beings and are involved in important physiological processes such as cell division and protein synthesis. Phytotoxicity of RENMs might be related to their genotoxicity and affect gene expression in plants. Some researchers have studied how RENMs affects plants at the genetic level. Lopez-Moreno et al. demonstrated the genotoxic effects of nano-CeO_2 to soybean plants, with the appearance of new bands in the random amplified polymorphic DNA (RAPD) assay, which can potentially detect a broad range of DNA damage and mutations (López-Moreno et al. 2010). Mattiello et al. (2015) investigated the genotoxicity of nano-CeO_2 on *Hordeum vulgare* L. seedlings by RAPD and mitotic index on root tip cells. The RAPD modified patterns at high concentrations of nano-CeO_2 (1000–2000 mg L^{-1}) indicated genotoxic effect, which could directly influence the cell cycle. This was further confirmed by the reduced mitotic index recorded in the samples treated with nano-CeO_2 (2000 mg L^{-1}), which clearly demonstrated the negative effect of high concentrations of nano-CeO_2 on the cell cycle. In addition, they inferred that signals of genotoxicity (RAPD banding patterns) and mitotic index in root cells (oxidative stress and chromatin modifications) resulted in a shortage of root elongation. Pagano et al. (2016) studied the different gene expression in zucchini and tomatoes treated with the nano-CeO_2, nano-La_2O_3, and nano-CuO. Nano-CeO_2 caused only small changes in gene expression in both plant species, which well agreed with the physiological responses that

nano-CeO_2 was significantly less toxic than nano-La_2O_3 and nano-CuO. Ma et al. (2016) evaluated the time-dependent transcription levels of three iron-regulating genes in *A. thaliana* shoots and roots upon exposure to nano-CeO_2 and nano-In_2O_3 for 96 and 120 h. Both NPs induced slight but not significant increases in ferritin (FER) expression in *A. thaliana* shoots upon exposure at 96 h, but the significant decreases in both treated *A. thaliana* shoots were evident at 120 h. However, nano-CeO_2 (1000 mg L^{-1}) did not affect the ferric chelate reductase (FRO) and iron-regulated transporter (IRT) regulation at 96 and 120 h.

The phytotoxic mechanisms of NMs are not well deciphered. The dissolution of RENMs in the biological environment may require particular attention. Some reports suggest that the phytotoxicity of RENMs results from the release of metal ions. Ma and Zhang studied the phytotoxicity of a series of RENMs on several species, and they thought that the observed phytotoxicity of nano-La_2O_3 and nano-Yb_2O_3 was mainly attributed to the released ions (Ma et al. 2011; Zhang et al. 2012a). In another study, Ma et al. (2015b) compared the different effects of nano-CeO_2 and nano-La_2O_3 on cucumber plants and found that the toxicity of the latter one was significantly higher than that of the former one. In the aerial parts, all of La was combined with phosphate or carboxylic groups implying nano-La_2O_3 acted as its ionic form, while CeO_2 displayed the behavior as a mixture of particles and ions. They concluded that the higher dissolution rate of nano-La_2O_3 than that of nano-CeO_2 might be the reason for their significant difference in phytotoxicity and transporting behaviors in cucumbers.

4.3 Uptake and Translocation

This section describes the uptake translation and accumulation of RENMs in plants, which are very important for understanding the interactions between RENMs and plants.

4.3.1 Factors that Influence the Uptake and Translation of RENMs

4.3.1.1 Size and Shape of RENMs

RENMs may adhere to the roots or leaf of the plants, and then they will affect the growth of plants. Because of the presence of cell walls in plants, the incorporation of NPs into plant cells requires that they pass through the cell wall, which has pore sizes of maximum 5 nm in most species (Miralles et al. 2012). Although the exact uptake mechanisms are not fully understood, it has been extensively proved that ENMs could pass through the barriers, enter into the xylem vessel, and finally be transported to the aerial parts via vascular bundles. Zhang et al. (2011) tracked the spread of two sizes of ceria NPs (7 and 25 nm) in cucumber plants using a radiotracer method and discovered radioactive ^{141}Ce was throughout the plants. Ce was found primarily around the edges of younger leaves and spread throughout the whole ones in old leaves (Fig. 4.1). Zhao et al. (2012) investigated the uptake of bare and coated

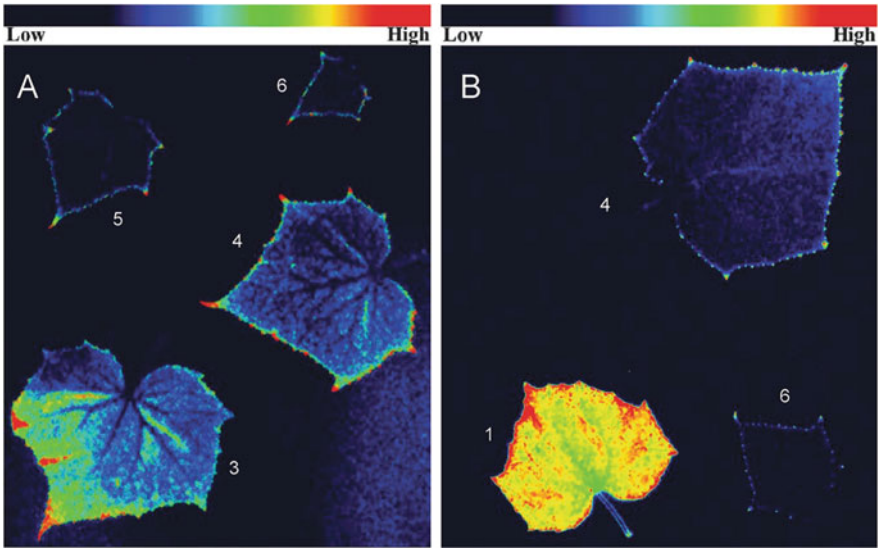

Fig. 4.1 Autoradiographs of ceria NPs in cucumber leaves: (**a**) 7 nm ceria, 3rd, 4th, 5th, and 6th leaves; (**b**) 25 nm ceria, 1st, 4th, and 6th leaves

CeO_2 NPs by corn plants grown in soil and found that soil organic matter and surface coating played important roles in the mobility and bioavailability of CeO_2 NPs. FITC-stained CeO_2 NPs were observed in cell walls of cortex and vascular cylinder, demonstrating that CeO_2 NPs can be taken up by plants. Franziska Schwabe et al. (2014) revealed that Ce was not only taken up in the form of NPs, but simultaneously to a significant degree also as dissolved Ce(III) ions, which then re-precipitated in the form of CeO_2 NPs inside the leaves.

It has been proved that the phytotoxicity of RENMs might be mainly due to the dissolution and release of RE^{3+} ions. The degree of dissolution varied with culturing conditions and particle sizes, with smaller particles being more easily to release RE^{3+} ions than the larger ones. Some researchers think that the dissolution of nano-CeO_2 depends on the ratio between Ce^{3+} and Ce^{4+} on the NP-surface layer. As NP size decreases, more and more oxygen vacancies occur in the ceria lattice, resulting in local reduction of Ce^{4+}. Franziska Schwabe et al. (2015) found that no Ce was translocated from roots to shoots when the applied CeO_2 NPs were bigger than 20 nm. The authors also suggested that the contribution of dissolved Ce uptake was particularly large for particles smaller than 10 nm due to their higher dissolution rate.

For foliar exposure, RENMs might be internalized in plants through the leaf stoma, entering into the vascular system of leaves, and then be transported to other parts through the phloem. At present, there are few reports about leaf absorption of RENMs. Birbaum et al. (2010) treated maize seedlings with 37 nm CeO_2 NPs by foliar exposure and found that maize did not absorb and transport nano-CeO_2 either as aerosol or as suspension. However, Hong et al. (2014) found that foliage applied

atmospheric CeO_2 NPs could be taken up and distributed within cucumber plant tissues.

The shape of RENMs is another critical factor that affects their uptake and translocation in plants. Zhang et al. (2017b) compared the translocation of octahedral, cubic, rod, and irregularly shaped nano-CeO_2 NPs in hydroponic cucumber plants. They found the Ce content in roots of each treatment group was close to each other, while the largest amount (153 mg kg^{-1}) of Ce accumulated in rod-like nano-CeO_2 treatment, which might be due to that rod nano-CeO_2 transformed faster and more than others.

4.3.1.2 Surface Charge and Modification of RENMs

Surface charge is an important property that can influence the uptake and transport of RENMs in the environment and biological systems. It is well established that plant cell walls and root surface are negatively charged because of the abundance of polysaccharides containing galacturonic acid residues. Therefore, due to electrostatic interactions, the positive charge particles were adhered significantly more to the roots than the negatively charged or neutral particles. Spielman-Sun et al. (2017) evaluated the influence of surface charge on nano-CeO_2 uptake by wheat seedling. Nano-CeO_2 was functionalized with positively charged, negatively charged, or neutral dextran coating. After 34 h, plants exposed to $CeO_2(+)$ NPs had higher Ce root concentrations than those exposed to $CeO_2(-)$ and $CeO_2(0)$ NPs. Further, surface charge could also influence the translocation of RENMs in plants. Although the leaves contained less than 1% of the total plant-associated Ce, $CeO_2(0)$ and $CeO_2(-)$ NP-exposed plants accumulated twice as much Ce in the leaves than did the $CeO_2(+)$. Ce was found mostly in the leaf veins of the $CeO_2(-)$ NP-exposed plant, while as clusters in the nonvascular leaf tissue of the $CeO_2(0)$ NP-exposed plants.

The complexation of RENMs surface atoms with organic acids (e.g., citrate) could change their physiological and biochemical properties. Zhao et al. (2012) investigated the effects of bare and alginate-coated CeO_2 NPs on corn plants grown in unenriched or organic soil and reported that surface coating and soil organic matter could promote the uptake of Ce in treated plants.

4.3.1.3 Plant Species

The uptake of RENMs by plants has been reported to vary with the plant species, with a higher translocation rate in dicotyledonous than in monocotyledonous plants. Such different uptake could be due to different vasculatures and structural features between them, that is, a tap root system in dicots vs. a fibrous root system in monocots. There is another possibility that the binding capacity of cations in dicots is greater than monocots (Guigues et al. 2014). By using radioactive isotopic tracer, Zhang et al. (2011) demonstrated the translocation of uncoated CeO_2 NPs from roots to shoots in hydroponically grown cucumber (dicotyledon) and detected that Ce was located in the nonvascular leaf tissue. In contrast, Spielman-Sun et al. (2017) found Ce was mostly in the leaf veins of wheat (monocotyledon) exposed to $CeO_2(-)$ NP, while Ce was as clusters in the nonvascular leaf tissue of wheat exposed to

CeO_2(0) NP. In addition, the total Ce concentrations in wheat roots were lower than those in the cucumber roots that were evaluated by Zhang et al.

4.3.1.4 Surrounding Medium

Plant roots can alter the chemical properties of the rhizosphere by taking up or releasing a wide range of exudates, such as organic acids, amino acids, and sugar, which are likely to change ionic concentrations, redox potential, and pH values. These exudates on the root surface could facilitate the adsorption and transport of the NMs in plants. Schwabe et al. (2015) reported that the presence of plant roots in nutrient solution led to a substantial increase in the dissolution of CeO_2 NP compared to plant-free medium. Experiments with Zr/CeO_x-NP revealed that Ce was not only taken up in the form of NPs, but simultaneously to a significant degree also as dissolved Ce(III) ions. This study highlighted that plant roots have a significant impact on the dissolution of CeO_2 NPs.

In addition, NMs tend to be adsorbed on soil matrix and aggregate in the natural environment which will modify their mobility and bioavailability. Using the ISO-standardized RHIZO test, Layet et al. (2017) treated tomato and fescue with nano-CeO_2, which was cultured in two soils with contrasted properties: a sandy soil poor in organic matter and a clay soil rich in organic matter. They found that the clay fraction reduced Ce uptake by enhancing the retention of CeO_2 NPs, whereas the organic matter content enhanced Ce uptake. Moreover, the organic citrate significantly enhanced the phyto-availability of the Ce by forming smaller aggregates and thus facilitating the transport of nanoparticles to the roots in the soil poor in organic matter.

4.4 Transformation

RENMs may undergo transformations under the environmental or biological conditions, which will modify their toxicity and ultimate fate. Transformation processes in plants are influenced by the properties of RENMs, exposure time, target plants, etc. A series of in-depth studies on the biotransformation of RENMs in plants have been performed, and the critical role of root exudates in the transformation process was highlighted. Ma et al. (2011) treated cucumber plants with nano-La_2O_3 for 5 days and found a large amount of needlelike $LaPO_4$ clusters in intercellular regions as well as in vacuole and cytoplasm of roots, indicating a significant biotransformation of La_2O_3 in plants. The in vitro experiment suggests that organic acids play a critical role in the transformation process by promoting the dissolution of NPs. Zhang et al. (2012a) reported that nanoparticulate Yb_2O_3 was more toxic than bulk Yb_2O_3 to cucumber plants. The authors found that organic acids in the rhizosphere greatly promoted the dissolution of nano-Yb_2O_3 and the Yb^{3+} concentrations in the rhizosphere solution were much higher than that in the exposure solution, which might be the reason for its higher toxicity. It was worth noticing that the transformation product was not only found in intercellular regions but also in vacuole and cytoplasm under the treatment of nano-Yb_2O_3 (Fig. 4.2), indicating that NPs probably crossed the cell wall, entered into the cytoplasm and vacuole, and were

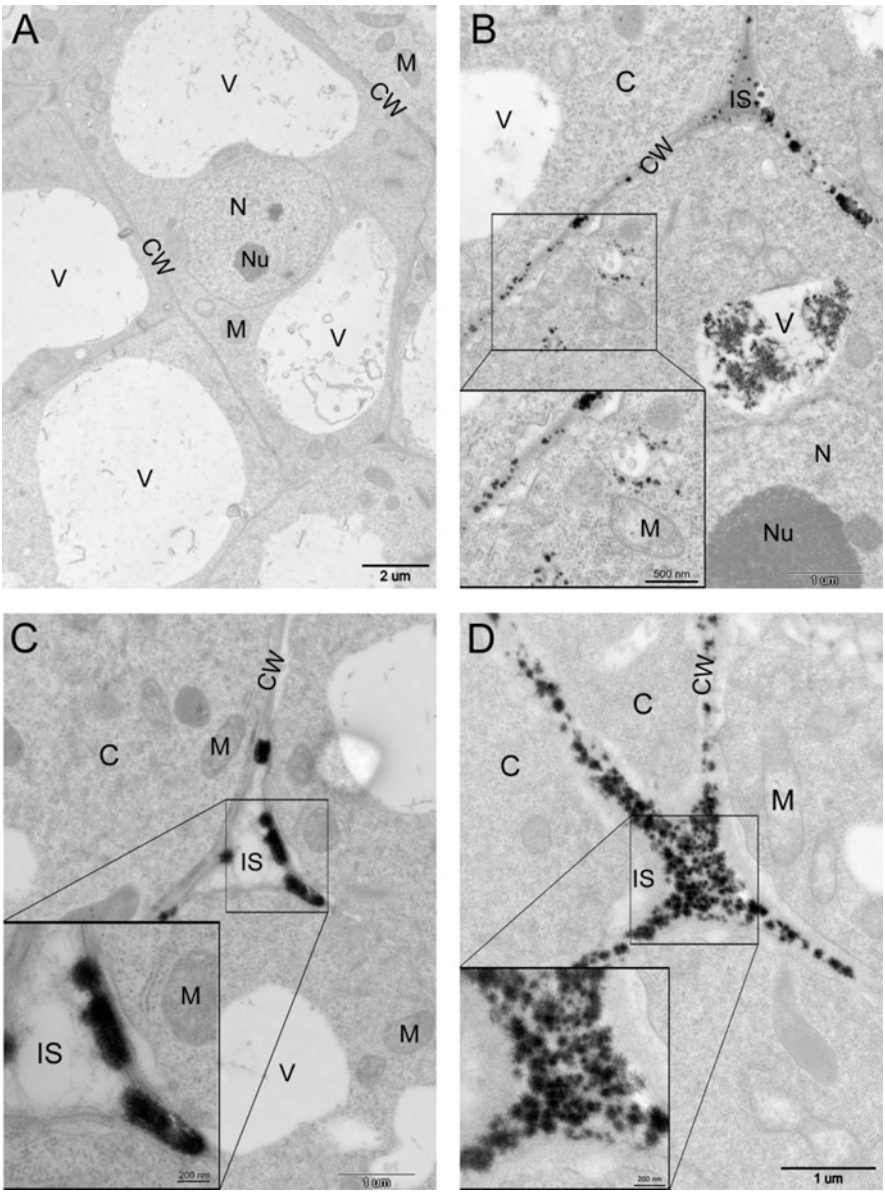

Fig. 4.2 TEM images of cross sections of cucumber root cells under the control (**a**), 2000 mg L^{-1} nm-Yb_2O_3 (**b**), 2000 mg L^{-1} bulk Yb_2O_3 (**c**), and 200 mg L^{-1} $YbCl_3$ (**d**). The insets are higher magnification of the rectangle areas. Cells walls (CW), nucleus (N) with nucleolus (Nu), intercellular space (IS), mitochondria (M), and vacuole (V)

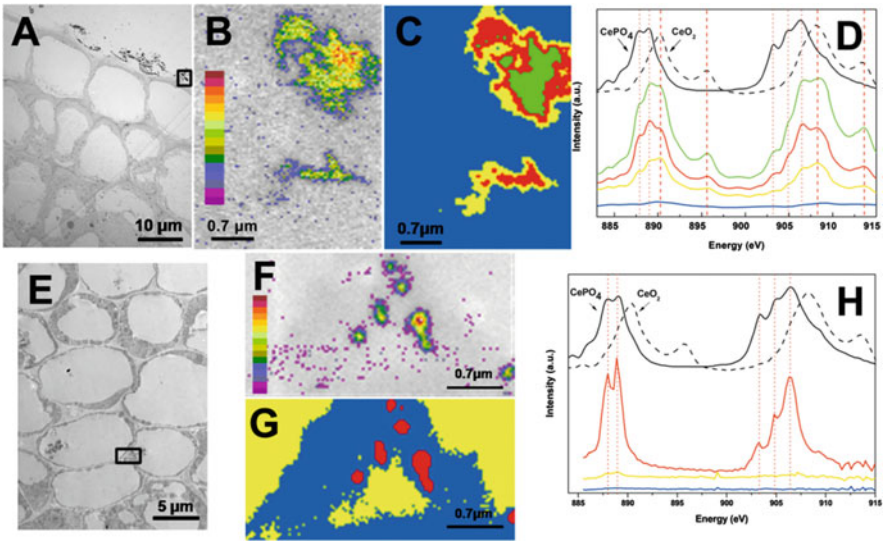

Fig. 4.3 (**a** and **e**) TEM images of root cells; (**b** and **f**) Ce maps of rectangle area in panels (**a**) and (**e**) obtained by a ratio of 886 and 888 eV images. Color bar values are estimated from Ce absorption coefficients and X-ray absorption measurements (in g cm^{-2}). The calculated surface densities are, respectively, between 1.1×10^{-5} to 6.4×10^{-5} g cm^{-2} and 2.4×10^{-6} to 2.8×10^{-5} g cm^{-2}; (**c** and **g**) color-coded maps of Ce components in panels (**b**) and (**f**) derived from an STXM Ce M edge stack analysis. The order of Ce contents is as follows: green > red > yellow; blue color represents the non-Ce regions; panels (**d**) and (**h**) are, respectively, the XAFS spectra extracted from the image sequences of panels (**c**) and (**g**). The black line spectra above belong to the standard compounds, and the colored spectra below belong to the root samples. The vertical red dotted lines indicate the characteristic peaks of $CePO_4$ and the dash lines indicate the characteristic peaks of CeO_2 NPs

transformed to $YbPO_4$. Yin et al. (2015) demonstrated that $NaYF_4$ upconversion nanoparticles were transferred to the stems and leaves of soybean via vascular bundles in the root-guided growth stage. They further indicated that a small amount of $NaYF_4$ was dissolved/digested and transformed into Y-phosphate clusters in the roots. Morphologies of the NPs obviously changed in the vessels, which are the main long-distance transport channel to all parts of the plants.

CeO_2 NPs were generally considered highly stable in the environmental and biological surroundings at early stage and had been used as model NMs in toxicology studies compared with other easily dissolved NMs, such as ZnO, Ag, etc. However, Zhang et al. found that CeO_2 NPs could be reduced and transformed to Ce(III) species in cucumber plants, with a large amount of needlelike clusters being observed in intercellular regions and epidermis in roots (Zhang et al. 2012b). TEM combined with EDS analyses suggested that these clusters contained Ce and P with an atom ratio of about 1:1, indicating that these clusters might be $CePO_4$, which was further evidenced by STXM and XANES analyses (Fig. 4.3). Bulk XANES studies suggested that Ce mostly presented as $CePO_4$ and CeO_2 in roots but as Ce

carboxylates and CeO_2 in stems and leaves. Combining these results and a further simulation study, the authors elaborated the transformation and translocation mechanism of CeO_2 NPs in cucumber plants. Nano-CeO_2 was reduced to Ce^{3+} with the assistance of the natural reducing substances and organic acids in the root exudates. One part of the released Ce^{3+} ions was immobilized by the phosphates which are abundant in nutrient solution and plant tissues, and the other part was translocated from the roots to shoots or immobilized by carboxyl compounds in xylem during the translocation process. A recent study that was carried out by Ma et al. (2015b) suggested that the transformation of nano-CeO_2 in plants exclusively occurred at the root surface, and interactions between the NPs and root exudates at the nano-bio interface were required for the transformation. Besides in hydroponic plants, CeO_2 NPs could be transformed in the plants grown in soil. Using micro X-Ray fluorescence (μ-XRF) and XANES spectroscopy, Hernandez-Viezcas et al. (2013) determined the distribution and chemical species of Ce in the soybean planted in soil amended with 1000 mg L^{-1} CeO_2 NPs and found that most of the Ce(IV) remained untransformed in the plant, while there was a small percentage of Ce(III) in the pod, indicating that RENMs could be transported into fruits and might result in trophic transfer through the food chain.

The transformation of RENMs in plants is one of the most effective methods to clarify the phytotoxicity of RENMs. Zhang et al. revealed that CeO_2 NPs have species-specific toxicity to *Lactuca* plants, the reason for which was that transformation of CeO_2 NPs occurred in roots and *Lactuca* plants were highly sensitive to the small-amount release of Ce^{3+} ions. Further studies carried out by the same research group demonstrated that the proportion of transformation of CeO_2 NPs in *Lactuca* plants and thus the different toxicity depended on the culture media (Cui et al. 2014; Zhang et al. 2017a). Additionally, the transformation and subsequent phytotoxicity of CeO_2 NPs were also significantly influenced by phosphates, which were widely present in the environment (Wang et al. 2017). Hence, we can see that understanding the transformation of ENMs is of critical importance when assessing their toxicity.

4.5 Conclusions and Perspectives

Phytotoxicity of RENMs is influenced by their physicochemical properties, exposure concentrations, surrounding medium, plant species, etc. These factors also determine the uptake, translocation, and transformation of RENMs in plants, which further affect the important physiological parameters of plants, such as enzyme activity, gene expression, biomass, root elongation, nutrients, and so on. These are the root causes of phytotoxicity on plants. Although many advances

have been made to explore the interactions between RENMs and plants, there are still many problems:

1. So far, most of the researches on RENMs are focused on Ce, La, and Y, and there are few studies on nanoparticles of other rare earth elements. The phytotoxicity of RENMs discussed in this paper is too limited.
2. In reality, the environment in which plants grow is extremely complex. But most of the experiments currently are conducted by hydroponics.
3. Most of the experiments were conducted using root exposure; there are less studies on the upper part of the ground exposed to RENMs.
4. At present, the mechanisms of uptake, transport, and transformation of RENMs in plants and the reason of some physiological phenomenon are still unknown and need further study.

Acknowledgments This work was financially supported by National Natural Science Foundation of China (Grant No. 11575208, 11375009, 11405183, 11675190, 11275215, and 11275218) and the Ministry of Science and Technology of China (Grant No. 2013CB932703).

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Interaction of Nano-sized Nutrients with Plant Biomass: A Review

5

Gea Guerriero and Giampiero Cai

5.1 The Impact of Nutrient Deficiency on Plant Biomass: General Aspects

Plant lignocellulosic biomass is an important natural resource providing (macro)molecules of industrial relevance (e.g. cell wall polysaccharides, secondary metabolites, sugars), as well as wood (Guerriero et al. 2014, 2016b). The synthesis of plant biomass depends, among other factors (e.g. light availability, photoperiod, temperature), on soil nutrient availability (Chatzistathis and Therios 2013), and the effects on plant cell wall synthesis can be quite strong in the case of both macro- and micronutrient deficiencies.

The impact of nanotechnology on agriculture has been a real revolution, an inspiration for innovative approaches, but also a source of controversies (*vide infra*). The exploitation of the wall pores of plant cells is at the base of nutrient nano-delivery (Liu and Lal 2015); despite the great potential of this innovation in agriculture, a thorough understanding of the relationship nanofertilizers/plants is still lacking.

In this section we will provide evidence for the impact of nutrient deficiency on plant cell walls (and therefore biomass production); more specifically, we will discuss the role of both macro- (N, P, Ca, Mg) and micronutrients (B, Cu, Zn, Fe). Additionally, we will include, in our survey, the effects of the metal Ti, the rare-earth metal Ce and C-based nanoparticles, which, despite not considered among the list of nutrients, are currently studied in relation to plant growth because of either their reported beneficial effect on plants (Lyu et al. 2017) or their release in the

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_5

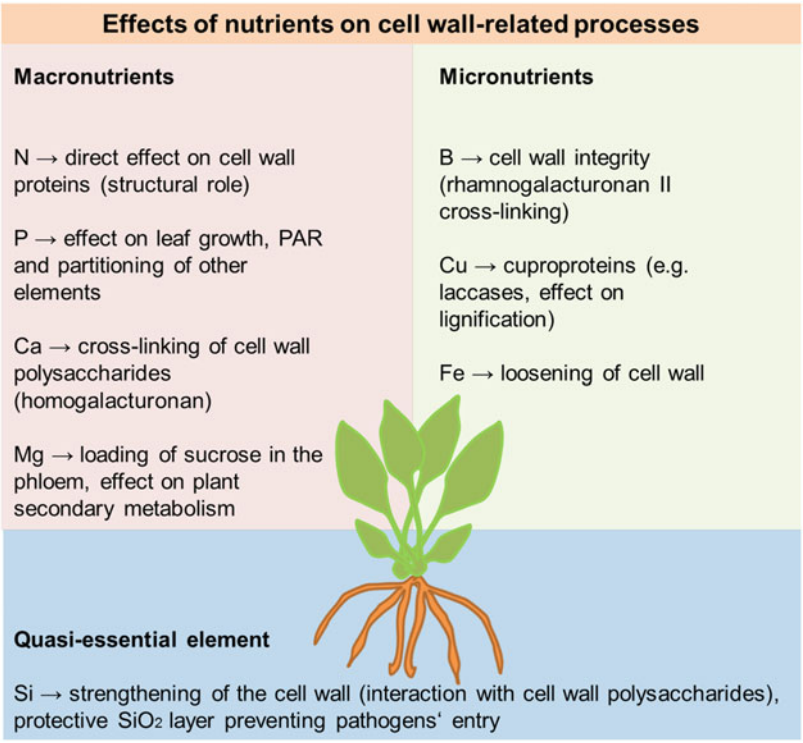

Fig. 5.1 Schematic summary of the effects that macro-/micronutrients and quasi-essential elements exert on cell wall-related processes

environment due to the nanotechnology industry (Maurer-Jones et al. 2013; Dahle and Arai 2015). We will present the major data available concerning the cell wall/plant biomass impact of different nutrient deficiencies, and for each nutrient described, we will provide, when possible, a survey of the respective nano-sized utilization (Fig. 5.1).

5.2 Macronutrients and Plant Biomass

The impact of nutrients on plant biomass is well evident in the case of essential elements (macronutrients), given their indispensable role in plant growth and development. Poplars fertilized with N (in the form of NH_4NO_3 5 mM) grew 1.4 times faster, displayed 20% thicker stems, and increased ca. 2 times the stem biomass (Euring et al. 2014). One of the potential routes through which N deficiency affects plant cell wall biosynthesis is via proline (Pro): indeed this amino acid is found in cell wall-associated proteins, e.g. hydroxyproline-rich glycoproteins, HGRPs, which comprise extensins and arabinogalactan proteins, AGPs (Kavi Kishor et al. 2015),

and has therefore an important structural role. In this respect it should be noted that in French bean plants, N deficiency triggered decreased Pro accumulation in both roots and leaves (Sánchez et al. 2002). This finding thereby confirms the existence of a relationship between N supplementation and Pro accumulation.

Urea is an excellent source of N; however its use as fertilizer is compromised by its rapid decomposition in the soil resulting in ammonia volatilization. To minimize this problem, a urea-based nano-fertilizer was recently devised by incorporation into a matrix of hydroxyapatite (HA) nanoparticles to reduce its solubility (Kottegoda et al. 2017). HA is a source of P and the interaction with urea is via amine and carbonyl group. The nano-hybrid (urea/HA ratio is 6:1) shows promising results, namely, a slow release of N (up to 1 week). Another recent study studied the properties of a nanocomposite composed of a matrix of extruded thermoplastic starch-urea (TPSUr) in which HA particles were dispersed at different ratios (50 and 20%) (Giroto et al. 2017): a controlled release of urea, together with a release of P in citric acid solution, was obtained. Moreover, a lower ammonia volatilization was observed, and the nanocomposite contributed to a reduced P immobilization (resulting in higher P availability) after 4 weeks of incubation in the soil (Giroto et al. 2017).

Similarly to N, P supplementation also has an important impact on plant growth and, ultimately, biomass production. In maize plants it was demonstrated that P deficiency affected biomass production principally via the negative effect exerted on leaf growth (reduced leaf area index) and the consequent lower photosynthetically active radiation (PAR) absorption (Plénet et al. 2000). One of the commonly reported symptoms of P deficiency is indeed a lower shoot/root biomass (Wissuwa et al. 2005). In addition to these effects, P deficiency has consequences on the accumulation and partitioning of other important plant nutrients: rice grown without P showed lower above-ground accumulation of N, K, Mg, Fe, Mn, Ca and Zn (Rose et al. 2016). Synthetic apatite (HA) nanoparticles have been used as an alternative to conventional P fertilizers (which can cause surface water eutrophication), and their application has been studied on *Glycine max* (Liu and Lal 2014). The plants treated with the HA nanoparticles showed increased growth rate (ca. 33% higher) and seed yield, as compared to plants supplemented with regular P fertilizer; the biomass increase was 41% below-ground and 18% above-ground (Liu and Lal 2014).

Ca is a key element involved in signal transduction, as well as pathogen resistance, fruit ripening via its involvement in cell wall polysaccharide cross-linking (Hocking et al. 2016). Indeed, Ca is involved in the cross-linking of homogalacturonan, with the "egg-box model" (Morris et al. 1982), via bridges with the carboxyl groups of galacturonic acid. A study on groundnut has shown that Ca oxide nanoparticles were translocated via the phloem after foliar application and corrected the symptoms of Ca deficiency: indeed the Ca content in roots, shoots and leaves increased after supplying the nanoparticles (Deepa et al. 2015). This indicates that Ca nanoparticles can eventually be used as nano-fertilizer.

Mg deficiency is a serious problem in agriculture: the phloem loading of sucrose is inhibited with consequent accumulation of C in source tissues (Guo et al. 2016); this leads to a feedback inhibition of photosynthesis, with clear effects on biomass

production. Mg deficiency has also effects on plant secondary metabolism: for example, putrescine and phenolic compounds increase (Guo et al. 2016). It would be interesting to study whether, under Mg deficiency, the lignin content increases in plant biomass.

5.3 Micronutrients and Plant Biomass

The impact of nutrient availability on plant biomass is evident also in the case of micronutrient: for example, the effects caused by B deficiency are particularly evident in dicots, where it plays a structural role by cross-linking the pectin in "type I" cell walls (Yokoyama and Nishitani 2004). Indeed, the morphology of cells growing in the absence of B is different: B-deprived *Chenopodium album* cells were more enlarged and detached, as compared to B-supplemented cells, and, importantly, they showed an increased cell wall pore size (Fleischer et al. 1998). The addition of B to the deprived cells triggered a reduction in the wall pore size within 10 min and the formation of borate cross-linked rhamnogalacturonan II dimer (absent in the B-depleted cells) (Fleischer et al. 1999). The impact of B deficiency on the integrity of the cell wall is therefore clear: a modified cell wall, where the pectin fraction shows an altered composition, results in a weakened cell wall with consequences on the physiology under normal and, even more dramatically, under stress conditions. The structural role of B is not only with plant cell walls but also with the plant cell membrane (Voxeur and Fry 2014): B contributes to the maintenance of the structural integrity of the lipid rafts by binding to glycosylinositol phosphorylceramides.

In woody species B deficiency causes organ deformity, and in both herbaceous and woody plants, the cell walls are thicker and brittle (Wang et al. 2015a). This is the result of the modification in the expression of genes involved in cell wall remodelling and lignin formation (Wang et al. 2015a).

Foliar sprays of chelated nano-B (average size 50 nm) increased the yield of pomegranate fruits by enhancing the number of fruits per tree (Davarpanah et al. 2016). The same result was obtained with nano-Zn applications alone or in combination with B. Interestingly, the more concentrated application of B and Zn led to an increased juice pH and also to increased total soluble solids in the juice. These results show the potential of using nano-B fertilizers (in combination with other microelements) to improve the yield of fruit trees; in this respect it should be noted that a patent is available on the manufacture of nano-fertilizers comprising B (Deb 2012): the nano-delivery form may overcome the problem of B low phloem mobility in higher plants.

Cu homeostasis is very important for proper plant development: it is a micronutrient which is important as cofactor for cuproproteins; however its excess can negatively impact plant growth (Printz et al. 2016b). An example of Cu-containing cell wall-related protein is the laccase: exposure of plant roots to Cu triggers an increase of ROS and, among other, laccase activity, resulting in increased lignin deposition and elongation impairment (Printz et al. 2016b and references therein).

Cu deficiency causes a decrease in expression of cell wall-related gene in alfalfa stems (e.g. cellulose synthases, phenylalanine ammonia lyase, peroxidases, sucrose synthase) (Printz et al. 2016a). In the literature, varying effects concerning the copper nanoparticles have been reported (reviewed in Kasana et al. 2017), which depend on the size and concentration of the nanoparticles; very recently, Cu-Zn nanoparticles were shown to activate the antioxidative system of winter wheat seedlings (Acveduc variety), by increasing SOD and catalase activities in the leaves under drought (Taran et al. 2017). In the light of these results, the combined actions of Cu and Zn nanoparticles may be beneficial in other crops subjected to drought and enhance the yield under environmental limiting conditions.

In another study, ZnO (zinc oxide) nanoparticles caused toxicity to thale cress via oxidative stress (Wang et al. 2016): genes involved in photosynthesis and chlorophyll biosynthesis were affected (decreased expression, however some carotenoid biosynthetic genes were induced), and biomass accumulation was reduced in both roots and shoots.

Fe is an important microelement for biomass production (Briat et al. 2015), as its deficiency triggers alterations in the structure of chloroplasts and photosynthesis, with consequences on productivity. Fe nanoparticles were shown to increase cell wall loosening (hence elongation) in roots of thale cress, by a non-enzymatic reaction, e.g. via the induction of OH-radical loosening (Kim et al. 2014). This is likely due to the oxidation of nZVI (nano zerovalent iron), which results in the release of H_2O_2, which in turn can soften the cell walls of root cells. Pectic polysaccharides are then degraded and the orientation of cellulose microfibrils also changes. All these effects could be particularly useful in specific stress conditions such as, for example, in the absence of water because iron nanoparticles could increase plant resistance to drought (Kim et al. 2014). Interestingly, the cell walls of the treated plants were thinner with a bias of the cellulose microfibrils in the transversal orientation; these anomalies also triggered an increase in endocytosis, as a result of asymmetrical distribution of tensional strength.

5.4 Silicon: The Plant Tonic

In this paragraph we will discuss the beneficial role of Si, a non-essential metalloid which, when supplied to plants (as orthosilicic acid $Si(OH)_4$), can confer enhanced immunity against biotic attack, increased resistance to abiotic stress and higher vigour (Guerriero et al. 2016a; Luyckx et al. 2017). With respect to Si accumulation, which in plants is under the form of amorphous silica (SiO_2), plants are classified into excluders, accumulators and intermediate types (Mitani and Ma 2005). Horsetail (*Equisetum* sp.) and the monocot rice are high silica accumulators (between 5 and 10% silica per dry weight). An emblematic example of the effects of Si in plants is represented by horsetail: it was reported that this high silicifying organism can grow well in the absence of added Si; however over time it becomes more susceptible to develop fungal infection (Guerriero et al. 2018; Law and Exley 2011). The role of Si in non-silicifying plants (e.g. many dicots) is even more obscure, given the latent

role of this metalloid in the absence of an external trigger (e.g. a stress) (Fauteux et al. 2006); nevertheless, some evidence is available to explain the protective effect of Si (Fauteux et al. 2005). In this respect, Si (in the form of biogenic silica) acts mechanically on plants, by associating with cell wall components, thereby forming an impenetrable barrier for pathogens; however, literature data have also proposed a role of second messenger for Si, whereby the metalloid can act on the plant metabolism (both primary and secondary metabolism) (Detmann et al. 2012; Gengmao et al. 2015). The effects of Si on cell wall-related processes are clear if one considers on one hand the mechanical role and on the other the effects on the secondary metabolism. A clear connection with the cell wall is given by the study on *Equisetum arvense* (Law and Exley 2011): callose was shown to "catalyse" biogenic silica deposition both in vivo and in vitro.

Foliar applications of nano-Si (prepared from Na_2SiO_3) increased the reduced glutathione and chlorophyll content in Cd-stressed rice and enhanced the activities of antioxidant enzymes, as well as the content of Fe, Mg and Zn (Wang et al. 2015b). Nanosilica (from rice husk ash) application was shown to increase maize seed germination and stem height/width, decreased the transpiration rate (thereby enhancing the water use efficiency) and increased the content of chlorophyll b and total chlorophyll (Yuvakkumar et al. 2011). Besides plants, nanosilica was shown to have also beneficial effects on soil bacterial population and nutrient content (Karunakaran et al. 2013; Rangaraj et al. 2014). This effect of nanosilica on soil microbiota is an element deserving more attention, considering the effect that soil bacteria play in seed germination and, ultimately, on plant productivity. For example, the effect of Si on spermosphere (the soil region surrounding the germinating seed) (Schiltz et al. 2015) would be very interesting to investigate, since this zone is crucial for the establishment of the interaction with microbial communities.

5.5 Pros and Cons Associated with the Use of Nano-sized Nutrients

Currently available data are sometimes controversial about the effects of nanoparticle-mediated delivery of nutrients, as described in some review papers (Aslani et al. 2014). There is no doubt that nanotechnology may play a critical role in food production and food safety. Indeed, the numerous applications of nanotechnology in agriculture include the use of nanofertilizers to increase both growth and yield of plants, as well as of nano-pesticides to manage pests and diseases and of nano-sensors to monitor plant health and soil quality. In addition, the technology of nanoparticles may mediate a better absorption of nutrients from the soil, thus contributing to the general health of plants (Servin et al. 2015). As discussed above, the literature is full of information on the effectiveness of nanotechnologies in mediating a better absorption of nutrients from the soil. In many cases, the available information is a description of the phenotypic effects of nano-based nutrients on some physiological parameters of plants, which however represent a good index of the physiological state of plants. As often happens in these cases, the

variety of plants analysed, the different experimental designs, the different doses of applied nanoparticles, the timing of treatment and the various parameters analysed are not uniform, and thus it is not simple to obtain a final verdict. All this must be added to the type of nanoparticle, its physical state and its ability to interact with the biotic and abiotic environment. Consequently, it is frequent to find information in the literature in favour or against the use of nano-based fertilizers for plant growth or on the impact of nanotechnology on plant biomass.

5.5.1 Pros

For example, TiO_2 nanoparticles could be a low-cost approach to deliver nutrients. In fact, the amendment of TiO_2 nanoparticles induces a significant improvement in root length of beans, as well as in chlorophyll content and total soluble proteins; the enzymatic activity of specific proteins (such as alkaline phosphatase, phytase and dehydrogenase) is also increased (Raliya et al. 2015). Similarly, Zn nanoparticles have been used as nanofertilizer to improve agricultural production in pearl millet. Even in this case, a significant improvement in plant root length and in chlorophyll content, total soluble proteins, biomass and enzyme activities of acid phosphatase, alkaline phosphatase, phytase and dehydrogenase was observed (Tarafdar et al. 2014). Similar results for Zn-based nanofertilizers were also found in sweet basil (El-Kereti et al. 2013).

Nano-Si can exert a marked improvement on corn crops by increasing seed germination, water efficiency utilization and total chlorophyll content (*vide supra*). Suitable Si levels can also increase the yield of rice and, at the same time, can attenuate the effects of biotic and abiotic stress; moreover, Si can improve grain quality by lowering the content of pollutants such as Cd and As. In fact, application of nano-Si results in less accumulation of Cd and even minor translocation of the metal. Nano-Si treatment can also reduce the oxidative stress of plants treated with Cd. Therefore, Si is considered as an essential element for optimum performance of plants and, therefore, for production of proper plant biomass. Silicon is incorporated into the cell walls thereby increasing their rigidity, providing physical protection against microbial infections as well as insects. In case of abiotic stress, silicon strengthens the overall structure of individual plant organs, thereby protecting against drought, high temperatures and UV rays, as well as against saline and metal stress (Meharg and Meharg 2015).

As previously described, nitrogen-containing inorganic nanoparticles are also important because they act as a major plant nutrient. Application of N-nanoparticles determines a significant increase in the activity of beneficial enzymes (such as phosphatase, esterase and dehydrogenase) as well as of the microbial population; of area, length and diameter of roots; as well as of total biomass. These observations strongly suggest that N-nanoparticles are effective when used as fertilizer (Thomas et al. 2016). It was suggested that N-nanoparticles could have significant effect particularly in developing countries where the cost of fertilizers is high and is a limiting factor for agriculture.

Similarly, C-based nanoparticles, which despite not considered nutrients are being released in the environment because of anthropogenic activities, can effectively be used as enhancer to carry nutrients. For example, C-based nanoparticles such as fullerol are efficiently absorbed, transported and accumulated. C-based nanoparticles are reported to have a number of positive different effects in melon seeds, by increasing biomass production, fruit as well as the contents of phytomedicines in fruits (Kole et al. 2013).

Even nanoparticles of cerium oxide (CeO_2-NP) have significant impacts on growth and productivity of plants in stressful situations, such as water deficiency. In *Brassica napus* L. (canola), moderate saline stress inhibits plant growth, but the same plants treated with CeO_2-NP have higher plant biomass, greater photosynthetic efficiency and less water stress (Rossi et al. 2016). Cerium oxide nanoparticles ($nCeO_2$) also have positive effects on the physiology, productivity and macromolecular composition of barley (*Hordeum vulgare* L.), but these effects must be carefully evaluated. $nCeO_2$-H promotes the development of plants in which biomass increased without apparent signs of toxicity. However, plants exposed to $nCeO_2$-H did not form grains. Conversely, $nCeO_2$-M determined the accumulation of Ce as well as of P, K, Ca, Mg, S, Fe, Zn, Cu and Al. Similarly, $nCeO_2$-M increased the levels of methionine, aspartic acid, threonine, tyrosine, arginine and linolenic acid in grains. It is evident that, in this particular case, the beneficial and harmful effects of $nCeO_2$ strictly depend on the type of nanoparticle used (Rico et al. 2015).

Nanoparticles of iron oxide (Fe_2O_3 NPs) are a clear example of controversial effects when used as fertilizers. Fe_2O_3 nanoparticles have positive effects on root lengths, plant height and plant biomass by probably acting at the level of phytohormones and antioxidant enzyme activity (Rui et al. 2016). In watermelon, magnetic iron oxide nanoparticles are absorbed by plants and translocated, thereby resulting in changes of several physiological indicators such as activity of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD), as well as of chlorophyll content. Treatment with nano-Fe_2O_3 also increased seed germination. Despite the positive data in favour of nano-Fe_2O_3, positive effects on plants decrease rapidly as soon as the concentrations of nano-Fe_2O_3 increase. Consequently, positive effects of nanoparticles (such as Fe in this case) are closely related to application of very specific doses, which might increase germination and development and improve resistance to environmental stresses (Li et al. 2013).

As a further point in favour of using nanoparticles in agriculture, this technology could be used to enhance resistance of plants to pathogens by conveying specific molecules for treatment of pathogens. In this case, the effect on plant biomass is secondary and not direct because nanoparticles would have a positive effect by preventing the toxic action of pathogens (Alghuthaymi et al. 2015).

5.5.2 Cons

Although many observations seem to suggest positive effects of nanoparticles on the plant biomass (especially in the case of nanonutrients), side effects of nanoparticles

on plants should be carefully evaluated, and negative effects should not be ignored. By comparing gene expression profiles after exposure to nanoparticles of TiO_2, silver and carbon nanotubes, in parallel to biotic or abiotic stress, *Arabidopsis* plants have shown changes in gene expression. For example, exposure to nanoparticles repressed transcriptional responses to pathogens thereby leading to increased bacterial colonization. Other adverse effects were observed on the development of root hair cells. Because the addition of salicylic acid reduced some phenotypic effects and post-transcriptional mechanisms related to nanoparticles, the negative effects of nanoparticles are closely related to hormone levels, and appropriate levels of specific hormones are likely to alleviate the side effects of nanoparticles (García-Sánchez et al. 2015). In other cases, the effect of Ti-based nanoparticles on plant biomass appears to be negligible although Ti nanoparticles are absorbed and translocated in the entire plant up to leaves (Larue et al. 2012).

CuO nanoparticles can inhibit the development of seeds of various *Arabidopsis thaliana* ecotypes as well as pollen germination; in addition, biomass accumulation is a parameter sensitive to CuO nanoparticles. Furthermore, after exposure to CuO nanoparticles, two genes were differentially expressed; these two genes regulate root growth and the production of reactive oxygen species, which suggests a relationship between inhibition of root growth and oxidative stress (Wang et al. 2016).

Negative effects of Zn nanoparticles on plant biomass were also described; this effect is fundamentally related to the absorption of nanoparticles on the root surface (Lin and Xing 2008). In many cases the negative effect on plant biomass appears to be a general trait of C-, Zn-, Cu- and Ag-based nanoparticles (Stampoulis et al. 2009). ZnO nanoparticles have phytotoxic effects on plant growth, bioaccumulation and antioxidant enzymatic activity of buckwheat. Biomass was significantly reduced in a wide range of concentrations of ZnO nanoparticles. The latter were observed in root cells as well as on their surface. In addition, the treatment induced the production of reactive oxygen species (Lee et al. 2013). Ag nanoparticles (AgNPs) have relatively negative effects on plant biomass as observed in wheat, where roots grow up slowly and often branch (Dimkpa et al. 2013). AgNPs are widely used as antimicrobial agents. Inevitably, their extensive use results in AgNP accumulation in the environment and therefore in plants. In Chinese cabbage (*Brassica rapa* ssp. pekinensis), AgNPs at low concentrations stimulate growth, while higher concentrations suppress growth and reduce root length and biomass. This was accompanied by increase of reactive oxygen species (ROS), production of malondialdehydes, anthocyanin biosynthesis and DNA damage. AgNPs treatment stimulates the expression of genes related to secondary metabolism and antioxidant activity (Baskar et al. 2015).

Although not closely related, the effect of nanoparticles on mycorrhiza associations must be taken into account because treatment with silver and iron nanoparticles can induce a drastic reduction of fungi association with roots; clearly, this has negative effects on the global biomass of plants (Feng et al. 2013). Therefore, although nanoparticles can counteract the effects of pathogens, negative effects on the associations of beneficial organisms must be considered. This is a very interesting research scope that must be carefully assessed in the future because it may

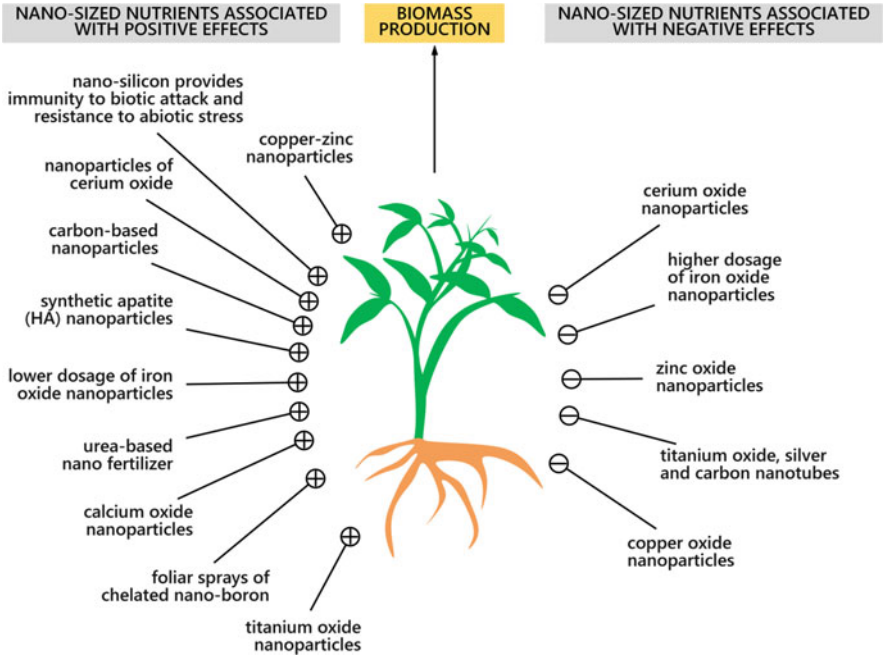

Fig. 5.2 Summary scheme of the two main effects caused by nanofertilizers on the biomass production of plants. On the left, the list of nanonutrients with positive effects (increase in biomass); on the right, the list of nanonutrients with negative effects (either direct decrease of biomass or toxicity that leads to the reduction of biomass). Note that in some cases the effect can be negative or positive depending on the concentration of the nanonutrient (such as in the case of copper-zinc nanonutrients)

represent an important method to monitor the association of both positive and negative microorganisms with plants.

Although the nanoparticles of cerium oxide (CeO_2 NP) can have positive interactions with plants, there is evidence of phytotoxicity of lettuce against CeO_2. Lettuce grows faster at medium-low doses by significantly increasing the nitrate content. While treatments with lower concentrations have no impact on plant growth, treatment with higher concentration greatly reduces the biomass of plants. Apparently, the ability of plants to respond to oxidative stress by means of superoxide dismutase (SOD), peroxidase (POD) and malondialdehyde (MDA) is inhibited after treatment at high dosages of CeO_2 nanoparticles (Gui et al. 2015) (Fig. 5.2).

It is paradoxical that the effects of nanoparticles can be more critical for biotechnologically transformed plants, as reported in a few case studies. In the specific case of CeO_2, Bt cotton plants have proven to be more sensitive to the treatment, thereby resulting in a smaller biomass, as well as a smaller accumulation of nutrients in Bt plants (Li et al. 2014). Although unfortunately sparse, these observations raise important questions on the relationship that may exist between genetically transformed plants and treatments with nanoparticles.

5.6 Future Perspectives

From the above, it is clear that the nanoparticle technology when applied to increase the contribution of micro- and macronutrients to plants is very promising. In the agricultural field, this technology most likely will allow increasing productivity of crops in the coming years. This is a major challenge as well as absolutely necessary because agriculture remains the most important source of food supply. Considering the expected increase in the world population, it becomes even more important to be able to adequately support growth and development of plants, especially of crop plants. Available data, as also discussed in this chapter, are still fragmentary, incomplete and sometimes contradictory. Nonetheless, they indicate that an adequate supply of nutrients mediated by nanoparticles can have significant effects on plant productivity in terms of biomass production. However, some critical aspects are to be understood and clarified; first, the most effective dimension of nanoparticles and their structure, because even the purely physical aspect of nanoparticles has considerable but unpredictable effects. Secondly, it is necessary to distinguish between direct or secondary (indirect) benefits. Direct benefits include the application of nanoparticles consisting of elements that are classified as macronutrients, such as N and P, and nanoparticles containing elements that are categorized as micronutrients (such as B and Zn). In the latter case, it might be very important to know the exact dispensation of micronutrient-based nanoparticles, because an incorrect dosage has often negative effects on plant growth. Concerning the secondary benefits, an increase in growth and plant biomass is due to the application of nanoparticles that do not contain macro- or micronutrients but that, however, have important positive effects on plant biomass. The most renowned examples of this category are Ti, Si and C-based nanotubes, which are not considered as nutrients, but whose contribution in the form of nanoparticles induces a significant increase in productivity (under certain experimental conditions). However, alongside a list of positive (direct or secondary) effects, the toxic effect of nanoparticles must be also carefully evaluated not only on the plants to which they are applied but also (and especially) on the environment in which these plants live (both nontarget plants and animals). Therefore, it remains to carefully define the parameters of standard analyses, molecular markers that are as broad as possible, but also targeted analyses for particular plant species, as well as the pathway of a given type of nanoparticles in cells or in the whole plant. Currently, detailed analysis of the genetic, protein or metabolic response of plants to nanoparticle treatment is unavailable. In practice, it remains to be understood the impact that the nanoparticle technology can have on the entire plant physiology; this is a very important aspect especially because of the high nutritional potential that nanoparticles have.

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Current Status of Nanoclay Phytotoxicity

6

Giuseppe Lazzara, Marina Massaro, and Serena Riela

6.1 Introduction

Nowadays, contamination of soils, with pesticides, fertilizers, and heavy metals, is the major problem in ecology. Therefore, the tendency of scientific community is to develop new systems that allow the efficient removal of pollutants using limited resources.

In the last few years, nanotechnologies are conceived as potential tools to revolutionize agriculture and food systems (Scott and Chen 2013). To support this innovative point of view, researchers have reported several studies about the properties of nanoscale biomaterials which enabled to apply them for innovative applications. For example, the development of engineering of nucleotides, led to a variety of nanoscale building blocks, could been exploited for several applications in medicine, biotechnology, and nanotechnology (Roh et al. 2011). Several studies have been focused on applications of nanomaterials on sensors and detection, drug carrier and delivery, and protein production. As far as is concerned the application in agriculture, a plethora of investigations have been reported. These studies are mainly focused on animal production input and the development of genetically modified crops.

Other interesting applications of nanomaterials have been focused on their use as biosolids for wastewater treatment fields, delivery of pesticides or fertilizers for agricultural purposes, and so on.

The increasing application of nanomaterials in agricultural field necessitates an improved understanding of their potential impact on environment because the industrial uses could lead on emissions of materials on nanometer scale into the

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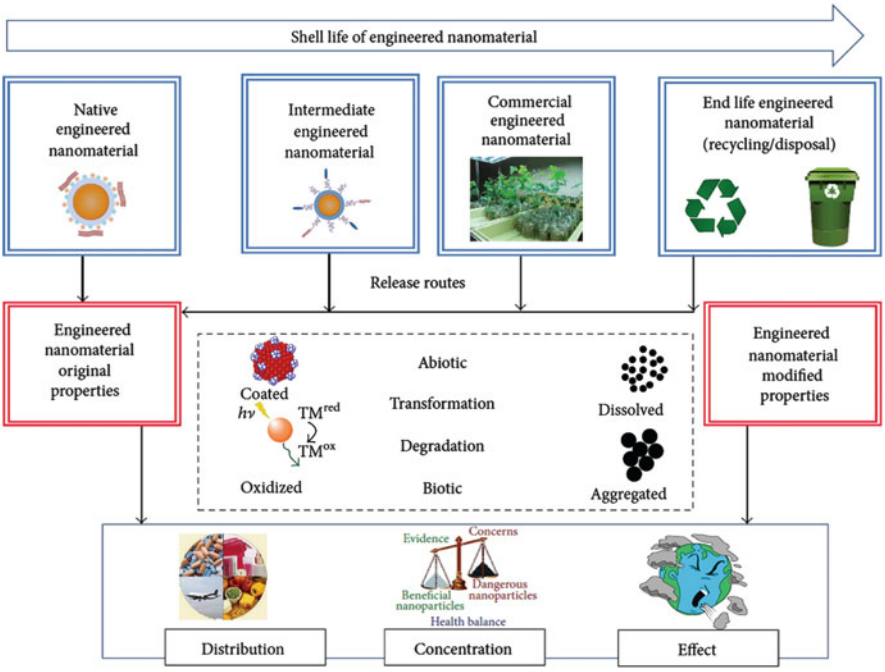


Fig. 6.1 Release routes of nanomaterials on living system. Reproduced from Aslani et al. (2014), with permission from Hindawi

atmosphere, hydrosphere, or geosphere (Fig. 6.1) (Chichiriccò and Poma 2015; Nair et al. 2010; Ruffini Castiglione et al. 2011; Shaymurat et al. 2012).

Therefore, there has been an increasing interest to test different nanomaterials against germination of seeds, crop production, and growth of shoot/root. Furthermore, it seems crucial to test their effects on flora and fauna. Owing to their sub-nanometer size, geometric structure, and unique properties, nanoparticles can indeed possess an intrinsic toxicity, modifying, for example, the plant growth depending on duration of exposure, nanoparticle size, surface structure, chemical composition, shape, and solubility (Bhabra et al. 2009; Nel et al. 2006).

Besides this, once the nanomaterials are released in the environment, they can pollute soil, migrate into surface/groundwater, and interact with biota. In addition, the nanomaterials can also be transported to an aquatic system by rainwater and/or wind runoff.

Carbon nanoparticles, for example, can penetrate plant cells (Khodakovskaya et al. 2011; Lui et al. 2009) and induce some phytotoxicity at high doses (Ghodake et al. 2010; Lin and Xing 2007; Stampoulis et al. 2009), leading to conclude that certain carbon nanoparticles are not 100% safe.

It was reported that the application of SiO_2NPs enhanced significantly the characteristic of seed germination and growth of tomato seedlings (Siddiqui and Al-Whaibi 2014). On the contrary it was demonstrated that the bioaccumulation of

SiO_2NPs in Bt-transgenic cotton represented a potential risk on food crops and human health (Le et al. 2014). Yang and Watts (2005) analyzed the phytotoxicity of Al_2O_3NPs on five plant species by root elongation experiments. They observed a reduction in root elongation due to toxic action related to the surface features of the NPs. The MWCNTs had no significant influence on germination percentage and root growth of six different crop species, including *Raphanus sativus* L. (Lin and Xing 2007). However, studies concerning the combined effects of NPs and metals on agricultural crops (Ahmed et al. 2013; Wang et al. 2014a) provided limited information on nanotubes' effects on plant development and cell differentiation.

Among the different nanomaterials in the last years, nanoclays have attracted a great interest for their intrinsic properties (Lazzara et al. 2017). Usually, clays are natural, nontoxic, and abundant in thousands of tons a low price. The use of nanoclay in nanotechnology not only diminishes the risks of ecotoxicity but also opens up enormous scope for employing nanotechnology in agriculture.

6.2 Nanoclays

Clays are fine-grained natural materials that may be found in sediments, soils, or rocks. Generally, the nanoclays are hydrous aluminosilicate minerals which present at least one dimension on the nanometric scale. From the mineralogical viewpoint, they belong to the family of phyllosilicates (Fig. 6.2) which are distinguished by layered structures composed of polymeric sheets of SiO_4 tetrahedra linked to

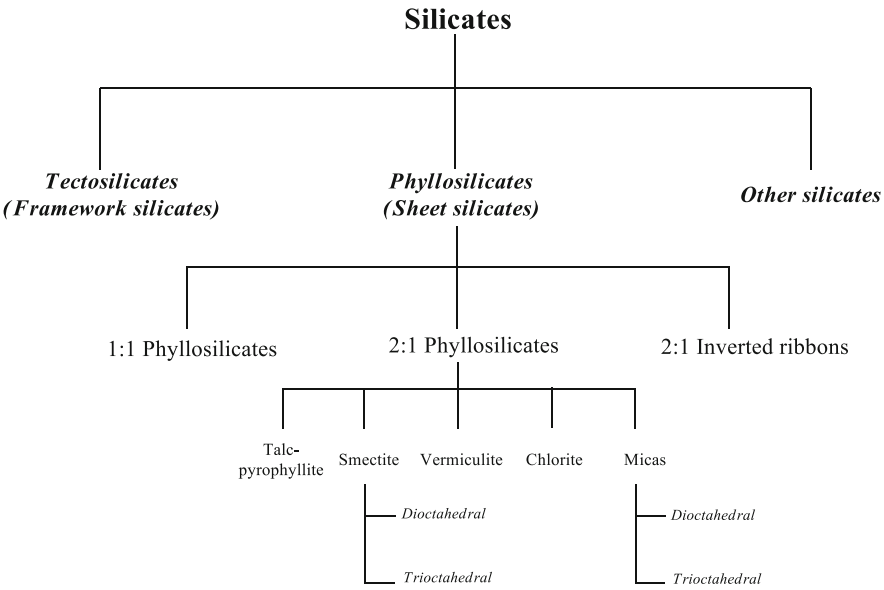


Fig. 6.2 Classification of silicates with the main subgroups of clays. Adapted from Maisanaba et al. (2015) with permission from Elsevier

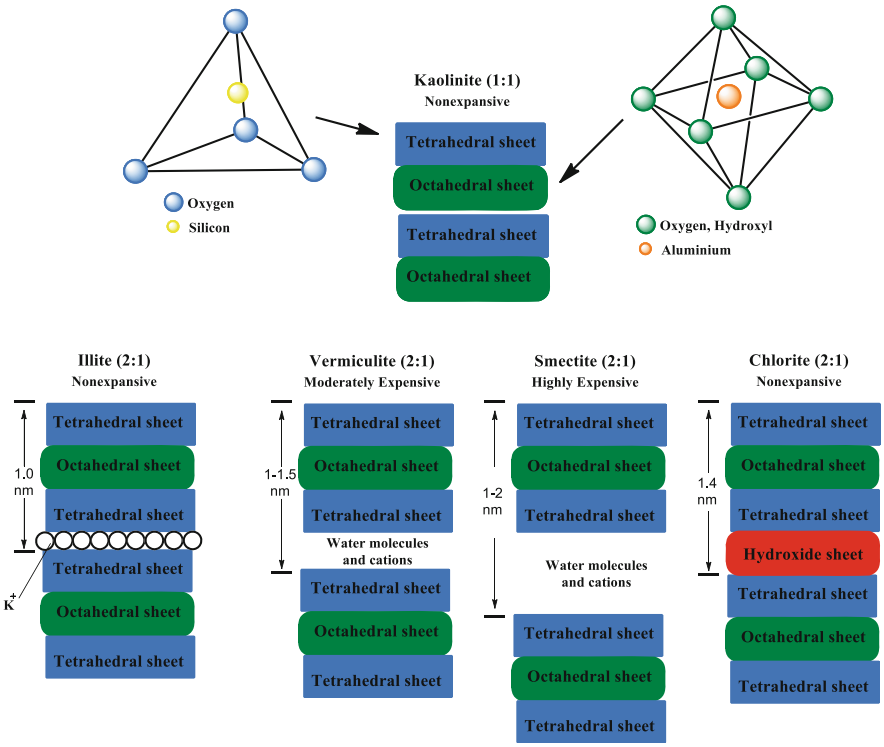


Fig. 6.3 Structure of clays

neighboring tetrahedra by sharing three corners. The combination of these structures results in a hexagonal network; the remaining fourth oxygen of each tetrahedron belongs also to adjacent octahedral sheet, usually constituted by aluminum, magnesium, or iron in sixfold coordination with oxygen from the tetrahedral sheet and with hydroxyl groups.

Clay minerals are layer-type aluminosilicates that are formed as products of chemical weathering of other silicate minerals at the earth's surface (Sposito et al. 1999). These minerals have a platy morphology because of the arrangement of atoms in the structure. The different arrangement of the tetrahedral and octahedral sheets allowed us to classify the clays in three categories: 1:1, 2:1, and 2:1:1 phyllosilicates. The 1:1 phyllosilicates, like kaolinite and halloysite, have one tetrahedral and one octahedral sheet per clay layer; as concerns the 2:1 clay minerals, each layer consists of one octahedral sheet sandwiched between the two tetrahedral sheets. Examples are given by montmorillonite, laponite, and illite. Finally, the 2:1:1 phyllosilicates, like cloisite, are composed of an octahedral sheet adjacent to a 2:1 layer (Fig. 6.3). Due to their different chemical composition, i.e., a succession of tetrahedral and octahedral sheets, clay minerals usually possess a charge, thanks to which they possess swelling and cation exchange properties. This specific charge can be a structural charge or a surface one. The first one is permanent and exists due to ion

substitutions, whereas the latter usually depends on the pH value (Eslinger and Pevear 1988). Owing to the fact that clay minerals are available in large amount at low cost, they have been used as raw materials for hundreds of industrial applications such as in engineering and construction applications, environmental remediation, food processing, and agricultural applications (Murray 2007). Currently, the use of several clays in the food industry is a reality for improving food packaging.

Certainly, the use of clay minerals in several applications involves outstanding advantages, especially when they are used as filler in polymeric matrices. However, up to now, limited studies are present in literature about the potential toxicological effects and impacts of unmodified or modified clay minerals and derived nanocomposites on human and environmental health.

Indeed, some of clay applications are for environmental purposes such as removal of pollutant from soil and water, animal feeding, algal blooms, removal of pathogens, and so on.

Therefore, clay minerals could be present in aquatic and terrestrial ecosystems as a consequence of their presence in different sources, mainly in consumer products, for example, pharmaceutical formulations, beauty therapy, and spas or as waste from the manufacturing process, landfills, and polymer degradation.

There are different scientific reports evaluating the toxic effects of clay minerals on different animal species but a few about clay impact on plant life and environment.

As far as is concerned the phytotoxicity of clay minerals, Asli and Neumann (2009) demonstrated that the presence of bentonite in water supplies could lead to an accumulation of this clay on the cell wall surfaces of the primary root of *Zea mays* seedlings and consequently induced an inhibition of the water transport capacity, leaf growth, transpiration, and cell wall pore size. Conversely, the addition of 0.5 wt % of bentonite in animal feeding results safe for all animals considered, as reported by EFSA (Additives and Products or Substances used in Animal 2013). Similarly, the sepiolite clay was found to be a safe nanomaterial in the animal feeding industry even at concentrations up to 2% (Additives and Products or Substances used in Animal 2013).

6.3 Halloysite Nanotubes

Halloysite (HNT) has a chemical formula for its cell unit $Al_2Si_2O_5(OH)_4 \times nH_2O$ that corresponds to kaolinite, a natural aluminosilicate clay. It is a dioctahedral 1:1 clay mineral present in soils. It is formed by weathering of several types of igneous and non-igneous rocks; thus, it can be found mainly in wet tropical and subtropical regions and weathered rocks. Of course, each deposit is characterized by different purity grade, characteristic sizes, and hydration state.

The term "halloysite" was employed for the first time by Berthier in 1826 and derived from d'Omalius d'Halloy, who found the mineral in Angleur, Liége, Belgium.

HNT has mainly a hollow tubular structure in the sub-nanometer range with an aspect ratio of ca. 20; the wall is constituted of 10–15 bilayers of aluminum and silicon oxide. Depending on the deposit, the halloysite dimensions can vary. Generally HNTs have a length in the range of 0.2–1.5 μm, while the inner and outer diameters of tubes are in the ranges of 10–30 nm and 40–70 nm, respectively (Abdullayev et al. 2013; Abdullayev and Lvov 2013; Konnova et al. 2013).

The special feature of halloysite clay tubes is the different surface chemistry at the inner and outer surfaces. In contrast to other clays, most of the aluminol groups are positioned into the HNTs' inner surface, whereas the external portions are primary siloxanes, while a few silanols/aluminols are exposed on the edges of the sheets (Fig. 6.4).

Dielectric properties of aluminum and silicon oxides are different. Similarly, they undergo to ionization in aqueous media in an opposite way generating tube with inner and outer surfaces oppositely charged. This charge separation occurs in water within a wide pH range from 3 to 8 (Veerabadran et al. 2007). Experimentally, the charge separation is predicted by comparing the negative and positive values for electrical ζ-potential of silica and alumina surfaces in water, respectively (Fig. 6.5). Therefore, the outermost layer of the halloysite tubes consisting silica possesses electrical ζ-potential of ca. –30 mV in the pH range abovementioned. Thanks to this nanotube surface charge, halloysite presents moderate 2–3 h colloidal stability in water. The superimposition of the negative silica outermost surface with the positive (alumina) inner lumen makes that the measured ζ-potential of HNTs is less than that of pure silica particles (–50 mV).

This charge separation is strictly dependent on the acid–base properties of HNTs. To understand the aqueous behavior of these peculiar nanotubes, Pettignano et al. studied the protonation/deprotonation equilibria of Si–OH and Al–OH groups by a ISE-H^+ potentiometric titration in variable pH, ionic strength, ionic medium, and concentration conditions (Bretti et al. 2016). In particular the authors determined one protonation constant for the Si−OH groups and two for the Al−OH groups. The protonation constant values increase with increasing of the ionic strength in all the ionic media suggesting the presence of a background electrolyte which stabilizes the protonated species through the formation of weak complexes between ions of the supporting electrolytes and the protonated species.

The different chemical composition of the surfaces allows for selective loadings of positive-charged molecules outside the nanotubes and negative molecules inside the lumen that consists in the 10–15 % in volume of the pristine tubes or till 30–40 vol% after etching with sulfuric acid (Abdullayev et al. 2012).

Therefore, in the inner lumen can be entrapped chemical agents such as macromolecules, including drugs, DNA, proteins, and other chemically active agents, e.g., anticorrosion for protective coating. Therefore, the empty lumen acts as a miniature container for processes which benefit from suitable molecules' sustained release (Dzamukova et al. 2015b; Fu et al. 2015; Lvov et al. 2014, 2016a, b; Massaro et al. 2015a, b, 2016a, c; Riela et al. 2014; Sanchez-Ballester et al. 2015; Tully et al. 2016; Wei et al. 2014; Wu et al. 2017).

Besides drug immobilization in HNTs' inner lumen, some inorganic salts can be loaded and released from halloysite lumen (Abdullayev and Lvov 2011). Therefore,

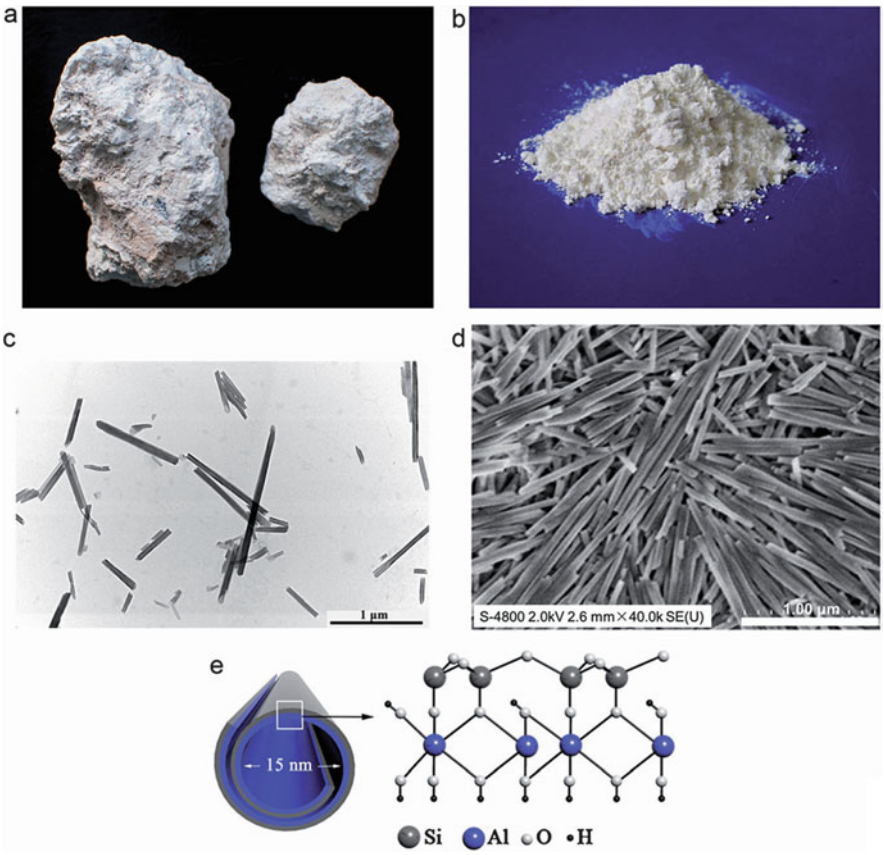


Fig. 6.4 (**a**) The raw halloysite and (**b**) ground halloysite; (**c**) TEM and (**d**) SEM photos of HNTs mined from Hunan Province, China; (**e**) schematic illustration of the crystalline structure of HNTs. Reproduced from Liu et al. (2014) and Yah et al. (2012) with permission from Elsevier and the American Chemical Society

ammonium molybdate ($NH_4{\cdot}6Mo_7O_{24}$), potassium permanganate ($KMnO_4$), sodium silicate (Na_2SiO_3), and sodium chromate (Na_2CrO_4) can be loaded into HNTs from their saturated solution in water via vacuum cycling. Complete release of the inorganic compounds from halloysite nanotubes is achieved within 1–2 h.

The negative surface potential in a wide pH range endows the external siloxane surface of HNTs to be modified by adsorbing specific cations. Lvov et al. adsorbed a monolayer of poly(ethyleneimine) (PEI) with a thickness of 54 nm and, then, alternately adsorbed HNTs forming a thin film with approximately 14 sets of HNT–PEI monolayers. Due to the loosely packed HNTs in the composite (ca. 50% is empty space), the material could be used to load and, subsequently, release guest molecules (Lvov et al. 2002).

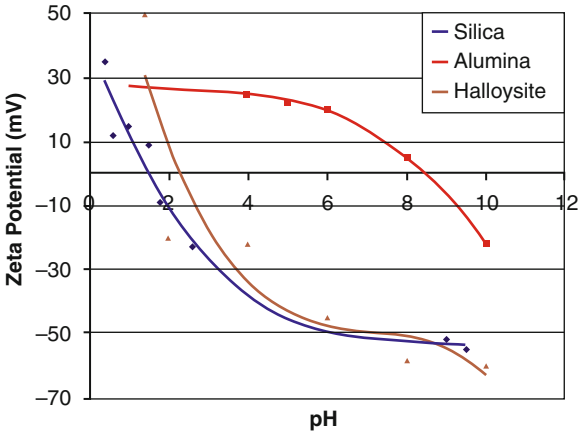


Fig. 6.5 ζ-potential of halloysite (blue dots), silica (red diamonds), and alumina (brown rectangle) nanoparticles. Reproduced from ref. Vergaro et al. (2010) with permission from the American Chemical Society

Abdullayev et al. exploited the charge difference in outer and inner surfaces of halloysite for loading benzotriazole (corrosion inhibitor), and the obtained material was mixed to paint coatings in the amount of 2–10 wt% (Abdullayev et al. 2009).

The most attractive feature of halloysite is its inner lumen with a diameter capable of entrapping chemical agents such as macromolecules, including drugs, DNA, proteins, and other chemically active agents, e.g., anticorrosion for protective coating. In this context, the empty lumen of halloysite acts as a miniature container for processes which benefit from suitable molecules' sustained release.

6.3.1 Halloysite Nanotoxicity

The increasing interest in these nanomaterials could involve, in the long term, release and accumulation of the nanoclay into the environment, and therefore this could bring such damage to human health or plants. Therefore, it is important to study the toxicity of halloysite nanotubes toward living organisms.

Several recent studies reported the investigation of halloysite nanotoxicity in vitro employing human cell cultures and microbial cells. The toxicity and cellular uptake of halloysite nanotubes were investigated using human breast cancer cells, epithelial adenocarcinoma cells, and anaplastic thyroid cancer cells (Massaro et al. 2015b; Vergaro et al. 2010). As a result, halloysite nanotubes have been found to be a safe and useful nanomaterial, applicable for fabrication of novel drug delivery systems or biomedical implants (Dzamukova et al. 2015a; Wei et al. 2014).

Moreover, the interaction of halloysite nanotubes with microscopic algae *Chlorella pyrenoidosa* was also investigated. Lvov et al. demonstrated that there was no penetration of the nanomaterials into cell interior due to electrostatic interactions between the cell wall surface and HNTs (Lvov et al. 2014). It was also reported that halloysite nanotubes were safe for freshwater ciliate protist *Paramecium caudatum* (Kryuchkova et al. 2016) and for the nematode *Caenorhabditis elegans* (Fakhrullina et al. 2015).

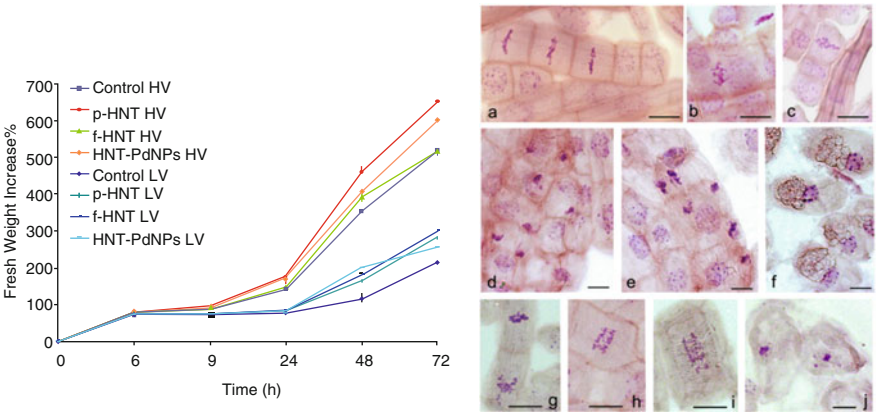


Fig. 6.6 (**a**) Effect of pristine halloysite nanotube (pristine HNT), functionalized halloysite nanotube (f-HNT), and halloysite nanotube-supported palladium nanoparticle (HNT–PdNP) treatments on fresh weight increase percentage of radish seedlings from high- and low-vigor seeds. Arrows indicate visible radicle emergence. Values marked by different letters indicate significant differences according to Tukey's test between observations at different imbibition times and between control and treated high-vigor and low-vigor seeds. *HV* high vigor, *LV* low vigor. (**b**) Effect of pristine halloysite nanotube (pristine HNT), functionalized halloysite nanotube (functionalized HNT), and halloysite nanotube-supported palladium nanoparticle (HNT–PdNP) treatments on mitotic figs. of radish roots' meristem 72-h imbibition from high-vigor and low-vigor seeds. High-vigor seeds treated with water (**A**), pristine HNT (**C**), functionalized HNT (**F**, **G**), and HNT–PdNPs (**H**). Low-vigor seeds treated with water (**B**), pristine HNT (**D**, **E**), and HNT–PdNPs (**I**, **J**). Arrows indicate cytological anomalies, described in the text. Scale bar = 10 μm. Reproduced from Bellani et al. 2016 with permission from Wiley

Similarly HNTs exhibit no toxicity toward *Escherichia coli* bacteria (Zhang et al. 2013) as well as in yeast cells (Konnova et al. 2013).

Recently, the first example of phytotoxic study on halloysite was reported (Bellani et al. 2016). The authors performed some experiments on *Raphanus sativus* L., to develop a quantitative risk assessment model for predicting the potential impact of HNT on plant life. Each experiment performed, such as seed germination, root elongation, chromosomic aberration, and mitotic index, showed that HNTs are safe materials even at high concentration (Fig. 6.6).

6.4 Removal of Organic Contaminants

Dyes are extensively used in several fields such as the plastic, textile, cosmetic, and paper industries; therefore, the possibility to discharge them in the environment, without a preliminary treatment, represents one of the major concerns, since it may cause various serious environmental problems, such as disturbance of aquatic photosynthesis and damage of the ecosystem. In addition, organic contaminants, including pharmaceuticals and organochlorine pesticides, are, also, an environmental concern because of their potential impact on aquatic organisms and capacity to

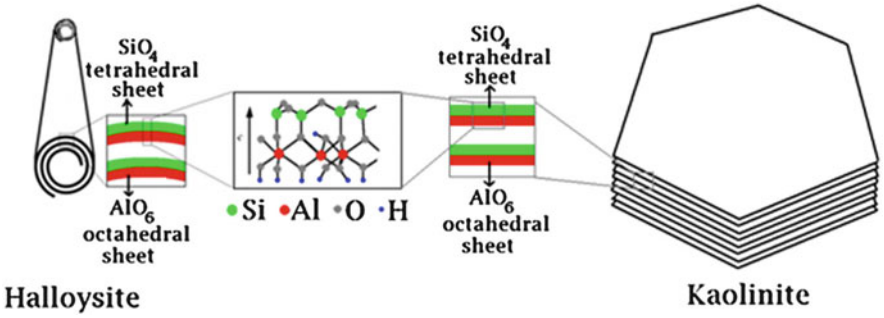


Fig. 6.7 Tubule halloysite and platy kaolinite structures. Reproduced from Zhao et al. (2013) with permission from Elsevier

bioaccumulate in the food chain (Kümmerer 2010; Loganathan et al. 2009; Nakata et al. 2002; Santos et al. 2010).

Therefore, the development and evaluation of new materials for the sorption of organic contaminants still remain in the focus of innovative applications in water, air, and soil treatment. Numerous approaches have developed cheaper and more effective materials especially composed of natural compounds like chitosan (Ding et al. 2009; Xie et al. 2013), starch (Kohli et al. 2012), zeolite (Perego et al. 2013), or clay (Barreca et al. 2014).

In this context, halloysite nanotubes, modified or not, present superior removal capacity for organic contaminant from contaminated wastewater. Indeed, with respect to other nanoclay, the possibility to adsorb pollutants both in HNT lumen and on HNT external surface increases the removal efficiency. For example, if compared to the platy kaolinite, halloysite has shown better adsorption ability toward organic dyes, such as Rhodamine 6G and chrome azurol S (Zhao et al. 2013). Indeed, the maximum adsorption capacity calculated from Langmuir model is 43.6 mg g^{-1} for Rhodamine 6G and 38.7 mg g^{-1} for chrome azurol S onto halloysite and 21.4 mg g^{-1} for Rhodamine 6G and 36.7 mg g^{-1} for chrome azurol S onto kaolinite. These differences, as abovementioned, could be explained taking into account the different structure, as shown in Fig. 6.7.

Recently, a number of studies on removal of cationic, neutral, and anionic dyes from aqueous solution onto natural HNTs have been reported (Chen et al. 2014; Kiani et al. 2011; Liu et al. 2011; Luo et al. 2010, 2011; Zhao and Liu 2008; Zhao et al. 2013). These works were based on the adsorption mechanisms including physical and chemisorption, site geometry, etc. and showed moderate adsorption capacity (26–113.46 mg g^{-1}) and reusability (Table 6.1).

Lazzara and Riela et al. reported (Massaro et al. 2017a), for the first time, a new class of inorganic–organic nanosponge hybrids obtained by the combination of the inorganic halloysite clay and organic cyclodextrin derivatives (HNT–CDs) with potentially complementary binding ability and additional nanochannels formed by the cross-link between CDs and HNT (Fig. 6.8).

Table 6.1 HNT nanomaterials used as adsorbent and/or catalyst for the photodegradation of organic pollutant

Nanomaterial	Organic pollutant	Mechanism	References
HNT–TiO_2–Fe_3O_4	Methylene blue	Adsorption	Zheng et al. (2016)
HNT-macroporous polymer foam	λ-Cyhalothrin	Adsorption	Chen et al. (2016)
HNT/magnetic polysulfone capsules	Sudan I	Adsorption	Pan et al. (2016)
HNT/alginate	Methylene blue	Adsorption	Liu et al. (2012)
HNT/Rose Bengal	4-*n*-Nonylphenol	Photodegradation	Bielska et al. (2015)
HNT pristine	Several pesticides	Coagulation/ adsorption	Shabeer et al. (2015)
Carbon-coated HNT/MNPs	Methylene blue	Photodegradation	Zhang et al. (2014)
HNT pristine	Methylene blue	Adsorption	Zhao and Liu (2008)
HNT/polymer	Chloramphenicol	Adsorption	Xie et al. (2016a, b)
HNT pristine	Malachite green	Adsorption	Kiani et al. (2011)
HNT-derived mesoporous silica nanotubes	Methylene blue	Adsorption	Shu et al. (2016)
HNT pristine	Methyl violet	Adsorption	Liu et al. (2011)
HNT/Fe_3O_4	Methyl violet	Adsorption	Duan et al. (2012)
HNT pristine	Azo dyes		
HNT/CB[8]	Toluene	Adsorption	Massaro et al. (2016b)
HNT/CB[8]	Pyrene	Adsorption	Massaro et al. (2016b)
HNT–CD hybrid	Rhodamine B	Adsorption	Massaro et al. (2017a)
HNT/surfactants	Hydrophobic compounds	Adsorption	Cavallaro et al. (2012)
HNT/alginate beads	Crystal violet	Adsorption	Cavallaro et al. (2013)
HNT/CeO_2/AgBr	Methyl orange	Photodegradation	Li et al. (2015b)
HNT/CdS	Tetracycline	Photodegradation	Xing et al. (2012)
HNT/AgNPs	Methylene blue	Photodegradation	Zou et al. (2012)
HNT/ZnO	Tetracycline	Photodegradation	Li et al. (2015b)
HNT/TiO_2	Rhodamine B	Photodegradation	Li et al. (2015a)
HNT/Au–Ni/Fe_3O_4	Congo red	Photodegradation	Jia et al. (2016)
HNT/TiO_2	Methylene blue	Photodegradation	Du and Zheng (2014), Jiang et al. (2015), Xianchu et al. (2006)
HNT/$LaFeO_3$	Chlortetracycline	Photodegradation	Li et al. (2016)

A potential multi-pocket nano-container was obtained by combining halloysite nanotube with cucurbit[8]uril (Massaro et al. 2016b). The physicochemical characterization highlighted that the CB[8] molecules interact in the hybrid both with external surface, by means of electrostatic interaction, and with HNT lumen by

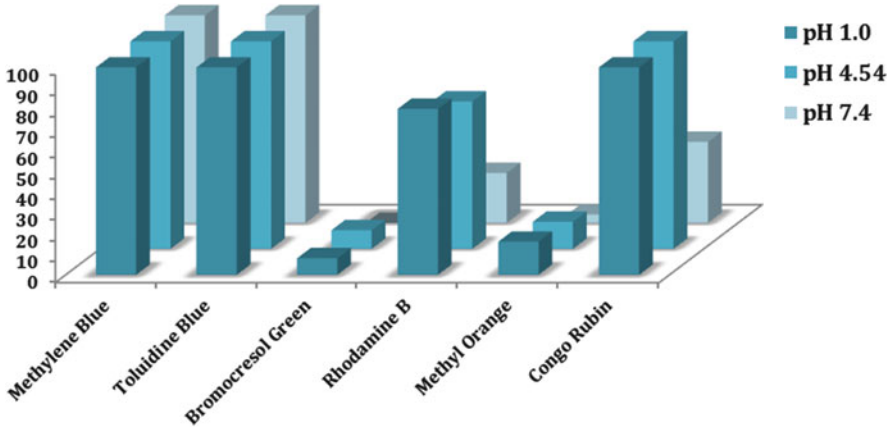


Fig. 6.8 Adsorption capacities of HNT–CD hybrid polymer for the different dyes at pH 1, 4.54, and 7.4. Reproduced from Massaro et al. (2017a) with permission from the American Chemical Society

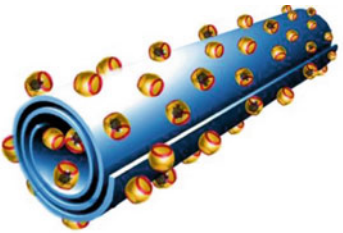
hydrogen bond formation between portal carbonyl groups of CB[8] and Al–OH groups of the HNT inner surface (Fig. 6.9a). In particular, by means of FTIR spectroscopy, it was observed that all stretching bands of CB[8] and the vibration bands of -OH inner surface groups of HNT are shifted toward lower values compared to the pristine compounds that indicate a more constricted vibration of CB [8] groups inside the confined spaces of halloysite lumen (Fig. 6.9b).

The obtained hybrids showed enhanced adsorption capability toward toluene and pyrene, with respect to the pristine halloysite.

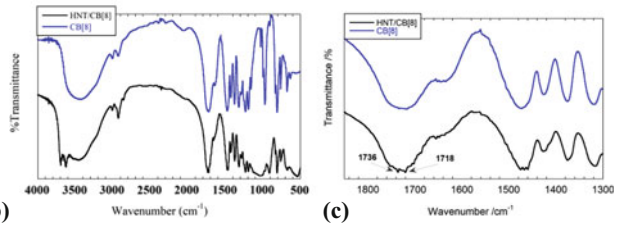
Non-covalent functionalization of both HNT lumens by selective adsorption of anionic and cationic surfactants as sodium dodecanoate and decyltrimethylammonium bromide was, also, reported (Fig. 6.10). It is demonstrated that the adsorption of anionic surfactant into the HNT lumen increases the net negative charge of the nanotubes enhancing the electrostatic repulsions and consequently the dispersion stability. On the contrary the cationic surfactant addition enhances the precipitation of the nanomaterial. The functionalization of HNT lumen with sodium alkanoate in addition generates a nanohybrid with a hydrophobic lumen. Due to this structure, this material behaves like a sponge to entrap hydrophobic compounds (Cavallaro et al. 2012).

Good adsorbent to dye based on alginate beads reinforced with HNT, with excellent physical and chemical properties, was obtained by Liu et al. This new system showed high removal efficiency of methylene blue (above 90%) even after ten successive adsorption–desorption cycles (Liu et al. 2012).

The modification of HNT external surface with silane, such as APTES, improves the dispersibility of the HNT in an organic system. In a recent work, Yang et al. functionalized the HNT (f-HNT) surface both with APTES and Fe_3O_4 (Zeng et al. 2016b). The magnetic modified halloysite was used as reinforcing agent of a supramolecular gel. The authors found that the introduction of only 4 wt% of f-HNT



(a)



(b) (c)

Time (h)	HNT/CB[8] W_{oil}	HNT W_{oil}	CB[8] W_{oil}
2	18.0 ± 0.2[a]; 72.0 ± 0.8[b]; 5.6[c]	0.37 ± 0.01	21.1 ± 0.3
3	36.9 ± 0.4[a]; 147.6 ± 1.6[b]; 6.9[c]	1.37 ± 0.04	23.3 ± 0.4
20	38.6 ± 0.4[a]; 154.4 ± 1.6[b]; 17.3[c]	10.7 ± 0.1	37.3 ± 0.4

W_{oil} mg toluene/g adsorbent; [a] experimental value; [b] experimental value expressed as mg toluene/g CB[8] in the hybrid [c] calculated value according to the rule of mixtures.

Fig. 6.9 (**a**) HNT/CB[8] nanosponge for volatile organic compound adsorption; (**b**) FTIR spectra of the HNT/CB[8] hybrid and pristine CB[8] (left), zoom in the carbonyl stretching region (right); (**c**) capture ability of vapor toluene at 25 °C. Adapted from Massaro et al. (2016b)

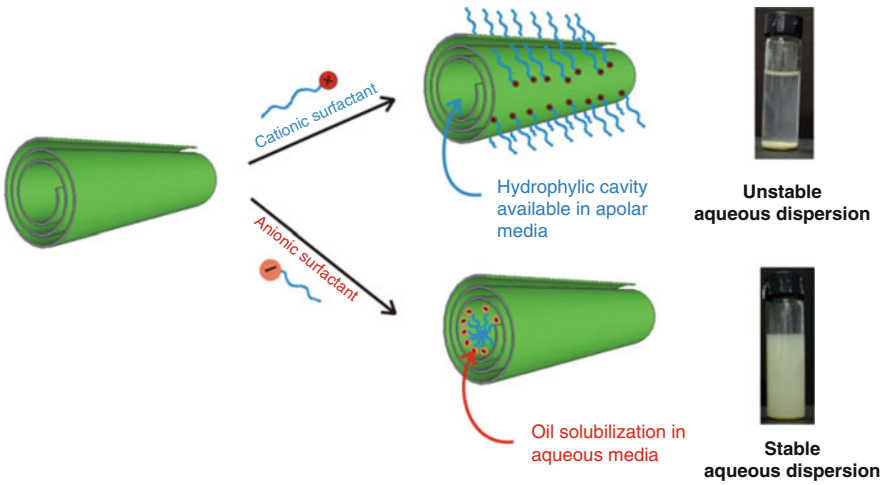


Fig. 6.10 Illustration of the hybrid surfactant/HNT materials. Adapted from Cavallaro et al. (2012)

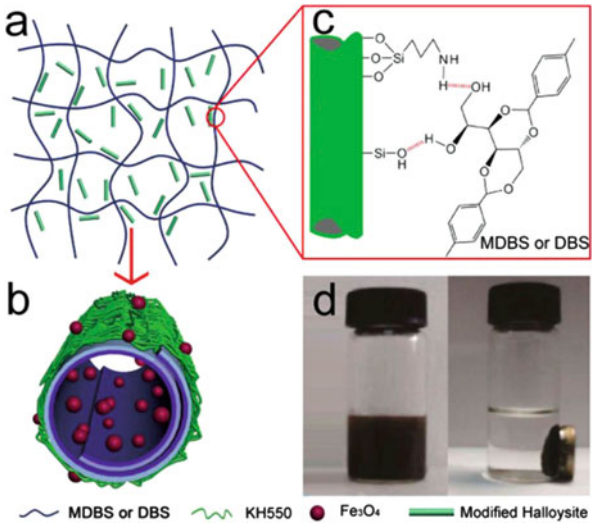


Fig. 6.11 Schematic illustration of the preparation of magnetic supramolecular gels containing hydrophobically modified HNTs loaded with Fe_3O_4 NPs. Reproduced with permission from Zeng et al. (2016b) with permission from Elsevier

remarkably increases the compressive strength of the reinforced supramolecular gel composite from 19 to 28 kPa. The magnetic supramolecular gel composite obtained was used as adsorbent for organic dyes exhibiting an excellent adsorption capability (Fig. 6.11).

Li et al. (2016) reported the immobilization of $LaFeO_3$, one of the most important perovskite-type semiconductors on HNTs' surface via facile sol–gel method. The catalytic performances toward degradation of antibiotics are evaluated under visible light using chlortetracycline as drug model. The decomposition studies revealed that pristine halloysite possesses no photocatalytic activity, whereas after irradiation of chlortetracycline in the presence of the pure $LaFeO_3$, the 74% of drug was degraded within 90 min. On the contrary, the degradation rate increases up to 87% when $LaFeO_3$/HNTs are used as catalyst. For similar purpose, graphitic carbon nitride ($g\text{-}C_3N_4$) which possesses high thermal and chemical stability and suitable bandgap was combined with halloysite and ZnO via a facile calcination method, in order to obtain promising nanocomposite photocatalyst for degradation of pollutant (Li et al. 2015b).

Phenol-based pesticides represent one class of important organic pollutant. They are widely used for agricultural purposes and are considered as one of the most important endocrine-disrupting chemicals present in the environment. For example, nonylphenol compounds are stable in water and exhibit aquatic toxicity and estrogenic activity even at very low concentrations; therefore, the development of adsorbent for these compounds is crucial. In this context HNTs were employed as basis materials to prepare some special adsorbents for adsorption/degradation of phenol-based pollutants.

A photocatalyst based on HNTs was prepared by Huo et al. combining CdS with the nanotube by hydrothermal synthesis method (Xing et al. 2015). This material

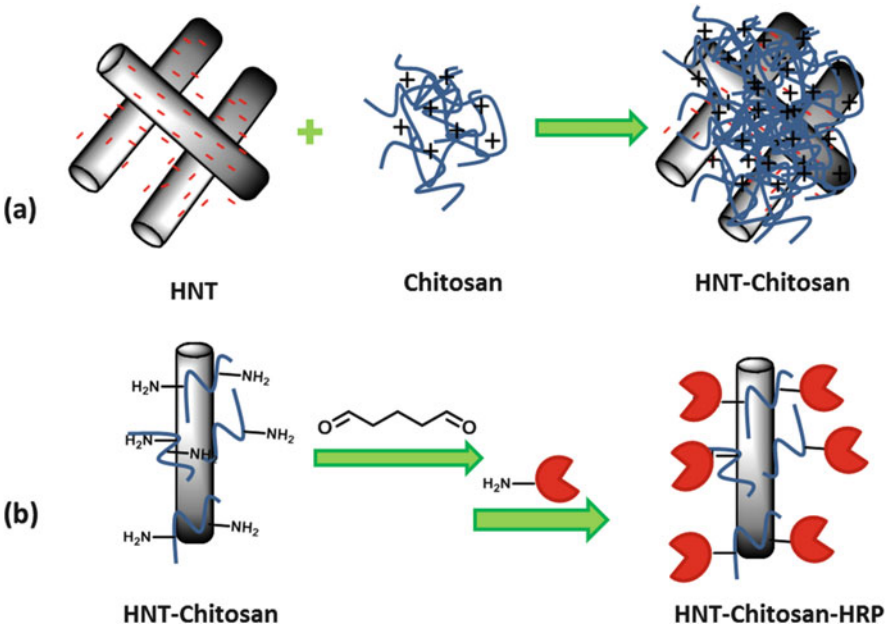


Fig. 6.12 Schematic illustration of (**a**) the preparation of chitosan–halloysite hybrid nanotubes and (**b**) the immobilization of horseradish peroxidase. Adapted from Zhai et al. (2013)

was used as core to fabricate a novel thermo-responsive surface molecularly imprinted photocatalyst, where the shell was created by introducing a thermo-responsive polymer such as poly(*N*-isopropylacrylamide) (Xing et al. 2013). The imprinted polymer layer provides the photocatalyst molecular recognition the ability to form the selective photodegradation of the target pollutant, and thanks to this, it might be used for the treatment and disposal dues of antibiotics from wastewater. Szczubiałka et al. (Bielska et al. 2015) synthetized a hybrid photosensitizer by the incorporation of Rose Bengal (RB) into HNTs for the photodegradation of phenol-based pesticide. The photosensitizer was found to be efficient for singlet oxygen generation and combined adsorption ability with photocatalytic properties.

Other work reports on HRP immobilized on biohybrid HNT–chitosan through cross-linking by glutaraldehyde that was used to remove chemicals, in particular phenols, from wastewater. It exhibited overall high removal efficiency and removal rate, which demonstrates that the HRP immobilized on HNT–chitosan systems could be promising in wastewater treatment (Fig. 6.12) (Zhai et al. 2013).

6.5 Heavy Metal Ions

As mentioned above, the lumens of HNTs could be loaded with various active chemicals, and the surfaces (both innermost and outermost surfaces) are readily non-covalently or covalently functionalized. Therefore, it can exploit the different surface charges to use HNTs as absorbents of pollutant (Jiang et al. 2015; Massaro et al. 2016b). In this context HNTs have been considered as ideal alternatives for the preparation of adsorbents for removal of different kinds of heavy metal ions from wastewater or from polluted soil (Hebbar et al. 2016; Kurczewska et al. 2015; Meng et al. 2017; Zeng et al. 2016a; Zhu et al. 2017).

Generally, pristine HNTs can remove heavy metal ions from aqueous media through the mechanisms of site geometry, physical and/or chemisorption, metal speciation, and so on. However, application of these materials as adsorbents of pollutant is limited by their low loading capacity, less metal ion-binding active sites, and low selectivity to specific metals (Zhu et al. 2017). To overcome to enhance the loading capacity and the affinity for heavy metal ions, HNTs can be functionalized with some interesting nanomaterials and/or functional groups (Massaro et al. 2017b, c) to endow them with the extra mechanism of complexation.

In Table 6.2 are reported some HNT-based nanomaterials used for the removal of heavy metal ions from aqueous or solid media.

Table 6.2 HNT nanomaterials used as adsorbent of heavy metal ions

Nanomaterial	Metal		References
HNT/alginate	Cu(II)	Water	Wang et al. (2014b)
Polydopamine-modified HNTs	Pb(II) and Cd(II)	Water	Hebbar et al. (2016)
Pristine HNT	Ni	Soil	Radziemska and Mazur (2016)
HNT–anilino/Fe_3O_4	Cr(VI) and Sb(V)	Water	Zhu et al. (2017)
A-HNTs@PVDF	Cu(II), Cd(II), and Cr (VI)	Water	Zeng et al. (2016a)
Alkaline-activated HNT	Several metals	Water	Meng et al. (2017)
HNT, pristine, calcined, and acid-activated	Pb(II), Cd(II), Zn(II), and As(V)	Water	Maziarz and Matusik (2016)
HNT/spirulina/chitosan	Cr(VI)		Tekay et al. (2016)
HNT–NH_2 or HNT–SH	Cd, As, Zn, Pb, Cu	Soil	Kurczewska et al. (2015)
HNT/triethanolamine/ diethanolamine	Pb(II), Cd(II), Zn(II), and Cu(II)	Water	Matusik and Wścisło (2014)
Pristine HNT	Ag(I)	Water	Kiani (2014)
HNT/ hexadecyltrimethylammonium bromide	Cr(VI)	Water	Jinhua et al. (2010)
HNT/alginate	Pb(II)	Water	Chiew et al. (2016)
Pristine HNT	Pd and Cr	Soil	Świercz et al. (2016)

Świercz et al. (2016) found that halloysite could be efficiently used for the phytoremediation of heavy metal-contaminated soils. Indeed, bioaccumulation factors indicated that the orchard grass, chosen as plant model, absorbs Zn and Cu more intensively than Pd and Cr in the presence of HNTs, which could indicate that these latter metals have limited bioaccessibility.

Matusik et al. (Matusik and Wścisło 2014) reported the functionalization of HNT by intercalation of diethanolamine and triethanolamine molecules in order to obtain promising nanomaterials with enhanced adsorption capacity toward Pb(II), Cd(II), Zn(II), and Cu(II). The authors also proposed an adsorption mechanism that considers a two-step gradual diffusion of the metals into the interlayer space and subsequent binding to the amine nitrogen of the grafted amino alcohol.

Radziemska and Mazur (2016) proposed a greenhouse study to evaluate the feasibility of using raw and modified halloysite for the remediation of simulated Ni-contaminated soil. The authors demonstrated that the combination of the phytoremediation process with the modification of the physicochemical properties of soil by introducing mineral reactive nanomaterials like halloysite is an effective way to drastically reduce the time required to complete the entire remediation process.

To increase the adsorption efficiency of pristine halloysite, Wang et al. (Zhu et al. 2017) reported the preparation of a new nanoadsorbent where Fe_3O_4 nanoparticles were selectively fabricated on surfaces of HNTs. Once obtained the HNTs/Fe_3O_4 nanocomposites, they were modified by silane coupling agents to increase the surface positive charge and functional groups (f-HNT/Fe_3O_4). The adsorption efficiency was tested for the simultaneous removal of Cr(VI) and Sb(V) from simulated wastewater. The authors found that the prepared f-HNTs/Fe_3O_4 adsorbent could be considered a promising candidate for simultaneous removal of Cr(VI) and Sb (V) from wastewater and natural surface water.

Compared to chemical methods for removing heavy metal ions from aqueous solutions, pristine HNTs and functionalized HNTs showed great advantages such as easy operation, lower cost, non-secondary, reusability, and so on.

6.6 Conclusions

The introduction of nanotechnology in agriculture is an important tool to improve the food industry. Unfortunately, many nanomaterials based on metal nanoparticles or carbon nanoforms possess several disadvantages related to their intrinsic toxicity.

Nanoclays are safe, biocompatible, and inexpensive nanomaterials that find application in several fields, in particular as agents for phytoremediation. Halloysite is an emerging nanoclay, with a tubular structure able to entrap in its lumen or adsorbed on its external surface pollutant and heavy metals.

To increase halloysite adsorption properties, a common strategy is to functionalize external surface of the tubes with specific organic molecules that can interact with organic dye or metal.

Therefore, the peculiar capacity of halloysite nanotubes to efficiently adsorb pollutant can diminish the risks of ecotoxicity in agricultural field.

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7 Mechanism and Interaction of Nanoparticle-Induced Programmed Cell Death in Plants

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7.1 General

Death is the destiny of the cells in every living organisms, and it is in balance with cell proliferation. In multicellular eukaryotes, genetically regulated cell death is an essential part of normal development. The term programmed cell death (PCD) was first used by Lockshin and Williams (1964), and it is contemporarily defined as a genetically controlled process which occurs in clonal prokaryotes and eukaryotes during normal development and environmental stress conditions (Wang et al. 2011a, b). In another way PCD is described as a genetically controlled mechanism, leading to elimination of retired, dysfunctional, overproduced, irregularly developed, or genetically damaged cells safely for the organism (Wang and Bayles 2013; Bayles 2014). The best characterized type of PCD is apoptosis in animal systems, and the term apoptosis was first used by Kerr et al. (1972) for apoptotic hepatic cells which morphologically differ from necrotic cells. Until recently, cell death was classified as apoptotic and necrotic cell death which is lack of a program. According to the last biochemical and molecular studies, PCD has been classified in three main groups (Table 7.1): apoptosis, autophagy, and necrosis (Green et al. 2011; Galluzzi et al. 2012).

Apoptosis is characterized as a disassembly of cells involving shrinkage of cell, contraction of cytoplasm, condensation of chromatin, fragmentation of DNA into 180–200 bp, cytosolic Ca^{2+} increase, phosphatidylserine migration, blebbing of the plasma membrane, and fragmentation of the cell into "apoptotic bodies" that are being engulfed by phagocytic cells (Hengartner 2000; Elmore 2007). Apoptosis is mediated by a class of cysteine proteases called cysteinyl aspartate-specific proteases (caspases) which cleaves their target protein only after an aspartic acid residue. Caspases are presented in the cell as inactive procaspases and once activated caspase functions as molecular switches to activate the apoptotic pathway

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_7

Table 7.1 Comparison of morphological and biochemical characteristics of cell death types

Apoptosis	Autophagy	Necrosis
180–200 bp DNA fragmentation	No fragmentation	No fragmentation
Occurrence of chromatin condensation	No chromatin condensation	No chromatin condensation
Formation of apoptotic bodies	No apoptotic bodies	No apoptotic bodies
No vacuolization	Vacuolization in cytoplasm	Vacuolization in cytoplasm
Intact cellular organelles	Degradation of cellular organelles in autophagosomes	Early/sudden degradation of cellular organelles
Rare mitochondrial swelling	Mitochondrial swelling in late stage	Early mitochondrial swelling
Shrinkage of cytoplasm	No apparent change of the cell volume	Swelling of cytoplasm
Intact plasma membrane	Intact plasma membrane	Early and sudden plasma membrane rupture
Active/continuous metabolic processes	Inactive/interrupted metabolic processes	Inactive/interrupted metabolic processes
Consumes ATP	No ATP consumption	No ATP consumption
Caspase activity	No caspase activity	No caspase activity
No inflammation	No inflammation	Inflammation in adjacent cells
Only single cell affected	Only single cell affected	A group of cells affected
Marker molecules: Bcl-2, Bax, Bad, Bak	Marker molecules: LC3, Beclin-1, Atg family, FIP200	Marker molecules: PARP1, TNF-α, NF-κß

Adapted from Galluzzi et al. (2011)

(Woltering et al. 2002; Shi 2002). Apoptosis is also regulated by Bcl-2 protein family by either inducing (pro-apoptotic) or inhibiting (anti-apoptotic) apoptosis. In apoptotic cell death, no cellular remnant remains inflicting the neighboring cells (Earnshaw et al. 1999; Denault and Salvesen 2002).

Autophagy is an evolutionary conserved catabolic process and characterized by the existence of double-membrane vesicles known as autophagosomes including isolated portions of cytoplasm and organelles. In this type of self-degradation process, the autophagosomes fuse with lysosomes or vacuoles for degradation and recycling by acid hydrolases (Gozuacik and Kimchi 2007). Autophagic cell death which is highly regulated by autophagy-related genes (ATGs) plays a critical pro-survival role in cell homeostasis during stress conditions such as nutrient starvation and hypoxia (Ouyang et al. 2012). In this type of cell death, caspases don't take part. Although the main targets are nucleus and DNA in apoptosis, autophagic cell death takes place in cytoplasm (Öz-Arslan et al. 2009).

Besides necrotic cell death exhibits neither apoptotic nor autophagic characteristics (Clarke 1990; Galluzzi et al. 2015). When the first cell death classification was formulated, necrosis was considered as an unprogrammed, unexpected, and pathological cell death (Galluzzi et al. 2012). Necrosis has peculiar morphological features including cell swelling, organelle dysfunction, chromatin condensation

into small and irregular patches, nuclear membrane dilatation, and cell lysis and inflammation. In necrotic cell death, cellular debris remains among adjacent cells as distinct from apoptosis and autophagy (Leist and Jaattela 2001; McCall 2010; Wu et al. 2012).

7.2 Plant Programmed Cell Death

It has been considered that PCD evolved from a common ancestral cell death process, and all of the living organisms may share common regulatory mechanism. Indeed, the cell death process in prokaryotes (*Bacillus*, *Streptomyces*, *Mycobacteria*, etc.) and unicellular eukaryotes represents analogy to PCD in multicellular eukaryotes (Woltering et al. 2002). Considering the ancestral form of PCD, plants are expected to have evolved their own pathways to manage the plant-specific characteristics such as the presence of cell walls that interfering apoptotic bodies with being phagocytosed by neighboring cells (Danon et al. 2004). Besides, molecular evidences suggest that plants are lack of homologs of animal apoptosis-related genes and there are no universal proteases identified analogous to the animal caspases in plant PCD (Kurusu et al. 2015). Thus, the molecular mechanisms of regulation and execution of PCD in plants are still unclear, despite PCD being well-documented in animals.

Although there are no caspase orthologues in plant genomes, caspase-like activities were detected in plant extracts. Recently some cysteine proteases and serine proteases which have structural homologies with animal caspases were identified in plant PCD. These proteases cleave their substrates from aspartic acid, asparagine, arginine, or lysine sides (Hatsugai et al. 2015). In several studies, it has been reported that plant extracts were effective on synthetic caspase substrates such as caspase-3 substrate DEVD and caspase-1 substrate YVAD. Similarly, animal caspase inhibitors inhibited plant cell death (Woltering et al. 2002; Danon et al. 2004). Afterward, vacuolar processing enzyme (VPE) which catalyzes caspase-1 substrate was defined during developmental and environmental PCD. VPE which plays a critical role in plant PCD is a cysteine protease cleaving their substrates from aspartic acid residues as in animal caspases and it exists in vacuoles. As soon as receiving pro-apoptotic signals, VPE activates vacuolar acidic hydrolases located in vacuoles inducing degradation of vacuolar membrane. After vacuolar collapse, hydrolytic enzymes are released, and cellular contents are degraded subsequently (Vercammen et al. 2004; Wituszyńska and Karpiński 2013).

An alternative to VPE, metacaspases (MCs) are identified in plants during PCD. Although MC is a cysteine protease, it cleaves their substrates from their arginine and lysine cites different from animal caspases (Hatsugai et al. 2004, 2015). In *Arabidopsis thaliana* nine MC-encoding genes have been identified and classified into two groups depending on the presence or absence of the N-terminal zinc finger domain. According to genetic evidences, it is hypothesized that proteins of MC family have both anti- and pro-apoptotic functions competing with each other to

Table 7.2 Morphological and biochemical characteristics of vacuolar cell death and necrotic cell death in plants

Cell death type in plants	Morphological and biochemical characteristics
Vacuolar cell death	Shrinkage of cell
	Formation of autophagosomes, small and large lytic vacuoles
	Reorganization of cytoskeleton
	DNA fragmentation
	Chromatin condensation
	Activation of vacuolar processing enzymes (VPEs)
	Intact organelles remain in the turgid cell until tonoplast rupture
	Tonoplast rupture
	No cellular remnant
Necrotic cell death	Swelling of the cell
	Swelling of the mitochondria
	Inhibition of the cellular respiration and ATP production
	Early and sudden plasma membrane rupture
	Cellular debris remains behind

Adapted from van Doorn et al. (2011)

decide between life and death (Vercammen et al. 2004; Watanabe and Lam 2005; Coll et al. 2010; Wituszyńska and Karpiński 2013).

Plants share some characteristic features with animals during cell death process such as cell and chromatin condensation, DNA fragmentation, and caspase-like activities (van Doorn et al. 2011). However, the general agreement is that plant PCD is not an apoptotic process (Gunawardena 2008). van Doorn et al. (2011) classified PCD into two main groups: vacuolar cell death and necrotic cell death (Table 7.2). Vacuolar cell death preserves some relationship with autophagic PCD in animal cells. In this type of cell death, plant vacuoles come forward. It has been known that there are two types of vacuoles in plant cells. One of them is storage vacuoles that store several types of substances such as organic acids, amino acids, and anthocyanin. The other type is lytic vacuoles including hydrolytic enzymes such as aspartate proteases, serine proteases, cysteine proteases, and nucleases (Huang et al. 2016). Vacuolar cell death is generally associated with an increase of the vacuolar volume according to the fusion of vesicles. These vacuoles also play a role in the turnover of some organelles and cytoplasm during autophagic cleanup in dying cells (Wituszyńska and Karpiński 2013). At the final step of cell death, tonoplast ruptures and vacuolar hydrolyses deliver into the cytoplasm. Until tonoplast rupture, nuclear fragmentation and intact organelles such as mitochondria and chloroplasts are visible in the cell. It has been certainly known that mitochondrion plays a central role in plant cell death, but its executioner role is still unclear (Diamond and McCabe 2011). After vacuolar collapse cellular destruction occurs. Besides, necrosis is characterized by early collapse of the plasma membrane and organelle disruption. In necrosis, cell and cellular organelles swell, plasma membrane disrupts, and because of the early mitochondria disruption, cellular respiration

ceases. In the meantime reactive oxygen species increase, but ATP production decreases. While vacuolar cell death is a physiological process, necrotic cell death is a pathological process under sudden and severe conditions. According to this classification, apoptotic cell death is not found in plant cells (van Doorn et al. 2011).

PCD which is an organized destruction (Lockshin and Zakeri 2004) controls the elimination of cells during development, defense (the hypersensitive response), and stress responses in plants (Kacprzyk et al. 2011). PCD plays a critical role in the regulation of vegetative and generative tissue development such as xylogenesis, embryogenesis, pollen maturation, seed development, seed germination, leaf morphogenesis, and leaf senescence (Delorme et al. 2000; Gunawardena et al. 2004; Galluzzi et al. 2012; Vardar and Ünal 2012). Plants have also evolved alternative adaptive mechanisms in which the cells and tissues are subjected to PCD under abiotic and biotic stresses, to enable the survival of whole organism during the evolution (Jackson and Armstrong 1999; Drew et al. 2000).

PCD also regulates the defense responses such as plant innate immunity against pathogen attack cell death process. Hypersensitive response (HR) is a rapid cell death at the infection site of plant. HR limits the propagation of the pathogen and initiates a potential systemic acquired response in adjacent plant cells (Coll et al. 2011).

Besides abiotic stress factors such as hypoxia, salinity, drought, cold, and heat UV light, pesticides and heavy metal also induce PCD in plants (Kacprzyk et al. 2011). It has been predicted that the rapid development of nanotechnology will cause an increase of nanoparticle presence and accumulation in the environment which is being considered as one of the abiotic stress factors in the last decade (Miralles et al. 2012).

7.3 Nanotechnology and Nanoparticles

Nanotechnology is the recent branch of science, dealing with design, production, and application of nanoscale products by using unique physical properties of nanomaterials. Nanotechnology has diverse applications in the field of electronics, energy, life sciences, and medicine of late years.

Particles are generally classified with regard to their diameters. While coarse particles cover a range of 2.5–10 μm, fine particles are sized between 2.5 and 0.01 μm. Ultrafine particles, or better known as nanoparticles (NPs), are sized between 1 and 100 nm (Buzea et al. 2007). In general NPs possess at least one dimension, increased relative surface area and quantum effects. NPs can change their chemical, electrical, magnetic, and optical features due to their high reactivity and large surface area to volume ratio (Roduner 2006).

NPs released to the environment have two main sources: natural and anthropogenic origin. Natural origin NPs are derived from natural events such as volcanic eruptions, desert surfaces, dust storms, forest fires, erosion, cosmic dusts, photochemical reactions, colloidal clays, mineral precipitates, dissolved organic matter (humic and fulvic acids), and herbal and animal remnants (pollen, skin, epithelium, feather,

etc.) (Buzea et al. 2007; Batley et al. 2011; Strambeanu et al. 2015). Anthropogenic origin NPs involve unintentionally released and engineered NPs. During daily activities such as cooking, car use, and engine combustion, NPs disperse to the environment unintentionally (Buzea et al. 2007). Engineered NPs are produced industrially using various types of materials such as metals, metal oxides, nonmetals, carbon, and polymers. Based on their chemical composition, NPs are classified into four groups: (1) carbon-based nanomaterials (fullerenes, nanotubes, etc.), (2) metal-based nanomaterials (nanogold, nanosilver, metal oxides, quantum dots, etc.), (3) dendrimers, and (4) composites (such as nano-sized clays) (EPA 2007).

These various types of engineered nanomaterials have special electrical, catalytic, magnetic, mechanical properties preferred in commercial fields (Subbenaik 2016). Physical properties of NPs such as size, shape, dimension, surface area, agglomeration, composition, surface morphology, and structure carry critical importance to control their uptake and detrimental impacts on living organisms (Nowack and Bucheli 2007; Subbenaik 2016).

Nanotechnology has wide range of applications in the fields of electronics, biomedical sciences, pharmaceutical industry, cosmetics, water filtration, catalytic systems, manufacturing, health care, and medical diagnosis (Nowack and Bucheli 2007). In addition, it is known that NPs make use of agricultural technologies, in the field of plant protection products, fertilizers, water/liquid retention, water purification, pollutant remediation, nanosensors, diagnostic devices, and plant genetic modification. The relevant applications of agricultural technologies have gained concerns about release of NPs to the environment. Besides plant-mediated green synthesis of NPs instead of synthetic methods nowadays has become a major focus by researchers (McMurray et al. 2006; Vamvakaki and Chaniotakis 2007; Torney et al. 2007; Alemdar and Sain 2008; Anjali et al. 2012; Milani et al. 2012; Parisi et al. 2015).

Nanotechnology in which rising investments have been made by governments and industries is considered to be one of the world's most promising technologies in the twenty-first century. According to "Global Nanotechnology Market Insights, Opportunity, Analysis, Market Shares and Forecast 2016–2022" report, it is forecasted that global nanotechnology market will grow at a compound annual growth rate (CAGR) of around 17.7% during 2016–2022, and it is expected that the technological advancements in nanotechnology market will be in the fields of health, agriculture, environment, and energy technologies. According to estimation of "Nanotechnology Market Outlook 2020" report, the global investment will reach US$75.8 billion by 2020. Increased investment in nanotechnology and widespread use of NPs require better understanding of their effects on the environment, since the potential toxic effects of released NPs on living organisms still remain unclear (http://www.researchandmarkets.com/reports/3841635/global-nanotechnology-market-insights#pos-0).

Gottschalk et al. (2009) reported that according to a wide range of usage of NPs, they could disperse and accumulate in aquatic, terrestrial, and atmospheric environment. Therefore, plants can take up NPs either through soils or foliar contact. It has been indicated that soil is the major environmental compartment which is face to

different forms of NP contamination (Nowack and Bucheli 2007; Cornelis et al. 2014). Plants hold on to soil with their roots and exposed to different contaminants such as toxic metals, pesticides, and NPs. Therefore, plants are vulnerable to harmful effects of soil contaminants (Anjum et al. 2016). Soil properties also affect the fate and behavior of NPs such as mobility and bioavailability with regard to NP uptake by plants (Cornelis et al. 2014). Plants being the essential base component of ecosystems have central roles in the food chain; thus, they are playing an important role for determination of potential effects during uptake and transfer of NPs (Miralles et al. 2012).

NP interaction with plants occurs via electrostatic adsorption, mechanical adhesion, and hydrophobic affinity (Wang et al. 2005; Zhang et al. 2011). When roots encounter with NPs in the soil, NPs tend to accumulate on epidermis and adhere surface tissues as individual particles or aggregates (Lin and Xing 2008; Wild and Jones 2009; Zhao et al. 2012a, Deng et al. 2014). When NPs are uptaken by roots, they penetrate into the cell wall (Tripathi et al. 2017). Plant cell wall consists of complex structures including a network of cellulose microfibrils cross-linked with hemicellulose and lignin and embedded in pectin (Serag et al. 2013). Plant cell wall has pores in the range of 5–20 nm; therefore, small NPs may diffuse through pores and enter apoplastic and/or symplastic flow (Tepfer and Taylor 1981; Deng et al. 2014).

NPs may pass through the cell walls of cortex cells and move to endodermis via apoplastic pathway (by osmotic pressure or capillary force) (Lin et al. 2009). According to the size of the NP, they may cross the cell membrane with symplastic pathway through binding to carrier proteins, aquaporins, ion channels, and endocytosis or by creating new pores (Ma et al. 2010; Rico et al. 2011; Deng et al. 2014). NPs which reached to the endodermis can traverse to vascular tissues and transport with xylem vessels to leaves (Tripathi et al. 2017). Several researches showed uptake, bioaccumulation, and translocation of NPs by different species of plant (González-Melendi et al. 2008; Corredor et al. 2009; Lin et al. 2009; Kurepa et al. 2010; Huang et al. 2011; Serag et al. 2011a, 2011b, Larue et al. 2012; Slomberg and Schoenfisch 2012; Hussain et al. 2013; Zhai et al. 2014; Dan et al. 2015; Koo et al. 2015; Yanik and Vardar 2015; Wang et al. 2016).

The NP interactions with plants in the soil can cause direct and apparent phytotoxicity with regard to morphological, physiological, and molecular symptoms (Deng et al. 2014). Initial studies on NP phytotoxicity focused on morphological and anatomical parameters such as germination index, root length, shoot/root biomass, and root morphology (Yang and Watts 2005; Lin and Xing 2007; Lee et al. 2008; Liu et al. 2010). The increased use of NPs reveals the need of much more detailed researches on their potential toxic effects and defining their toxicity mechanism (Ma et al. 2010; Miralles et al. 2012; Tripathi et al. 2017).

The mechanisms of NP phytotoxicity are suggested in five steps:

1. Dissolution and release of toxic ions
2. Size–shape-dependent mechanical damage and blockage

3. Reactive oxygen species (ROS) production with redox cycling and Fenton reaction
4. Binding and interaction with surfaces
5. Oxidation of biomolecules (Asli and Neumann 2009; Nel et al. 2009; Shen et al. 2010; Dietz and Herth 2011; Atha et al. 2012; Wang et al. 2016)

Recent experimental studies have described phytotoxic symptoms subsequent to NP treatment in different plant species including reductions in crop quality, germination rate, biomass, root and shoot elongation, photosynthetic capacity, transpiration rate, and mitotic index. Besides, damage in root cap and epidermal cells, DNA and chromosomal damage, up- and downregulation of various stress-related genes, antioxidant enzyme activation, lipid peroxidation, and cell death due to the formation of reactive oxygen species (ROS) were also reported. It has been also reported that the toxic effects of NPs may vary with size, type, shape, structure, and surface charge in plants (Lin et al. 2009; Stampoulis et al. 2009; Arora et al. 2012; Poborilova et al. 2013; Jacob et al. 2013; Rico et al. 2013; Nair and Chung 2014; Chen et al. 2015; Nagaonkar et al. 2015; Hossain et al. 2016; Tripathi et al. 2017). ROS which are important source of defense signaling molecules have been known as key modulators of programmed cell death (PCD) as well as many other biological processes such as growth, development, and stress adaptation (Gechev et al. 2006; Deng et al. 2014).

7.4 Nanoparticle-Induced Oxidative Stress and ROS

Triplet oxygen being as a free radical with two impaired electrons is the ground state of the oxygen. Triplet oxygen can react with molecules in a doublet state to form reactive oxygen species (ROS) (Apel and Hirt 2004; Gill and Tuteja 2010). ROS are continuously produced in chloroplast during photosynthesis, mitochondria during respiration, and peroxisomes during photorespiration. Besides, ROS are also produced during other enzymatic activity processes such as plasma membrane NADPH oxidases, cell wall peroxidases, and apoplastic amine oxidases (Mittler et al. 2004; Reape et al. 2015; Caverzan et al. 2016). ROS are highly reactive and toxic free radicals formed via transfer of electron(s) to oxygen. As it is presented in Fig. 7.1 and Table 7.3, in plant cells the most occurring free radicals are superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), perhydroxyl radical ($HO_2^{\bullet}$), as well as non-radical molecules such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Sharma et al. 2012a). Under steady conditions, ROS are eliminated by antioxidant defense system, and this provides equilibrium with ROS production and scavenging. The antioxidant defense system contains several enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione peroxidase (GPx), guaiacol peroxidase (GPX), and glutathione *S*-transferase (GST). Furthermore the toxic effects of ROS are also counteracted by nonenzymatic antioxidants such as ascorbic acid (ASH), glutathione (GSH), oxidized

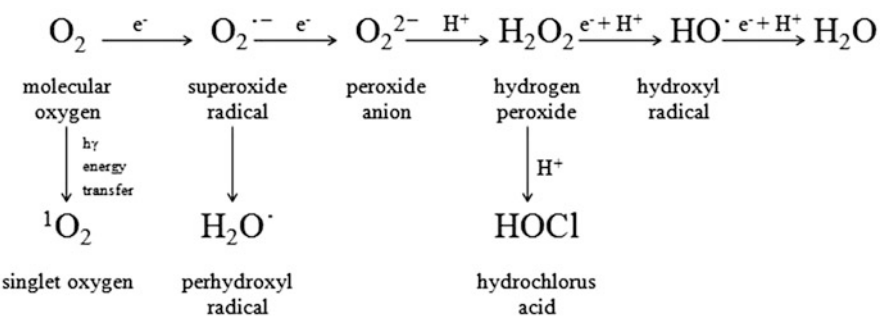

Fig. 7.1 Generation of different reactive oxygen species (Adapted from Gill and Tuteja 2010)

Table 7.3 Most important ROS and their properties

Type of ROS	Half-life	Location in the cell	Characteristics	Scavengers	Form of activity
Superoxide ($O_2^{\bullet -}$)	10^{-6}	Membranes, chloroplasts, mitochondria	Unstable, signaling molecule	SOD	Reacts with double-bounded biomolecules, Fe–S clusters of proteins, reacts with $NO^{\bullet}$
Hydroxyl radical ($^{\bullet}OH$)	10^{-9}	Membranes, chloroplasts, mitochondria	Unstable, very reactive	Flavonoids and proline	Extremely reacts with protein, DNA, lipid, and biomolecules in the cell
Hydrogen peroxide (H_2O_2)	Stable	Membranes, chloroplasts, mitochondria, peroxisomes	Toxic, signaling molecule, generation of other ROS	CAT, peroxidases, flavonoids	Oxidizes proteins, reacts with superoxide, and forms $HO^{\bullet}$
Singlet oxygen ($^1O_2$)	10^{-6}	Membranes, chloroplasts, mitochondria	Excited oxygen molecule, radical and non-radical form	Carotenoids and α-tocopherol	Oxidizes proteins, polyunsaturated fatty acids, and DNA

Adapted from Domej et al. (2014) and Das and Roychoudhury (2014)

glutathione (GSSG), phenolic compounds, alkaloids, carotenoids, and α-tocopherols (Gill and Tuteja 2010).

Because of their potential toxicity, lower doses of ROS have a crucial role as a signaling molecule modulating acclimation of stress conditions or activation of PCD. ROS level is balanced with enzymatic and nonenzymatic antioxidants. Overproduction of ROS as a result of environmental stresses causes an imbalance between production and scavenging of ROS that is called oxidative stress. Oxidative stress influences various cellular functions and structure by oxidizing proteins,

causing lipid peroxidation, damaging nucleic acids, inhibiting enzyme, and activating PCD pathway (Shah et al. 2001; Verma and Dubey 2003; Meriga et al. 2004; Sharma and Dubey 2005; Maheshwari and Dubey 2009; Srivastava and Dubey 2011; Mishra et al. 2011; Sharma et al. 2012a, Reape et al. 2015).

In the consequence of outer membrane damage, cytochrome c (cyt c) is released from the mitochondrial inner membrane under stress conditions. Cyt c release is a key event in cell death both in animal and plant cells. The release of cyt c causes disruption of electron transport in mitochondria inducing the generation of toxic levels of ROS. Cyt c can also interact with H_2O_2 and superoxide radicals to form most reactive and mutagenic hydroxyl radicals leading to raise ROS increase. Although specific ROS receptors are still not clear, downstream components of H_2O_2 and ROS signal transduction networks including protein kinases, protein phosphatases, and transcription factors have been identified during regulation of plant PCD. As a result of permeability transition pore (PTP) opening, mitochondrial signals are transported to the nucleus leading to alterations in gene expression (Balk et al. 2003; Vacca et al. 2006; Reape et al. 2015). It has been suggested that ROS has two fundamental roles in plant PCD:

1. It acts as a signaling molecule leading to PTP opening which would cause generation of more ROS.
2. It causes a feedback amplifying PCD-inducing stress signal (Jabs 1999; Reape et al. 2015).

Nanotoxicity studies with relation to oxidative stress demonstrated that exposure to NPs induce production of ROS and increase of antioxidant enzymes in several plants (Table 7.4), such as *Triticum aestivum*, *Oryza sativa*, *Allium cepa*, *Zea mays*, *Arabidopsis thaliana*, *Nicotiana tabacum*, and *Vigna radiata* (Shen et al. 2010; Panda et al. 2011; Dimkpa et al. 2012; Zhao et al. 2012b, Mirzajani et al. 2013; Poborilova et al. 2013; Nair and Chung 2014, 2015).

Detailed analysis indicated that NP-induced ROS production targets various cellular components such as chromosomes and DNA. Studies with different species revealed that NPs have genotoxic effects. NPs reduced mitotic activity and increased chromosome aberrations consisting stickiness, bridges, breakages, micronuclei, and laggards (Reddy et al. 2016).

Although there are several research subjecting NP-induced ROS accumulation and antioxidant enzyme activities, the limited number of studies directly related to NP-induced PCD is available.

To the best of our knowledge, the first research that directly mentions programmed cell death term was performed by Shen et al. (2010). The researchers treated leaf protoplast of *Arabidopsis* and rice with different concentrations (5, 25, 100 μg mL^{-1}) of single-walled carbon nanotubes (SWCNTs). They also tested the effects of SWCNTs on *Arabidopsis* leaves. Based on their results, SWCNTs induced cell aggregations, chromatin condensation, ROS accumulation, and oxidative stress leading to programmed cell death. The researchers concluded that whereas NPs have broad advantages in many industrial areas, their adverse effects still need to be

Table 7.4 Effects of NPs on plant antioxidant defense systems

Plant	NPs	Size (nm)	Concentrations	Oxidative stress-related effects	References
Oryza sativa	MWCNT	10–30	20, 40, 80 mg L^{-1}	Increased ROS production, decreased cell viability	Tan et al. (2009)
Brassica oleracea var. *capitata*, *Lycopersicon esculentum*, *Amaranthus tricolor*, *A. lividus*, *Lactuca sativa*	Graphene	~1(h)	500, 1000, 2000 mg L^{-1}	Concentration-dependent increase in ROS and membrane leakage except *L. sativa* in which no significant effects observed	Begum et al. (2011)
Lolium perene, *Cucurbita mixta*	Fe_3O_4 (magnetite)	25	30, 100 mg L^{-1}	Increased SOD and CAT activity, increase in MDA content	Wang et al. (2011a, b)
Triticum aestivum	Cuo ZnO	<50 <100	500 mg kg^{-1}	Increased POD and CAT activities, increment in MDA and GSSG contents	Dimkpa et al. (2012)
Zea mays	CeO_2	10 ± 1	400, 800 mg kg^{-1}	Increased accumulation of H_2O_2, increased CAT and APX activity, upregulation of the HSP70	Zhao et al. (2012b)
Brassica juncea	Ag	~29	25, 50, 100, 200, 400 ppm	Decreased in H_2O_2 and MDA content, activities of APX, GPX, and CAT, decrease in proline content	Sharma et al. (2012b)
Lycopersicon esculentum	TiO_2	27	50, 100, 500, 1000, 5000 mg L^{-1}	Increased SOD activity	Song et al. (2013)

(continued)

Table 7.4 (continued)

Plant	NPs	Size (nm)	Concentrations	Oxidative stress-related effects	References
Oryza sativa	CeO_2	8 ± 1	62.5, 125, 250, 500 mg L^{-1}	H_2O_2 generation and alteration of antioxidant enzymes, enhanced lipid peroxidation and electrolyte leakage, decreased lignin content	Rico et al. (2013)
Coriandrum sativum	CeO_2	8	62.5, 125, 250, 500 mg kg^{-1}	Increased activity of CAT and APX	Morales et al. (2013)
Oryza sativa	CuO	<50	0.5, 1, 1.5 mM	Enhanced MDA, proline, and H_2O_2 contents, increased activity of APX and GR	Shaw and Hossain (2013)
Phaseolus vulgaris	CeO_2	8 ± 1	62.5, 125, 250, 500 mg L^{-1}	Reduced root antioxidant enzyme activity, increased root lipid peroxidation, increased root-soluble protein	Majumdar et al. (2014)
Pisum sativum	ZnO	10	125, 250, 500 mg L^{-1}	Reduced CAT and APX activity, increased H_2O_2 generation and lipid peroxidation in higher concentration	Mukherjee et al. (2014)
Brassica juncea	Au	50	100, 200, 300, 400 ppm	Increased activity of APX, CAT, GR	Gunjan et al. (2014)
Vicia narbonensis	TiO_2	<100	0.2, 1, 2, 4 ‰	Production of H_2O_2, SOD, CAT, GPX,	Castiglione et al. (2014)

(continued)

Table 7.4 (continued)

Plant	NPs	Size (nm)	Concentrations	Oxidative stress-related effects	References
				and POD activity, enhanced lipid peroxidation, increased activity of antioxidants, dose-dependent increased in situ DNA fragmentation	
Hordeum vulgare	CuO	<50	0.5, 1, 1.5 mM	Increased H_2O_2 and MDA content, alterations of antioxidant enzyme activities, H_2O_2 accumulation, and membrane integrity	Shaw et al. (2014)
Brassica juncea	CuO	<50	20, 50, 100, 200, 400, 500 mg kg^{-1}	Increased H_2O_2 and MDA content, alterations in POD and SOD activity, decline in CAT activity	Nair and Chung (2015)
Allium cepa	Al_2O_3	<50	0.01, 0.1, 1, 10, 100 μg mL^{-1}	Dose-dependent decrease in mitotic index, chromosomal aberrations, and increased activity of SOD	Rajeshwari et al. (2015)
Brassica rapa	CeO_2	10–25	10, 100 mg L^{-1}	Increased H_2O_2 generation, no significant changes in CAT and SOD activities	Ma et al. (2016)

(continued)

Table 7.4 (continued)

Plant	NPs	Size (nm)	Concentrations	Oxidative stress-related effects	References
Solanum tuberosum	Ag	20	2, 10, 20 mg L^{-1}	Increased ROS generation, increases in the activities of SOD, CAT, APX, and GR, reduced GSH, ASA, GSSG, DHA	Homaee and Ehsanpour (2016)
Lycopersicon lycopersicum	$CoFe_2O_4$	12 ± 2	62.5, 125, 250, 500, 1000 mg L^{-1}	Decreased CAT activity	López-Moreno et al. (2016)
Brassica juncea	CuO	<50	200, 500, 1000, 1500 mg L^{-1}	Production of H_2O_2, increase in APX CAT and SOD activity, increased proline content, alterations in MDA	Rao and Shekhawat (2016)
Brassica juncea	TiO_2	<25	200, 500, 1000, 1500 mg L^{-1}	H_2O_2 generation, alterations in antioxidant enzyme activity, increase in proline content, decrease in MDA	Rao and Shekhawat (2016)
Solanum lycopersicum	Ag	<100	10, 20, 40, 80 mg L^{-1}	Increased SOD, APX activity, and MDA, reduced chlorophyll content, increment polymorphic bands with ISSR	Cekic et al. (2017)

clarified. Faisal et al. (2013) reported PCD symptoms after different concentrations (0.025, 0.05, 0.1, 0.25, 0.5, 1, 1.5, 2 mg mL^{-1}) of nickel oxide NPs (NiO-NPs) treatment in tomato roots. The researchers indicated that NiO-NPs induced oxidative

stress, mitochondrial dysfunction, caspase-3-like activities, and antioxidant enzyme activation. The flow cytometric analyses revealed that NiO-NPs caused both apoptosis and necrosis depending on the application dose. The researchers concluded that NiO-NPs may trigger intrinsic pathway of apoptosis depending on the dose. Following this research Andón and Fadell (2013) put emphasis on nanomaterial-induced PCD in their review. The researchers tried to assess different pathways of cell death related to nanomaterial toxicity in living organisms. Faisal et al. (2016) revealed that with different concentrations (0.025, 0.05, 0.1, 0.25, 0.5, and 1 mg mL^{-1}) of cobalt oxide (Co_3O_4), NPs caused PCD due to ROS accumulation, DNA fragmentation, and mitochondrial disruption in eggplant roots. The researchers monitored the DNA fragmentation which is one of the most sensitive parameters of PCD by comet assay and flow cytometry. Besides vacuolization, mitochondrial damage and NO increase were also indicated. Yanik et al. (2017) reported PCD events in wheat roots after Al_2O_3 NPs treatment. The researchers observed defects in nucleus morphology, microtubule disorganization, loss of plasma membrane integrity, and chromosomal aberrations. Moreover, TUNEL-positive reaction which labels the 3′OH ends of DNA breaks and caspase-3-, caspase-8-, and caspase-9-like activities was also observed. In their previous study, the researchers also indicated vacuolization and DNA fragmentation revealed by agarose gel electrophoresis in wheat roots (Yanik and Vardar 2015). Although it is clear that NPs have potential effects on inducing PCD, execution and regulation of PCD also remain to be unclear.

7.5 Conclusion

The development of nanotechnology increased the usage of NPs which pose a risk of a new type of waste (nano-waste). They accumulate in the form of aggregates and/or colloids and contaminate the soil and water. Because of their sessile nature, plants are facing NPs' toxicity affecting their growth and development. Although some types of NPs have been proved to have a beneficial role in plant development, most types of NPs have been demonstrated to have a destructive role regarding induction of PCD in plants. The uptake, accumulation, and translocation of NPs by plants are still not clear. Besides, execution and regulation of NP-induced PCD also remain to be elucidated. The phytotoxicity mechanisms of NPs involved in the regulation and execution of PCD are important for crop yield stability, and up-to-date limited literature is available.

Considering the fact that NPs have exclusive properties, evaluating the effects of NPs on the environment and living organisms should be performed by assessing risk association with each type of NPs independently. For sustainable development of nanotechnology, it is important to understand the phytotoxic effects of NPs. A more comprehensive research needs to be designed to assess NPs' role in regulating and executing PCD that would be helpful for current and future research to progress the long-term effects of NPs on ecosystems.

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Metal-Based Nanomaterials and Oxidative Stress in Plants: Current Aspects and Overview

8

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8.1 Introduction

Nanotechnology is a new field of the technological sciences, dealing with materials at the nanometer scale (Whatmore 2006). The definition of nanomaterial (NM) has been changing throughout the years, being currently defined as "a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm" (Rauscher et al. 2014). Although nano-based products are found in the nature, nanotechnology only started to emerge as a promising science since the final years of the twentieth century, as a tool to face the massive dependence of properties (electronic, magnetic, optical, mechanical, etc.) on particle size and shape (Alkilany and Murphy 2010). Nowadays, nanotechnology occupies a pivotal place among the scientific community, given the global desire to produce materials with improved performance, which can be applied to different areas of knowledge, such as physical, chemical, biological, and health sciences (Arruda et al. 2015). Indeed, the current decade is already stated as the nano-era, where thousands of NMs are applied on several consumer and cosmetic products (Hansen et al. 2008). Actually, the application of nano-based products is also positively affecting different economy sectors, from pharmaceuticals and cosmetics to energy and agriculture businesses (Roco 2003; Nowack and Bucheli 2007). Based on estimations, in the year of 2013, there were more than 1600 available products containing NM, and it is predicted that this number will grow even more (Kurwadkar et al. 2014). Additionally, a previous reference suggested that nanotechnology market is expected to reach $30 billion in 2020, with an annual production of nano-based products of 58,000 tons (Gubbins

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_8

et al. 2011); however, more recent findings propose that the global market of nanoscience reached the $1 trillion mark in 2015. With effect, the USA is listed as the largest producer of nano-based products, but several European countries, like Germany, the UK, and Switzerland, are also present in this survey (Kurwadkar et al. 2014).

The fast-growing increased application of NM will inevitably lead to their accumulation across the major environmental matrices. Thereby, it is not surprising that, paired with the development of this innovative science, serious concerns about the possible fate and accumulation of NM in the environment have also become a reality among scientists. In fact, great controversy surrounding this issue has been generated, debating on the risks and benefits of NM's wide application (Agency 2007). It is now considered that risk assessment of NM should be performed as brief as possible, in attempts to minimize their potential hazards. The accumulation of NM in the environment is already a reality (Arruda et al. 2015; Tripathi et al. 2017a and references therein). The contamination of ecosystems by these nanomaterials can occur at different stages of their life cycle, from their production until their recycle, with short or long emissions. Once into the environment, NM can remain unchanged, but can also experience some modifications, changing their form, aggregation/agglomeration, chemical composition, and possible biological consequences. Accordingly, studies conducted at 2010 found out that the geometry of gold-containing NM mediates its interactions with biological systems (Albanese et al. 2010; Hutter et al. 2010). Given the widespread application of NM all over the world, different studies have already detected their presence in different environments, like water and soil (Gottschalk and Nowack 2011; Nowack et al. 2012; Sun et al. 2014), though not much is known about their fate and biological effects, especially regarding their interaction with living organisms, at the molecular and cellular levels (Barrena et al. 2009; Khot et al. 2012). Thus, aiming to clearly understand the mode of action of several engineered nanomaterials (ENM), as well as their potential toxic effects on ecosystems and human health, new research efforts are necessary and urgent.

The introduction of NM in the environment can occur by different ways and as a result of unintentional practices, such as atmospheric emissions and the runoff of different industrial wastes (Helland et al. 2008; Klaine et al. 2008; Bhatt and Tripathi 2011). However, the intentional delivery of NM on the environment could also become a reality. Actually, several remediation programs announce the direct application of different NM on the environment, for treating contaminated soils and waters. For instance, remediation of contaminated groundwater can be performed by nanoparticle treatment (Tratnyek and Johnson 2006; Klaine et al. 2008). Also, nanotechnology is already used for the detection of toxins in water and air (Dionysiou 2004). In this sense, soil is one of the main matrices affected by this problem, and NM can attain the soil by multiple ways. One of them is closely linked to the direct deposition of airborne NM, through pluviometric precipitation transport. Furthermore, according to Mueller and Nowack (2008), the application of biosolids in agricultural lands represents another main source of soil contamination by NM. Indeed, the presence of ENM has already been confirmed in urban sewage sludge, which has been commonly applied in agriculture practices. Regarding this

matter, data from a recent study reported that almost 30% of the total amount of NM released to the environment reached the soil through sludge application in Europe (Sun et al. 2014), and other significant part is attained by irrigation water. Taking into account this scenario, there is an increasing demand for quantifying NM levels in soil, along with the responsible evaluation of their ecotoxicity significance. At the moment, most of the available data regarding this issue is based on mathematical predictive estimations (Sun et al. 2014). Thus, although these reports provide valuable scientific information, their accuracy cannot be entirely assumed. In this way, improved procedures for quantification of NM in the environment should be investigated and properly implemented (Handy et al. 2008; Bour et al. 2015).

ENM, or manufactured nanomaterials, are one of the main classes of nanoparticles, with particular physicochemical properties, since these nanomaterials are specifically designed to a certain application. ENM can be subdivided in five classes: carbonaceous nanomaterials, metal oxide-based nanomaterials, semiconductors, metal-based nanomaterials, and nanopolymers (Handy et al. 2008; Monica and Cremonini 2009; Ma et al. 2010; Bhatt and Tripathi 2011), all of them with great applications in the global market. Indeed, different society sectors, including engineering, public health, and food industry, take advantage of these new technologies, applying them in a wide range of products, such as electronic gadgets, textiles, medical devices, cosmetics, and food packages (Biswas and Wu 2005; Monica and Cremonini 2009; Kurwadkar et al. 2014; Arruda et al. 2015). Among the different groups of ENM, the metal-based materials were classified as one of the main elements of nanoscience, largely due to their unique characteristics and their great potential applications. Indeed, different metal-based nanomaterials are available, differing in their chemical composition, size, shape, and crystalline structure. Among all, metal-based ENM of TiO_2, CeO_2, ZnO, and CuO are the most widely used and, perhaps by this reason, those for which there are more available data about their ecotoxicity.

Even though in the last years, the number of studies regarding the effects of ENMs in plants has been increasing, little is acknowledged about the potential phytotoxicity of the main groups of metal-based ENMs. Nevertheless, according to several references, these nanomaterials, as their bulk counterparts, can be uptaken by plants, inducing a wide range of metabolic responses that culminate in several physiological adjustments, including those related to tolerance and toxicity mechanisms. In this way, it is also suggested that these NMs can interfere with the normal cellular redox homeostasis, being able to induce or reduce the occurrence of oxidative stress (Tripathi et al. 2017a). Indeed, oxidative stress appears to be one of the commonest features of all types of abiotic and biotic stress, being regarded as a multifunctional event that takes place when the levels of reactive oxygen species (ROS, e.g., superoxide anion, $O_2^{\cdot -}$; hydrogen peroxide, H_2O_2; hydroxyl radical, OH·; oxygen singlet, $^1O_2$) exceed the cellular threshold. In order to avoid ROS-induced damage, plants possess a powerful antioxidant (AOX) system, efficient at removing and/or neutralizing ROS, by the employment of both enzymatic and nonenzymatic mechanisms (Gill and Tuteja 2010). Among all, particular importance is attributed to the activity of superoxide dismutase (SOD), which is

considered the first enzymatic line of defense against ROS, catalyzing the disruption of $O_2^{\cdot -}$ into H_2O_2 and water. Besides SOD, catalase (CAT) and ascorbate peroxidase (APX), both involved in the intracellular detoxification of H_2O_2, are also two of the most important AOX enzymes. Moreover, the involvement of several classes of peroxidases, such as APX, guiacol peroxidase (GPX), and other enzymes, like glutathione S-transferase and glutathione reductase, also play an important role against oxidative stress, contributing to the maintenance of the cellular redox balance. Regarding the nonenzymatic component, several low molecular weight molecules are included, being involved in the direct removal of ROS and/or serving as substrate for different AOX enzymes. Given their abundance and essential roles, special attention has been given to ascorbate (AsA), glutathione (GSH), and proline. Thus, it is the homeostatic balance that is established between the production of ROS and its removal by the AOX system that allows the maintenance of the cellular redox state, preventing the occurrence of oxidative damages that can induce injuries in proteins, nucleic acids, and lipids, ultimately culminating in cell death (Gill and Tuteja 2010; Sharma et al. 2012).

In this perspective, this chapter intends at bringing together the current knowledge about the relationship between metal-based NM and oxidative stress in plants. The following sections will review the findings of the main works in the nano-phytotoxicity area, shedding some light about the effects of the most used metal nanomaterials on plants. Furthermore, in order to facilitate and organize the data, a set of tables (Tables 8.1, 8.2, 8.3, 8.4, 8.5, and 8.6) was developed for each metal-based NM, where information regarding the particle size, the plant species, the exposure mode and time, as well as the main effects on the oxidative stress can be found.

8.2 Titanium Dioxide (TiO_2) Nanomaterials (Nano-TiO_2)

Nowadays, TiO_2 nanomaterials are among the most used, with innumerous applications in different economic sectors. Nano-formulations of this metal oxide can be found in personal care products, cosmetics, sunscreens, foodstuffs, and agrochemicals, such as nano-fertilizers and nanosensors (Shi et al. 2013; Cox et al. 2016; Grande and Tucci 2016). Moreover, nano-TiO_2 is also used as a bactericide agent and applied for wastewater treatment approaches. With effect, given its great industrial and economic dispersion, nano-TiO_2 levels in the environment are highly and quickly growing, accompanied by an increase in the studies regarding its potential phytotoxicity. According to several publications, the effects of nano-TiO_2 are mainly related to their nanostructure and not to the release of Ti^{4+} ions (Du et al. 2011; Conway et al. 2015). However, data regarding the phytotoxicity of this NM are consensual, with a great variability in what concerns (1) the dimension of the NM, (2) the experimental concentrations tested, (3) the plant species used, and (4) the exposure time and conditions. To the best of our knowledge, there have been studies conducted on monocot and dicot terrestrial plant species, as well as on some macrophytes (Table 8.1).

Table 8.1 Summary of nano-TiO_2-induced effects on the oxidative stress and antioxidant responses of different plant species

Particle size (nm)	Species	Exposure media	Exposure time	Concentration	Biomarkers	NOEC for MDA	LOEC for MDA	Oxidative stress	AOX system	Citation
30	*Hordeum vulgare* (barley)	Turf	21 days	5, 10, 20, 40, and 80 mg kg^{-1}	SOD, CAT, APX, GSH, and proline	–	–	–	↑	Doğaroğlu and Köleli (2017)
–	*Spinacia oleracea* (spinach)	Foliar spray	7 days	0.25%	SOD, CAT, POD, H_2O_2, and MDA	–	–	↓	↑	Hong et al. (2005)
21	*Triticum aestivum* (wheat)	Nutrient solution	20 days	5, 50, and 150 mg L^{-1}	Cell death, EL, MDA, and TAC			↓	–	Silva et al. (2017)
27	*Solanum lycopersicum* (tomato)	Soil	7 days	1000 and 5000 mg L^{-1}	SOD and TAC	–	–	–	↑	Song et al. (2013)
27	*Cucumis sativus* (cucumber)	Soil	150 days	250, 500 and 750 mg kg^{-1}	CAT and APX	–	–	–	↓↑	Servin et al. (2013)
10	*Allium cepa* (onion)	Nutrient solution	1 day	0.1, 1, 10, 100, and 1000 mg L^{-1}	MDA, CAT, GPX, APX, and GR	100 mg L^{-1}	>100 mg L^{-1}	=	=	Koce et al. (2014)
<30	*Hydrilla verticillata*	Nutrient solution	0, 24, 48, 96, and 168 h	0.1 and 10 mg L^{-1}	H_2O_2, GSH/GSSG, CAT, and GR	–	–	↑	↑	Spengler et al. (2017)

Green and red arrows represent positive and negative effects, respectively

Table 8.2 Summary of nano-CeO_2-induced effects on the oxidative stress and antioxidant responses of different plant species

Particle size (nm)	Species	Exposure media	Exposure time	Concentration	Biomarkers	NOEC for MDA	LOEC for MDA	Oxidative stress	AOX system	Citation
8	*Phaseolus vulgaris* (green bean)	Nutrient solution	15 days	62.5, 125, 250, and 500 mg L^{-1}	MDA, CAT, APX, and GPX	500 mg L^{-1}	>500 mg L^{-1}	↓	↑↓	Majumdar et al. (2014)
10–30	Green bean	Soil and foliar spray	15 days	250, 500, 1000, and 2000 mg L^{-1}	Proline, CAT, and POD	–	–	↑ (spray) = (soil)	↓ (spray) ↑ (soil)	Salehi et al. (2018)
–	*Lactuca sativa* (lettuce)	Soil	30 days	50, 100, and 1000 mg kg^{-1}	MDA, SOD, and POD	100 mg kg^{-1}	1000 mg kg^{-1}	↑	↓	Gui et al. (2015)
8	Tomato	Soil	210 days	62.5, 125, 250, and 500 mg kg^{-1}	CAT and APX	–	–	–	↓	Barrios et al. (2016)
8	*Coriandrum sativum* (coriander)	Soil	30 days	62.5, 125, 250, and 500 mg kg^{-1}	CAT and APX	–	–	–	↑↓	Morales et al. (2013)
231	*Oryza sativa* (rice)	Nutrient solution	10 days	62.5, 125, 250, and 500 mg L^{-1}	H_2O_2, EL, MDA, thiols, SOD, CAT, APX, GPX, DHAR, GR, and AsA	250 mg L^{-1}	500 mg L^{-1}	↑ (high doses) ↓ (low doses)	↑↓	Rico et al. (2013a)
231	Rice	Nutrient solution	10 days	62.5, 125, 250, and 500 mg L^{-1}	H_2O_2, EL, MDA, thiols, SOD, CAT, APX, GPX, DHAR, GR, and AsA	62.5 mg L^{-1}	125 g L^{-1}	↑ (high doses) ↓ (low doses)	↑↓	Rico et al. (2013b)
8	Wheat	Soil	8 months	100 and 400 mg kg^{-1}	MDA, SOD, and CAT	400 mg kg^{-1}	>400 mg kg^{-1}	↓	↑	Du et al. (2015)
10–30	*Arabidopsis thaliana*	Nutrient solution	5 days	250 and 1000 mg L^{-1}	$O_2^{\cdot -}$, H_2O_2, SOD, CAT, APX, POD, GST and GR	–	–	↑	↑	Ma et al. (2016)

10	*Zea mays* (maize)	Soil	20 days	400 and 800 mg kg^{-1}	Cell death, MDA, EL, H_2O_2, CAT, and APX	800 mg kg^{-1}	>800 mg kg^{-1}	=	↑	Zhao et al. (2012)
15–30	*Arabidopsis thaliana*	Nutrient solution	20 days	100, 200, 500, 1000, and 3000 mg L^{-1}	H_2O_2 and MDA	500 mg L^{-1}	1000 mg L^{-1}	↑	–	Yang et al. (2017)
25	Cucumber	Nutrient solution		0.2, 2, 20, 200, and 2000 mg L^{-1}	H_2O_2 and cell death	–	–	=	–	Ma et al. (2015)
8	*Raphanus sativus* (radish)	Soil	40 days	62.5, 125, 250, and 500 mg kg^{-1}	Phenols, Flavonoids, DPPH, TAC, FRAP, CAT, and APX	–	–	–	↑	Corral-Diaz et al. (2014)
8	*Helianthus annuus* (sunflower)	Soil	35 days	100, 200, 400, and 800 mg kg^{-1}	SOD, CAT, APX, and GR	–	–	–	=	Tassi et al. (2017)

Green and red arrows represent positive and negative effects, respectively

Table 8.3 Summary of nano-ZnO-induced effects on the oxidative stress and antioxidant responses of different plant species

Particle size (nm)	Species	Exposure media	Exposure time	Concentration	Biomarkers	NOEC for MDA	LOEC for MDA	Oxidative stress	AOX system	Citation
<100	Onion	Nutrient solution	4 h	25, 50, 75, and 100 mg L^{-1}	MDA	25 mg L^{-1}	50 mg L^{-1}	↑	–	Kumari et al. (2011)
44.46	*Fagopyrum esculentum*	Nutrient solution	5 days	1, 5, 10, 100, 1000, and 2000 mg L^{-1}	GSH and CAT	–	–	–	↑	Lee et al. (2013)
10	Maize	Soil	30 days	100, 200, 400, and 800 mg kg^{-1}	CAT and APX	–	–	–	↓	Zhao et al. (2013)
90	Maize	Soil	8 weeks	400, 800, 1600, and 3200 mg kg^{-1}	$O_2^{\cdot -}$ and SOD	–	–	↑	↑	Wang et al. (2016a)
10	*Pisum sativum* (pea)	Soil	25 days	125, 250, and 500 mg kg^{-1}	H_2O_2, MDA, CAT, and APX	250 mg kg^{-1}	500 mg kg^{-1}	↑	↓	Mukherjee et al. (2014)
15	Tomato and wheat	Nutrient solution	15 days	100 and 200 mg L^{-1}	H_2O_2, MDA, Proline, SOD, CAT, APX, and POD	<100 mg L^{-1}	100 mg L^{-1}	↑	↑	Amooaghaie et al. (2016)
<100	*Brassica nigra* (black mustard)	Nutrient solution	30 days	1, 5, 10, and 20 mg L^{-1}	Phenols, flavonoids, DPPH, TAC, and FRAP	–	–	–	↑	Zafar et al. (2016)

Green and red arrows represent positive and negative effects, respectively

Table 8.4 Summary of nano-CuO-induced effects on the oxidative stress and antioxidant responses of different plant species

Particle size (nm)	Species	Exposure media	Exposure time	Concentration	Biomarkers	NOEC for MDA	LOEC for MDA	Oxidative stress	AOX system	Citation
10–100	Lettuce and *Medicago sativa* (alfalfa)	Nutrient solution	15 days	5, 10, and 20 mg L^{-1}	CAT and APX	–	–	–	↑	Hong et al. (2015)
<50	Rice	Nutrient solution	31 days	2.5, 10, 50, 100, 1000 mg L^{-1}	MDA, Proline, AsA, SOD, and APX	1000 mg L^{-1}	>1000 mg L^{-1}	=	↑	Da Costa and Sharma (2016)
<50	Wheat	Sand	14 days	500 mg kg^{-1}	MDA, GSH, CAT, and POD	–	–	↑	↑	Dimkpa et al. (2012)
<50	Rice	Nutrient solution	14 days	0.5, 1.0 and 1.5 mM	$O_2^{\cdot-}$, H_2O_2, MDA, cell death, Proline, GSH, AsA, SOD, APX, GR, MDHAR, DHAR	<0.5 mM	0.5 mM	↑	↑	Shaw and Hossain (2013)
30	*Arabidopsis thaliana*	Nutrient solution	21 days	0.5, 1, 2, 5, 10, 20, 50, and 100 mg L^{-1}	$O_2^{\cdot-}$, H_2O_2, MDA, APX, and CAT	2 mg L^{-1}	5 mg L^{-1}	↑	↑	Nair and Chung (2014a)
20–40	*Arabidopsis thaliana*	Nutrient solution	96 h	20 and 50 mg L^{-1}	ROS and SOD	–	–	↑	↑	Wang et al. (2016a, b)
<50	*Arabidopsis thaliana*	Nutrient solution	14 days	10 mg L^{-1}	Gene expression	–	–	↑	↑	Landa et al. (2017)

(continued)

Table 8.4 (continued)

Particle size (nm)	Species	Exposure media	Exposure time	Concentration	Biomarkers	NOEC for MDA	LOEC for MDA	Oxidative stress	AOX system	Citation
50	*Glycine max* (soybean)	Nutrient solution	14 days	20, 50, 100, 200, 400, and 500 mg L^{-1}	H_2O_2, MDA, SOD, APX, and POD	50 mg L^{-1}	100 mg L^{-1}	↑	↑↓	Nair and Chung (2014b)
50	*Brassica juncea* (mustard)	Nutrient solution	14 days	20, 50, 100, 200, 400, and 500 mg L^{-1}	H_2O_2 and POD	–	–	↑	↑	Nair and Chung (2015)
50	*Brassica napus* (rapeseed)	Nutrient solution	14 days	20, 50, 100, 200, 400, and 500 mg L^{-1}	ROS, MDA, SOD, CAT, and APX	20 mg L^{-1}	50 mg L^{-1}	↑	↑	Nair and Chung (2017)
47	*Stevia rebaudiana* (Stevia)	Nutrient solution	27 days	0.1, 1, 10, 100, and 1000 mg L^{-1}	Phenols, Flavonoids, TAC, FRAP, and DPPH	–	–	–	↑↓	Javed et al. (2017)

Green and red arrows represent positive and negative effects, respectively

Table 8.5 Summary of nano-Ag-induced effects on the oxidative stress and antioxidant responses of different plant species

Particle size (nm)	Species	Exposure media	Exposure time	Concentration	Biomarkers	NOEC for MDA	LOEC for MDA	Oxidative stress	AOX system	Citation
47	*Brassica* sp.	Nutrient solution	–	1 and 3 mM	H_2O_2, $O_2^{\cdot -}$, CAT, and APX	–	–	↑	↑	Vishwakarma et al. (2017)
22.9	*Spirodela polyrhiza*	Nutrient solution	72 h	0.5, 1, 5, and 10 mg L^{-1}	ROS	–	–	↑	–	Jiang et al. (2017)
100	Tomato	Nutrient solution	14 days	10, 20, 40, and 80 mg L^{-1}	MDA, SOD, CAT, APX, and GR	<10 mg L^{-1}	10 mg L^{-1}	↑	↓	Çekiç et al. (2017)
25	Barley	Nutrient solution	21 days	0.5 and 1 mM	MDA, Phenols, and POD	<0.5 mM	0.5 mM	↑	↑	Fayez et al. (2017)
–	Cucumber	Nutrient solution	14 days	0.5 and 1 mM	H_2O_2, $O_2^{\cdot -}$, and MDA	<0.5 mM	0.5 mM	↑	–	Tripathi et al. (2017b)
79	*Lemma minor*	Nutrient solution	14 days	0.05, 0.130, 0.320, 0.800, and 2.00 mg L^{-1}	CAT, GPX, and GST	–	–	–	↑	Pereira et al. (2017)
17	Wheat	Nutrient solution	48 h	1.0 mg L^{-1}	H_2O_2 and MDA	–	–	↑	–	Li et al. (2016)
20	Pea	Nutrient solution	15 days	1 and 3 mM	H_2O_2, $O_2^{\cdot -}$, MDA, GSH, SOD, APX, and GR	<1 mM	1 mM	↑	↑↓	Tripathi et al. (2017c)

Green and red arrows represent positive and negative effects, respectively

Table 8.6 Summary of other metal-based NM-induced effects on the oxidative stress and antioxidant responses of different plant species

Nanomaterial	Particle size (nm)	Plant species	Exposure media	Exposure time	Concentration	Biomarkers	NOEC for MDA	LOEC for MDA	Oxidative stress	AOX system	Citation
Nano-Al_2O_3	22.9	*Arabidopsis thaliana*	Nutrient solution	10 days	98 μM	H_2O_2, MDA, SOD, CAT, and POD	–	–	=	=	Jin et al. (2017)
Nano-Al_2O_3	50	Onion	Nutrient solution	4 h	0.01, 0.1, 1, 10, and 100 mg L^{-1}	SOD	–	–	=	↑	Jiang et al. (2017)
Nano-$CoFe_2O_4$	17	Tomato	Nutrient solution	15 days	62.5, 125, 250, 500, and 1000 mg L^{-1}	CAT	–	–	–	↓	López-Moreno et al. (2016)
Nano-Fe_3O_4	40–53	*Fragaria* × *ananassa* Duch (strawberry)	Nutrient solution	–	0.08 and 0.8 mg L^{-1}	H_2O_2, MDA, SOD, and POD	–	–	↓	↑	Mozafari et al. (2017)
Nano-γ-Fe_2O_3	300	Rapeseed	Nutrient solution	5 days	0.5, 0.8, 1.0, and 2.0 mg L^{-1}	H_2O_2 and MDA	–	–	↓	–	Palmqvist et al. (2017)
Nano-Fe_3O_4	80	Mustard	Nutrient solution		500 mg L^{-1}	H_2O_2, MDA, SOD, CAT, and APX	–	–	↓	↓	Praveen et al. (2017)
Nano-NiO	100	Barley	Soil	14 days	87.8, 131.7, 197.5, 296.5, 444.4, 666.7, and 1000 mg kg^{-1}	H_2O_2, $O_2^{\cdot -}$, MDA, and cell death	<87.8 mg kg^{-1}	87.8 mg kg^{-1}	↑	–	Soares et al. (2016)
Nano-NiO	100	Barley	Soil	14 days	120 mg kg^{-1}	H_2O_2, $O_2^{\cdot -}$, MDA, thiols, AsA, proline, SOD, CAT, and APX	–	–	↑	↑↓	Soares et al. (2018)
Nano-NiO	<50	Tomato	Nutrient solution	10 days	0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 1.5, and 2.0 g L^{-1}	ROS, MDA, GSH, SOD, and CAT	0.25 g L^{-1}	0.5 g L^{-1}	↑	↑	Faisal et al. (2013)
Nano-NiO	<50	*Allium* sp.	Nutrient solution	6 h	10, 25, 50, 62.5, 125, 250, and 500 mg L^{-1}	MDA, SOD, CAT, and POD	<10 mg L^{-1}	10 mg L^{-1}	↑	↑	Manna and Bandyopadhyay (2017)

Green and red arrows represent positive and negative effects, respectively

The exposure of barley plants to increasing concentrations (0–80 mg kg^{-1}) of nano-TiO_2, with a medium size of 30 nm, resulted in an increase of SOD and CAT activities, but did not significantly altered APX levels, though a tendency for a decreased activity of this enzyme in response to nano-TiO_2 was observed. In what regards the nonenzymatic component, Doğaroğlu and Köleli (2017) have found that proline and GSH levels did not show a regular trend as concentrations increased, with an enhancement of proline content in the 5 mg kg^{-1} treatment and a decrease in GSH in both 5 and 40 mg kg^{-1} treatments. A positive response of the plant AOX system was also reported in spinach plants grown in the presence of 0.25% nano-TiO_2 (Hong et al. 2005). In this study, isolated chloroplasts were simultaneously exposed to nano-TiO_2 and light radiation, and the authors found that the NM was efficient at stimulating an AOX response, by the upregulation of SOD, CAT, and POD that, in turn, allowed the maintenance of redox homeostasis, reducing the levels of $O_2^{\cdot -}$, H_2O_2, and MDA over time and slowing the chloroplast aging. Recently, Silva et al. (2017) reinforced the idea that nano-TiO_2 (21 nm; 0–150 mg L^{-1}) effects are dependent on the organ, tested concentration, and time of exposure. The growth of wheat plants in the presence of nano-TiO_2 in hydroponics revealed that its effects were more pronounced in leaves than in roots, where lipid peroxidation and AOX activity were reduced. In the photosynthetic organs, although lipid peroxidation remained unchanged independently of concentration and time, an enhancement of AOX activity was observed for the highest nano-TiO_2 concentration. Other recent studies, performed in pumpkin fruit, tomato, and a macrophyte species, corroborate that this metal-based NM is able to modulate the plant AOX performance, by upregulating the activity of different AOX enzymes, such as CAT, GR, and SOD (Servin et al. 2013; Song et al. 2013). Furthermore, according to Spengler et al. (2017), TiO_2-based NM (<30 nm; 10 mg L^{-1}) did not change the levels of H_2O_2 in *Hydrilla verticillata*, but led to a decrease of GSH/GSSG ratio, suggesting the disturbance of the redox balance and the occurrence of oxidative stress that seemed to be enough to activate the response of the plant AOX system, stimulating the activities of CAT and GR. In contrast, Koce et al. (2014), using onion plants, did not detect any alteration in MDA levels nor AOX enzymes in response to the nano-TiO_2 (particle size 10 nm) treatments. However, it should be noted that the concentrations tested in this last study are much lower (1 mg L^{-1}) than those of the most part of the available studies.

8.3 Cerium Dioxide (CeO_2) Nanomaterials (Nano-CeO_2)

Cerium oxide nanomaterials (nano-CeO_2), along with nano-TiO_2, represent one of the most common nanomaterials currently used and applied to a variety of economic sectors (Keller et al. 2013; Andreescu et al. 2014). Apparently, the global spread of these NMs is tightly linked to their special properties, in terms of electrical, optical, and thermal features. CeO_2-based nanomaterials can be of great interest for environmental remediation practices, mechanical polishing, sensing, catalysis, and biomedicine, being used as catalysts, UV-radiation protectants, and polishing agents (Andreescu et al. 2014). According to a previous report, nano-CeO_2 production is

somewhere around 10,000 metric tons per year and is one of priorities of the Organisation of Economic Co-operation and Development (OECD), which considers the ecotoxicological relevance of this NM of particular importance in the present days. Indeed, nano-CeO_2 is known to be stable and insoluble in biological and environment systems, making them a persistent substance in different matrices. Moreover, knowing that nanoscience is only in the beginning of its expansion, nano-CeO_2 release to the environment is already—and will be even more—an issue of extreme importance, since the effects of these NMs on different types of organisms remain poorly understood.

Up to date, the relevance of nano-CeO_2 on plants has been moderately studied, though the largest number of studies just focused on biometric, growth, and productivity approaches, without exploring the effects of this NM at the physiological, biochemical, and molecular levels. Yet, when compared to other metal-based NM, nano-CeO_2 appears to be one of the most studied in plant systems, especially regarding its influence on the antioxidant metabolism (Zuverza-Mena et al. 2017 and references therein) (Table 8.2). Based on different bibliographic reports, the modulation of nano-CeO_2-induced responses in plants relies on distinct factors, including the exposure doses and the plant species (Zuverza-Mena et al. 2017).

According to a study conducted by Majumdar et al. (2014), *Phaseolus vulgaris* L. plants were able to tolerate nano-CeO_2 (8 nm; 0, 62.5, 125, 250, and 500 mg L^{-1}), by activating the main AOX defense mechanisms. In this work, the applied treatments led to differential responses between studied organs (roots, stems, and leaves) and the exposure period. After 7 and 15 days of growth, the MDA content, as an indicator of lipid peroxidation, was, generally, kept under the levels of the control, but the modulation of different AOX defenses was observed. At the 7th day, rises in APX and GPX activity were found in response to 250 and 125 mg L^{-1} nano-CeO_2 in roots and stems, respectively. However, after 15 days of exposure, nano-CeO_2 induced a negative response in CAT, APX, and GPX activities in roots, except in the 250 mg L^{-1} situation, where GPX was not affected by nano-CeO_2. Also, in leaves, APX was downregulated by 250 mg L^{-1} nano-CeO_2, while GPX activity was enhanced at 62.5 and 125 mg L^{-1}. Another study, also conducted with kidney bean, revealed that the plant responses to nano-CeO_2 (10–30 nm; 0, 250, 500 1000, and 2000 mg L^{-1}) were dependent on the given dose and the application method. In general, the foliar spraying of nano-CeO_2 was more toxic than the soil application (Salehi et al. 2018). *P. vulgaris* grown in the presence of this NM increased the accumulation of proline, an important AOX molecule, when nano-CeO_2 was foliar sprayed; however, the opposite behavior was detected when nano-CeO_2 was applied directly to the soil. The AOX enzyme activity was also modulated by treatments, though CAT and peroxidase did not show a regular trend when plants were foliar sprayed with this NM (CAT activity was always higher than the control for concentrations above 500 mg L^{-1}, where it reached the maximum value; peroxidase was diminished at the lowest and highest dose and enhanced at the 500 and 1000 mg L^{-1}). Regarding soil contamination, overall, both enzymes were efficient at scavenging H_2O_2 at the highest concentration, suggesting a positive response of the AOX system.

The dose-dependent effects of nano-CeO_2 on plant species were also observed when lettuce was exposed to concentrations ranging from 0 to 1000 mg kg^{-1} nano-CeO_2 (Gui et al. 2015). At low concentrations (100 mg kg^{-1}), nano-CeO_2 had a beneficial effect on the AOX system, namely, in the activities of SOD and POD, which helped to maintain the levels of MDA unchanged from the control. However, at 1000 mg kg^{-1}, nano-CeO_2 highly inhibited the activity of these two AOX enzymes, imposing the occurrence of oxidative stress by the enhancement of MDA content (Gui et al. 2015). In line with these results, the inhibition of AOX enzyme activity was previously reported for different plants (Morales et al. 2013; Rico et al. 2013a, b; Barrios et al. 2016), though there are also a plenty number of records showing a positive correlation between nano-CeO_2 treatments and AOX metabolism (Zhao et al. 2012; Morales et al. 2013; Rico et al. 2013a; Du et al. 2015; Ma et al. 2015). For instance, Du et al. (2015) reported that the exposure of wheat to 400 mg kg^{-1} (the highest applied concentration) nano-CeO_2 (8 nm) gives rise to an increase in SOD and CAT activities, which prevented the high levels of $O_2^{\cdot -}$ and H_2O_2, respectively, and thus limited occurrence of lipid peroxidation. This upregulation of the enzymatic AOX system was also described by Ma et al. (2016), using *Arabidopsis thaliana* as a model species. In this work, the authors found that nano-CeO_2 (30 nm) applied at 250 and 1000 mg L^{-1} led to increased activities up to 50% of SOD, CAT, POD, glutathione *S*-transferase (GST), and GR, especially under the highest treatment. Nevertheless, in this case, the activation of the plant AOX system was not totally efficient at ROS scavenger, since H_2O_2 and $O_2^{\cdot -}$ remained higher than the control (Ma et al. 2016). Similar results for H_2O_2 content in the same plant species were described elsewhere (Yang et al. 2017). Contrasting findings were observed for rice plants under nano-CeO_2 (8 nm; 0–500 mg L^{-1}) contamination, where levels of H_2O_2 were always lower or identical to the control situation in both leaves and roots (Rico et al. 2013b). Also in cucumber, the levels of H_2O_2, along with cell death, did not change in response to nano-CeO_2 (25 nm; 0–200 mg L^{-1}) (Ma et al. 2015).

The modulation of plant physiology by nano-CeO_2 was also investigated in *Coriandrum sativum*, by the evaluation of CAT and APX activity (Morales et al. 2013). The response of CAT and APX was different in roots and shoots—CAT activity was enhanced in shoots but repressed in roots (at 250 and 500 mg kg^{-1}); APX activity was greatly inhibited in the aerial parts and only stimulated in roots exposed to 125 mg kg^{-1}. Based on these results, Morales et al. (2013) concluded that nano-CeO_2 led to the establishment of oxidative damage, since CAT and APX were significantly affected by different concentrations in both organs. Plants of *Raphanus sativus* cultivated in soil treated with nano-CeO_2 (0, 62.5, 125, 250 and 500 mg kg^{-1}) did not change the levels of nonenzymatic AOX, such as flavonoids and total phenols in both tubers and shoots. However, nano-CeO_2 (250 mg kg^{-1}) significantly affected the antioxidant capacity of tubers, evaluated in terms of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reducing potential, ferric reducing antioxidant power (FRAP), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\cdot -}$) AOX potential, with increases up to 85% in relation to the control. CAT and APX activities were also increased in tubers and leaves exposed to 125 and 500 mg kg^{-1}

nano-CeO_2, respectively (Corral-Diaz et al. 2014). Furthermore, these authors hypothesize that the higher activity of CAT and APX results from nano-CeO_2-mimetized SOD activity, leading to a higher H_2O_2 content to be detoxified by these enzymes (Corral-Diaz et al. 2014). However, according to Tassi et al. (2017), nano-CeO_2 (particle size 8 nm; 100, 200, 400, and 800 mg kg^{-1} soil) was not able to induce any change in SOD, CAT, APX, and GR activities in sunflower grown in contaminated soil for 35 days. Based on the work of Zhao et al. (2012), in which *Zea mays* plants were exposed to 400 and 800 mg kg^{-1} nano-CeO_2 (10 nm) for 20 days, the levels of H_2O_2 were increased in both concentrations after 10 days of exposure, but these differences in relation to the control were reduced and totally disappeared at the end of the assay. The authors suggest that this pattern can be related to an adaptive response of corn plants against nano-CeO_2. In line with this hypothesis, lipid peroxidation was not changed throughout the experiment, and CAT and APX activity were only increased after 10 days of exposure (Zhao et al. 2012).

8.4 Zinc Oxide (ZnO) Nanomaterial (Nano-ZnO)

Nano-sized zinc oxide form (nano-ZnO) represents one of the largest applications of nanoindustry, being extensively used in the areas of cosmetics, textiles, and dermatology. Given its ability to absorb UV radiation, it is expected that the application of zinc oxide nanomaterial (nano-ZnO) will exceed that of other NM, and are already being used in the manufacture of fabrics to avoid bad odors, given their antimicrobial properties. In addition, ZnO NM are also applied in ceramics, rubber processing, and wastewater treatment facilities (Ghodake et al. 2011). Overall, it is estimated that nano-ZnO production will exceed 500 tons/year, with more than 300 companies involved in its production. As a result, the increased use of these NMs inevitably promotes their release and subsequent accumulation in the environment (Kumari et al. 2011).

Zn is an essential micronutrient, and so in moderate amounts, it is beneficial for all organisms including plants but, when present in excess, can be phytotoxic. Zn-induced toxicity is associated with inhibition of growth and interference in several metabolic processes, being capable of inducing oxidative stress, compromising the redox state of the plant (Tsonev and Lidon 2012). Although toxicity mechanisms of metal-based nanomaterials are dependent on its features as particles (e.g., size and shape), it is recognized that, once inside plant cells, some of their effects and toxicity are identical to their bulk counterparts and zinc salts (Ma et al. 2013; Soares et al. 2018). In the case of nano-ZnO, it seems that the nano-sized oxide induces more toxicity/stress compared to bulk-ZnO (Mukherjee et al. 2014; Amooaghaie et al. 2016), and it was also found that individual Zn NMs may form secondary-sized NMs (aggregates) in the cell that could have a more toxic effect than that of the individual ZnO NMs (Lee et al. 2013). Despite all this, the toxicity of ZnO NMs may be mediated by multiple mechanisms or modes of action, including the incorporation of NMs, accumulation in root surface and root tissues, and dissolution of Zn ions from MPs along with other physicochemical properties as well as

exposure conditions (Ma et al. 2011, 2013). Nevertheless, depending on plant species, ZnO NMs can cause both negative and positive effects on plant metabolism, namely, they affect plant architecture, physiology, and biochemistry (Zafar et al. 2016) as documented by reports about the interaction of Zn-based NMs with plant systems (Kumari et al. 2011; Prasad et al. 2012; Zhao et al. 2013; Mukherjee et al. 2014; Wang et al. 2016a).

One of the primary negative effects of plant exposure to ZnO NMs is the enhanced production of ROS (Table 8.3). The phytotoxic effects of ZnO NMs have been evaluated in several plants including rape, radish, ryegrass, lettuce, cucumber (Lin and Xing 2007), zucchini (Stampoulis et al. 2009), garden cress and broad bean (Manzo et al. 2011), wheat (Du et al. 2011; Amooaghaie et al. 2016), tomato (Amooaghaie et al. 2016), *Allium cepa* (Kumari et al. 2011), buckwheat (Lee et al. 2013), *Pisum sativum* (Mukherjee et al. 2014), *Brassica nigra* (Zafar et al. 2016), and *Zea mays* (Lin and Xing 2007; Zhao et al. 2013; Wang et al. 2016a), but only some of these studies have focused at determining the impact of ZnO NMs on production of ROS and antioxidant response. Kumari et al. (2011) demonstrated that exposure of root cells of *A. cepa* to ZnO NMs (particle size <100 nm; 0, 25, 50, 75 and 100 μg mL^{-1}) caused cytotoxicity and genotoxicity and concluded that ZnO NMs were more toxic than bulk ZnO, though not due to the dissolved zinc ions alone, but probably by the presence of the nanoparticles/aggregates. These researchers also showed a direct relationship between the increase in the concentration of Zn NMs and TBARS formation, used as indicator of lipid peroxidation in consequence of oxidative stress, suggesting that the higher phytotoxicity of ZnO NMs when compared to zinc ions was due to a higher ROS generation and oxidative damage of biological membranes. When buckwheat plant (*Fagopyrum esculentum*) was exposed to Zn NMs (nano-ZnO: particle size of 44.46 ± 4.84 nm and ZnO MPs particle size 2~5 μm; 0, 1, 5, 10, 100, 1000, and 2000 mg L^{-1}) (Lee et al. 2013), an increase in GSH and CAT activity was detected compared to control situation, but at higher doses of ZnO NMs (1000 and 2000 mg L^{-1}), GSH concentration and CAT level were lower than that recorded at low NM doses (1–100 mg L^{-1}). The authors explained these results based on the excessive ROS formation and release in response to the treatment with high doses of ZnO NMs. Similarly, Zn NM (particle size 10 nm; 400 mg L^{-1}) caused reduced CAT and APX activity in corn-treated plants compared with controls (Zhao et al. 2013). A study using the same species found a rise in superoxide free radical ($O_2^{-\cdot}$) at Zn NM (particle size 90 ± 10 nm) doses ranging from 400 to 3200 mg kg^{-1} that was accompanied by a significant increase in SOD activity at the highest concentration (Wang et al. 2016a). It was also evidenced the importance of plant root arbuscular mycorrhizal fungi (AMF) symbiosis in the rhizosphere, since AMF helped to alleviate the phytotoxicity induced by nano-ZnO by decreasing ROS production while increasing antioxidant capacity. The treatment of *Pisum sativum* with nano-ZnO (particle size 10 nm; 0, 125, 250, and 500 mg kg^{-1}) in organic matter-enriched soil led to reduced CAT and APX activity at all NM concentrations, while at 500 mg kg^{-1} treatment, induced more ROS (H_2O_2) and lipid peroxidation (Mukherjee et al. 2014). Recently, Amooaghaie et al. (2016) reported that the exposure of tomato and wheat to Zn-based NM (Zn NM particle size 25 nm and ZnO NM particle size 15 nm) also

increased MDA content and accumulation of H_2O_2, consequently causing significant oxidative stress. In addition, NM treatments led to a rise in proline and enhancement of antioxidant enzyme activities, including POD, SOD, APX, and CAT, contributing to the maintenance of the redox homeostasis and the integrity of cellular components. The effect of ZnO NM (particle size <100 nm; 0, 1, 5, 10, and 20 mg L^{-1}) was also studied in *Brassica nigra* stem explant culture (Zafar et al. 2016). The presence of NM in the culture medium resulted in elevated DPPH radical scavenging activity, total antioxidant and reducing power potential, as well as increased levels of total phenolics and flavonoids.

8.5 Copper Oxide (CuO) Nanomaterial (Nano-CuO)

Similarly, to Zn, copper (Cu) is an essential nutrient for plants, being necessary for growth and development, performing important functions in photosynthesis, mitochondrial respiration, ethylene sensing, reactive oxygen metabolism, protein trafficking, hormone signaling, and cell wall remodeling (Burkhead et al. 2009). Plants need only trace amounts of Cu, and its increased concentrations are toxic for them. In fact, exposure of plants to excess Cu results in chlorosis, necrosis, stunting, and inhibition of root and shoot growth (Yruela 2009). The redox property of Cu also contributes to its toxicity, since as a redox-active transition element, Cu can catalyze the overproduction of ROS by Haber-Weiss or Fenton reactions (Halliwell and Gutteridge 1984), resulting in oxidative stress injury. Due to the important role of Cu in the growth and development of plants, several studies were done focusing on the evaluation of the effects of exposure to excessive or insufficient levels of Cu (Adrees et al. 2015 and references therein; Yruela 2009), and in recent years, significant research focused on studying the effects of Cu-based NM in plants have gained enormous importance.

Copper (nano-Cu) and copper oxide nanomaterials (nano-CuO) are widely used in the solar cells and lithium-ion batteries, lubricant oils, polymers, inks/ceramic pigments, gas sensors, catalysts, and electronics (Anjum et al. 2015). Additionally, due to its antimicrobial properties, its use has been intensifying in recent years in agricultural practices, where they are used in formulations of fungicides, pesticides, and herbicides. Regarding this procedure, Cu-based NMs were proved to be more effective against pathogenic fungi than the corresponding bulk forms, thus allowing the application of lower doses of Cu in Crop Prot (Giannousi et al. 2013). Concerning the mechanisms of the potential nanotoxicity of Cu-based nanomaterials, there are no conclusive studies, with reports showing a diversity of data (Table 8.4). In fact, certain reports attribute the toxicity of nano-CuO to the nanosize-specific effects, whereas some studies explained toxicity as a direct effect of the released Cu^{2+} ions (Landa et al. 2017 and references therein). On the other hand, comparative studies aimed at discriminating the effects of metal and metal oxide NMs with the corresponding ionic form or bulk particles are limited. Regarding higher plants, according to the literature published to date, the size effects combined with the effect of ionic Cu^{2+} seem to be responsible for the observed

toxicity; however, the available results are ambiguous. For instance, Dimkpa et al. (2012) reported that nano-CuO were more toxic compared with bulk particles to wheat cultivated in sand growth matrix and concluded that the released Cu^{2+} were only partly responsible for the toxicity of the NM. Recently, Wang et al. (2016b) showed higher growth inhibition of *A. thaliana* seedlings caused by nano-CuO (20–40 nm) than by ionic Cu^{2+}, whereas the effect of bulk particles was much lower. Studies conducted with maize (Wang et al. 2012) also showed that CuO NM (20–40 nm) at 100 mg L^{-1} induced visible chlorosis and had significant inhibition on seedling growth; however, no equivalent phytotoxicity of the dissolved Cu^{2+} or corresponding bulk particles was evident. On the other hand, Perreault et al. (2014) showed increased toxic effects of nano-CuO (particle size 97 nm) in *Lemna gibba* (duckweed), mainly due to particle solubilization into toxic metal ions.

As it was already reported, the uptake of NM by plants depends on the properties of NM, the dispersion conditions, and the tested plant species (Wang et al. 2011), cell walls being the primary site for interaction and a barrier for the entrance of MN into the cells (Masarovičová and Kráľová 2013). According to Le Van et al. (2016), in roots of cotton, most of the nano-CuO aggregates were found on the root outer epidermis, and only a small amount was detected in intercellular spaces. In turn, Wang et al. (2012) showed that nano-CuO (particle size 20–40 nm) in maize roots could pass through the epidermis and cortex, reaching the stele, and also demonstrated root−shoot−root redistribution of CuO NMs within maize. After entering root cells, nano-CuO or Cu compounds and the released Cu ions may cause ROS formation and accumulation, thus resulting in oxidative damage. An increasing number of studies clearly show evidences of Cu-based NM toxicity via interference with ROS production, oxidative stress, and activation of the main AOX defense mechanisms. For example, Hong et al. (2015) found that when lettuce and alfalfa plants were exposed to Cu-based NM or compounds (particle size 10–100 nm; 0, 5, 10, and 20 mg L^{-1}), CAT was reduced in alfalfa but was not affected in lettuce, whereas APX activity increased in roots of both species, without effects on shoots. Oxidative stress in nano-CuO (particle size <50 nm)-treated wheat plants was also evidenced by increased lipid peroxidation and oxidized glutathione with significant higher peroxidase and catalase activities (Dimkpa et al. 2012). Exposure of rice to CuO NMs (particle size <50 nm; 0, 2.5, 10, 50, 100, and 1000 mg L^{-1}) caused increased proline and ascorbate levels but showed negligible effect on lipid peroxidation, while gene expression of antioxidant enzymes (APX and SOD) increased with nano-CuO concentration. In another study (Shaw and Hossain 2013) also using rice exposed to nano-CuO (particle size <50 nm; 0, 1.0 and 1.5 mM), the researchers found significant oxidative stress with higher levels of MDA and H_2O_2, but with enhanced levels of ascorbate and proline as well as antioxidant enzyme activity. Similarly, *A. thaliana* exposed to CuO NMs (particle size 30 nm; 0, 0.5, 1, 2, 5, 10, 20, 50, and 100 mg L^{-1}) responded through the production of excess ROS (H_2O_2 and superoxide), enhanced levels of MDA and proline, as well as upregulation of genes coding for enzymatic and nonenzymatic antioxidant defense mechanisms (Nair and Chung 2014a). Increased ROS generation and significant induction of genes related to oxidative stress responses were also

observed in *A. thaliana* by others (Wang et al. 2016b, particle size 20–40 nm at 0, 20, and 50 mg L^{-1}; Landa et al. 2017, particle size <50 nm at 10 mg L^{-1}). In addition, nano-CuO (particle size 50 nm; 0, 50, 100, 200, 400, and 500 mg L^{-1}) also caused significant oxidative stress in *Glycine max* (Nair and Chung 2014b), *Brassica juncea* (Nair and Chung 2015), and *Brassica napus* (Nair and Chung 2017), with higher ROS and MDA content, but with variable responses of the antioxidant enzymes—while some of them showed increased activity, no change or even inhibition of enzyme activity was observed. The effect of CuO NMs (particle size 47 nm; 0, 0.1, 1.0, 10, 100, and 1000 mg L^{-1}) on oxidative stress response has also recently been studied in in vitro-grown medicinal plant *Stevia rebaudiana* (Javed et al. 2017). These researchers observed an enhanced production of antioxidant molecules (phenolics and flavonoids) up to 10 mg L^{-1} of NPs, but with a higher level of phytotoxicity at 1000 mg L^{-1} of CuO NMs.

8.6 Silver Nanomaterial (Nano-Ag)

Along with other metals, silver (Ag) nanomaterial (nano-Ag) is gaining particular attention due to its unique features, like antibacterial properties. Currently, nano-Ag can be found in a great variety of products of different commercial sectors, such as cosmetics, pharmaceuticals, food technology, and wastewater treatments (Boxall et al. 2008; Rai et al. 2009; Wijnhoven et al. 2009). Based on estimations of 2012, nano-Ag production reached the mark of 2500 tons per year only in the United States of America, with more than 200 tons ending up in the environment (Khaydarov et al. 2009; El-Temsah and Joner 2012). In contrast to other metal-based NM, nano-Ag has a high solubility in water, potentially aggravating its deleterious effects on different biota. Indeed, previous works unequivocally demonstrated that nano-Ag exposure can induce toxicity at multiple levels to different types of organisms (Jiang et al. 2017 and references therein). Moreover, it is well established that nano-Ag toxicity is not only dependent on the release of Ag^{+} ions (Zhao and Wang 2011; Kaveh et al. 2013) but also on the shape and size of the NM and their ability to induce oxidative damage (Choi and Hu 2008; Kim et al. 2012; Yin et al. 2013; Gorka et al. 2015; Osborne et al. 2015; Sun et al. 2016). However, regarding plants, only limited data is available concerning the interaction between nano-Ag and the oxidative status of plant cells (Li et al. 2016; Çekiç et al. 2017; Jiang et al. 2017; Pereira et al. 2017; Tripathi et al. 2017b; Vishwakarma et al. 2017) (Table 8.5). From the available data, increased ROS production seems to be one common feature of nano-Ag phytotoxicity (Vishwakarma et al. 2017). Indeed, the treatment of an aquatic plant (*Spirodela polyrhiza*) with different concentrations of Ag (0, 0.5, 1, 5, and 10 mg L^{-1}) for 72 h led to a higher generation of ROS, as a result of Ag+ internalization in both bulk and nano-exposures (Jiang et al. 2017). The exposure of tomato plants to nano-Ag (particle size 100 nm; 0, 10, 20, 40 and 80 mg L^{-1}) caused both oxidative and genotoxic damages (Çekiç et al. 2017). In this study, after 2 weeks of exposure, plants exhibited higher MDA levels up to 80%, accompanied by a downregulation of SOD, CAT, and APX activities for almost treatments,

especially in the higher concentrations tested. In line with this, nano-Ag (particle size 25 nm; 0, 0.5, and 1 mM) treatment also impaired the redox homeostasis of barley plants, by an overproduction of MDA up to 107%. In this case, the effect of nano-Ag was even more harmful than the dissolved ions, which only caused an increase of 26% in lipid peroxidation in relation to the control (Fayez et al. 2017). However, contrasting findings were reported by Tripathi et al. (2017b), where *Cucumis sativus* L. growth and oxidative homeostasis were more impaired by $AgNO_3$ than by nano-Ag. By employing histochemical and spectrophotometric methods, these authors reported that both 500 and 1000 μM bulk and nano-sized Ag (20 nm) induced the accumulation of ROS (H_2O_2 and $O_2^{\cdot -}$), as well as an increase of lipid peroxidation, though the effects were more pronounced in response to $AgNO_3$. Another study, conducted in *Brassica* sp., also revealed that nano-Ag was lesser toxic than its bulk counterpart. Indeed, although both nano-Ag and $AgNO_3$ led to oxidative damages, the effects of nano-Ag were not so evident, since plants exposed to the NM accumulated less Ag in their tissues, and the AOX system performance was more efficient than those exposed to $AgNO_3$ (Vishwakarma et al. 2017). The involvement of the AOX system in the plant responses against nano-Ag was also studied in *Lemma minor*, by the evaluation of GPX, GST, and CAT activities (Pereira et al. 2017). In this study, the exposure of *L. minor* to nano-Ag (particle size 79 nm; 0, 0.05, 0.130, 0.320, 0.800, and 2.0 mg L^{-1}) resulted in an increase of GPX activity throughout all treatments. However, data concerning the activity of CAT indicated that this enzyme was not changed in response to nano-Ag, and GST was only activated in the highest concentration tested. Moreover, this study also concluded that Ag^+ was more toxic than nano-Ag, supporting some of the above-cited references.

From a different perspective, Li et al. (2016) tried to understand the possible interaction between extracellular polymeric substances (EPS), produced by different bacteria, and nano-Ag (particle size 17 nm; 0, 0.5, 1.0, 1.9, 3.3, and 9.2 mg L^{-1}) on the physiological performance of wheat plants. Based on their results, it was possible to recognize that nano-Ag-induced phytotoxicity was greatly alleviated by EPS treatment, reducing the levels of lipid peroxidation and H_2O_2 and contributing to a higher tolerance of plants to Ag-based NM. From a parallel angle, the potential of nitric oxide (NO) for enhancing plant tolerance to nano-Ag was recently investigated (Tripathi et al. 2017c). As expected, nano-Ag (particle size 20 nm; 1000 and 3000 μM) disrupted the redox homeostasis of *P. sativum*, by an overproduction of H_2O_2 and $O_2^{\cdot -}$ and an upsurge of LP. However, upon NO co-treatment, this negative effect was strongly counteracted, with a significant reduction of the Ag-induced oxidative damage, most likely due to its influence on the AOX system (SOD, APX, GR, ascorbate, and GSH), whose efficiency was much more pronounced in response to both NO and nano-Ag treatments.

8.7 Other Metal-Based Nanomaterials

Besides the abovementioned NM, other nano-sized metals, such as nickel (Ni), aluminum (Al), and iron (Fe), are also in the front line of nanotechnology applications. However, their molecular and physiological interactions with plant systems are less explored than those of Ce, Ti, Zn, Cu, and Ag. Nevertheless, in this section, we will summarize the principal findings concerning the effects of these NMs on the redox balance and homeostasis of different plant species (Table 8.6). As reported for the other classes of metal-based NM, the phytotoxicity and/or beneficial effects of Ni-, Al-, and Fe-based NM are dependent on several factors, including the particle size and the release of the metal ions.

Although Al is known for its high phytotoxicity under acidic soils, the beneficial effects of its nano-sized counterpart (aluminum oxide NM—nano-Al_2O_3) were recently explored by Jin et al. (2017), by treating *Arabidopsis thaliana* roots with 98 μM nano-Al_2O_3 (particle size 22.9 nm) and with an Al salt at the equivalent molar concentration ($AlCl_3$, 196 μM). According to their results, the exposure of *Arabidopsis* roots to nano-Al_2O_3 did not trigger a significant oxidative damage (MDA and H_2O_2 levels) nor changed the AOX response (POD, SOD, and CAT), even contributing to a higher growth rate in terms of root length and weight. However, although these authors suggest that nano-Al_2O_3 can be a potential tool for use in agriculture and biotechnology, especial caution must be given to this issue, since Al is known for its high phytotoxicity. Moreover, contrast findings to the ones of Jin et al. (2017) were reported for onion plants exposed for 4 h to a solution of nano-Al_2O_3 (particle size 50 nm) at 0, 0.01, 0.1, 1, 10, and 100 mg L^{-1} (Rajeshwari et al. 2015), in which nano-Al_2O_3 led to higher activities of SOD in a dose-dependent manner, accompanied by an upsurge of chromosomal aberrations and a decrease of mitotic cell index. However, it should be stressed that these two contrasting works were performed in different plant species, under different experimental conditions, and testing different concentration ranges; hence, no extrapolations regarding the effects of nano-Al_2O_3 can be made without further studies.

Iron-based NMs (nano-FeOx) are one of the most applied compounds in the environment sciences, since as part of several formulations, these NMs are important scavengers of different metals, such as As, Cr, Cd, Zn, Pb, and Cu. Moreover, it has been postulated that, generally, FeOx NMs are nontoxic to the environment, since these NMs are narrowly uptaken by plants with a minimal translocation for the aerial parts (Tripathi et al. 2017a and references therein). Yet, in the last years, several studies have been conducted to uncover the physiological consequences of nano-FeOx on plant systems. As its bulk counterpart, it seems that nano-FeOx is able to modulate plant's AOX metabolism, as reported by López-Moreno et al. (2016), whose work demonstrated that the exposure of tomato plants to $CoFe_2O_4$ NM (particle size 17 nm; 0, 62.5, 125, 250, 500, and 1000 mg L^{-1}) resulted in an inhibition of CAT activity throughout treatments. The response of the plant AOX system under nano-Fe_3O_4 was investigated using strawberry (*Fragaria* × *ananassa* Duch.) plants submitted to drought stress (Mozafari et al. 2017). In this case, when

drought-stressed plants were treated with nano-Fe_3O_4 (40–53 nm; 0.0, 0.08, and 0.8 mg L^{-1}), the enzymatic activity of POD and SOD was significantly higher than that of plants not treated with Fe, along with a reduction of lipid peroxidation and H_2O_2 levels. Thus, it seems that the exogenous application of nano-Fe_3O_4 was efficient at mitigating drought-induced oxidative stress in *Fragaria*, by the upregulation of the AOX performance and the reduction of the oxidative damage. Equivalent findings were reported by the team of Palmqvist et al. (2017), which evaluated the potential of maghemite (γ-Fe_2O_3) NM (0, 0.5, 0.8, 1.0, and 2.0 mg L^{-1}) supplementation to overcome the effects of drought in soil-grown *Brassica napus*. Furthermore, the metal chelating ability of iron-based NM was recently explored in *Brassica juncea* exposed to high levels of As (150 μM) for 96 h. Through the assessment of both oxidative stress markers and the AOX enzymatic response, this work helped to support the potential of nano-Fe_3O_4 (80 nm; 500 mg L^{-1}) to enhance plant tolerance to abiotic stress, such as metal excess. Indeed, based on the results of H_2O_2 and MDA quantification, it was possible to observe that the co-treatment of plants with As and nano-Fe_3O_4 led to a lower oxidative damage than those of plants growing in the absence of the NM. Furthermore, since it was observed that the co-treatment with nano-Fe_3O_4 led to a reduction of the activities of SOD, CAT, and APX compared to the As treatment alone, the authors suggested that the protective role of this NM may be related to the restriction of As uptake and mobility inside plant tissues (Praveen et al. 2017).

Lastly, nickel oxide nanomaterial (nano-NiO) remains as one of the less studied NMs, with only a few available records regarding its toxicity to plants. However, there is strong evidence that nano-NiO releases more Ni ions than its bulk material, hence aggravating its potential hazards (Horie et al. 2011). To the best of our knowledge, the work of Faisal et al. (2013) was the first one to study the detrimental effects of nano-NiO (particle size <50 nm; 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 1.5, and 2.0 g L^{-1}) on the early development of *Solanum lyopersicum* seedlings. The exposure of tomato to increased concentrations of nano-NiO led to significant increases of CAT and SOD activity and GSH levels, but the response of the plant AOX system was not fully sufficient to minimize the oxidative damage, since LP remained higher than the control situation. Moreover, the detection of ROS by flow cytometry revealed the generation of ROS was enhanced from concentrations above 0.250 mg L^{-1}, reaching the maximum at the highest applied dose (2.0 mg L^{-1}). Accordingly, Soares et al. (2016) also reported that increasing concentrations of nano-NiO (particle size 100 nm; 0, 87.8, 131.7, 197.5, 296.5, 444.4, 666.7, and 1000 mg kg^{-1}) resulted in a severe oxidative imbalance in barley plants, with significant boosts in $O_2^{\cdot -}$ and MDA levels in dose-dependent manner, accompanied by the stimulation of programmed cell death and the decrease of plant growth. The same pattern was already described for different *Allium* species exposed to a range of nano-NiO (particle size <50 nm) concentrations (0, 10, 25, 50, 62.5, 125, 250, and 500 mg L^{-1}) (Manna and Bandyopadhyay 2017). By assessing different oxidative stress-related parameters, it was possible to perceive that nano-NiO increased the lipid peroxidation in all of the studied species, thus triggering the occurrence of oxidative damage, even though the activities of SOD, CAT, and POD were generally

increased among treatments. In the same way, nano-NiO negatively affected the growth performance and the cellular redox homeostasis of barley plants grown for 14 days under nano-NiO (particle size 100 nm; 120 mg kg^{-1}) soil contamination (Soares et al. 2018). This work combined different approaches and provided robust data regarding the cross talk between the generation of ROS and the response of the enzymatic and nonenzymatic AOX system. Plants growing under nano-NiO stress showed an overproduction of superoxide anion ($O_2^{\cdot -}$), which favored the occurrence of oxidative stress and the enhancement of lipid peroxidation (LP). Regarding the AOX defense response, nano-NiO induced the accumulation of proline but led to a higher oxidation of AsA in leaves. These authors also observed that nano-NiO was able to increase SOD activity and that CAT and APX had differential responses between organs, with CAT being more active in leaves and APX in roots.

8.8 Concluding Remarks

Tables 8.1, 8.2, 8.3, 8.4, 8.5, and 8.6 summarize all the data provided by the studies reviewed in this chapter. Several remarks are easily highlighted when data is compiled, what can be useful to guide future studies aimed in assessing the risks of metal-based NM. Up to date, the studies addressing the phytotoxicity of NM have almost exclusively targeted species with agronomic interest, and it was demonstrated that the sensitivity to the NM is species-dependent. Although this approach is clearly justified by the easier acquisition of seeds, easier seedling, and growth in the laboratory, in parallel with the anthropogenic and economic importance of the species, the information obtained is insufficient to protect wild species and, subsequently, not enough to mitigate the impacts of nanotechnology on the biodiversity of agroecosystems, which have already been managed to be less biodiverse. Therefore, taking this into account, the diversity of species used in phytotoxicity studies must increase and should include species used for cover crops, soil fertilization purposes, hedgerows, green pastures, as well as macrophyte plants that could be exposed through runoffs from agriculture soils. The protection of these species is also relevant if the success of agri-environment measures recommended for pursuing the sustainability of agriculture is to be met.

When looking at the tables, it is also noticed that a great majority of studies aimed in assessing the phytotoxicity of NM is still performed under hydroponic conditions. Although extremely useful to study the mode of action of NM, their utility to estimate environmental risks is very limited, since exposures are not made under environmental relevant conditions, and, thus, plant responses may be overestimated. With effect, under hydroponic conditions, the role of the interactions between soil mineral and organic components in the availability of NMs does not play its role. For all of these reasons, the information is still limited, making the estimation of risk limits difficult for almost all the NMs, including those that are more used and that may represent a great environmental risk. This aspect becomes even more relevant if one takes into account that there is a great variety in terms of the size and chemical composition of the NM and the exposure conditions and duration tested in each

study (Tables 8.1, 8.2, 8.3, 8.4, 8.5, and 8.6). To generate useful information for risk assessment, the use of standard protocols and recommended experimental designs must be stimulated, even when the studies aim to address other relevant scientific questions. The application of these protocols should also include the evaluation of endpoints, at different biological levels of organization. From the data analyzed in this study, there are no doubts that the metal oxide NMs are able to induce oxidative stress in plants, especially at higher concentrations. However, it cannot be inferred if the oxidative stress will have consequences in growth and yield parameters and even less in the sustainability of populations or even communities. Exposure conditions and duration also need to be rethought, as it is important to take into account the likely pathway of the NM to soil, in order to decide the most appropriate exposure procedure and the expression of the concentrations. Although the exposure to the NM added to soil should always be considered, foliar spraying can also be relevant for those NMs that can be used to prepare phytopharmaceutical formulations and used from an agronomic perspective, such as Cu- and Fe-based NM.

The concentrations tested in the great majority of studies have been criticized for their lack of ecological relevance. Although this criticism is, at least in part, relevant, it is also true that the maximum concentrations tested should always be up to 1000 mg kg^{-1} (e.g., OECD 2006). Above this concentration, standard protocols assume that the compounds are not toxic. The large range of concentrations that can be tested up to this level also allows the finding of different potential effects, for which ecotoxicological data (NOEC, no observed effect concentrations; EC_x, effect concentration for a x% of effect) can be obtained to apply deterministic or probabilistic methodologies to derive risk limits, like PNEC values (EC 2003). Although the application of the former ones is limited due to the lack of data, PNEC values can be obtained for oxidative stress using assessment factors (AF). The European Community (EC 2003) proposed the use of an AF of 100, when at least one NOEC value is available, for a long term-test. Considering that almost all the exposure periods described in Tables 8.1, 8.2, 8.3, 8.4, 8.5, and 8.6 were long, and that oxidative stress is a sublethal endpoint, if this AF is used to estimate the PNEC for each NM (PNEC = lowest NOEC/AF), thresholds up to 10 mg kg^{-1} of soil will be obtained, thus indicating the absence of oxidative stress in plant species exposed to concentrations lower than these PNEC values. This information can be indicative for a preliminary risk assessment, but still needs more data to reduce the uncertainty associated with its estimation. In fact, and considering that each study tested a different NM (e.g., in terms of size), this makes the data available truly insufficient for risk assessment purposes, even for the application of AF to derive PNECs. Further, and taking into account that these NMs are composed of metal oxides, additive effects with other forms of metal oxides already present in the soil matrix cannot be neglected.

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Biological and Phytotoxic Impacts of a Nanomaterial

9

Usha Rani Pathipati and Prasanna Laxmi Kanuparthi

9.1 Introduction

The increasing production and use of nanomaterials both natural and engineered (ENPs) inevitably result in their higher concentrations in the environment. This may lead to undesirable environmental effects and thus warrants risk assessment. The fate of nanoparticles (NPs) in the environment, their mobility in ecosystems, and their interactions with different living organisms including plants remain relatively unknown. Nanoparticles (NPs) are mostly engineered type of particles (ENPs), and since they have different chemical and physical properties, they also possess diverse biological activities and at times are even suspected to have toxicity too. The noble metal nanoparticles (MNP) and their nano-sized agglomerates have gained much importance in research due to their unique electronic, mechanical, chemical, optical, and magnetic properties which are due to their tiny size compared with large-sized particles of their bulk materials (Bhattacharyya et al. 2010). Interestingly, the synthesis of nanoparticles whether by chemical and physical procedures or by using biological materials (green synthesis) also often causes differences in their activities. The extracellular production of silver and gold NP was carried out using plant materials such as eggplant, *Solanum melongena* L.; datura, *Datura metel* L.; coat buttons, *Tridax procumbens* L.; bitter orange, *Citrus aurantium* L.; papaya, *Carica papaya* L.; Calotropis, *Calotropis gigantea* L.; and Barbados nut, *Jatropha curcas* L. Leaves of these plants were extracted by sunlight exposure (Rajasekharreddy et al. 2010; Song and Kim 2008).

The unique structural, electronic, electrical, and optical properties of nanomaterials in combination with biomolecules will result in newer and potent technologies that are often used in medical diagnostics and also in medical treatments. One significant character of nanomaterials is possessing a wider surface area which

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_9

enables them to hold to other molecules easily and cause the formation of aggregates that further may lead to biotic uptake. Of late, nanotoxicology is being established as a new field, with its major focus on human and animal studies. Even though nanomaterials have found numerous applications on a day-to-day basis, their unique properties enable them to more effectively penetrate the cells and cause nanotoxicity to various life forms such as microorganisms, plants, and animals.

In a study conducted on zebrafish (*Danio rerio*), it was observed that as the number of exposure days increased, the number of viable embryos significantly reduced under TiO_2 NPs exposure (Ramsden et al. 2013). Few studies were published on positive/negative effects of NPs on toxicity of various organisms. *Piper betle* L. leaves acted as good reducing agents of Ag^+ ions, and the biosynthesized AgNPs showed less toxicity to *Daphnia magna* than the chemically synthesized AgNPs (Usha Rani and Rajasekharreddy 2011). In *Lumbricus rubellus*—an earthworm species—a shortness of development and reduction of growth were observed after exposure to C60 fullerene-NPs. A study by Wan et al. (2012) and Wang et al. (2012) has shown that the cytotoxicity of cobalt (Co) and CuO NPs to human lung epithelial A549 cells is due to the induction of reactive oxygen species (ROS) upon NPs penetration of A549 cells, causing subsequent irreversible DNA damage. Copper oxide nanoparticles (CuO NPs) are known to cause membrane damage by K+ leakage in *Escherichia coli* as demonstrated by Zhao et al. (2013). Monodispersed flavonoids loaded silver nanoparticles, prepared by green synthesis using castor (*Ricinus communis* L.) plant leaves, showed great potential against *Staphylococcus aureus* in vitro and in vivo studies against the infected silkworm, *Bombyx mori* L. larvae (Rajasekharreddy et al. 2017). Though the antimicrobial properties of nanomaterials are investigated to a large extent, insects are also found to be sensitive to the exposure of NPs. Green synthesized silver and lead nanoparticles using *Avicennia marina* mangrove plants extracts exhibited pesticidal activity against one of the major stored product pests, *Sitophilus oryzae* (Sankar and Abideen 2015), showing their probable role in future pest control operations. Another good example of entomological effects of NP is on *Drosophila melanogaster*. Exposure to AgNPs from early development caused demelanization of adult cuticle in these flies. As a result, all adult insects appeared totally bleached due to the lack of melanin pigments (Panacek et al. 2011). Chakravarthy et al. (2012) used CdS, nano-Ag, and nano-TiO_2 inorganic nanoparticles against *Spodoptera litura* F. and have observed that these NPs cease active larval movements and caused stiffness of the skin and entire body and oozing of the body contents (lysis). AgNPs synthesized using aqueous leaf extract of *Aristolochia indica* exhibited antifeedant and larvicidal activities against third instar larvae of *Helicoverpa armigera* (Siva and Kumar 2015).

Plant cells possess cell walls that constitute a primary site for interaction and a barrier for the entrance of NPs. Studies done on mechanisms allowing NPs to pass through cell walls and membranes are still in rudimentary stages. The published research on the impact assessment of NPs showed that they definitely have some impact on ecological terrestrial species, particularly plants. Hence, this study is aimed at providing brief knowledge on the environmental behavior and ecotoxicity

of nanoparticles (NPs) on plant community. Plants are critical to ecosystem function and food supply integrity and hence form an essential component in the environment. Based on the findings of recent laboratory studies, abiotic and oxidative stresses caused by NPs exposure in plants were described at physiological and biochemical levels (Asli and Neumann 2009; Dimkpa et al. 2012; Ma et al. 2013).

The tiny size of nanoparticles and their hydrophobic nature mediate the transport that might lead to bioaccumulation of nanoparticles. All these emphasize the importance and necessity of the deeper understanding of nanoparticles accumulation in the environment. More research is desired in this area of bio-uptake of nanoparticles to understand this phenomenon, particularly the surface chemistry and sizes of nanoparticles and also on the specific molecules that may play important role in bio-uptake by binding. The world's first report on nanotechnology research projects and issues to human health, environment, and safety was released in March 2009 by the United Kingdom who launched a worldwide survey on this.

9.2 Nanoparticle Absorption by Leaves and Effects on Plant Growth and Physiology

Most cells of the plants possess cell walls that constitute a primary site for interaction and a barrier for the entrance of ENPs into their cells. The functional groups, such as hydroxyl, carboxylate, amine, phosphate, sulfhydryl, and imidazole, confined in these biomolecules offer a range of distinct effective functional sites (Vinopal et al. 2007). The plant cell walls mainly consist of cellulose and are semipermeable, allowing the passage of small molecules while limiting the passage of larger molecules. Thus, only nanoparticles and nanoparticle aggregates with smaller size than the pores are expected to pass through the cell wall and reach the plasma membrane, making it necessary to investigate the impact of the NPs reaching the inner parts of the cell. The potential entry routes of ENPs through this bilayer lipid membrane have been discussed in detail by Pérez-de-Luque (2017). As soon as the ENPs enter the cell, they may bind with different types of organelles (e.g., endoplasmic reticulum, Golgi, and endo-lysosomal system) and interfere with the metabolic processes there, possibly as a result of the production of reactive oxygen species (ROS). Some plants reject the nanoparticles and some accept or store them. Absorption and accumulation of nanoparticles by plants are also influenced by the functionalization and coating of the nanomaterial surfaces (Judy et al. 2012). Also the presence of other organisms in the plant niche such as bacteria or fungi can influence the plant absorption of nanoparticles, especially with the microorganisms such as mycorrhizal fungi which are associated in symbiosis with plants (Feng et al. 2013; Wang et al. 2016a).

Plants are essentially exposed to NPs in both atmospheric and terrestrial environments. The airborne NPs will be attached to aerial parts of plants such as leaves and tender stems, while the waterborne or soil-associated NPs may reach the roots and interact with them. Therefore, it can be expected that plant communities with higher leaf area indexes (LAI) will also have a higher interception potential for

airborne ENPs, thus increasing their entry into trophic webs. Forests can function as very efficient traps for airborne ENPs, for example, total leaf area of single trees of spruce (*Picea abies*) forests in Southern Germany reached up to 750 m^2 for which LAIs ranged between 5.3 and 7.9 (Tenhunen and Mauser 2001). Also under canopy vegetation, LAI ranging from 0.27 to 3.3 (Kostner 2001) may act as an efficient ENPs trap.

It is interesting that accumulation of airborne iron particles on plants having waxy and smooth leaf surfaces has been shown to be reduced, while the accumulation increased on the non-waxy and wrinkled surfaces (Da Silva et al. 2006). NPs which reach the leaf surface might penetrate the plants through stomata or trichome bases and will be translocated from there to different plant tissues. The NPs accumulation on leaf and other photosynthetic surfaces often cause enhanced foliar heat due to stomatal obstruction and might alter the gas exchange, thus affecting the plant physiology (Da Silva et al. 2006).

Nanoparticles attached to agrochemicals or other substances could reduce the damage to plant tissues and the amount of chemicals released into the environment. It is essential to find the penetration, localization, route, and transport of the nanoparticles into plants for exploring the benefits of applying nanotechnology to agriculture. It is also essential to study different plant species and various nanoparticles used. This bio-uptake may result in significant environmental consequences. The way nanomaterials move inside plants is really important, because it can give indications about what parts of the plant they can reach and where they might accumulate. Nanoparticles should be applied to the roots in order to get a good distribution throughout the plant, whereas if they show good translocation through the phloem, application should be done via foliar spraying. However, nanomaterials moving through the phloem will mostly accumulate in plant organs such as fruits and grains, which is a major consideration when trying to avoid human or animal ingestion of nanomaterial (Pérez-de-Luque 2017). Also it is necessary to develop methods for detection and analysis of nanoparticles introduced into plants and their concentration in selected plant tissues. Usually different microscopy techniques are used for the detection and analysis of the magnetic nanoparticles, ranging from conventional light microscopy to confocal and electron microscopy. The nanoparticles can be charged with different substances, introduced within the plants, and, if necessary, concentrated into localized areas by using magnets which can be further assessed by simple or more complex microscopical techniques.

Nanotechnology use in crop protection has just begun. Recently application of NPs is widespread in the form of growth stimulators, nanopesticides, nanofertilizers, and sensors for monitoring different agricultural parameters in the field (Fraceto et al. 2016; Wang et al. 2016b). Silver has wider application values than other metals, and, hence, it is more appropriate to first focus on studying the effects of nano-silver on environment. Very little is known about the adverse effects of silver nanoparticles to human health and their fate in ecological systems. Through sludge and surface water, the AgNPs may easily reach the plant ecosystems. In a study on *Oryza sativa*, AgNPs of 25 nm size at high concentration caused toxic effect by breakage of the cell wall and damaging the vacuoles of root cells (Mazumdar and

Ahmed 2011), while Mirzajani et al. (2013) observed that the same AgNPs were unable to penetrate the root cells of *O. sativa* when present in low concentration (up to 30 μg mL^{-1}) and at 30 μg mL^{-1}, root growth was accelerated compared to restricted growth at 60 μg mL^{-1}. These observations also indicate that the penetration of AgNP is necessary to cause a toxic effect, whereas when present in surrounding, it may have a positive impact on plants. The AgNPs of 200–800 nm size were observed to accelerate the plant growth (Jasim et al. 2016), while 35–40 nm of AgNPs were observed to positively influence the root and shoot growth of *Vigna sinensis* and *Brassica juncea* (Pallavi et al. 2016). The exact reason behind different sensitivities of different plants toward NPs is still unclear, and hence more such studies are important not only from the point of view of the application of nanoparticles in plants but also for understanding the toxic effects if any on plants and the possibilities of their reach and accumulation in fruits and grains for further entry into the food chain.

Of late, nanotoxicology is being established as a new field, with its major focus on human and animal studies. However, very few studies have been conducted to assess the toxicity of nanomaterials to ecological terrestrial species, particularly plants. Several plants exposed to the industrial pollution by lead metal have been affected severely, and it leads to the causes of species to completely extinct. High lead concentration induces oxidative stress by increasing the production of ROS in plants (Reddy et al. 2005). The study on the effects or impact generated by bio-nanoparticles on physiology of the plant can contribute vastly to the new and exciting areas of bio-nanotechnology. The irrational use of nanoparticles without understanding the toxic effects on plants may sometimes result in mutations damaging both the plants and ecosystem.

Till now many reports stating the importance of nanomaterials in various industrial sectors and also quite a few studies hinted that some nanoparticles could have adverse environmental health effects. The main gateway of the nanomaterial being the sewage treatment plants from where they reach aquatic environment. It is interesting to see the effects these nanomaterials produce on the sewage associated aquatic plants. There is a necessity to focus on how nanoparticles behave in wastewater and how that gateway might be closed off. We have evaluated the effect of synthesized AgNPs [chemically (S-AgNPs) and/or biologically (B-AgNPs)] on the growth and physiology of an aquatic plant, water hyacinth—*Eichhornia crassipes* (Mart) Solms. Fifth day after the treatment, a decreased growth was recorded with S-AgNPs treatment alone but not for B-AgNPs. The atomic absorption spectroscopy results (at 100 mg L^{-1} concentration) showed a higher accumulation of S-AgNPs over the B-AgNPs in various parts of the treated plants. Significant changes in activities of antioxidative enzymes have occurred due to the nanoparticle treatment (Usha Rani et al. 2016).

Another most useful utilization of nanoparticles that makes them all the more important is their potential use in smart delivery systems. The minute size and their variation in nature make them good carriers for the specific targets in living organisms such as plant or animal cells. Several studies already depicted the role of nanoparticles in smart delivery system. They were mostly explored for their medical use in human

cells or animal cells during the experimentation. However, in plants too nanoparticles can be used for a broad range applications. Metalloid nanoparticles such as mesoporous silicon nanoparticles have been used to deliver DNA, proteins, and other chemicals in plants (Torney et al. 2007; Martin-Ortigosa et al. 2014).

9.3 Nanoparticle Absorption by Seeds and Effects on Plant Seed Germination

Phytotoxicity in higher plants should be investigated in order to develop a comprehensive toxicity profile for nanoparticles (USEPA 2005). Seed germination and root elongation test is sensitive, simple, and low cost, and its suitability for unstable chemicals or samples makes it a rapid and widely used acute phytotoxicity test (Munzuroglu and Geckil 2002; Wang et al. 2001). In soils, a potential ENP entrance mechanism in plants is via endocytosis, which was observed during the growth of root hair cells (Ovecka et al. 2005). Published studies on nanoparticles effects on plants till now indicate that they have both positive and negative impacts on plant germination, growth, and development. The negative impact of nanoparticles on plants has been depicted in several studies in the form of decreased plant growth and reduction in productivity and pigments (Landa et al. 2016; Tripathi et al. 2017).

The effects produced due to the application or absorption of silver nanoparticle (AgNPs) and their bulk counterpart silver nitrate ($AgNO_3$) on seed germination, root, and shoot length of castor bean, *R. communis* L., plant were measured. Silver nanoparticles did not show any significant effects on seedling growth even at higher concentration of 4000 mg L^{-1}, while the silver in bulk form as $AgNO_3$ applied on the castor bean seeds inhibited seed germination (Fig. 9.1). We also confirmed through atomic absorption spectroscopy the silver uptake in seedlings of the castor seeds on treatment with both the forms of silver. The silver nanoparticle and silver nitrate application to castor seeds also caused an enhanced enzymatic activity of ROS enzymes and phenolic content in castor seedlings. High-performance liquid chromatography analysis of individual phenols indicated enhanced content of parahydroxy benzoic acid (Jyothsna and Usha Rani 2013). These kinds of studies are of great interest in order to unveil the movement and accumulation of nanoparticles in plant tissues for assessing future applications in the field or laboratory. We studied the impact of α-pinene and linalool terpenes absorption onto SNPs and the treatment effects on insect antifeedant activity. The combination of SNP and the bioactive molecules increased their longer shelf life and had better stability and higher antifeedant activities against *Spodoptera litura* and *Achaea janata* (Usha Rani et al. 2014). The impact of silica nanoparticles on the plant physiology and plant growth was studied using germination experiments with SNPs in the range of 100–500 mg L^{-1} on *Gossypium hirsutum* seedlings. The plant cells did not respond to the presence of a high density of SNPs which is evident from the absence of changes in their subcellular organization as compared to the control root sections (Usha Rani and Jyothsna, unpublished data).

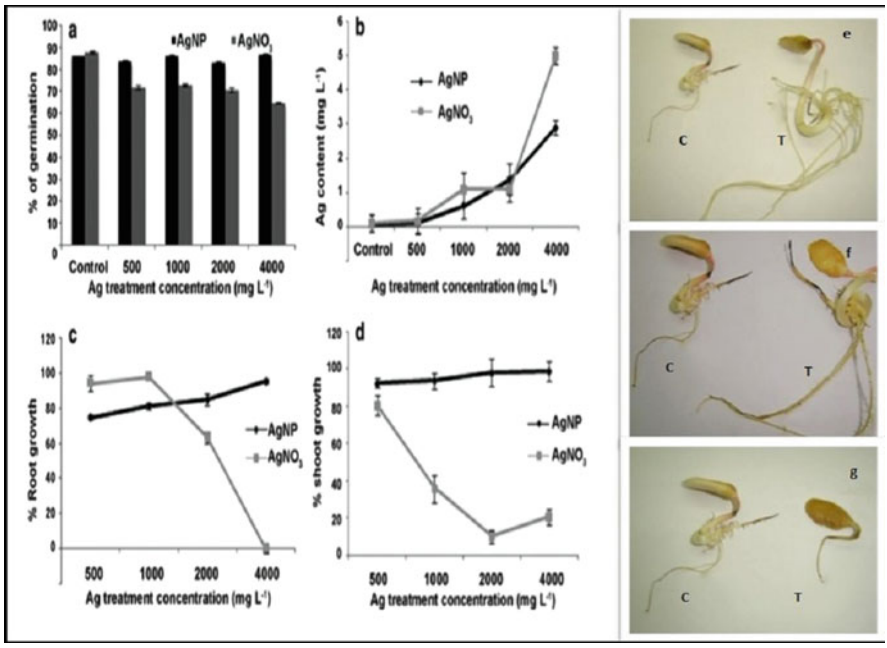

Fig. 9.1 Effect of AgNP and $AgNO_3$ treatments on germination of castor seeds. (**a**) Seed germination, (**b**) Ag content, (**c**) percent of root growth, (**d**) shoot growth in control and treated castor seedlings. Growth of castor seedlings at (**e**) 1000 mg L^{-1}, (**f**) 4000 mg L^{-1} AgNPs, and (**g**) 4000 mg L^{-1} $AgNO_3$ due to seed treatment (T) compared with control (C) (Jyothsna and Usha Rani 2013)

9.4 Nanoparticle Absorption by Leaves and Effects on Plant ROS Enzymes

Reactive oxygen species (ROS) are signaling molecules which are the product of aerobic metabolism in an ordinary plant (Thannickal and Fanburg 2000). The excess ROS levels which can surpass defense mechanisms would lead to oxidative stress and induce DNA damage, protein oxidation, electrolyte leakage, lipid peroxidation, and membrane damage, ultimately causing cell apoptosis (Gill and Tuteja 2010; Sharma et al. 2012). For example, ZnO caused increased chromosomal aberration indices and lipid peroxidation in onion (Kumari et al. 2011). A common finding from plant nanotoxicity studies is that excess amounts of ROS are produced upon NP (CuO NPs, Ag NPs, CeO_2 NPs) exposure to terrestrial plant species such as wheat (*Triticum aestivum*), rice (*O. sativa*), onion (*Allium cepa*), and corn (*Zea mays*) (Panda et al. 2011; Zhao et al. 2012; Mirzajani et al. 2013). In order to understand gene regulation in plants to NPs exposure, microarrays were used to analyze gene regulation in Arabidopsis treated with ZnO and TiO_2 NPs. Although both NPs disrupted gene regulation involved in response to abiotic stresses, genotoxicity

was highly NP specific, with ZnO NPs inducing a much greater molecular response than TiO_2 (Landa et al. 2012). The understanding on the ROS activation is still debatable, if the activity of these particles arises from intact particles or, rather, from ions released from NPs. Recent studies support that activation is in the form of ions, for example, in *Spirodela polyrhiza*, internalized Ag, both in form of Ag^+ ions or AgNPs, exhibited same capacity to generate ROS, thus supporting the hypothesis that intracellular AgNPs dissociate into highly toxic Ag^+ ions (Jiang et al. 2017).

Nanoparticle instigated stress is known to activate plant's antioxidant enzymes, superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). Briefly, SOD catalyzes detoxification of $O^{\cdot}{}_2^-$ into either ordinary molecular oxygen (O_2) or H_2O_2 and, which POD and CAT further detoxifies (Zhang et al. 2005). Analysis of exposure of rare earth elements on *Arabidopsis thaliana* indicated that activities of SOD, CAT, ascorbate peroxidase (APX), and POD were significantly elevated upon exposure to CeO_2 NPs, whereas only SOD and POD activities in the In_2O_3 NP treatments were elevated (Ma et al. 2016). The expression of various antioxidant enzyme genes was increased upon exposure to the low concentration of AgNPs but was not induced at high concentration of AgNPs in *Arabidopsis* plants (Qian et al. 2013).

We observed a significant increase ($P < 0.05$) in the activity of three antioxidative enzymes SOD, POD, and CAT in SNP treated cotton, *G. hirsutum*, seedlings compared to the untreated seedlings at all the treatment concentrations, and highest increase for all the three enzymes is observed at 400 mg L^{-1} concentration. Even at 300 mg L^{-1}, a drastic increase in the activities of these enzymes is recorded, and these values are greater than the activities observed with the treatments at 500 mg L^{-1} concentration (Usha Rani and Jyothsna, unpublished). In another study performed on *R. communis,* it was observed that SOD activities were enhanced in castor seedlings grown in AgNPs and $AgNO_3$ treated soil and were highest at 1000 and 4000 mg L^{-1} concentrations (Jyothsna and Usha Rani, Unpublished data). SOD activity also significantly differed among treatments on *Lycopersicon esculentum* in response to both nano-TiO_2 and AgNPs (Song et al. 2013), and CAT activity was significantly decreased in maize plants treated with 1000 mg Zn kg^{-1} (Cui and Zhao 2011). Findings of Sharma et al. (2012) indicate that silver nanoparticles promote the growth of *B. juncea* seedlings by modulating their antioxidant status and that APX and CAT activities were increased under high AgNPs concentrations. Few more examples of effects of nanoparticles on plant antioxidative enzymes are given in the Table 9.1.

Overall, it is clear that NPs can cause toxicity to biota in the environment. Regardless of the pathway of NPs are released or discharged in the environment, potential risks need to be fully characterized so as to avoid negative impact on environmental and human health.

Table 9.1 Summary of the plant antioxidant enzymes upon NPs exposure

Nanoparticle size (nm)	Concentration	Exposure	Plant	Effect	References
TiO_2 NPs (<25 nm)	0.01, 0.1, 1 10 mg L^{-1}	Hydroponic	*Hydrilla verticillata*	Increase in CAT and glutathione reductase activity	Okupnik and Pflugmacher (2016)
ZnO NPs (~85 nm)	200, 400, 800 mg L^{-1}	Hydroponic	*Allium cepa*	Increase in ROS and glutathione peroxidase production whereas a decrease in CAT	Ghosh et al. (2016)
Ag NPs (6 and 20 nm)	0.5, 5, 10 mg/L	Hydroponic	*Spirodela polyrhiza*	Increase in levels of ROS, SOD, POD, and glutathione activity	Jiang et al. (2014)
CuO NPs (40 nm)	10, 50, 100, 150, 200 mg L^{-1}	Hydroponic	*Lemna minor*	Increase in POD and CAT activity	Song et al. 2016
CeO_2 NPs	250 mg L^{-1}	Hydroponic	*Arabidopsis thaliana*	Excessive ROS production, increased SOD, POD, CAT, PAL-phenylalanine ammonia lyase, and polyphenol oxidase (PPO)	Ma et al. (2016)
In_2O_3 NPs	1000 mg L^{-1}	Hydroponic	*Arabidopsis thaliana*	Increase in SOD and POD	Ma et al. (2016)
CuO NPs (50 nm)	500 mg kg^{-1}	In soil	*Triticum aestivum*	Increase in CAT	Dimkpa et al. (2012)

9.5 Conclusion

In conclusion, nanotechnology has tremendous scope in every aspect life. The tremendous usage of these new nanotechnologies may consequently leave their impact on environment. Hence, all these require risk assessment followed by developing methodologies for risk reduction. The emerging technologies should introduce the environmental perspective in early research, as minor adjustments sometimes lead to more harmless inventions. There is vast amount of data that has to be explored, detected, and invented. At the same time, we have to find the consequences of the nanoparticle usage, whether positive or negative on environment, and non-target organisms to proceed and fully take the advantage of nanotechnology.

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Nanoparticle-Associated Phytotoxicity and Abiotic Stress Under Agroecosystems

10

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10.1 Introduction

There is no doubt that nanomaterials and nanoparticles have occurred in the nature several centuries ago but the nanotechnology is nearly a new science. It is reported that both nanoparticles of silver and gold have been used in fabricating ceramic glazes in order to provide a lustrous or iridescent effect in Persia in the ninth century BC (Ghorbanpour and Hadian 2017; Joo and Zhao 2017). In general, there are the natural nanoparticles, which well known also particulate nanomaterials. These engineered nanoparticles may be penetrated and diffused in the biosphere as well as the agroecosystems through different multipurpose applications. Therefore, this very wide spreading will raise the global concern about the fate and behavior of these nanoparticles on the health of human being as well as the environment (Peijnenburg et al. 2016; Cecchin et al. 2017; Luo et al. 2018). Among different agroecosystem compartments, soil and terrestrial plants are the most important components of these systems, which interact directly with nanoparticles, water, and atmospheric

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_10

environmental compartments controlling the distribution of different engineered nanomaterials (Van Koetsem et al. 2017; Joo and Zhao 2017). Therefore, the study of nanoparticles in soil–plant system could be considered one of the most important issues including understanding the ability of nanomaterials to be nanofabricated, understanding the fate and behavior of nanomaterials in soils as well as their interaction with plants, enhance nutrients use efficiency and their availability for plants, and biosafety and environmental compliance (Thiruvengadam et al. 2015; Mukhopadhyay and Kaur 2016).

Higher plants were and still the main subject to the exposure of engineered nanomaterials from different sources as well as the humans (Miralles et al. 2012). Therefore, the interactions between these vascular plants and different nanoparticles could shed light on different environmental consequences of nanotechnology (Ghorbanpour and Hadian 2017). Even at the same type of nanomaterials, both positive and negative effects could be occurred on higher plants. These effects include the physiological, molecular, and biochemical processes on different plant species (Siddiqui et al. 2014, 2015; Ghorbanpour 2015; Baiazidi-Aghdam et al. 2016; Ghorbanpour and Hadian 2017; Jośko et al. 2017a, b; Khan et al. 2017; Siddiqi and Husen 2017a, b; Tripathi et al. 2017a). Therefore, a great concern has been raised regarding the phytotoxicity of nanoparticles on plants on one side (e.g., Watson et al. 2015; Rao and Shekhawat 2016; Tripathi et al. 2016b, 2017a; Boddupalli et al. 2017; Jain et al. 2017; Tassi et al. 2017) and using of nanomaterials in ameliorating plant stress on the other hand (Mehrian et al. 2015; Rico et al. 2015; Da Costa and Sharma 2016; Abdel Latef et al. 2017; Faizan et al. 2017; Ghorbanpour and Hadian 2017).

Several adverse effects may be caused by engineered nanoparticles on edible plants in agroecosystems. These nanoparticles could enter into plants, water, and soils thereby affecting the whole food chain (Jha and Pudake 2016; Belal and El-Ramady 2016; El-Ramady et al. 2015a, 2016b). Therefore, engineered nanoparticles should be treated as stimulators for plant growth under stress conditions as well as a new group of contaminants. These pollutants may pose serious threats to the agroecosystems and human health. Hence, it is necessary to evaluate the environmental fate of engineered nanoparticles and their potential toxicity through developing appropriate risk assessment methods (Jacobs et al. 2015; Jha and Pudake 2016). Concerning the potential effects of nanoparticles on plants, there is still little information known about the side effects of these nanoparticles. It is reported that a huge number of nanoscale materials has been shown to be absorbed by plant cells and translocated to various tissues and plant organs (Nair et al. 2010; Khodakovskaya et al. 2011; Jha and Pudake 2016; Jośko et al. 2017b; Tripathi et al. 2017a). Therefore, this book chapter mainly focuses on engineered nanoparticles and their phytotoxicity under agroecosystem conditions. This chapter concludes and interprets the current knowledge status of nanoparticles in soil–plant system, nanomaterials and soil–microbes interaction, nanoparticles and abiotic stress on plants, and phytotoxicity of nanoparticles in polluted lands.

10.2 Nanoparticles in Soil–Plant System

10.2.1 Soil–Plant System: More than a Treasure

The rhizosphere area as the plant root–soil interfaces could be characterized (Baetz 2016; Kumar et al. 2016; Jia et al. 2017). This zone is the complex, most important, and an active zone in the soils for different soil microbial activities, soil biodegradation of pollutants, as well as the plant nutrition aspects (Oyelami and Semple 2015; Schlic and Hund-Rinke 2015; Jia et al. 2017; Li et al. 2017). It is a real treasure in plant nutrition due to its significance in fate, behavior, and uptake of nutrients by plants (Kayler et al. 2017; Rugova et al. 2017). The role of rhizosphere and its significance have been increased day by day controlling by the change in climatic attributes including temperature, moisture content, precipitation, etc. In rhizosphere area, several biological and ecological processes (or transformations) take place controlling microbial activity and plant growth as well as nutrients uptake by plants (Callesen et al. 2016; Huang et al. 2016; Shen et al. 2017). Definitely, these processes differ in case of the bulk soil comparing with the rhizosphere due to the physical, chemical, and biological processes and resulting from different plant and microbial activities (Ibekwe et al. 2017; Rugova et al. 2017). The rhizosphere zone has very dynamic interfaces including (1) the interface between soil and plant roots, (2) the plant root systems, soil, and its microorganisms, (3) and soil, plant roots, and invertebrates (e.g., Singh et al. 2016; Rugova et al. 2017). The rhizosphere is more that treasure because the fate and behavior of organic compounds, which released by the plant roots and soil microorganisms is the dominant process in the rhizosphere (Cai et al. 2017). The rhizosphere zone is very rich in several compounds including root exudates (low molecular weight) and humic substances, i.e., high molecular weight like mucilage or polysaccharides and proteins (Jha et al. 2015).

Therefore, the soil–plant system was and still one of the most important part in the agroecosystem. This system may represent the rhizosphere including dynamic and fate of different nutrients as well as the microbial activity in soils. Several studies have been conducted on this zone searching for more information about this treasure (e.g., Loh et al. 2017; Machado et al. 2017; Shao et al. 2017). There are several applications of nanotechnology in agricultural sector especially soil–plant system including nanofertilizers, nanopesticides, nano-stimulators, etc. (Solanki et al. 2015; Banerjee and Kole 2016; Monreal et al. 2016; Mukhopadhyay and Kaur 2016; Shalaby et al. 2016; Ghorbanpour et al. 2017; Jośko et al. 2017a). Furthermore, several engineered nanoproducts could enter into soil–plant system causing a lot of troubles. Many successes of nanotechnology applications in soil–plant system have been achieved in biosafety of engineered nanomaterials, improve nutrient use efficiency and soil fertility (Monreal et al. 2016), and transport mechanisms of nutrient and water in soil–plant system (Mukhopadhyay and Kaur 2016).

More than a century, several studies have been published about the soil–plant interactions (Hinsinger and Marschner 2006). These studies could be considered one of the most important challenges facing the scientific society of the present century. Furthermore, these studies also include a lot of topics ranging from chemical to

molecular biology themes on one side and from the rhizosphere to ecosystems on the other hand (Lambers et al. 2007). In the last decade, soil–plant interactions also have been focused on the global problems including (1) soil erosion, degradation, and desertification; (2) soil salinization and sodification; (3) nutrient deficiency; (4) inefficient water use, drought, and low-temperature stresses (Liang et al. 2010), whereas these topics have been shifted towards new global issues related to climate changes including greenhouse gas emission from soil–plant systems and soil carbon management (Lambers et al. 2007; Bai et al. 2017). Several benefits could be gained from the soil–plant system, where many treasures could be discovered as follows:

1. Plant nutrition: All interactions for plant nutrition nearly take place in the rhizosphere or soil–plant system, which represents the basis of different terrestrial ecosystems. Furthermore, the uptake of nutrients by plant roots and transformations of different nutrients in soils mainly take place in this very active zone or rhizosphere (Schnepf et al. 2011; Gómez-Merino et al. 2015; El-Ramady et al. 2016b; Cai et al. 2017).
2. Biogeochemistry: The main crossroads for different biogeochemical cycles at the lithosphere–biosphere interface or soil–plant system represent the second treasure (Vimal et al. 2017). Several aspects of different nutrients' biogeochemistry in the soil–plant system have been reviewed including silicon (Liang et al. 2015; Matychenkov et al. 2016), selenium (Sharma et al. 2015; El-Ramady et al. 2014, 2016b; Statwick and Sher 2017), arsenic (Anawar et al. 2013), cadmium (Shahid et al. 2017a), phosphorus (Kirkby and Johnston 2008; White Philip and Hammond 2008; Yadav et al. 2012), copper (Perlatti et al. 2016), sulfur (Prasad and Shivay 2016), etc. or trace elements (Amrhein and Doner 2014; Zhu et al. 2014; Rinklebe et al. 2017).
3. Pedosphere: This sphere represents the soil with its abiotic and biotic components and is very essential in understanding different interactions between soil and plants in particular the rhizosphere (Osman 2013; Blume et al. 2016). This pedosphere has very strong link with plant nutrition and the biogeochemistry of nutrients (Bech 2014; Zech 2016). This sphere also includes different transformation processes in the uppermost part of the lithosphere, which is influenced by different fluctuations of temperature, precipitation, atmospheric gases, aerosols, and radiation (Zech 2016).
4. Phyto- or bioremediation and biofortification: Almost all interactions in the frame phyto- or bioremediation and biofortification may happen within the soil–plant system reflecting the significance of this area for such interactions. It is reported that all nutrients that humans consume are derived from the soil–plant system and could overcome the deficiency of micronutrients in the diet through increasing the density and bioavailability of micronutrients in edible parts of plants through biofortification (Yang et al. 2007; El-Ramady et al. 2014, 2016a). On the other hand, understanding the interactions among soil–plant–microbe is needed in frame bioremediation in order to monitor the fate of different contaminants in the soil–plant ecosystem (Karthikeyan and Kulakow 2003; Sushkova et al. 2016).

5. Soil fertilization: Definitely soil fertilization and its interactions in soil–plant system are in closed relationship. Factors controlling the soil fertilization mainly depend on the characterization of both soil and plants. Different kinds of fertilization like organic and microbial inoculants could show strong potential in improving plant growth in agroecosystems (Bashan et al. 2014; de Souza et al. 2015; Larsen et al. 2015, 2017; Mahmood et al. 2016). Concerning different functional groups of microorganisms in the rhizosphere, these groups could be used as biofertilizers including arbuscular mycorrhizal fungi (Davaran Hagh et al. 2016), the saprotrophic fungus *Trichoderma harzianum* (Contreras-Cornejo et al. 2016), and the diazotrophic bacterium *Azospirillum brasilense* among others (Pereg et al. 2016; Larsen et al. 2017).
6. Sustainability: The sustainability of soil–plant system itself will guarantee to overcome many global problems occurring during the low productivity and degradation of soils worldwide including soil erosion, nutrient deficiency, soil degradation and its desertification, soil salinization and its sodification, and water and soil insecurity (Liang et al. 2010; Shang et al. 2014; Baum and Thiet 2016; Bahadur et al. 2016; Bharti et al. 2017).

Therefore, it could be concluded that soil–plant interactions definitely represent several beneficial fields. These interactions could focus on the most recent scientific issues including these fields such as (1) the biochemical and microbial processes of nutrient cycling in soil–plant systems, (2) biofortification of different crops, (3) phyto- and bioremediation of polluted lands, (4) nutrient and water transport mechanisms, (5) nutrient and water management in arid ecosystems, (6) physiological and molecular mechanisms of plant adaptation to stressed environments, (7) monitoring different biogeochemical cycles for different elements at the lithosphere–biosphere interface or soil–plant system, and (8) nutrient and/or metal bioavailability in agro-ecosystems.

10.2.2 Nanomaterials and Soil–Microbes Interaction

As mentioned before, the soil–plant zone has great treasures, which could help us to maximize the harvested benefits from it. One of the most important fields, which applied to this zone, is the nanotechnology. Nanotechnology, as well known, has "magic" tools nowadays in facing many global problems. Nearly, nanotechnology has touched all our life sides including agricultural fields. Therefore, many reports confirmed the release of engineered nanoparticles through direct and/or indirect routes into terrestrial environments (e.g., Gardea-Torresdey et al. 2014; Bour et al. 2015; Schaumann et al. 2015a; Thul and Sarangi 2015; Kwak and An 2016; Rodrigues et al. 2016; Servin and White 2016; Wang et al. 2016; de la Rosa et al. 2017; Goswami et al. 2017). Concerning the direct sources of engineered nanoparticles, they include nanofertilizers, nanopesticides, soil nanoremediators, and nanowastes from consumer products and manufacturing, whereas the indirect sources represent runoff from agro-chemicals and disposal of solids and biosolids

from wastewater treatment in landfills (Kwak and An 2016; Terekhova et al. 2017). Hence, several investigations also have been focused on different adverse effects regarding the terrestrial species including soil microorganisms (Gladkova and Terekhova 2013; Terekhova and Gladkova 2013; Gajapathi et al. 2015; Karimi and Fard 2017; Subramanian and Thirunavukkarasu 2017), plants (Thul and Sarangi 2015; Hatami et al. 2016; Reddy et al. 2016; de la Rosa et al. 2017; Tolaymat et al. 2017; Zuverza-Mena et al. 2017), and earthworms (Antisari et al. 2015a; Carbone et al. 2016; Yadav 2017).

It is well known that soil microbes are very important in maintaining the functions of soil, where they are the main key in several soil processes including soil organic matter and its decaying, bioremediation of pollutants through removing different soil toxins, restoring the biogeochemistry of different elements and nutrients, forming the soil structure, suppressing different soilborne plant diseases, and promoting plant growth (Cong et al. 2015; Hegde et al. 2016; Kwak and An 2016; Mukherjee et al. 2016a). So, several studies have been published regarding these roles of soil microbes under different conditions (e.g., Grandy et al. 2016; Romero-Olivares et al. 2017; Vimal et al. 2017; Xie et al. 2017). Therefore, the protection of different environmental and beneficial soil microbes from undesirable conditions or stresses like nanotoxicity is very important due to the previous potential of soil microbes. Moreover, further studies concerning the interaction between nanomaterials and soil microbes as well as different transformations for these nanomaterials in soils should be conducted.

Many reports have been confirmed that the concentration of engineered nanoparticles or nanomaterials in soil generally is higher comparing with its content in air or water indicating that the soil is the main sink for leakage and release these nanomaterials into different environmental compartments (Klaine et al. 2008; Tiede et al. 2009; Cornelis et al. 2014; Bour et al. 2015; Schaumann et al. 2015a, b; Hegde et al. 2016; Kwak and An 2016; Peijnenburg et al. 2016; Rodrigues et al. 2016; Karimi and Fard 2017; Xie et al. 2017). Concerning different effects of nanomaterials on soil microbes, they may include the structure and biomass of soil microbial community, extracellular enzymes, and mineralization (Kwak and An 2016; Khan et al. 2016). It is found that the leakage of different nanomaterials into soil environment is considered one of the most serious threats to microbial communities in these ecosystems (Hegde et al. 2016; Terekhova et al. 2017). Thus, great concern has been raised regarding the potential adverse of nanomaterials and their toxicological effects on soil microbial community. Definitely, more studies are needed for monitoring and evaluating the fate of nanomaterials in soils as well as their effects on soil living organisms. Therefore, several studies of risk and safety assessment should be conducted to evaluate the fate of different nanoparticles in soil environment. Due to the significant balanced harmony of the soil ecosystem, the study of the interaction between nanomaterials and the soil microbial communities is very important (Schlich and Hund-Rinke 2015; Hegde et al. 2016; Fernandes et al. 2017).

Concerning different impacts of engineered nanoparticles or nanomaterials on soil microbial communities, these impacts include many mechanisms like the direct

toxic effects of nanomaterials. These direct toxic effects have exact mode of toxicity including (1) the oxidation of proteins and nucleic acids, (2) the damage of cell membrane and cell death, (3) the genotoxicity, (4) the interaction with respiratory chain and local proton depletion, and (5) the production of reactive oxygen species and/or apoptosis (Dinesh et al. 2012; Hegde et al. 2015a, 2016; Thit et al. 2015). Regarding the indirect effects of nanomaterials, the mode of action is resulted from their interactions with different natural organic compounds in soils. There are also two mechanisms for the impacts of nanomaterials on microbial communities including the interaction between them enhancing the toxicity of persistent organic pollutants in both soil and water and by changing the bioavailability of toxins or nutrients in the environment (Dinesh et al. 2012; Hegde et al. 2015b, 2016). Therefore, the exact mode of toxicity of these nanomaterials on microbial communities needs more and further investigations and is still not completely understood.

10.2.3 Interactions Among Nanomaterials–Plant–Soil System

Inescapable release of engineered nanoparticles into the agroecosystem has been recorded due to the extensive production and use of them as well as growing number of different nanoproducts in the consumer market (Nowack and Bucheli 2007; Fernandes et al. 2017; Jośko et al. 2017b). Thereby, these engineered nanoparticles may enter different natural ecosystems including soils *via* diverse pathways and plants. So, this soil–plant system is very complicated and could be characterized through the following considerations (1) plants are the essential component of all ecosystems and have a very strong relationship with the phytotoxicity of nanoparticles, (2) soils are the main sink and a critical pathway for nanoparticles fate in the agroecosystem, (3) and any changes in different soil chemical or/and biological properties will be bound to have impact on the system of plants (Anjum et al. 2013, 2015, 2016; Jośko et al. 2017a). Therefore, based on our available literatures, a multidisciplinary integrated approach is strongly recommended for further researches concerning the fate of nanoparticles, transformation, accumulation, and phytotoxicity potentials in soil–plant systems as well as their cumulative impact on the environment and human health (Anjum et al. 2013).

As mentioned before, several soil characteristics have the ability to influence on the bioavailability, solubility, and toxicity of engineered nanoparticles to soil biota including the pH value, soil mineralogy or mainly the clay content, soil organic matter content, and ionic strength. Soil organic matter has a notable effect on the solubility, dissolution, and uptake of these nanoparticles (Bradfield et al. 2017). On the other hand, higher plants have a great role in soils in dealing with nanoparticles owing to their throng interactions with these nanoparticles. Therefore, many pathways could be resulted from the exposure of terrestrial plants to nanoparticles in soils including (1) intentional subsurface release for environmental nanoremediation, (2) nano-land applications of contaminated biosolids, (3) potential leaching from nano-enabled products, (4) irrigation using contaminated surface

water with nanoparticles, (5) surface run-off for nanoparticles and wastewater contaminated with nanoparticles effluent discharge (Pokhrel and Dubey 2013; Anjum et al. 2015). So, there is a crucial need for a good understanding of the interactions of nanoparticles with plant system for assessing the phytotoxicity and trophic transport (Anjum et al. 2013, 2015; Hegde et al. 2016; Goswami et al. 2017) as well as the researches of nanotoxicology concerning nanoparticles uptake and their accumulation in plants should be addressed under soil–plant system (Anjum et al. 2013, 2015, 2016; Goswami et al. 2017).

Therefore, it could be concluded that both soils and plants are very closed under the soil–plant system due to the direct effect of soil-associated engineered nanoparticles on plants and thereby the consumers like human and animals. Moreover, the understanding of different potential environmental effects of engineered nanoparticles and exploring the potential toxicity of the interactions among nanoparticles, soil, and plants have been become very important. The interactions between these engineered nanoparticles with other nanoparticles might affect their toxicity. It is found that the toxicity of inorganic mixtures for different nanoparticles may differ from the sum of their effects caused by their individual components.

10.3 Nanoparticles and Abiotic Stress on Plants

It is well known that all living organisms can ideally grow and develop under normal conditions, but under unfavorable conditions many problems can take place including stresses and/or environmental constrains. Concerning plant stresses, they could cause losses in crop production. Thereby, this crop productivity may face a lot of environmental stresses including abiotic stresses such as drought, salinity, heavy metals, flooding, chilling, freezing, heat, ozone, and ultraviolet radiation (Tripathi et al. 2016a, b, c; Wani et al. 2016; Abiri et al. 2017). Whereas, the biotic stress may include different pathogens like bacteria, virus, fungi, etc. (Abiri et al. 2017; Calanca 2017; Lu et al. 2017; Khan et al. 2017). Plant abiotic stresses have been investigated including many studies such as drought (Kaushal and Wani 2016; Xuan et al. 2016; Aslam et al. 2017; Lu et al. 2017; Nxele et al. 2017; Sheikh Mohammadi et al. 2017), salinity (Kaushal and Wani 2016; Xuan et al. 2016; Khan et al. 2017; Jiang et al. 2017; Nxele et al. 2017), heavy metals (Lemtiri et al. 2016; Shahid et al. 2017b), flooding (Kamal and Komatsu 2016; Loreti et al. 2016; Azizi et al. 2017), chilling (Xu et al. 2016; Ding et al. 2017), heat (Ohama et al. 2017; Buchner et al. 2017; Prasad et al. 2017), ozone (Alves et al. 2016; Łabanowska et al. 2016; Li et al. 2016a), and ultraviolet radiation (Pérez et al. 2016; Ren et al. 2016; Verdaguer et al. 2017).

Plants may be exposed to different combinations of abiotic and biotic stresses at the same time under field conditions. It is reported about common stress combinations that they may include drought and salinity, drought and pathogen, and salinity and heat, (Rossini et al. 2016; Li et al. 2017; Nxele et al. 2017). This encouraged many researchers nowadays to focus on these different interactions among combined abiotic and biotic stress under biochemical, molecular,

physiological, and morpho-anatomical basis (Nankishore and Farrell 2016; Sinha et al. 2016; Tripathi et al. 2017a). Therefore, different tailored molecular and physiological responses by plants have been recorded under these combined stresses. Thus, the responses of plants to abiotic and biotic stresses are very dynamic and complex. Concerning multiple and integrated omics studies, they could be used in discovering new areas of interactions and regulation as well as response of stress kinetics and identification of multiple response phases (Suzuki et al. 2014; Rossini et al. 2016). Several studies have been conducted on the role of nanoparticles in ameliorating the stress effects on plants (e.g., Mohammadi et al. 2014; Rico et al. 2015; Da Costa and Sharma 2016; Abdel Latef et al. 2017; Faizan et al. 2017).

In general, the excessive application of nanoparticles may cause real problems for plants through the phytotoxicity and other plant damage. On the other hand, some nanoparticles or nanomaterials could be used in ameliorating the stress resulting from abiotic and biotic stress on plants. Concerning the first case, many studies have been confirmed that different engineered nanomaterials could induce oxidative stress or produce reactive oxygen species (ROS) in plants (e.g., Oukarroum et al. 2012; Ghorbanpour and Hadian 2015; Mehrian et al. 2015; Ghorbanpour et al. 2015; Rico et al. 2015; Rani et al. 2016; Siddiqi and Husen 2017b). This effect also includes the plant defense system through antioxidative enzyme activities (e.g., superoxide dismutase, catalase, peroxidases, ascorbic peroxidase, and glutathione peroxidase) and nonenzymatic antioxidants including glutathione, carotenoids, ascorbic acid, alpha-tocopherol (vitamin E), and proline (Ghorbanpour and Hatami 2015; Ghorbanpour et al. 2015; Hatami et al. 2016; Rao and Shekhawat 2016; Ghorbanpour and Hadian 2017). The exact mechanism of plant defense against the phytotoxicity of nanomaterials has not been fully explored (Siddiqi and Husen 2017b).

Concerning the role of nanoparticles in stressed plants, it is reported that nanoparticles could help plants against the abiotic stresses including drought stress (Martínez-Fernández et al. 2015; Aghdam et al. 2016; Dimkpa et al. 2017; Taran et al. 2017), salinity (Abdel-Haliem et al. 2017; Abdel Latef et al. 2017), cold stress (Mohammadi et al. 2013, 2014; Hasanpour et al. 2015; Amini et al. 2017), UV-B stress (Tripathi et al. 2017b), and flooding (Mustafa et al. 2015, 2016). Under drought conditions, it was found that copper- and zinc nanoparticles decreased the negative effects of drought action upon wheat plants. Concerning the mode of action of nanoparticles under drought stress, it includes (1) increasing in the activity of antioxidative enzymes in leaves (e.g., superoxide dismutase and catalase), (2) increasing the relative water content in leaves, (3) reduction in the level of accumulation of thiobarbituric acid reactive substances (like lipid peroxidation), and (4) stabilization of the content of photosynthetic pigments (Taran et al. 2017).

Therefore, it could be concluded that engineered nanoparticles could influence the growth and development of higher plants including the enhancement (positive effects) or reduction (negative effects) in several physiological and biochemical activities like the germination of seeds, shoot/root growth, and the production of plant biomass. Hence, further investigations are needed in order to draw a

comprehensive picture of the interactions between plant and engineered nanomaterials at different levels including cellular and molecular.

10.4 Phytotoxicity of Nanoparticles in Polluted Lands

10.4.1 Nanoparticles Interaction with Plants

Engineered nanoparticles may reach to different environmental compartments including soil, air, freshwater, plants, and humans through their handling or accidently during their manufacture for sewage sludge, landfills, and waste water (Cornelis et al. 2014; Gardea-Torresdey et al. 2014; Aziz et al. 2015; Hegde et al. 2015a, b, 2016; Bour et al. 2015; Prasad et al. 2016; Goswami et al. 2017; Khan et al. 2017; Joo and Zhao 2017; Joško et al. 2017a, b; Tripathi et al. 2017a). These engineered nanoparticles may be toxic to the soil fauna as well as flora (plants). The bio-uptake and accumulation of nanoparticles in plants may have the positive (stimulator) and negative sides (phytotoxicity). This phytotoxicity response mainly depends on the nanoparticles size and shape as well as their concentration (Siddiqi and Husen 2016a). On the other hand, the uptake and translocation of engineered nanoparticles in different plant parts also depend mainly on their bioavailability, solubility, concentration, and exposure time (Siddiqi and Husen 2017b). Furthermore, Siddiqi and Husen (2017b) reported that the phytotoxicity of the free metal ions has been shown to be greater than that of the nanoparticles of these metals. Concerning the role of nanoparticle under plant stress, several studies have demonstrated that nanoparticles could improve the scavenging potential of free-radical and antioxidant enzymatic activities regulating different physiological, morphological, and metabolic processes in plants as well as altering micro-RNAs expression (Siddiqi and Husen 2016a; Dimkpa et al. 2017; Taran et al. 2017).

Concerning the interaction between plants and nanoparticles, day by day a great concern has been increased. The effect of nanoparticles on plants generally depends on many factors including nanoparticle types, exposure methods, their physicochemical properties, presence of dispersants (e.g., surfactants or natural organic matters), plant species, and the plant growing media like soil, hydroponics, or culture medium (Aslani et al. 2014; Deng et al. 2014; Prasad 2014; Yadav et al. 2014; Arruda et al. 2015; Bakshi et al. 2015; Chichiricco and Poma 2015; Hossain et al. 2015; Ma and Gao 2015; Schwab et al. 2015; Shukla et al. 2016; Boddupalli et al. 2017; Pacheco and Buzea 2017). The phytotoxicity of nanoparticles through some biological and morphological measurements such as root elongation, germination index, shoot and root biomass, and root tip morphology could be determined (Deng et al. 2014; Boddupalli et al. 2017; Pacheco and Buzea 2017). Therefore, many studies have investigated different effects of nanoparticles on crops starting from the uptake, translocation, accumulation, and toxicity of nanoparticles in crop plants as well as different morphological, physiological, and genetic consequences and their potential trophic transfer (Miralles et al. 2012; Deng et al. 2014; Ma and Gao 2015; Zhang et al. 2015; Ebbs et al. 2016a, b; Shukla et al. 2016; Bradfield et al. 2017;

Ghorbanpour and Hadian 2017; Jośko et al. 2017b; Pacheco and Buzea 2017; Tolaymat et al. 2017; Tripathi et al. 2017a).

Natural soils have the ability to reduce the negative and may be also the positive effects of engineered nanoparticles on different cultivating plants. It is also found that foliar application may seem to be less detrimental for plants comparing with root exposure depending on the age of plants (Ma and Gao 2015). The entrance mechanisms of engineered nanoparticles in tissues of plant are still not totally understood, where some nanoparticles may have the ability to form complexes with the proteins of membrane transporter or the exudates of roots and thereby translocated into the plant systems (Yadav et al. 2014; Pacheco and Buzea 2017). Therefore, there are two main ways for the uptake of nanoparticles by plant roots including symplastic and apoplastic route (Deng et al. 2014; Pacheco and Buzea 2017). Concerning the apoplastic pathway, the nanoparticles may pass through the pores of plant cell walls, which range from 5 to 20 nm (Deng et al. 2014). Therefore, the nanoparticles larger than the size of these pores will be prevented from passing to endodermis because of the root epidermal cell wall (Deng et al. 2014). These nanoparticles can diffuse between cell walls and plasma membrane after crossing porous cell walls and may be subjected to both capillary forces and osmotic pressure (Lin et al. 2009; Pacheco and Buzea 2017). On the other hand, the symplastic pathway of nanoparticle includes their entrance through the inner side of the plasma membrane considering more important than the apoplastic one.

Therefore, different pathways could be concluded regarding the interaction between nanoparticles and plants as follows:

1. Higher plants have the ability to interact with released nanoparticles in the rhizosphere through several pathways including the uptake and accumulation into plant biomass. There are many other pathways including the transportation of nanoparticles and their transformation in the agroecosystem (Nair 2016; Tripathi et al. 2017b).
2. Nanoparticles can get adsorbed through foliar application on different plant surfaces, and their subsequent uptake occurs through different plant openings including nanometer- and micrometer-scaled plant openings (Nair 2016).
3. The airborne and engineered nanoparticles may get dispersed by wind reaching the plant leaves or the aerial part of plants. This facilitates the interaction of these nanoparticles with plant shoot surfaces and to get a chance for a portion of nanoparticles to release into the environment (Espinosa and Oliva 2006; Nair 2016).
4. Plants may interact with atmospheric nanoparticles through the stomata of plant leaves, and this stomatal pathway is highly capacitive because of its large size (above 10 nm) as well as its high transport velocity, but because of the variability in permeability may make this pathway highly unpredictable (Eichert et al. 2008; Nair 2016).
5. Nanoparticles may also get deposited on the cell walls of sub-stomatal cavity or nearby cells, and nanoparticles may be associated with the plant aerial parts including bark surfaces, cuticle, and stigma of flowers (Nair 2016).

6. The size of nanoparticles and its concentration definitely play an important role in the uptake of nanoparticles and their distribution within plant system (Tarafdar et al. 2012; Nair 2016).

On the other hand, some studies have been conducted on the combined mixtures of nanoparticles and their effects on plants (e.g., Ebbs et al. 2016a, b; Yu et al. 2016; Bradfield et al. 2017; Dimkpa et al. 2017; Jośko et al. 2017b). The interaction between different nanoparticles may result in more toxic nanoparticles. For more details concerning the role of combined mixtures of nanoparticles and their toxicity on plants as pollutants, this will be illustrated in the next subsection. Therefore, it could be concluded that the interaction between the nanoparticles and plants may include several changes like morphological, physiological, and genotoxic levels. Furthermore, the understanding of these interactions is very important regarding the effective uses of nanotechnology in the agricultural sector. It is found that both negative and positive responses of different nanoparticles on plant growth and its development depending upon the properties of nanoparticles and their mode of action as well as plant species. Further studies on the bioavailability, uptake, translocation, and biotransformation of different nanoparticles as well as the application risks on different important crops are also needed.

10.4.2 Nanoparticles and Plant Growth

As mentioned before, higher plants are the most important component of the agroecosystem compartments. Concerning the role of nanoparticles in growing the plants, several studies have been suggested that application of nanoparticles can stimulate plant growth for different crop plants starting from seed germination till harvesting at lower doses (e.g., Zhao et al. 2014; Mousavi Kouhi et al. 2015; Mukherjee et al. 2016b; Nair 2016; Venkatachalam et al. 2017). This stimulation effect resulted from the alterations of different biochemical pathways affecting the regulation of plant gene expression. These alterations or induction effect of nanoparticles is the major biochemical changes following nanoparticles exposure mediating the plant oxidative stress. These nanoparticles can induce the antioxidant defense enzymes of plants including catalase, superoxide dismutase, and peroxidase through inducing reactive oxygen species and modulating changes in plant cells (Mousavi Kouhi et al. 2015; Venkatachalam et al. 2017).

Several metal or metal oxide nanoparticles have been used in studying the effects of these nanoparticles on plant growth and its development. These metal or metal oxide engineered nanoparticles include Ag, Co, Se, and Ni as well as Al_2O_3, CeO_2, CuO, Fe_3O_4, MnO, SnO_2, TiO_2, and ZnO, respectively (Antisari et al. 2015b; Landa et al. 2016; Siddiqi and Husen 2016b, 2017b). Positive (beneficial at lower doses) and negative (adverse at higher ones) effects of these previous nanoparticles have been recorded regarding plant growth including germination of seeds, growth of plant biomass production, physiological and biochemical activities, or photosynthetic activities (Fig. 10.1; Tripathi et al. 2017; Siddiqi and Husen 2017b). The main

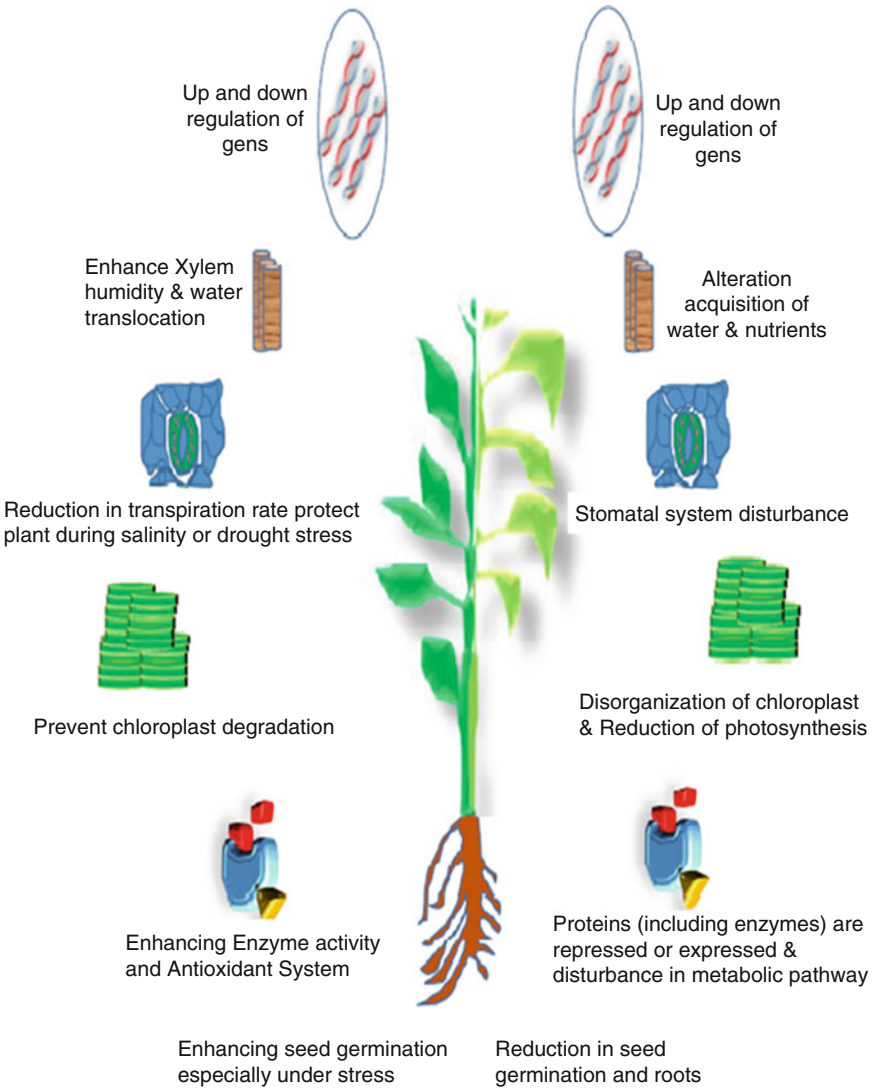

Fig. 10.1 A comparison between effects of nanoparticles on plants in general, where two cases could be distinguished including (1) the lift side of this drawing represents the positive or the beneficial effects under lower doses and (2) the right side represents the negative or the adverse at higher ones. Both cases include many effects on plant growth including germination of seeds, growth of plant biomass production, physiological and biochemical activities, or photosynthetic activities

problem in these previous studies is represented in the short time of experimentations. In other words, the most of these investigations have been carried out on the early developmental stages, whereas these studies require a full-term

study to reach satisfied final results of the plants (Siddiqi and Husen 2017b). Therefore, more detailed research investigations are required in order to examine different impacts of these nanoparticles on the entire environment and human health.

10.4.3 Phytotoxicity of Nanoparticles in Polluted Soils

It is well known that the global agriculture is one of the main engines of the global economic and its development. As a result of industrialization and urbanization, the pollution of agricultural soils has become a growing concern in countries worldwide (Saha et al. 2017). The main problem of polluted soils is represented in the potential threat to health of humans and their food safety (Abhilash et al. 2013a, b). Due to the great demand for all arable lands (including polluted lands) for agricultural and food production, using polluted lands for agriculture will address (1) the increased food demand, (2) the cultivation of biomass and biofuel crops to meet growing energy demand, and (3) improving the nutrient content of agricultural products through the biofortification (Zhao and McGrath 2009; Edrisi and Abhilash 2016; Abhilash et al. 2016; Singh 2017). Therefore, it could be used in the polluted soils in the production of bioenergy crops and thereby after a few years these soils will be converted into the production of strategic food crops. Furthermore, the suitable plant cultivars should be carefully selected through molecular and genetic breeding seeking for the optimized cropping on these polluted lands. These selected plants should be targeted toward low-accumulating cultivars or phyto-excluders, reducing the bioavailability of pollutants in soils and restricting the uptake and thereby the translocation of pollutants into edible parts (Ye-Tao et al. 2012; Tripathi et al. 2014a, b, 2015a, b; Abhilash et al. 2016; Singh 2017). Several approaches have been confirmed in remediation of polluted soils such as using marble sludge (González et al. 2017); clay minerals including bentonite, palygorskite, and sepiolite (Xu et al. 2017); microbiological methods including bioaugmentation, biostimulation, and composting (Cycoń et al. 2017); phytoremediation methods like phytoextraction, phytostabilization, and phytodegradation (El-Ramady et al. 2015b, 2016b, Idaszkin et al. 2017); aromatic plant–microbe associations (Verma et al. 2017); and nanomaterials (Emadi et al. 2016; Li et al. 2016b; Sun et al. 2016; Mansouri et al. 2017).

The application of different nanoparticles to decontaminate soil pollutants is one of the most important challenges facing researchers of environmental nanotechnology. This process mainly depends on the reactivity, stability, mobility, and transport of engineered nanoparticles. The most widely used engineered nanoparticles in soil decontamination include nano iron (nano zero-valent iron, bimetallic iron nano-particles and magnetite nanoparticles), titanium dioxide nanoparticles (nano-TiO_2), nano carbon such as fullerene, graphene and carbon nanotubes, as well as group of nanoparticles such as gold, palladium, silver and amphiphilic polyurethane nanoparticles (Li et al. 2016b). Concerning the decontamination of pollutants by nanoparticles, it has many issues including the effects of these nanomaterials on soil biology (Fernandes et al. 2017), the phytotoxicity of these nano-sized materials (Jain et al. 2017; Jośko et al. 2017b), and

the effects of nanoparticles on soil pollutants—even single or combined mixture of pollutants (Jośko et al. 2017b; Li et al. 2016b).

Therefore, nanoparticles may interact with various soil components including soil matrix, pollutants, and other agroecosystem compartments. The coexistence of nanoparticles with other contaminants in soils may translate into the behavior of nanomaterials and consequently into their phytotoxicity as well as the accumulation of these nanomaterials and contaminants. It is reported that carbon-based nanomaterials exhibited a decrease in the uptake of contaminants (pesticides or heavy metals) by 21–80 % by maize, ryegrass, soybean, tomato, and zucchini (Sun et al. 2016; Ghorbanpour and Hadian 2017; Jośko et al. 2017b). It is also confirmed that nanoparticles have been used in remediating different soils contaminated with some pollutants including pesticides, herbicides, organic pollutants, and heavy metals (Pulimi and Subramanian 2016). Regarding different possible reaction mechanisms for nanoparticles (like hydroxyapatite nanoparticles) for the immobilization of heavy metals, they may include the following processes of surface complexation, ion exchange, and precipitation as new metal phosphates. Therefore, it could be summarized that nanoparticles (e.g., hydroxyapatite) could be used as effective materials in immobilizing pollutants (e.g., heavy metals like Cu and Zn) in contaminated soils (Sun et al. 2016). Concerning multiple pollutants and nanoparticles, further studies are needed for more emphasizing the interactions among different pollutants and nanomaterials in soils and the role of plants under different conditions.

10.5 Conclusion

Depending on the available information, it is clear that engineered nanoparticles interfere with different agroecosystem compartments including soils and plants at both molecular and physiological levels. This interface could have both the inhibitive and enhancive effects for the same engineered nanoparticles, and the exposure concentration appeared to be critically very important. Furthermore, the safe threshold exposure concentration for different engineered nanoparticles in agriculture should be established. Therefore, phytotoxicity of engineered nanoparticles could be considered an important issue. Moreover, long-term investigations are also needed to be conducted to assess the role of engineered nanoparticles in regulating different plant physiological processes under stress. This would be very helpful in establishing a global database concerning the setting of a global nano-agro-database accessible and useful for current and future researchers all over the world. Great benefits could be gained from remediating polluted soils using nanomaterials including production of bioenergy, reduction of pollution risks for human health, and increasing the arable lands for strategic crops production.

Acknowledgement Authors thank the outstanding contribution of STDF research teams (Science and Technology Development Fund, Egypt) and MBMF/DLR (the Federal Ministry of Education and Research of the Federal Republic of Germany), (Project ID 5310) for their help. Great support from this German-Egyptian Research Fund (GERF) is gratefully acknowledged.

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Phytotoxic Assessment of Nickel Oxide (NiO) Nanoparticles in Radish

11

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11.1 Introduction

The recent rapid expansion of nanotechnologies-based consumer products has raises questions regarding the accumulation of nanoparticles in the environment and their possible health risks on environment and biota. Therefore, release of nanoparticles into the environment is likely inevitable (Landa et al. 2012, 2016). Nanoparticles can enter plant cells across plasma membrane either through endocytosis or non-endocytic penetration (Lin and Xing 2008; Lin et al. 2009). The absorption of nanoparticles by plant roots and its transportation to shoots through vascular tissues depends on the composition, size, and shape of nanoparticles and plant anatomy. The interaction between plant cell and nanoparticles could lead to the modification of plant gene expression and associated biological pathways; therefore it influences the plant development (Lin et al. 2009). Cellular uptake of Ag nanoparticles is reported in *Arabidopsis thaliana* (Geisler-Lee et al. 2014; Qian et al. 2013); aluminum oxide (Al_2O_3) nanoparticles in *Nicotiana tabacum* (Burklew et al. 2012); SiO_2 nanoparticles in *Raphanus sativus* L. (Zhang et al. 2015c); ZnO nanoparticles, bulk ZnO, and $ZnCl_2$ in *Medicago sativa* (Bandyopadhyay et al. 2015); ZnO in *Brassica napus* (Mousavi Kouhi et al. 2015); nickel oxide (NiO) nanoparticles in *Lycopersicum esculentum* (Faisal et al. 2013); and NiO nanoparticles in *Allium cepa* (Manna and Bandyopadhyay 2017). Wang et al. (2016) have studied the effect of ZnO nanoparticles on biomass accumulation and photosynthesis in *Arabidopsis thaliana* (L.), and they found that 200 and 300 mg L^{-1} ZnO nanoparticles treatments reduced *Arabidopsis* growth by ~20% and 80%, respectively. At 300 mg L^{-1}, the chlorophyll a and b contents, leaf stomatal conductance, net rate of photosynthesis, intercellular CO_2 concentration, and transpiration rate were

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_11

reduced more than 50%. Servin et al. (2013) found that the rate of catalase (CAT) and chlorophyll increased in *Cucumis sativus* L. when treated with TiO_2 nanoparticles, while the ascorbate peroxidase (APX) decreased. In another study, at 15 days, the CAT and APX activities in kidney bean roots reduced sharply when treated with 500 mg L^{-1} CeO_2 nanoparticles compared with control group, and at the same time, the root soluble protein increased by 204% (Majumdar et al. 2014). Zhang et al. (2015b) showed that ZnO nanoparticles decreased root length of *Zea mays* and *Cucumis sativus* by 17% and 51%, respectively, but showed no effects on the germination. Nair and Chung (2015) have reported that CuO nanoparticles reduced the shoot and root growth and increased reactive oxygen species (ROS) generation and lipid peroxidation in *Cicer arietinum*. Landa et al. (2016) have tested the toxicity of various metal oxide nanoparticles (Al_2O_3, CuO, Fe_3O_4, MnO, TiO_2, and ZnO) and metal ions on germinating seeds of *Sinapis alba*. The study showed that the toxicity of metal ions was more than the toxicity of metal oxide nanoparticles at the same concentrations. Gui et al. (2015) observed that the abscisic acid (ABA) and indole-3-acetic acid (IAA) content in the roots of transgenic and non-transgenic rice rose in response to Fe_2O_3 nanoparticles. Nhan le et al. (2015) reported that CeO_2 nanoparticles had no significant effect on IAA, ABA, and gibberellic acid (GA) in the leaves of Bt-transgenic and conventional cotton compared with the control group, while trans-zeatin riboside (t-ZR) content in the conventional cotton leaves decreased by 25% when exposed to 500 mg L^{-1} CeO_2 nanoparticles. ZnO nanoparticles increased the ROS generation and activities of antioxidant enzymes, while it reduced the shoot and root length and biomass in *Brassica juncea* (Rao and Shekhawat 2014). The effect of nanoparticles on plants depends on several factors such as type, size, and source of the nanoparticles, the plant species, and the exposure period of nanoparticles to crops (Rizwan et al. 2017). Metal and metal oxide nanoparticles toxicity can potentially include at least three different mechanisms: (1) particles may release toxic substances into exposure media, (2) surface interactions with the media may give toxic substances, and (3) particles or their surfaces may interact directly with biological targets and disrupt them (Ma et al. 2013). The interaction of nanoparticles with organisms or factors present in the environment (e.g., UV radiation) may produce ROS (Navarro et al. 2008). ROS generation depends on many properties of nanoparticles such as physicochemical properties, biotransformation, size, shape, and metal ions released from metal and metal oxide nanoparticles (Yang et al. 2017). ROS are regarded as key factor in inducing DNA damage (Mehrabi and Wilson 2007); also they play important roles in the toxicity of metal oxide nanoparticles (Wang et al. 2017). There is a wide evidence showing that the ROS play an important role in the exposure of plants to abiotic and biotic stress (Yang et al. 2017); also ROS contribute as important signaling molecules for controlling plant programmed cell death (PCD) (Gechev and Hille 2005) and stomatal closure (Kwak et al. 2003). They also organize the activities of many components by signaling, including transcription factors, protein kinases, and protein phosphatases (Cheng and Song 2006). ROS include the superoxide radical ($O^{\cdot}{}_2^-$), hydroxyl radical (·OH), hydroperoxyl radical (HO_2), hydrogen peroxide (H_2O_2), alkoxy radical (RO), peroxy radical (ROO), singlet oxygen ($O_2\ {}^1\Delta_g$), and excited carbonyl ($R{=}O^*$), all of which are cytotoxic to plants (Faisal et al. 2013). Reduced levels of ROS induce Ca^{2+} influx into the cytoplasm and activate NADPH oxidase. Plant

NADPH oxidases generate O^{-}_{2}, which gets converted to H_2O_2 by SOD, and the peroxide diffuses through the cell wall to the extracellular medium and enters into the cell (Hammond-Kosack and Jones 1996). In the present study, we examined the effect of NiO nanoparticles in radish to assess the (1) phytotoxicity, (2) translocation of nanoparticles in root tissues, (3) lipid peroxidation and membrane damage, and (4) ROS generation and antioxidant activities. Radish plant is very sensitive to nanoparticles and is considered as a model plant in this type of studies (Khodakovskaya et al. 2009). Radish is an important vegetable of the Brassicaceae family cultivated and consumed worldwide. The swollen underground root of the plant is being mostly eaten raw as a crunchy salad. Plants with edible roots are more susceptible to uptake the nanoparticles from soil in comparison to leafy vegetable or plants.

11.2 Materials and Methods

11.2.1 Characterization of NiO Nanoparticles

Transmission electron microscopy (TEM) was used for characterization of NiO nanoparticles. Ultrasonicated NiO nanoparticles suspension was left to dry on TEM copper grid at room temperature. The average primary particle size was determined via analyzing six different TEM samples with at least ten micrographs from the field emission transmission electron microscope (JEM-2100F, JEOL, Japan) at 200 keV.

11.2.2 Effect of NiO Nanoparticles on Seed Germination and Growth

The seeds of *Raphanus sativus* (radish) var. *radicula* Pers. procured from the local seed market were washed in running water for 10 min. The seeds were surface sterilized with 5% sodium hypochlorite solution for 10 min, and the sterilized seeds were washed thoroughly with sterile pure water. Different concentrations of NiO nanoparticles ($\leq$50 nm) (catalog no. 637130, Sigma Chemical Company, St. Louis, MO, USA) suspensions were used, namely, 0.25, 0.5, 1.0, 1.5, and 2.0 mg mL^{-1}. For each concentration, 20 sterilized seeds were treated with the assigned suspension on rotary shaker at room temperature for 4 h. After treatment, seeds were rinsed thoroughly with deionized water and cultured in Petri dishes on filter paper for germination. Seeds were placed in growth chamber at 25 $\pm$ 2 °C and left for 4 days.

11.2.3 Translocation of NiO Nanoparticles

TEM analysis was performed to examine the uptake and translocation of NiO nanoparticles in the roots of radish according to the method of Corredor et al. (2009). Glutaraldehyde solution (10%) was used to fix the roots of control and

nanoparticles treated seedlings in 0.1 M cacodylate buffer (pH 7.4) for 20 min. After fixation, 1% OsO_4 solution was used to suspend roots in 0.1 M cacodylate buffer (pH 7.4) for 1 h at 4 °C. Roots were, then, incubated at room temperature in aqueous uranyl acetate (2%) for 1 h. After dehydrating roots in ethanol series, they were embedded in araldite resin with low viscosity to prepare sections with thickness of 80 nm. Sections were visualized using JEOL-1011 Electron Microscope (JEOL, Tokyo, Japan) under high vacuum (100 kV).

11.2.4 Lipid Peroxidation and Changes in Membrane Potential ($\Delta\Psi m$) by NiO Nanoparticles

For quantitative evaluation of lipid peroxidation in root tissues of both control and NiO treated seedlings, the level of lipid peroxidation product malondialdehyde (MDA) was measured by the method described by Cakmak and Horst (1991). In brief, 0.2 g of root tissues were grounded in 5 mL (0.1% w/v) of trichloroacetic acid (TCA) and centrifuged at 14,000 rpm for 5 min. After centrifugation the supernatants (1.0 mL) were transferred to a fresh tube containing 4.0 mL of 0.5% (w/v) TBA prepared in 20% (w/v) TCA, and the mixture was incubated at 95 °C for 30 min. After rapid cooling, the mixture was centrifuged at 12,000 rpm for 5 min, and absorbance of the supernatant was measured using UV-visible spectrophotometer (Jenway 6705, UK). Concentration of MDA was calculated according the following formula:

$$\text{MDA concentration}\left(\text{nmol g}^{-1}\text{ fresh weight}\right) = (\text{A532} - \text{A600})\, V \times 1000/\varepsilon \times W$$

where A532 and A600 are the absorbance at 532 nm and 600 nm wavelength, respectively, V is the volume of extraction medium, ε is the specific extinction coefficient (=155 mM^{-1} cm^{-1}), and W is the fresh weight of root tissues.

To examine the visual changes in membrane potential ($\Delta\Psi m$) induced by the NiO nanoparticles, the root tissues of treated and non-treated seedlings were stained for 30 min with 1 μg mL^{-1} Rh123, and images were observed and captured using a fluorescence microscope (Nikon Eclipse 80i, Japan).

11.2.5 Intracellular ROS Generation and Antioxidant Activities

Roots were stained for 15 min with 0.25 μM 2′,7′-dichlorofluorescin diacetate (DCF-DA) and captured using a fluorescence microscope to analyze ROS production qualitatively as described by Hernandez et al. (2010). Roots of 20 different seedlings in both control and NiO treated groups were used to extract the superoxide dismutase (SOD), catalase (CAT), and total glutathione (GSH) using different assay kits according to manufacturer's instructions (Cayman Chemicals, MI, USA) to examine the level of oxidative stress. Multiwell microplate reader (Multiskan Ex,

Thermo Scientific, Finland) was used to measure the colored compound which resulted from enzyme extraction at 550, 405, 450, and 500 nm, respectively.

11.2.6 Statistical Analysis

Twenty different seeds/concentrations were used and the data were obtained from three different experiments (at least). Values were reported as mean ± SD. Data was subjected to one-way analysis of variance (ANOVA) by using the software IBM SPSS version 24, and mean values were compared using Duncan's multiple range test ($P \leq 0.05$).

11.3 Results and Discussion

11.3.1 Characterization of NiO Nanoparticles

Morphology and size of NiO nanoparticles were studied in solid state using TEM. The average size of the particles was 23.3 nm (Fig. 11.1). The results of TEM analysis showed the morphology of polyhedral crystallite spheres with agglomerates of nanoparticles. The primary and secondary sizes of nanoparticles are among the most important parameters controlling their toxicity to plant cells (Faisal et al. 2013). Our findings regarding the characterization of nanoparticles

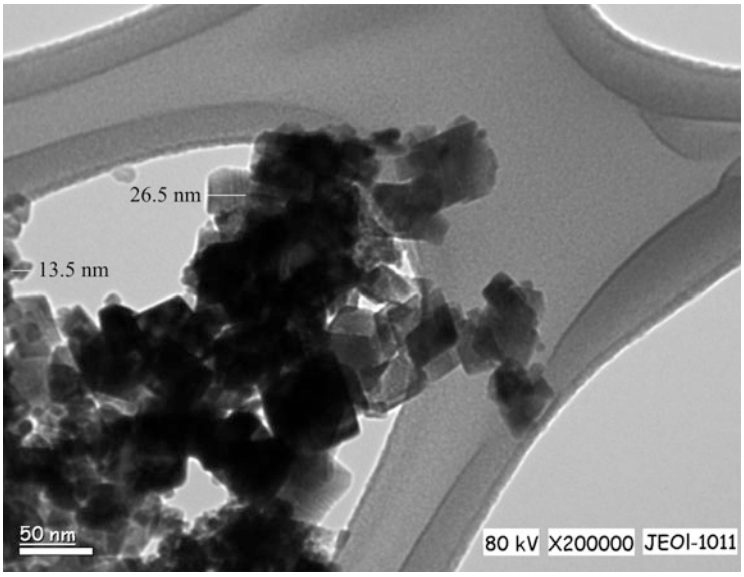

Fig. 11.1 TEM image of NiO nanoparticles at ×200,000 magnification

(indicating their aggregation) are in accordance with several reports which studied the hydrodynamics of ZnO, TiO_2, and superparamagnetic FeO nanoparticles (Saquib et al. 2012; Sharma et al. 2012; Singh et al. 2012).

11.3.2 Effect of NiO Nanoparticles on Seed Germination and Growth

The effects of different concentration of NiO nanoparticles on seed germination and root growth were examined and compared with the control plants. In the present study, it was observed that NiO nanoparticles showed a tendency to get adsorbed on the seed coat of the radish seeds (Fig. 11.2A). The nanoparticles showed more adsorption to seed coats with increased concentration. This could, mainly, be attributed to the physical attachment of nanoparticles to the rough surface of radish seeds and the hydrophobic and electrostatic attractions between nanoparticles and seeds. Wu et al. (2012) stated that adsorption of nanoparticles on seed coat may stimulate generation of ions released from nanoparticles and thus increase the phytotoxicity of such nanoparticles. Application of NiO nanoparticles for 4 h on radish seeds led to significant reduction in percent seed germination and root length (Fig. 11.2B). After 4 days of incubation, seed germination of $39.3 \pm 4.04\%$ with root length of 0.46 ± 14 cm was documented from seeds treated with 2 mg mL^{-1} of NiO nanoparticles. Comparatively, untreated seeds exhibited $91.4 \pm 4.50\%$ germination with average root length of 2.53 ± 17 cm. Earlier reports on different nanoparticles such as Fe_2O_3, Zn, ZnO, Cu, SWCNTs, and TiO_2 in plants (radish, perennial ryegrass, tomato, oilseed rape, pumpkin, and Asian rice) showed the same pattern of results (Boonyanitipong et al. 2011; Cañas et al. 2008; Lin and Xing 2008; Stampoulis et al. 2009). In *Arabidopsis thaliana*, it was found that fullerene when dissolved in water showed negative effects on root elongation and led to loss of gravitropism of roots (Liu et al. 2010). On the other side, TiO_2 and MWCNT nanoparticles penetrated seed coats and enhanced the germination rates and root elongation of tobacco and spinach at concentrations ranging from 10 to 40 and 55 to 500 μg mL^{-1}, respectively.

11.3.3 Translocation of NiO Nanoparticles

Uptake and translocation of NiO nanoparticles into the cytoplasm were confirmed by the analysis of ultrathin sections of root tissues using TEM (Fig. 11.3). The nanoparticles were localized in the cytoplasm and aggregates in cell vacuoles observed in the form of black dots (Fig. 11.3B). The observed TEM data by the treatment with NiO nanoparticles in radish are in accordance with earlier reports on *Schoenoplectus tabernaemontani* (Zhang et al. 2015a) and *Solanum melongena* (Faisal et al. 2016). Subcellular anomalies like mitochondrial fission, abundance of peroxisomes, and excessive vacuolization were observed as compared to untreated samples.

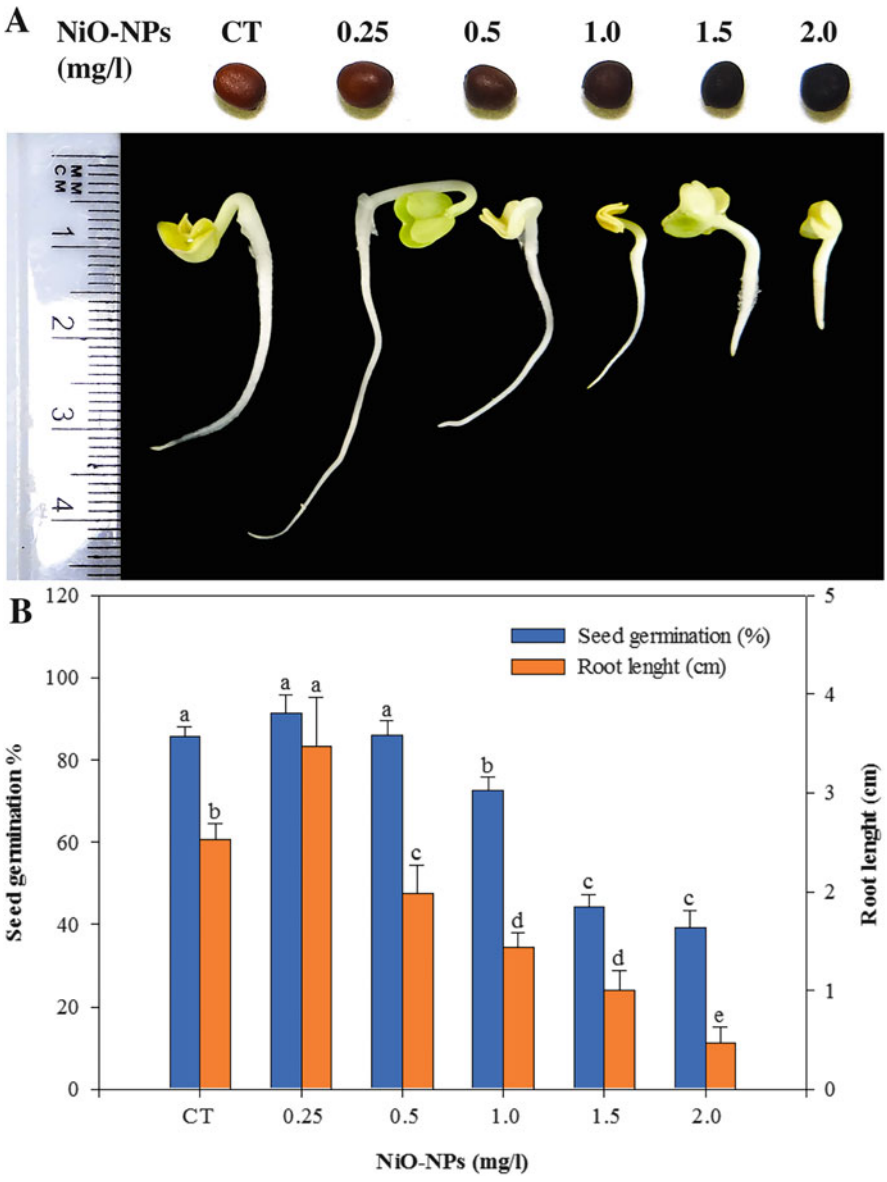

Fig. 11.2 (**a**) Adsorption of NiO nanoparticles on radish seeds and phenotypic changes showing stunting and thickening of radish seedling after 4 days of exposure. (**b**) Concentration dependent repression of root length after 4 days of exposure NiO nanoparticles. Bars represent the mean ± SD. Bars denoted by the same letter are not significantly different ($p \leq 0.05$) using Duncan's multiple range test

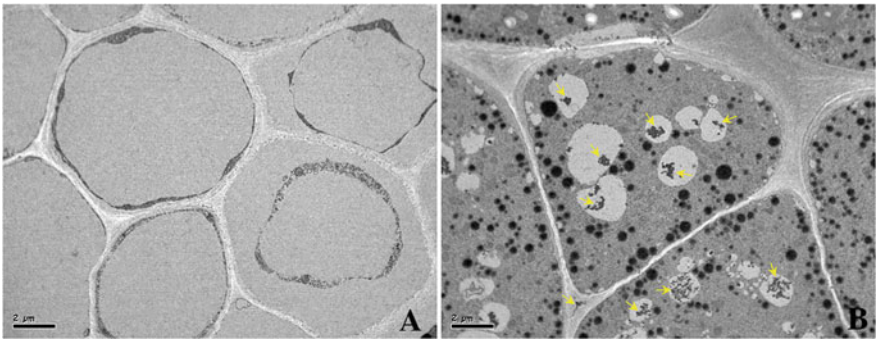

Fig. 11.3 (**a**) Ultrastructure of control roots showing the parenchymal cells and mitochondria with integrated cristae and no appearance of peroxisomes and vacuoles. (**b**) Ultrastructure of root tissue from treatment group of 2.0 mg/mL NiO nanoparticles showing the presence of NPs, abundance of peroxisomes and excessive vacuolization

11.3.4 Lipid Peroxidation and Changes in ΔΨm Induced by NiO Nanoparticles

Lipid peroxidation is considered as a potential indicator for cellular membrane damage owing to oxidative stress. ROS promotes the lipid peroxidation causing membrane damage and other lipid components in the cell generating several by-products that cause harsh damage to DNA (Girotti 1998; Tuteja et al. 2001). In this study, the level of lipid peroxidation product malondialdehyde (MDA) was measured. The concentration of MDA in seedlings treated with NiO nanoparticles was 6.0, 9.3, 16.0, 17.1, and 19.3 μM at 0.25, 0.5, 1.0, 1.5, and 2.0 mg mL^{-1} concentrations, respectively (Fig. 11.4), which is significantly higher when compared to control plants (3.2 μmol g^{-1} FW). Wang et al. (2011) found that perennial ryegrass and pumpkin plants exposed to Fe_3O_4 nanoparticles showed increase in lipid peroxidation by 218 and 259%, respectively. Furthermore, exposure of onion roots to TiO_2 nanoparticles led to increase in lipid peroxidation by 4.5-folds compared to control plants (Ghosh et al. 2010). Increased oxidative stress above the threshold of plant tolerance affects the functions of different cellular components via lipid peroxidation of cellular and organelle membranes (Gill and Tuteja 2010; Montillet et al. 2005) which results in membrane damage and as well as severe damage to DNA (Tuteja et al. 2001).

Furthermore, Rh123 was used to assess the qualitative changes in $\Delta\Psi m$ to validate the membrane damage which resulted from ROS generation. Roots treated with 0.25 and 0.5 mg mL^{-1} NiO nanoparticles showed remarkable reduction in Rh123 fluorescence as compared to control roots (Fig. 11.5A–E). On the other hand, a gradual increase in Rh123 fluorescence was observed with increasing concentration (Fig. 11.5B–E), which could be attributed to diffusion into the cytoplasm of cells. Increase in fluorescence intensity at higher concentrations could be due to the

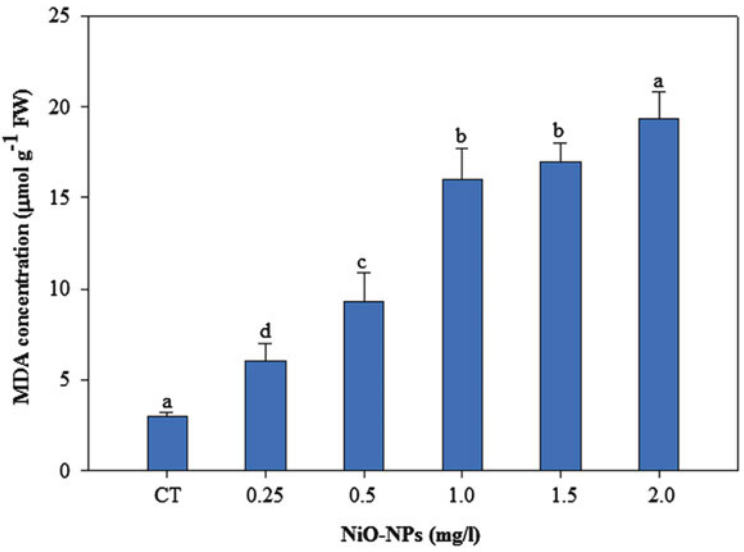

Fig. 11.4 Effect of NiO nanoparticles on MDA concentration. Bars represent the mean ± SD. Bars denoted by the same letter are not significantly different ($p \leq 0.05$) using Duncan's multiple range test

mitochondrial ability to dwindle and expand with the changes in $\Delta\Psi m$. After changes in shape, mitochondria are not able to retain Rh123 anymore, and it is leaked out to the cell cytoplasm mainly via bulge (Ouédraogo et al. 2000). On the other hand, at the lower concentrations (0.25 and 0.5 mg mL^{-1}), $\Delta\Psi m$ is dispersed via inner membrane permeability. Our results supported the earlier findings about the strong relation between increased ROS generation and changes in $\Delta\Psi m$ (Liu et al. 2010). Based on these results, it could be established that lower concentrations of NiO nanoparticles (0.25 and 0.5 mg mL^{-1}) disperse $\Delta\Psi m$, while higher concentrations (1.0, 1.5, and 2.0 mg mL^{-1}) bulge the mitochondria causing leakage of Rh123 to the cell cytoplasm which led to hyperpolarization of the dye. It was reported that $\Delta\Psi m$ dispersion is an early indicator of cell apoptosis in plants (Yao et al. 2004). Mitochondrial dysfunction could result from ROS generation in the complex I and II of electron transport system as these ROS react with different lipids and proteins inside the mitochondria and hinder their functions (Indo et al. 2007).

11.3.5 Effect of NiO Nanoparticles on Intracellular ROS Generation and Antioxidant Activities

Generation of ROS by NiO nanoparticles in root tissue of radish plants was examined using DCF-DA staining (Fig. 11.6A–F). DCF fluorescence was considered as an indicator for oxidative stress in the plant cell. In this study, at higher

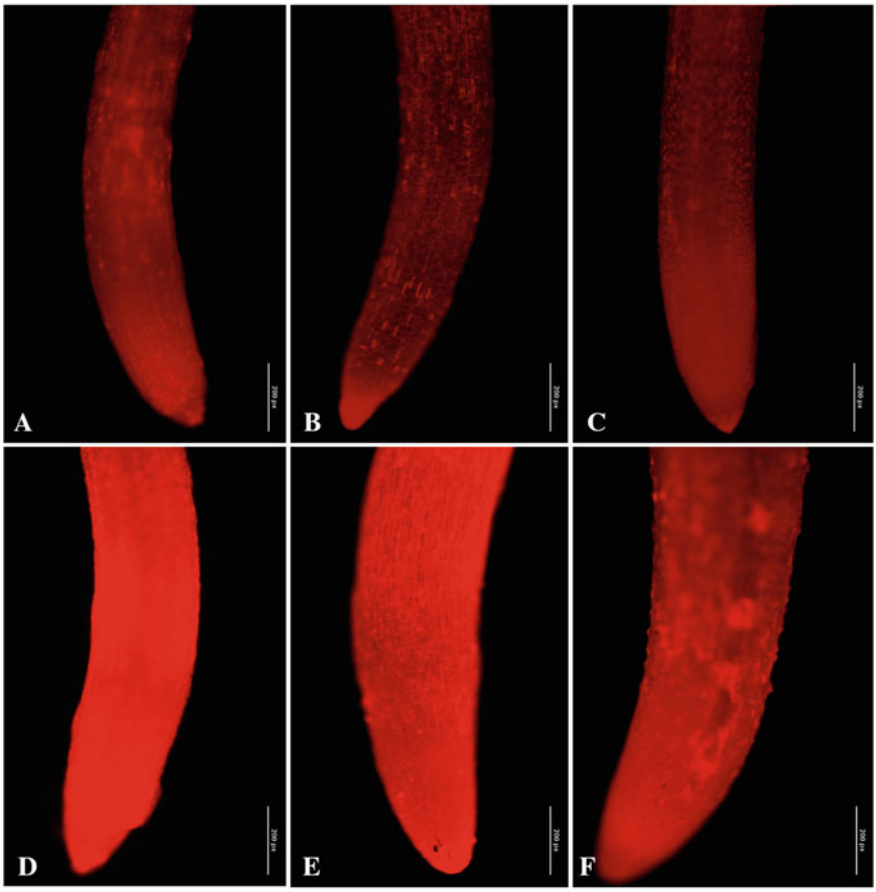

Fig. 11.5 Radish roots stained with Rh123 showing fluorescence enhancement upon NiO nanoparticles exposure

concentrations of NiO nanoparticles, enhancement in the fluorescence of DCF was observed (Fig. 11.6B–E). The results are considered as a great evidence for induced oxidative stress by exposure to NiO nanoparticles. Oxidative stress was found in the roots of cabbage and Asian rice exposed to salinity stress and/or MWCNT stress (Hernandez et al. 2010; Tan et al. 2009). Heavy metal stress may cause damage to plant cells both directly and indirectly. It was suggested that such damage may be attributed to the indirect generation of ROS (Kumari et al. 2009; Zhang et al. 2005). It was approved that ROS caused DNA damage, protein modifications, and purine oxidations as they have the ability to react with different cellular components (Beckman and Ames 1997; Berlett and Stadtman 1997). NiO nanoparticles may cause programmed cell death via cellular damage in which ROS play a major role.

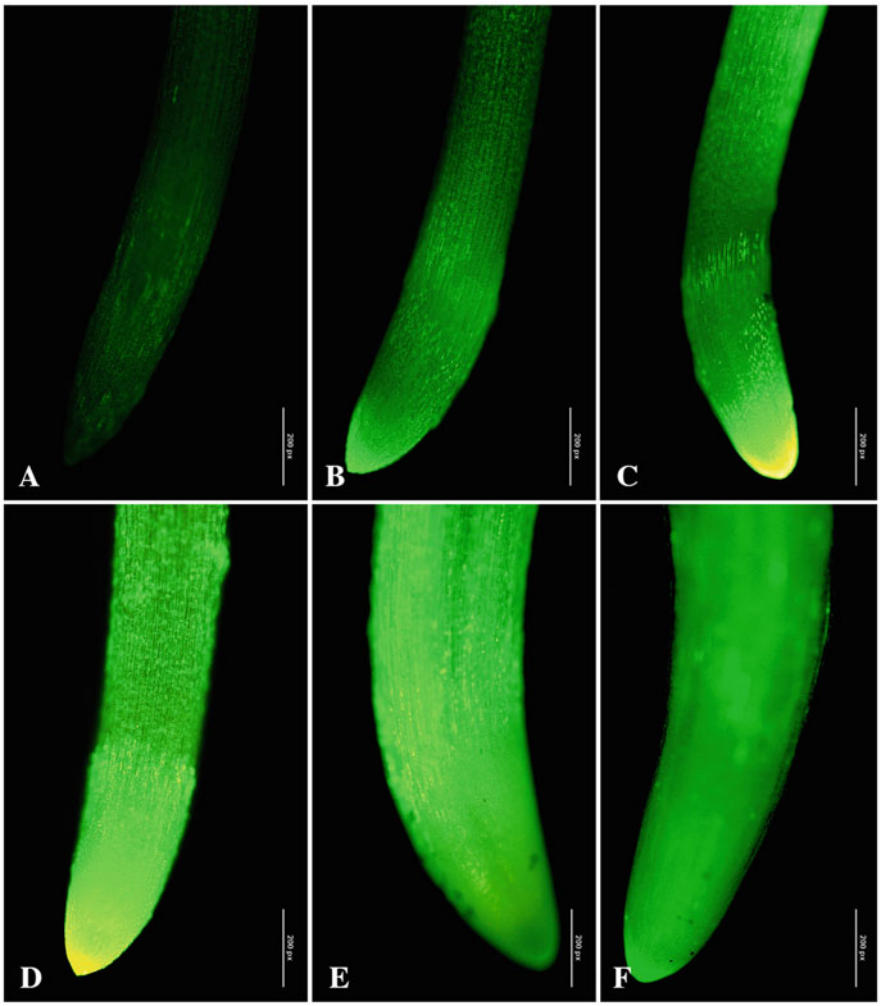

Fig. 11.6 DCF fluorescence in radish roots showing localization of ROS in root tip and area of elongation upon NiO nanoparticles exposure

Superoxide dismutase (SOD), catalase (CAT), and total glutathione (GSH) were also measured to examine the level of oxidative stress in the radish seedlings treated with NiO nanoparticles (Fig. 11.7A–C). CAT activity in control plants was 12.16 $nmol^{-1}$ min^{-1} mL^{-1}; however, it was increased up to 41.16 $nmol^{-1}$ min^{-1} mL^{-1} at 1.5 mg L^{-1} (Fig. 11.7). On the other hand, at the highest concentration of 2.0 mg mL^{-1} NiO nanoparticles, activity of CAT was reduced to 23.67 $nmol^{-1}$ min mL^{-1} (Fig. 11.7A). Activity of SOD was higher in NiO nanoparticles treated plants as compared to control plants (0.87 U mL^{-1}). SOD activity was 1.81, 2.10,

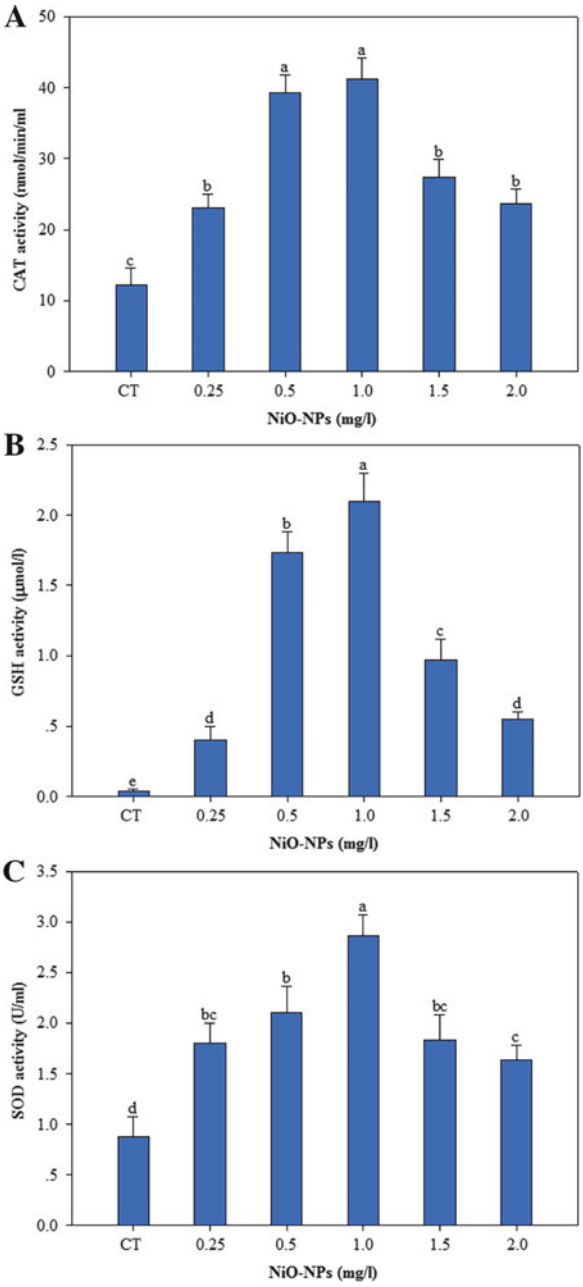

Fig. 11.7 Effect of NiO nanoparticles on antioxidant enzymes, CAT (**a**) GSH (**b**), and SOD (**c**) in root tissues of radish. Bars represent the mean ± SD. Bars denoted by the same letter are not significantly different ($p \leq 0.05$) using Duncan's multiple range test

and 2.86 U mL^{-1} at 0.25, 0.5 and 1.0 mg mL^{-1} concentrations of NiO nanoparticles, while it started to decrease to 1.83 and 1.64 U mL^{-1} at higher concentrations (1.5 and 2.0 mg mL^{-1}) (Fig. 11.7B). Similarly, the total accumulated GSH in the root tissues of plants treated with NiO nanoparticles was higher as compared to that accumulated in the roots of control plants (Fig. 11.7C), while less accumulation (0.55 + 0.15 μmol L^{-1}) was observed at higher concentrations of 2 mg mL^{-1} NiO nanoparticles (Fig. 11.7C). Similar results were reported regarding the activity of SOD, CAT, and GSH in plants exposed to different nanoparticles (Estrella-Gómez et al. 2012; Kim et al. 2012; Wang et al. 2011). To mitigate the adverse effects of ROS generated under abiotic stress, plant cells increase the activity of different antioxidant enzymes, especially SOD, CAT, and GSH as they constitute the first line of defense against oxidative stress and toxicity inside the cell (Gill and Tuteja 2010). When the level of oxidative stress exceeds the threshold of plant tolerance, plant antioxidant system and the activity of different antioxidants are compromised and weakened (Lee et al. 2013), which is the case in our study at higher concentrations (1.5 and 2.0 mg mL^{-1}) of NiO nanoparticles.

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Nanosilicon Particle Effects on Physiology and Growth of Woody Plants

12

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12.1 Introduction

Total silicon is a very important element of the earth's crust (Sommer et al. 2006), and it consists up to 10% of the many plants (Hodson et al. 2005). Si is not accepted as an essential mineral element for plants; but the beneficial effects for growth and production of many plants, especially in terms of tolerance of biotic and abiotic stresses, have been examined and documented in the literature (Chalmardi et al. 2014; Ma and Yamaji 2006, 2015). Interestingly, even if silicon accumulates

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_12

excessively in plants, it is the only element that has no serious detrimental effects (Ma and Yamaji 2015).

Researchers think that absorption of nanoparticles (NPs) in plants is greater than the same chemicals applied to the plant as bulk particles (Braunack 1995; Suriyaprabha et al. 2012b; Shukla et al. 2016). Further, Zarafshar et al. (2015) reported nontoxic effects of silicon oxide SiO_2 NPs on pear seedlings even when the seedlings were irrigated with high concentrations of SiO_2 NPs. In recent years, SiO_2 NPs are regarded as important to examine growth and developmental aspects in plant biology; nevertheless these studies are limited. In this regard, Bao-shan et al. (2004) immersed roots of Changbai Larch (*Larix olgensis*) seedlings in nanosilicon solution at concentrations from 62 to 2000 μl.1 L^{-1} for 6 h. Results exhibited positive effects of silicon nanoparticles on growth and quality of the seedlings. Haghighi and Pessarakli (2013) showed that application of silicon in nano and bulk size was beneficial for improving salinity tolerance of tomato plants. Also, Karimi and Mohsenzadeh (2016) applied SiO_2 NPs at concentrations of 50, 100, 200, 400, and 800 mg L^{-1} on *Triticum aestivum* L. seedlings and concluded that nanosilicon application displayed negative effects along with some positive effects in wheat seedlings. In addition, SiO_2 NPs improved seed germination and prompt growth of lentil (*Lens culinaris* Medik) seedlings under salinity stress (Sabaghnia and Janmohammadi 2015).

Nano-materials confirm particle size between 1 and 100 nm and implicate new physical, chemical, and biological properties compared to bulk size materials (Monica and Cremonini 2009). Nano-sciences have led to the development of a wide range of applications for enhancing plant growth (Nair et al. 2010). Carbon-based, metal oxides, quantum dots, nano-sized polymers, and biocomposite materials are being fabricated using plants as natural factories (Khot et al. 2012). Application of nanotechnology in order to improve crop productivity especially woody plant is completely raising science, and, therefore, more extensive investigations are required. However, mechanisms for NPs uptake, accumulation in plants, and their effects on growth and development of woody plants are still unclear (Nair et al. 2010). To evaluate the enigmatic behavior of silicon nanoparticles, an experiment was conducted to study the effects of SiO_2 NPs on water status, gas exchange, and growth parameters of hawthorn (*Crataegus aronia* L.) and mahaleb (*Prunus mahaleb* L.) seedlings.

12.2 Materials and Methods

12.2.1 Preparation of Materials

In late winter, 108 dormant (uniformly sized) 1-year-old bare roots from each one mahaleb (*Prunus mahaleb* L. syn. *Cerasus mahaleb* L. Mill. Rosaceae) and hawthorn (*Crataegus aronia* L.) seedlings were obtained from an Iranian forest nursery and transferred to the experimental garden facility at the Faculty of Natural Resources and Marine Sciences of Tarbiat Modares University, Noor, Mazandaran, Iran. Seedlings were transplanted to plastic pots (7 L) containing a mixture of forest

Table 12.1 Characteristics of the used soil in the study

Characteristics	Silt (%)	Sand (%)	Clay (%)	Bulk density	pH	Organic carbon (%)	Available phosphorus (ppm)	Exchangeable potassium (ppm)
	28	46	26	1.26	8.04	0.875	30.33	274.6

brown soil, river sand, and clay (2:1:1, v/v/v; see soil characteristics in Table 12.1) and grown in a greenhouse with day/night average temperatures of 30/21 °C.

12.2.2 Nanoparticles Treatments

After potting, SiO_2 NPs procured from TECNAN (Tecnología Navarra de Nanoproductos S.L., Spain; see characteristics in Table 12.2) were applied at three different concentrations (i.e., 10, 50, and 100 mg L^{-1}) for 45 days, whereas a treatment without SNPs was taken as control. Pots were irrigated to field capacity (300 mL pot^{-1}) with SiO_2 NPs suspensions every 3 days. There were 27 seedlings in each SiO_2 NPs treatment.

12.2.3 Plant Physiological Parameter Measurements

Net photosynthesis (A, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mmol m^{-2} s^{-1}), and transpiration rate (E, mmol m^{-2} s^{-1}) were measured at 10, 20, 30, and 40 days after the SiO_2 NPs treatments. All gas exchange parameters were taken in triplicate from six randomly selected plants in each treatment. Measurements were done on sunny days (between 09:00 and 11:00 h) at temperatures ranging from 22 to 28 °C, using a portable infrared gas analyzer (Model LCpro+, ADC BioScientific Ltd., Hertfordshire, UK). Average values of leaf temperature and internal CO_2 concentration were 27.5±3.1 °C and 340 ± 11.9 ppm, respectively.

At the end of the experiment (i.e., day 45), xylem stem potential (ψ stem, MPa) was measured with a pressure chamber system (Skye, SKPM 1400, UK). Complementarily, relative water content (RWC) of leaves was determined according to the following description: four leaves (from similar positions) were removed from randomly selected plants from each treatment, weighed immediately (W_f), and incubated in tubes with deionized water for 24 h at room temperature under low light. Afterward, individual leaves were reweighed to determine their turgid weights (W_t). Finally, the samples were placed in an oven at 60 °C for 48 h and then reweighed to obtain their dry weights (W_d). RWC was calculated by the following equation:

$$\mathrm{RWC} = \frac{W_f - W_d}{W_t - W_d} \times 100$$

Table 12.2 Characteristics of nanoparticles are used in the study

Nanostructure	Silica nanoparticles
Chemical formula	SiO_2
Color	White
Morphology	Amorphous
Size range	10–15 nm
Specific surface area	180–270 $m^2\ g^{-1}$
Purity	99.999%

12.2.4 Plant Morphological Parameters and Growth

At the end of the experiment, primary stem length, collar diameter, longest root, and root volume of all seedlings were measured. Root length was measured using a scaled ruler, and root volumes were measured through water displacement in graduated cylinders. Afterward, seedlings were harvested separating roots and shoots (i.e., aerial organs), and then all tissues were oven dried for 48 h at 70 °C to obtain their corresponding dry weights.

12.2.5 Microscopic Observations

At the end of the experiment, fresh root sections were taken for microscopic analysis. The adsorption of SiO_2 NPs to fresh roots was observed by scanning electron microscopy (SEM) (KYKY-EM3200) in the laboratory of Tarbiat Modares University.

12.2.6 Measurements of Leaf Nutrient Elements

Oven-dried leaves were pulverized in an electric mill. The powdered leaf tissues were transmitted to the atomic energy organization of Iran (AEOI). The concentrations of Si, N, P, and K were detected by X-ray fluorescence analysis (XRF) (ED 2000 Oxford Instruments Corporation).

12.2.7 Statistical Analysis

Physiological data were analyzed through repeated measures ANOVA (rmANOVA). All other variables were assessed using one-way ANOVAs. For comparison between groups, Duncan's multiple range tests were applied at 0.05 probability level. In case of percentage data, arcsin transformation was applied before ANOVA analyses. All data were tested for normality, homogeneity of variance, and Mauchly's test prior to ANOVAs. Statistical analyses were performed using SPSS software (IBM SPSS Statistics).

12.3 Results

12.3.1 Confirmation of the Presence of SiO_2 NPs in Treated Roots

SEM analysis confirmed presence of SiO_2 NPs of consistent size on the root surface of treated seedlings of both the plant species whereas not found on the root surfaces of untreated seedlings (Fig. 12.1). Observation of the root system of treated plants revealed the presence of nanoparticles aggregates attached to the roots at the highest SiO_2 NPs concentration (100 mg L^{-1}) (Fig. 12.1), while fewer nanoparticles were found adhered on the roots treated with lower concentrations of SiO_2 NPs (i.e., 10 and 50 mg L^{-1}) (images not shown).

12.3.2 Effect of SiO_2 NPs on Gas Exchange Parameters

Results revealed that SiO_2 NPs application showed positive response for photosynthesis rate (A), stomatal conductance (g_s), and transpiration rate (E) in both species (repeated measures ANOVA; treatment and treatment x time effect, $P < 0.001$; data are not shown). The study further indicated that the positive effect of SiO_2 NPs on A, g_s, and E was evident after 20 days; this difference was maintained until the end of the experimental period. At the end of the experiment, seedlings treated with 50 and 100 mg L^{-1} SiO_2 NPs registered significantly higher values for such parameters than those of control plants (i.e., without SNPs treatment) (Fig. 12.2). However, more profound effects for SNPs treatments were observed in hawthorn for photosynthesis rate (A) and in mahaleb for stomatal conductance (g_s) and transpiration rate (E).

12.3.3 Effect of SiO_2 NPs on Water Relation Parameters

Relative leaf water content (RWC) decreased with increasing concentrations of SiO_2 NPs treatments in mahaleb but not in hawthorn seedlings (Fig. 12.3). Xylem water potential (XWP) was also negatively affected by SiO_2 NPs treatment in both species. On this note, both species showed decreasing XWP with increasing concentrations of SiO_2 NPs treatments. Nevertheless, the adverse effects of SiO_2 NPs for RWC and XWP were more prominent in hawthorn in comparison to mahaleb.

12.3.4 Effect of SiO_2 NPs on Growth, Root Morphology, and Biomass Allocation

Irrigation with SiO_2 NPs did not affect hawthorn and mahaleb seedling height and root collar diameter (data not shown). Results, in general, established a positive correlation between the SiO_2 NPs treatments and growth parameters studied, viz., root length, root volume, root biomass, stem biomass, leaf biomass, and biomass allocation. The root length was greater in SiO_2 NPs-treated seedlings in comparison

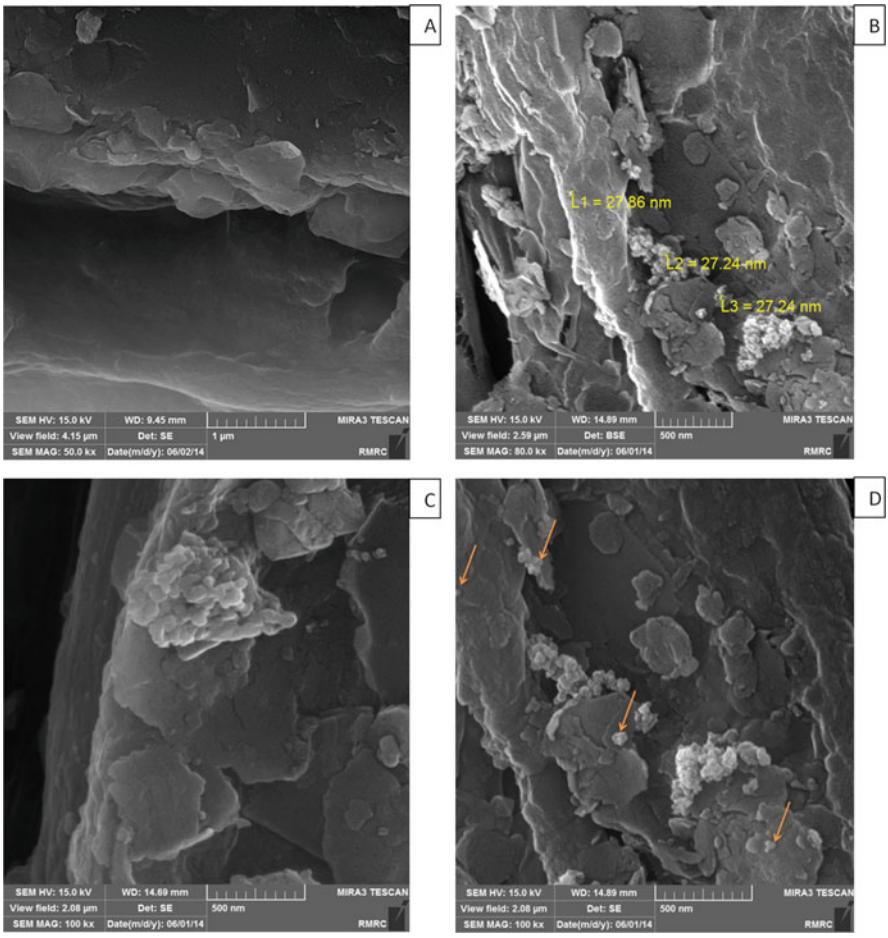

Fig. 12.1 Scanning electron microscopy (SEM) images of root surface of mahaleb [(**a**) for 0 mg L^{-1} and (**b**) for 100 mg L^{-1} treatments] and hawthorn seedlings [(**c**) for 0 mg L^{-1} and (**d**) for 100 mg L^{-1} treatments]

to control seedlings for both species. The positive effects of SiO_2 NPs on root volume were evident because root volume progressively increased with increase of SiO_2 NPs concentration; but the increasing root volume was not significant in mahaleb seedlings. Generally, positive effects of SiO_2 NPs on the whole dry weight of the plant were recorded. Among three plant components, root biomass was improved in a higher extent by SiO_2 NPs treatments in comparison with stem and leaf biomass (Fig. 12.4). More pronounced effects of SiO_2 NPs treatment were seen in hawthorn when compared to mahaleb.

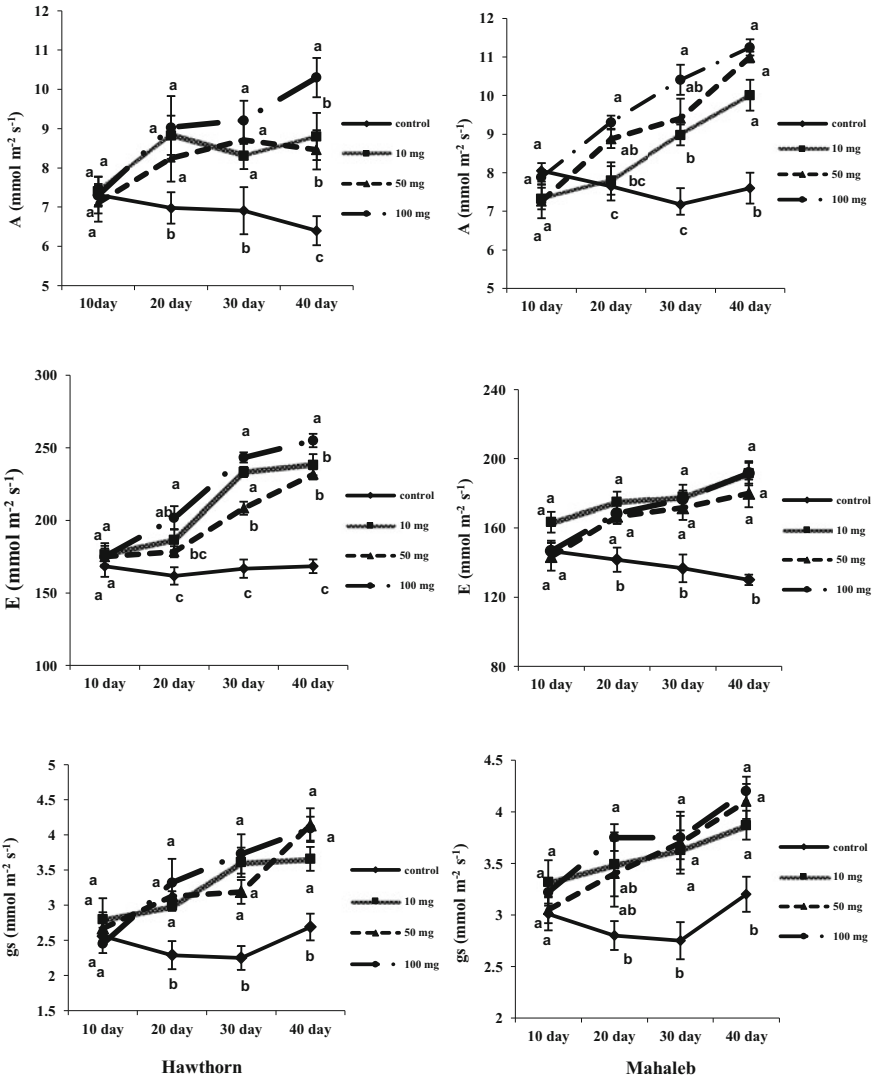

Fig. 12.2 The effects of SNPs on photosynthesis rate (A), stomatal conductance (g_s), and transpiration rate (E) in hawthorn and mahaleb plants. The gas exchange was assayed at days 10, 20, 30, and 40 on the recent fully expanded leaves of six randomly selected plants in similar positions on the plant (multiple leaves per plant) in hawthorn and mahaleb seedlings (mean ± SE; $n = 6$). *SNPs* silica nanoparticles

12.3.5 Effect of SiO_2 NPs on Nutrients

As evident, Si concentration in leaf tissues was significantly higher at increasing SiO_2 NPs concentrations in both species (Fig. 12.5). Concentration of N in leaves

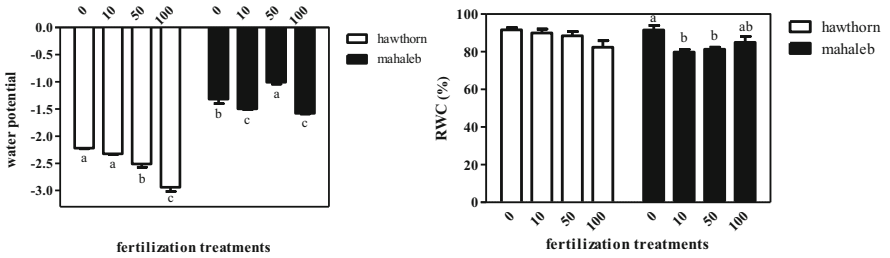

Fig. 12.3 Relative water content and xylem water potential of hawthorn and mahaleb seedlings treated with different concentration of SNPs. Different letters indicate significant differences ($P < 0.05$) among treatments based on the Duncan tests (mean ± SE; $n = 6$)

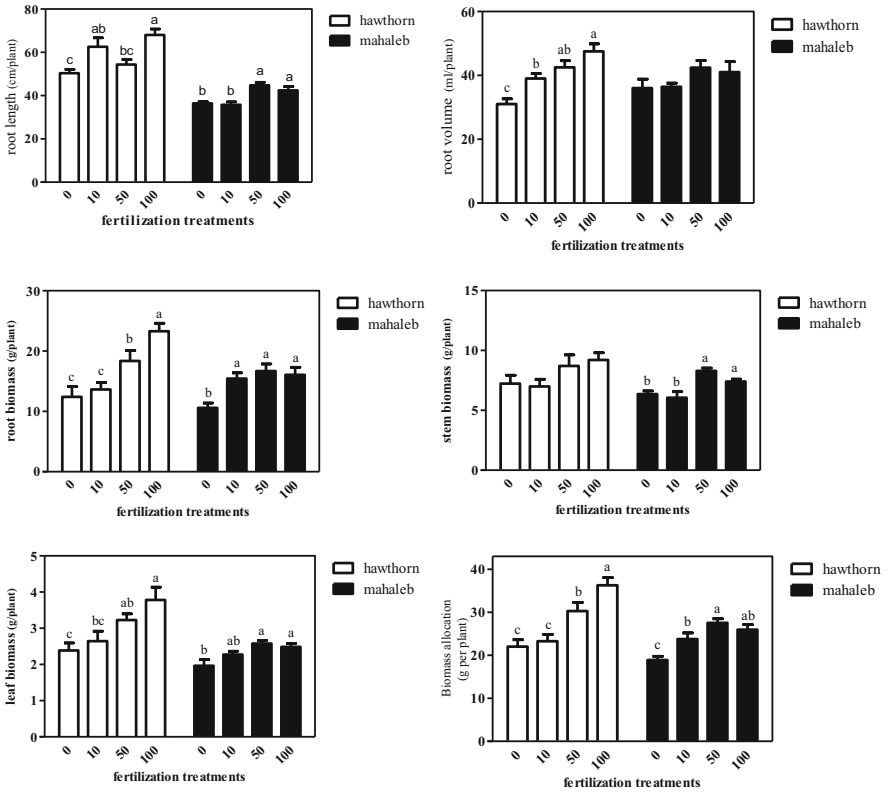

Fig. 12.4 Root length, root volume, and biomass allocation of mahaleb and hawthorn seedlings treated with different concentration of SNPs. Different letters indicate significant differences ($P < 0.05$) among treatments based on the Duncan tests (Mean ± SE; $n = 6$)

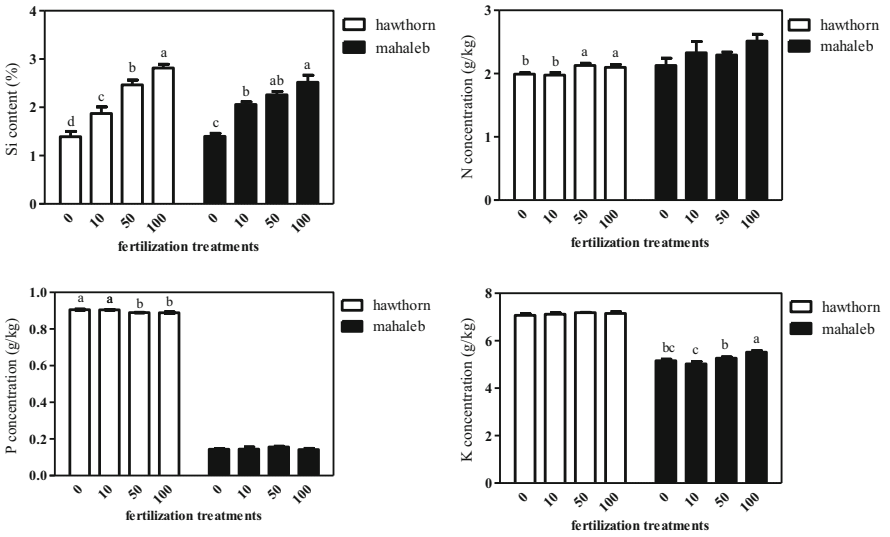

Fig. 12.5 Concentration of Si, N, P, and K in leaf tissues of mahaleb and hawthorn seedlings (on dry weight basis) treated with different concentration of SNPs. Different letters indicate significant differences ($P < 0.05$) among treatments based on the Duncan tests (mean ± SE; $n = 6$)

was higher in seedlings treated with 50 and 100 mg L^{-1} SNPs compared to the untreated in hawthorn seedling (Fig. 12.5); but the increasing N concentration under the influence of SiO_2 NPs was not significant in mahaleb seedlings. On the other hand, in the case of hawthorn, seedlings had slightly higher leaf P concentrations under control conditions, and also the effects of SiO_2 NPs on P concentrations were not significant in mahaleb seedlings. Finally, SiO_2 NPs application did not affect the concentration of K in hawthorn leaves, while in mahaleb, K concentrations were slightly higher in SiO_2 NPs-treated seedlings (Fig. 12.5).

12.4 Discussion

Silicon (Si) is ubiquitously present both in oceans and on terrestrial areas (Basile-Doelsch et al. 2005). Plant Si plays a pivotal role in regulation of growth and development and, therefore, can be considered a "quasi-essential" element for plants because its deficiency can cause several irregularities in plant's functional biology. Nevertheless, its functions in plant biology have been poorly understood, and the efforts to associate Si with metabolic or physiological activities have been unclear (Epstein 1994; Epstein and Bloom 2005).

The present study witnessed the presence of nanoparticles on the root surfaces of treated plants. Occurrence of SiO_2 NPs in roots of treated plants was confirmed by SEM. Similarly in a previous report, Lin and Xing (2008) described interaction of ryegrass with NPs where scanning electron microscopy studies confirmed the

adsorption and aggregation of the NPs on the root surface. TEM images of root cross sections of the ryegrass also showed the presence of particles in the apoplast, cytoplasm, and nuclei of the endodermal cells. Similar results were reported by Lin et al. (2009) where uptake, accumulation, and the translocation of natural organic matter (NOM), suspended fullerene C_{70}, and MWCNT were studied in rice plants. C70 accumulation in the form of black aggregates was observed. These aggregates were found more in the seeds and roots as compared to the stems and leaves of the rice. In the present study, Si accumulation pattern varied in both species. Si accumulation among different species have attributed to variations in the root density (Ma and Yamaji 2008). Si uptake and transport by the root systems have shown to be either unspecific or mediated by special groups of aquaporins (Ma et al. 2004; Ma and Yamaji 2006).

In the present study, application of SiO_2 NPs resulted in improved root growth, stem, and leaves in plants of both the species. Several reports endorsing the roles of nanoparticles for enhancement of yield, plant biomass, have recently been examined and documented in the literature (Parveen and Ashraf 2010; Misra et al. 2016; Gautam et al. 2016a, b; Rajoriya et al. 2016). In a research it was observed that leaf area development and chlorophyll content increased as result of silica availability to plants under saline stress, which resulted in increased fresh and shoot dry weight. This increase was attributed to increased turgor pressure, RWC, and improved water use efficiency due to application of silica nanoparticles (Rawson et al. 1988). Similarly, exogenous application of SiO_2 NPs mediated seedling growth enhancement and improvement of quality characters, viz., mean height, root collar diameter, main root length, and the number of lateral roots in Changbai larch (*Larix olgensis*) seedlings, as well as increased the synthesis of chlorophyll (Bao-shan et al. 2004). Tamai and Ma (2008) stated the importance of silicon for growth and high production of rice, which can accumulate Si to over 10% of shoot dry weight, and under scarce concentration of Si, the yield is reduced. Similar effects were also reported by Suriyaprabha et al. (2012a) where SiO_2 NPs amendment to soil increased the synthesis of total protein and facilitated the uptake of nutrients, favoring growth of maize plants. In contradiction to our findings, significant decrease in plant height, shoot, and root biomasses was found when transgenic cotton plants were treated with SiO_2 NPs (Le et al. 2014).

Nanotechnology has the potential to improve function of photosynthetic machinery. Studies on the impacts of NPs on plant photosynthetic activities have gained much attention (Falco et al. 2011; Giraldo et al. 2014). In the present study, SiO_2 NPs application showed positive response for photosynthesis rate (A), stomatal conductance (g_s), and transpiration rate (E) in both species. Prior studies have provided similar reports where positive effects of NPs on photosynthesis and transpiration were recorded. Samuels et al. (1993) showed that the presence of silicon resulted in high light absorption and consequently increased photosynthetic capacity of the plant, as well as it improved mechanical strength of stems and leaves. Potential of silicon to enhance chlorophyll concentrations per unit leaf area and enzyme ribulose bisphosphate carboxylase in leaf tissue was already reported (Adatia and Besford 1986). This enzyme regulates CO_2 metabolism and promotes

improved carbon assimilation by plants, leading to higher rate of photosynthesis. SiO_2 NPs enhance the plant growth and development by increasing gas exchange and chlorophyll fluorescence parameters, such as net photosynthetic rate, transpiration rate, stomatal conductance, PSII potential activity, effective photochemical efficiency, actual photochemical efficiency, electron transport rate, and photochemical quenching (Xie et al. 2011; Siddiqui et al. 2014).

Our findings are in agreement with findings by Shi et al. (2013) who also reported that application of silicon has the potential to improve the net photosynthetic rate, stomatal conductance, and transpiration rate in seedlings of rice. These positive effects of Si nanoparticles were shown by Singh et al. (2015) where chlorophyll a, chlorophyll b, and carotenoid concentration increased in leaves of treated plants irrespective of nanoparticles concentrations applied (as compared to controls). SiO_2 NPs increase photosynthetic rate by changing the activity of carbonic anhydrase and synthesis of photosynthetic pigments (Xie et al. 2012; Siddiqui et al. 2014). Nano mesoporous silica compound (SBA) bound with photosystem II and increase activity of photosynthetic oxygen evolving reaction (Noji et al. 2011). Xie et al. (2012) stated enhancement in the photosynthetic activity of mesophyll cells in *Indocalamus barbatus* (bamboo) after foliar spraying with Si nanoparticles. Siddiqui et al. (2014) reported that SNPs could increase both chlorophyll content and net photosynthetic rate in squash (*Cucurbita pepo* L.). Similarly, Sun et al. (2016) reported positive effect of mesoporous silica nanoparticles (MSNs) on photosynthetic machinery in wheat and lupin where photosynthetic capacity was analyzed in isolated wheat and lupin chloroplasts, evaluating the Hill reaction. MSN treatments at concentrations of 500 and 1000 mg L^{-1} greatly promoted oxygen release rate of both wheat and lupin (Sun et al. 2016). In addition, the photosynthesis activity of wheat plants exposed to 2000 mg L^{-1} MSNs was also significantly enhanced. For both lupin and wheat, the maximum photosynthetic activity efficiency was reported at 500 mg L^{-1} MSNs compared with the control, enhanced by 53.9 and 44.6%, respectively. Intense increase in the total proteins as well as both chlorophyll a and b pigments was correlated with enhanced photosynthetic capacity.

Chlorophyll biosynthesis is an end point of tetrapyrrole metabolism which is executed via a series of cooperative reactions catalyzed by a number of enzymes, and the production of chlorophyll is influenced by short-term environmental changes (Wang and Grimm 2015). Silicon plays an important role in the synthesis of intracellular organic compounds and for the maintenance of normal biochemical reactions (Matichenkov et al. 2008). Recent studies have demonstrated that the application of Si significantly enhanced the expression levels of genes such as HemD and PsbY, which were related to chlorophyll biosynthesis and degradation. It has been suggested also that stimulation of expression of these genes improves the activity of photosystem II and the electron transfer rate (Song et al. 2014; Li et al. 2015).

Si plays an important role in keeping a high moisture condition at decreasing transpiration rate range under drought stress condition, and Si in rice leaves was involved in water relations of the cells, such as mechanical properties and water permeability, which are important factors for normal development of plants (Ma 2004). Parveen and Ashraf (2010) found that exogenously applied Si enhanced

plant water use efficiency (WUE) of maize and slightly increased photosynthetic rate under saline stress condition in wheat cultivars and maize. These responses are likely due to the role of silicon in the enzyme activities and biochemical processes in plant tissues commented before. Reduction of plant water uptake with salinity could be related to reductions of stomatal conductance and transpiration. There is a correlation between Si uptake and the amount predicted from water loss and Si concentration in soil solution. So, Si accumulation in the shoot dry mass may therefore be a suitable parameter for calculation of water use efficiency (WUE) in cereals grown under rain-fed conditions (Walker and Lance 1991). However, even in plants where close correlations between transpiration and Si accumulation are found, it should be emphasized that roots are not freely permeable to the radial transport of Si (Ma and Yamaji 2006). Rad et al. (2014) reported decrease in RWC in *Zea mays* plants when treated with SiO_2 NPs at 400 mg L^{-1}, 2000 mg L^{-1}, and 4000 mg L^{-1} concentrations (respectively) in comparison to controls, but this decrease was relatively low at 4000 mg L^{-1}. Such low decrease at 4000 mg L^{-1} can be likely due to unusual increases of root growth at this concentration and the resulting high water uptake. Transpiration from leaves occurs mainly through stomata and partly through the cuticle. As Si is deposited beneath the cuticle of the leaves, transpiration through the cuticle may decrease at increasing Si concentration (Okuda and Takahashi 1965; Tamai and Ma 2008). So, the better RWC at the high SiO_2 NPs application might be related to higher water uptake and a slightly reduced transpiration due to the changes commented on cuticle characteristics.

The ample Si supply from soil to plants exceeds uptake of essential nutrients in several species including cereals (Epstein 1994). The SiO_2 NPs also affected the contents of Cu, Mg in shoots, and Na in roots of transgenic cotton; and SOD activity and IAA concentration were significantly influenced by SiO_2 nanoparticles (Le et al. 2014).

12.5 Conclusions

Nanotechnologies, and its applications, specially related to agriculture and forestry, are under development. The scientific community thinks that it will surely arise as an exciting and powerful discipline of science in the next years. Si, in many documentations, is reported to upsurge physiological efficiency of many plants, and it is even considered to be *quasi*-essential for certain taxa, particularly grasses, although studies on silicon effects on woody plants are very scarce. Therefore, *in planta*, behavior of silicon nanoparticles (SiO_2 NPs) toward plants' functional biology is a newfangled area for research. In this research, two woody plants, namely, hawthorn and mahaleb, were treated with different concentrations of SiO_2 NPs, and growth and physiological plant performance were assessed. Here, SiO_2 NPs accumulated on the root surface (i.e., adsorption) as revealed by our scanning electron microscopy (SEM) observations. Some adverse effects of SiO_2 NPs were observed in the water status (xylem water potential and RWC) of both plant species (more clearly notorious in hawthorn), but contrary to expected, these were opposite

in our study in terms of plant growth (i.e., root length and plant dry masses were higher when plants were treated with Si nanoparticles). Furthermore, despite the worse water status of SiO_2 NPs-treated plants, all gas exchange parameters (i.e., photosynthesis rates, stomatal conductance, and transpiration rate) were improved in plants treated with SiO_2 NPs in both species. Accordingly to this leaf physiological behavior, all growth parameters studied also showed a positive correlation with SiO_2 NPs treatments. From our investigation, it can be stated that silica nanoparticles pretreatments have promising potential to ameliorate to some extent the deleterious effects of drought on plant growth. Further investigation is required to address the impact of nanotechnology in a mechanistic approach related to internalization and mobilization of these within cells but also to strengthen our understanding of their physiological impact on the functioning of woody plants.

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Phytotoxicity of Nanoscale Zerovalent Iron (nZVI) in Remediation Strategies

13

Mar Gil-Díaz and M. Carmen Lobo

13.1 Introduction

The origin of nanotechnology dates back to the lecture titled "There's plenty of room at the bottom" given by Richard Feynman in 1959 at the annual meeting of the American Physical Society. In that lecture Feynman stated that the manipulation of matter on the atomic scale could lead to materials with exceptional properties (Feynman 1960). However, the term nanotechnology was not coined until 1974, when Taniguchi referred to the ability to engineer materials (driven by electronics industry needs) at the nanometer scale (Taniguchi 1974; Hunt 2004). Granqvist et al. (1976) referred to a broad range of new very tiny structures of only a few nanometers in size, able to be described with the new microscopic technologies, as ultrafine particles. In this regard, nanomaterials refer to material with one dimension smaller than 100 nm, whereas nanoparticles are materials with at least two dimensions between 1 and 100 nm. These have higher surface area than bulk materials and different quantum effects which induce higher reactivity and unique properties (Klaine et al. 2008; The Royal Society and The Royal Academy of Engineering 2004; USEPA 2014). In the last 20 years, the global market of nanotechnology has grown considerably, and a wide range of applications in a variety disciplines including biotechnology, electronics, medicine, chemical synthesis, manufacturing, agriculture, food, personal care, and environmental remediation have been developed (Klaine et al. 2008; PEN 2017a). Currently, more than 1800 nanotechnology-based consumer products have been inventoried (PEN 2017a; Vance et al. 2015). However, increasing production and use of nanoparticles can imply potential issues on the environment and human health. Thus, to achieve a suitable development of nanotechnology, research which evaluates its environmental risk are highly essential including studies on transformations and interactions between nanoparticles and

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_13

environmental particles, ecological effects, impact of exposure, environmental fate and transport, human health effects and life-cycle analysis is highly essential (Thomas et al. 2006). Among the different nanotechnological applications, nanoremediation involves the use of nanoparticles for environmental remediation; this implies adding them in soil and groundwater for cleanup proposes.

Pollution is a worldwide problem due to its harmful effects on human health and the ecosystem. The most frequent contaminants are mineral oils and metal(loid)s, and they reach the environment mainly through anthropogenic activities such as industrial processes, manufacturing, mining, road transport, military activities, use of agrochemicals, and land application of domestic sludge (van Liedekerke et al. 2014). In the EU, there are about 2.5 million potentially contaminated sites, of which about 14% (340,000 sites) require urgent remediation (van Liedekerke et al. 2014). Annual national expenditures for the management of contaminated sites are on average about 10 € per capita and range from approximately 2 € in Serbia to more than 30 € in Estonia. This corresponds to an average of 0.4 per million euros of national gross domestic product (GDP) (van Liedekerke et al. 2014). In the USA, an EPA report (USEPA 2004) estimated that it will cost up to $250 billion to clean up that nation's hazardous waste sites. With regard to the developing world, studies show that, to date, more than 2000 contaminated sites have been identified in 47 countries exposing an estimated population of 71,500,000 to risk (Ericson et al. 2013). According to WHO, in 2012, 12.6 million people died as a result of living or working in an unhealthy environment, representing 23% of all deaths. As regards the low- and middle-income countries, the regions of Southeast Asia and Western Pacific have the largest environment-related disease burden, with a total of 7.3 million deaths in 2012 (WHO 2016).

This chapter presents an overview of the state-of-the-art technology on different types of nanoscale zerovalent iron (nZVI) particles and their reactivity. It includes applications in site remediation and a discussion on the current knowledge related to the impact of nZVI on plants.

13.2 Nanoscale Zerovalent Iron (nZVI) Characteristics

The most important iron nanoparticles are nZVI, nano-magnetite (Fe_3O_4), nano-maghemite (γ-Fe_2O_3), and nano-goethite (α-FeOOH). Though they are magnetic nanoparticles, they display different chemical properties as well as different reactivity and behavior to contaminants. At field scale, nZVI is currently the most commonly used nanomaterial for remediation purposes (60% of the iron nanoparticles), whereas iron oxide nanoparticles represent only 2% (Karn et al. 2009).

Iron typically exists in the environment as Fe(II) and Fe(III) oxides, whereas metallic iron (Fe^0) also referred to as zerovalent iron (ZVI) is a manufactured product. Zerovalent iron is well known for being highly susceptible to corrosion

when exposed to dissolved oxygen or aqueous media according to the following electrochemical reactions (13.1) and (13.2):

$$2Fe0(s) + 4H^{+}(aq) + O_2(aq) \rightarrow 2Fe^{2+}(aq) + 2H_2O(l) \qquad E^{\circ} = 1.67\ V \quad (13.1)$$

$$Fe0(s) + 2H_2O(l) \rightarrow Fe^{2+}(aq) + H_2(g) + 2OH^{-} \qquad E^{\circ} = -0.39V \quad (13.2)$$

$$2Fe^{2+}(s) + 2H^{+}(aq) + 1/2O_2(aq) \rightarrow 2Fe^{3+} + H_2O\ (l) \qquad E^{\circ} = 0.46V \quad (13.3)$$

$$2Fe^{2+}(s) + 2H_2O(l) \rightarrow 2Fe^{3+} + H_2(g) + 2OH^{-}\ (aq) \qquad E^{\circ} = -1.60V \quad (13.4)$$

The consumption of protons and the production of hydroxyl ions induce an increase of solution pH. Further oxidations of Fe^{2+} to Fe^{3+} can be produced according to the reactions (13.3) and (13.4) (Crane and Scott 2012; Tosco et al. 2014). Oxygen and water are common electron acceptors in the environment; however other substances, including many environmental contaminants, can also act as electron acceptors and be reduced. The corrosion of iron produces various iron precipitates including $Fe(OH)_2$, $Fe(OH)_3$, Fe_3O_4, Fe_2O_3, and FeOOH which surround the Fe^0 core (Crane and Scott 2012). As a consequence, micro ZVI or nanoscale ZVI has a core-shell structure, with a Fe^0 core responsible for the reduction processes and a shell composed of iron oxides and hydroxides, and can have metallike or ligand-like coordination properties depending on the solution chemistry. When pH is below the isoelectric point (by 8.1), iron oxides are positively charged and attract ligands such as phosphate, nitrate, and arsenate; when the pH is above the isoelectric point, the oxide surface becomes negatively charged and can form surface complexes with metal cations (Li et al. 2007; Li and Zhang 2007; Crane and Scott 2012; Zhang 2003). Consequently, halogenated organic compounds can accept the electrons from iron oxidation and be reduced to compounds of lower toxicity. The core-shell structure of ZVI nanoparticles shows exceptional properties for concurrent sorption and reduction of metal(loid) ions, and the specific removal mechanism depends on the standard redox potential (E°) of the metal(loid). In this sense, for Zn^{2+} and Cd^{2+}, with E° very close to or more negative than that of Fe (−0.41 V), the main removal mechanism is sorption or surface complex formation; for meta(loid)s with E° slightly higher than Fe, such as Ni^{2+} and Pb^{2+}, they can be immobilized by both mechanisms, sorption and reduction; and for metal(loid)s with E° much more positive than Fe (Cu^{2+}, Ag^{+}, Hg^{2+}), the interaction mechanism is preferentially reduction and precipitation (Li and Zhang 2007; Mu et al. 2017; O'Carroll et al. 2013). Mu et al. (2017) described the interaction mechanisms of the oxide shell with organic and inorganic pollutants. Under natural conditions, the overall reaction mechanisms strongly depend on the environmental conditions, presence of different pollutants, as well as the type and dose of nZVI.

Zerovalent iron nanoparticles present a small particle size (1–100 nm in diameter), resulting in higher reactivity than granular iron (Grieger et al. 2010; Karn et al. 2009; Li et al. 2006, 2007; O'Carroll et al. 2013). In this sense, nZVI with a diameter of 50 nm has a specific surface area of approximately 15,000 $m^2\ kg^{-1}$, whereas

conventional granular iron with diameter of 1 mm has a theoretical surface area of 0.77 $m^2\ kg^{-1}$ (Li et al. 2007).

Depending on the synthesis and stabilization process, nZVI nanoparticles differ in particle size, surface area, degree of crystallinity, thickness, and composition of oxide shell. These differences lead to different reactivity and aggregation properties; thus, the remediation efficiency as well as the toxicity is also likely to be different (Crane et al. 2015; El-Temsah et al. 2016; Gil-Díaz et al. 2017a; Lefevre et al. 2016; Ma et al. 2010; Mu et al. 2017; Mueller and Nowack 2010; O'Carroll et al. 2013; Tosco et al. 2014; USEPA 2005; Wang et al. 2017; Zhuang et al. 2012).

13.2.1 Synthesis of nZVI

There are several methods to synthesize nZVI for remediation purposes (Crane and Scott 2012; Kharisov et al. 2012; Mueller and Nowack 2010; O'Carroll et al. 2013; Stefaniuk et al. 2016):

- Physical method: ZVI nanoparticles are produced by milling bulk metallic iron with steel shot in a high-speed rotary chamber to break down the micro iron particles (Jamei et al. 2014; Li et al. 2009; Stefaniuk et al. 2016). This method does not use solvents and is scalable to large-scale manufacturing. Golder Associates Inc. produces nZVI using this method (Crane and Scott 2012).
- Chemical method: This is the most frequently used method for synthesizing nZVI for remediation purposes (O'Carroll et al. 2013; Stefaniuk et al. 2016). The chemical methodology consists of the reduction of a ferrous or ferric salt with sodium borohydride ($NaBH_4$) as a reducing agent. Sodium aluminum hydride ($NaAlH_4$) and lithium aluminum hydride ($LiAlH_4$) can also be used as reducing agents. These nanoparticles are amorphous, with a diameter between 10 and 100 nm and with an α-Fe core and a superficial shell of iron oxides and hydroxides (O'Carroll et al. 2013 and references therein). Another chemical method for nZVI synthesis is based on the gas-phase reduction of iron oxides, goethite (α-FeOOH) or hematite (α-Fe_2O_3), at high temperatures with a reducing gas, such as H_2, CO_2, or CO. The nanoparticles thus obtained have a diameter between 40 and 70 nm, a relatively large α-Fe core and an outer Fe_3O_4 shell.
- Sonochemical method: In this method nZVI is obtained by applying ultrasonic waves on solutions of $FeSO_4$ and $NaBH_4$. The characteristics of these nZVIs strongly depend on the frequency of the ultrasound used. Under high ultrasonic power, the morphology of nZVI changes from a spherical type to plate and needle types, the particle size decreases, and the surface area increases compared to other methods of synthesis (Jamei et al. 2014; Stefaniuk et al. 2016).
- Electrochemical method: This produces nZVI by electrolysis, using a solution with ferrous and/or ferric salts, a cathode, an anode, and electric current. A method to disperse Fe0 deposited at the cathode is also necessary. This method is considered cheaper and faster than chemical reduction methods (Crane and Scott 2012; Stefaniuk et al. 2016). According to Chen et al. (2004), the obtained ZVI nanoparticles have a diameter between 1 and 20 nm.

- Electrical wire explosion method is based on the vaporization of a specific part of iron wire through "electric explosion" in an inert atmosphere (with Ar, CO, CO_2, or N_2), followed by the condensation of the iron vapor to form spherical nanoparticles. This is a simple, efficient, and effective method; it produces pure weakly aggregated nanoparticles with a diameter of 5–100 nm (Kotov 2009; Pustovalov and Zhuravkov 2015; Seyedi et al. 2017).
- Green synthesis method uses different biomaterials including bacteria, algae, fungi, and plants for the synthesis of nZVI or iron oxide nanoparticles (Machado et al. 2013; Saif et al. 2016). Since it does not require high temperatures or pressures, this method is low-cost, energy-efficient, and environmentally friendly (Crane and Scott 2012; Stefaniuk et al. 2016; Saif et al. 2016). Most of the green synthesis researches carried out so far have been with plant extracts, mainly green tea. The plants have different organic reducing compounds that led to the synthesis of nanoparticles of more homogeneous size, and the process shows higher rate than using microorganisms (Dhillon et al. 2012; Saif et al. 2016). Green synthesis using different parts of the plant such as leaf, stem, seed, and root is a simple, reproducible, and cost-effective strategy (Kalaiarasi et al. 2010). The method is based on the extraction of a phenolic solution by heating plant extracts in water; the extract is mixed with a solution of ionic iron which is reduced to Fe0 by the polyphenols (Hoag et al. 2009; Saif et al. 2016).

In spite of the advantages of the green method, its use is not yet widespread at the industrial scale, being the chemical methods of gas-phase reduction of iron oxides and those based on electrolysis the most extensively used by nZVI companies. In fact, this method is applied by Toda Kogyo Corp. for the synthesis of ZVI nanoparticles (USEPA 2005; Tosco et al. 2014). The method of reduction with sodium borohydride is quite expensive due to the high price of reducing agent ($NaBH_4$), and it is difficult to scale to large-scale manufacturing (Li et al. 2009; Yan et al. 2013). Thus, the methods of reduction with $NaHB_4$, green synthesis, and sonochemical and electrical wire explosion are more widespread at the academic level.

13.2.2 Stabilization of nZVI

Zerovalent iron nanoparticles are colloidal and exhibit strong attractive interparticle forces (mainly magnetic and van der Waals forces). They can therefore quickly agglomerate forming aggregates of micron size, thereby decreasing their reactivity and mobility. In addition, the volume of the particles can also be increased due to the precipitates formed by the iron corrosion or by their tendency to adhere to the surfaces of natural materials such as soil and sediment. This behavior makes it difficult for nZVI to move in groundwater and soils maintaining its reactivity. Various particle-stabilizing coatings such as surfactants, polymers, and solid substrates have been employed to improve the stability and mobility of ZVI nanoparticles and thus increase their effectiveness. These coatings also offer

protection against iron oxidation. The chosen stabilizer should be nontoxic, environmentally friendly, low-cost, easily available, and stable toward changes that can occur in the sites in which it is to be used (Singh and Misra 2015).

13.2.2.1 Surfactants

Surfactants are amphiphilic molecules which reversibly change the surface charge of nanoparticles to negative over a wide range of pH, the hydrophobic tails adsorbed on the surface of nZVI, while the hydrophilic heads inhibit flocculation, allowing suspension of nZVI in aqueous medium for relatively longer periods (Singh and Misra 2015). In addition, the amphiphilic nature of surfactants can contribute to solubilize organic pollutants absorbed on soil particles (Esumi 2002). Surfactants can be anionic (sodium oleate, sodium laurate, sodium dodecyl phosphonate, sodium dodecyl sulfate, polyvinyl alcohol-co-vinyl acetate-co-itaconic acid), cationic (cetylpyridinium chloride, hexadecyltrimethylammonium, alkyldimethyl amine oxides), and nonionic (alcohol ethoxylates, alkylethanolamides, Tween 80) (Saleh et al. 2007; Singh and Misra 2015; Wang et al. 2017; Wei et al. 2012). The choice of a surfactant to stabilize the nZVI depends on the type of contamination as well as the environmental conditions. The main disadvantage of the use of surfactants is that the surface adsorption is reversible and the desorption can be favored when nanoparticles are transported through surfactant-free medium (Singh and Misra 2015).

13.2.2.2 Polymers

Different polymers including synthetic polymers and biopolymers have been tested with varying success for nZVI stabilization (Crane and Scott 2012; O'Carroll et al. 2013; Singh and Misra 2015; Stefaniuk et al. 2016; Thomé et al. 2015 and references therein). The stabilization capacity of a polymer is mainly influenced by the functional group, molecular structure and weight, adsorbed mass, and thickness layer of the polymer (Singh and Misra 2015). Anionic polymers such as polyacrylic acid and carboxymethyl cellulose have proven effective for nZVI stabilization (Crane and Scott 2012; Kharisov et al. 2012; O'Carroll et al. 2013; Singh and Misra 2015; Stefaniuk et al. 2016). Their negatively charged functional groups allow electrostatic stabilization, and their large molecular weight produces steric hindrance, reducing the aggregation phenomena. The main disadvantage of anionic polymers is that they can be complex with divalent cations present in the medium, as Ca^{2+}, resulting in higher cross-linking grade of the polymers. In turn, the particle cluster forms a network which favors the aggregation and reduces the mobility of ZVI nanoparticles (Singh and Misra 2015).

Among synthetic polymers are polyacrylic acid, polystyrene sulfonate, butyl methacrylate, polyaspartate, polymethacrylic acid, and polymethyl methacrylate, as well as mixtures of different polyelectrolytes as triblock polymers. Carboxymethyl cellulose is the most used biopolymer (El-Temsah et al. 2013; Ševců et al. 2017; Wang et al. 2014), although good results were also found with starch, guar gum, xanthan gum, calcium alginate, and extract of sineguelas (Fan et al. 2015; Crane and Scott 2012; Singh and Misra 2015; Wijesekara et al. 2014). Starch, guar gum, and

xanthan gum are polysaccharides soluble in water, neutrally charged under a broad range of pH (pH 5–9) or ionic strength. They stabilize nZVI by steric hindrance and increasing the viscosity of the suspension, which decelerates the aggregation process (Comba and Sethi 2009; Singh and Misra 2015; Tiraferri et al. 2008; Tiraferri and Sethi 2009; Xue and Sethi 2012). Their main advantage compared with anionic polymers is that guar gum and xanthan gum have not available sites for complexation with divalent cations, which limits the cross-linking and polymer bridging processes (Singh and Misra 2015).

13.2.2.3 Solid Substrates

An alternative to stabilizing nZVI is supporting them on a solid material such as porous materials including silica (Qiu et al. 2011), mesoporous carbons (Schrick et al. 2004; Qu et al. 2017; Zhu et al. 2009), biochar (Dong et al. 2017; Peng et al. 2017), chelating resins (Park et al. 2009; Toli et al. 2016), and graphene (Chen et al. 2016; Lv et al. 2014). Inorganic clay minerals such as bentonite (Chen et al. 2011; Shi et al. 2011), kaolinite (Chen et al. 2012; Wang et al. 2015), zeolite (Arancibia-Miranda et al. 2016; Kim et al. 2013; Zhou et al. 2015), and clay (Li et al. 2017) have also been successfully tested. The use of solid supports reduces nZVI oxidation, controls the aggregation phenomena, and approaches the contaminant near the nanoparticles by adsorption to the substrate (Singh and Misra 2015; Zou et al. 2016). Another way of stabilizing nZVI is by using nanocomposite of Ca (Wei and Li 2013) or Mg (Liu et al. 2015), loading nZVI onto $Ca(OH)_2$ and $Mg(OH)_2$, respectively. This is a low-cost and environmentally friendly method.

Due to the difficulty of knowing the lifetime of these coatings, especially after injection into the subsurface (Kim et al. 2009), it is currently not yet clear which coating agent may be dominant to stabilize at field-scale nZVI (Grieger et al. 2010). In addition, consideration of these agents based on their lifetime is relevant to avoid the introduction of a potential additional contamination load to the site (Yirsaw et al. 2016). Some of these coating agents could be biodegraded by the microorganisms in subsurface environment (Gerlach et al. 2000).

13.2.3 Other Types of nZVI

The use of bimetallic ZVI nanoparticles is another method used to increase the effectiveness of nZVI. It is based on the addition of a small amount of a transition metal on the nZVI surface (doping) which acts as a catalyst increasing the reactivity of nanoparticles. Metal catalysts used to obtain bimetallic ZVI nanoparticles are Pd, Pt, Ag, Ni, and Cu, although Pd is the most commonly used, especially in dehalogenation reactions (Crane and Scott 2012; Stefaniuk et al. 2016). The reduction reactions of halogenated contaminants can take place either by electron transfer with the noble metal or through reaction with H_2 generated by Fe0 oxidation (Crane and Scott 2012). Bimetallic ZVI nanoparticles have been successfully used for the removal of nitro and azo compounds by nitro and azo hydrogenation (Liu et al. 2014). As disadvantage, bimetallic nanoparticles have a higher reactivity, the

oxidation of Fe0 is accelerated, and consequently, iron nanoparticles show a shorter lifetime. Furthermore, the use of bimetallic nanoparticles can induce toxicity problems due to the characteristics of the noble metal. In this sense, they have not been used at field applications in Europe (Mueller et al. 2012; PEN 2017b), whereas in the USA, about 40% of nanoremediations used bimetallic iron nanoparticles (Karn et al. 2009; Mueller and Nowack 2010; PEN 2017b).

An alternative to improve the mobility of nZVI for the treatment of dense nonaqueous phase liquids (DNAPL) is to emulsify the iron nanoparticles in an oil–water suspension (emulsified nZVI, E-nZVI) using food-grade surfactant and vegetable oil. The degradation of contaminants by E-nZVI is achieved by combining the sequestration of contaminants into vegetable oil and degradation with nZVI. Zerovalent iron nanoparticles are encapsulated in oil, and they cannot interact with water; this produces a reduction of aggregation and a passivation of the nZVI, limiting its corrosion during delivery (Berge and Ramsburg 2009; Quinn et al. 2005; Singh and Misra 2015; Stefaniuk et al. 2016). Thus, E-nZVI can move through the subsurface and is miscible with DNAPL so it favors contaminant degradation. In addition, vegetable oil and surfactants act as electron donor and promote anaerobic degradation (Singh and Misra 2015). Emulsified ZVI nanoparticles have been successfully used for the treatment of chlorinated solvents at lab (Berge and Ramsburg 2009; Dong et al. 2015), pilot, and field scale (Hara et al. 2006; Karn et al. 2009; Quinn et al. 2005; Su et al. 2012).

13.3 Nanoscale Zerovalent Iron for Site Remediation

Different physicochemical and biological strategies are being performed for the remediation of polluted sites. In situ technologies are directly performed on the same site, whereas the ex situ ones imply the removal of the polluted soil or groundwater to be treated in the polluted area (*on-site*) or transported to a treatment plant (*off-site*). The choice of a strategy will depend on the characteristics of the area, type of contamination, risk assessment, and decontamination objectives. The application of in situ strategies is preferred over ex situ techniques, and this is because the former are simpler to apply and cost less (Azubuike et al. 2016); moreover in situ techniques reduce the risk of losing the soil resource (Lobo et al. 2009). In situ remediation techniques such as bioremediation strategies, thermal processes, and physicochemical stabilization are increasingly being used so as to avoid excavation and landfilling or surface treatment of groundwater from "pump and treat" projects (Bardos et al. 2015). In this sense, the use of iron nanoparticles for environmental remediation broadens the range of available in situ remediation technologies and provides an interesting alternative to ex situ treatments (Bardos et al. 2015; Karn et al. 2009).

The introduction of nZVI in remediation scenarios led to a broader range of applications as direct injections or recirculation in contaminated areas to degrade a wide range of pollutants (Grieger et al. 2010). Previously, other authors (Wang and Zhang 1997; Zhang et al. 1998) proposed the use of nZVI as an alternative to ZVI in

permeable reactive barriers in order to overcome the limitations of granular iron (Tosco et al. 2014). The use of iron nanoparticles for site remediation will lead to them being released to the environment. Depending on the environmental conditions and type of nanoparticle, this may produce unknown effects such as their accumulation in soils, water, or living organisms. Thus, to evaluate the risks of the use of nZVI, it is necessary to know its composition, size, reactivity, mobility, availability, persistence, and toxicity. Due to variations between source zone, architecture, contaminant plume size, contaminant characteristics, hydrogeological site conditions, etc., the concentration used should be site-dependent (Grieger et al. 2010).

Laboratory research performed in the last 15 years has confirmed that a wide variety of environmental contaminants can be remediated with nZVI including halogenated organic solvents (Diao and Yao 2009; He and Zhao 2005; Kim et al. 2010; Lien and Zhang 2001; Liu et al. 2005; Song and Carraway 2005; Taghavy et al. 2010; Zhang 2003; Zhuang et al. 2012; Zou et al. 2016), metal(loid)s (Gil-Díaz et al. 2014a, b, 2016a, b, 2017a, b; Li et al. 2014; Li and Zhang 2007; Vítková et al. 2017; Wang et al. 2014; Yan et al. 2010; Zhang et al. 2014), radionuclides (Crane et al. 2015; Klimkova et al. 2011), inorganic anions (Chen et al. 2004; Wu et al. 2013), pesticides (Bezbaruah et al. 2009; Elliott et al. 2009; El-Temsah et al. 2013, 2016; Singh et al. 2011), and pharmaceutical compounds (Fang et al. 2011; Hanay and Türk 2013; Jarosova et al. 2015). In addition, nZVI displays antimicrobial properties against viruses and bacteria including gram-negative *Escherichia coli*, cyanobacteria, denitrifying bacteria *Alcaligenes eutrophus*, gram-positive *Bacillus subtilis* var. *niger*, and gram-negative *Pseudomonas fluorescens* (Dong et al. 2012; Kharisov et al. 2012; Li et al. 2010; Marsalek et al. 2012).

Whereas most of the research has been performed on water samples (Bardos et al. 2015; Grieger et al. 2010; Karn et al. 2009; Mueller and Nowack 2010; Mueller et al. 2012), in the last few years, studies with polluted soils have attracted attention (Gil-Díaz et al. 2014a, b, c, 2016a, b, 2017a, b; Madhavi et al. 2014; Vítková et al. 2017; Wang et al. 2014; Zhang et al. 2010). With regard to the field applications of nZVI, in the USA, nanoremediations have increased rapidly and replaced many ex situ remediation technologies, whereas in Europe a precautionary behavior has been observed, and only a few full-scale applications have been carried out yet (Karn et al. 2009; Mueller and Nowack 2010; Mueller et al. 2012). This is mainly because many polluted sites in the USA are in remote areas far from population centers with limited risk of human exposure (Mueller et al. 2012). The Project on Emerging Nanotechnologies (Kuiken 2010; PEN 2017b) and Karn et al. (2009) compiled comprehensive details of 44 applications of nZVI performed in seven countries, 36 of them were located in 12 US states. The main pollutants were chlorinated compounds (PCE, DCE, TCE, PCBs, and vinyl chloride), Cr and nitrate. With respect to type of medium, 60% of the site remediations targeted groundwater, and 18% treated groundwater and soil simultaneously. Mueller et al. (2012) and Kvapil et al. (2010) compiled the pilot studies and full-scale applications carried out in Europe (Czech Republic, Germany, Italy, and Slovakia). As in the USA, the target pollutants were chlorinated compounds (PCE, TCE, DCE, PCBs, and vinyl

chloride), other organic pollutants (BTEX and hydrocarbons), Cr, Ni, and nitrate. Recently, in the framework of a demonstrative project (NanoRem) funded through the European Commission FP7, different full-scale actions have been performed in Europe. These include Nitrastur (Spain) (Otaegi et al. 2016), Spolchemie (Czech Republic) (Kvapil et al. 2010; Stejskal et al. 2017), Balassagyarmat (Hungary) (Laszlo and Szabo 2017), and Solvay (Switzerland) (Bitsch et al. 2017). In general, the studies evaluated the efficiency of the nanoremediation process, and in some cases, the impact on soil properties was also evaluated. Changes in soil electrical conductivity or in soil pH due to the high pH of the nanoparticles solution should be considered in the application of these materials (Gil-Díaz et al. 2014b). On the other hand, the use of surfactants, polymers, or solid substrate as biochar or chelating resins as well as the incorporation of other metals in the case of bimetallic nZVI can condition the nZVI properties as well as their potential use in soil. Currently very little is recorded in the literature that addresses these effects in the soil. Thus, it makes studies on the impact of nZVI on both physicochemical characteristics of the site and on its microbiota and biota very necessary.

It is noteworthy that the use of nZVI for the treatment of organic pollutants can lead to their total degradation, whereas for metal(loid) pollution, the efficacy of the strategy is measured by the reduction of the available metal(loid) or its immobilization. In this latter case, the stability of the immobilization process should be controlled and monitored. In addition, at each contaminated site, criteria including cost, time, efficiency, environmental disturbance, public acceptance, etc. should be the principle factor in deciding the most appropriate remediation option (Lemming et al. 2010). Regarding price, nZVI suspension with weight content in Fe0 of 14–18% commercialized by NANO IRON and Toda companies oscillates between 24 and 30 € kg^{-1}, although the price can increase depending on the type of nZVI, as bimetallic and emulsified ZVI nanoparticles have a higher price. In comparison, granular iron is available for less than 1 € kg^{-1} (Mueller and Nowack 2010). The increase in number of companies commercializing nZVI would lead to a decrease in the price.

13.4 Impact of nZVI on Plant Phytotoxicity

Nanoscale zerovalent iron particles are considered by USEPA as a potential emerging nanoscale contaminant (USEPA 2014). This is because their release to the environment may produce different effects depending on the environmental conditions and type of nanoparticle, as their accumulation in soils, water, or living organisms. Also, iron nanoparticles can react with environmental constituents, aggregate, spread, migrate to large distances, or settle on bottoms of water reservoirs. The impact of different types of nanoparticles (carbon, metal, and metal oxide nanomaterials) on plants has been discussed in several reviews (Capaldi Arruda et al. 2015; Lei et al. 2018; Martínez-Fernández et al. 2017; Rico et al. 2011; Siddiqi and Husen 2017; Zuverza-Mena et al. 2017). To date, available data regarding the

effects of nZVI on plants, its uptake and accumulation, and the physiological response of plants is limited.

Table 13.1 lists current published studies on the impact of nZVI on plant development, including studies carried out under different experimental conditions with different exposure time, different types of nZVI, doses, and plants. The heterogeneity of the conditions makes it difficult to make general conclusions. Most of the studies are germination assays which suppose a starting point to evaluate the toxicity of nZVI. Some studies evaluate the effect of nZVI directly on seeds in aqueous medium, whereas other studies consider the effect of nZVI on seeds or plants in soil assays. In the latter, soil properties could lessen the impact of this material. Different studies showed the nZVI effect whether in contaminated or uncontaminated soil; this fact is relevant because the presence of pollutants can mask other effects caused by nZVI (Martínez-Fernández et al. 2017).

13.4.1 Phytotoxicity Evaluation in Uncontaminated Medium

Most of the available studies on the impact of nZVI on plants were performed using uncontaminated medium including hydroponics, sand, or soil (El-Temsah and Joner 2012; Ghosh et al. 2017; Kim et al. 2014, 2015; Lebedev et al. 2014; Li et al. 2015; Libralato et al. 2016; Ma et al. 2013; Marsalek et al. 2012; Trujillo-Reyes et al. 2014; Wang et al. 2016). The results obtained at hydroponic conditions are difficult to extrapolate to natural conditions because soil has a decisive effect on iron nanoparticle behavior. In this sense, in hydroponic conditions, the dissolved elements are available for plant uptake, whereas in soil they can be partially immobilized by soil particles. El-Temsah and Joner (2012) studied the impact of nZVI for the germination of flax, barley, and ryegrass in water and two contrasting soils. Differences between the species were detected. Inhibitory effects on seed germination were evident at 500 mg L^{-1} of nZVI for barley and ryegrass and at 1000 mg L^{-1} for flax in aqueous medium. No seed germination of ryegrass was observed at concentrations $\geq$1000 mg L^{-1}, whereas for flax and barley, it occurred at $\geq$ 2000 mg L^{-1}. However, a decrease of root and shoot elongation was detected at lower nZVI concentrations. Flax was the most sensitive to nZVI in soil germination assays, and at nZVI concentration higher than 500 mg kg^{-1}, no seed germination was observed for any of the species. In the same way, Marsalek et al. (2012) found low toxicity in a germination assay performed in aqueous medium using *Sinapis alba* seeds at nZVI dose between 0 and 1000 mg L^{-1}. Trujillo-Reyes et al. (2014) performed a hydroponic experiment with 18-day-old grown lettuce seedlings which were treated with nZVI doses of 10 and 20 mg L^{-1} for 15 days and compared with the effect of $FeSO_4$. The authors concluded that under experimental conditions, nZVI did not negatively affect the physiological parameters of the lettuce plants. They observed accumulation of iron in roots, regardless its size. Gil-Díaz et al. (2014a) evaluated the impact on germination of an nZVI suspension at doses of 1 and 10%, and no negative effects were observed on the germination of barley and vetch plants at any doses assayed; in fact, nZVI had a phytostimulant effect on vetch germination. These authors evaluated

Table 13.1 Compilation of published studies on the effect of nZVI on plants

Plant	Origin of nZVI	Surface stabilizer	Particle size	Dose of nZVI	Type of assay	Medium	Exposure time	Effects	References
Barley (*Hordeum vulgare*), flax (*Linum usitatissimum*)	Synthesized by $NaBH_4$ method	Carboxymethyl cellulose	20–100 nm	1 g kg^{-1}	Germination test for leachates and soil	DDT-polluted soil + nZVI, column experiment	4 and 5 days for leachates and soil, respectively	After leaching with four pore volumes, no negative effects neither for soil nor leachates. Differences between barley and flax	El-Temsah et al. (2013)
Flax (*Linum usitatissimum*), ryegrass (*Lolium perenne*), barley (*Hordeum vulgare*)	Synthesized by $NaBH_4$ method	Polyacrylic acid	Data not shown	100, 250, 500, 1000, 2000, 5000 mg L^{-1}	Germination test	Aqueous suspension	5 days	Growth inhibition at concentrations ≥250 mg L. Barley and ryegrass decreased germination rate at 500 mg L^{-1}; flax at 1000 mg L^{-1}. Ryegrass showed no seed germination ≥1000 mg L^{-1}; flax and barley at ≥2000 mg L^{-1}	El-Temsah and Joner (2012)
Flax (*Linum usitatissimum*), ryegrass (*Lolium perenne*), barley (*Hordeum vulgare*)	Synthesized by $NaBH_4$ method	Polyacrylic acid	Data not shown	100, 250, 500, 1000, 2000, 5000 mg kg^{-1}	Germination test, seedling elongation	Soil	5–7 days	Growth inhibition depended on soil properties, highest inhibition in soil with higher sand and lower organic matter contents. In this soil, complete inhibition at	El-Temsah and Joner (2012)

								250 mg kg^{-1} for flax, 500 mg kg^{-1} for ryegrass, and 1000 mg kg^{-1} for barley	
White mustard (*Sinapis alba*), duckweed (*Lemna minor*)	Nanofer 25 (NANO IRON)	Bare	70 nm	5, 10, 50, 100, 500, 1000 mg L^{-1}	Germination assay	Aqueous suspension	*S. alba* 72 h, *L. minor* 7 days	Low toxicity for the two plants species, lower than that found for cyanobacterium *Microcystis aeruginosa*	Marsalek et al. (2012)
Cattail (*Typha latifolia*), hybrid poplars (*Populus deltoids* × *Populus nigra*)	Synthesized by $NaBH_4$ method	Bare	300–400 nm	25, 50, 200, 500 and 1000 mg L^{-1}	Pot experiment in greenhouse	Hydroponics, solutions replaced once a week with new nZVI solutions	4 weeks	Toxic effects at concentrations > 200 mg L^{-1}. Irregular aggregates of nZVI on root surface; nZVI internalization by poplar root cells, but not for *Typha*	Ma et al. (2013)
Wheat (*Triticum vulgare*)	Synthesized using the method of high temperature condensation	Data not shown	80 nm (average)	2.0, 0.5, 0.125, 0.001, and 10–6 g L^{-1}	Germination test	Hydroponic conditions	3 and 7 days	nZVI showed stronger inhibition of germination than nFe_3O_4; leaf elongation and content of photosynthetic pigments depended on exposure time	Lebedev et al. (2014)
Barley (*Hordeum vulgare*), vetch (*Vicia sativa*)	Nanofer 25S (NANO IRON)	Polyacrylicacid	60 nm (average)	1%, 10% (w:w)	Germination test	Aqueous suspension	72 h	nZVI did not induce phytotoxicity; phytostimulant effect in the case of vetch	Gil-Díaz et al. (2014a)

(continued)

Table 13.1 (continued)

Plant	Origin of nZVI	Surface stabilizer	Particle size	Dose of nZVI	Type of assay	Medium	Exposure time	Effects	References
Barley (*Hordeum vulgare*), vetch (*Vicia sativa*)	Nanofer 25S (NANO IRON)	Polyacrylic acid	60 nm (average)	1%, 10% (w:w)	Germination test	As-polluted soil + nZVI	72 h	nZVI application reduced soil phytotoxicity, higher germination index for plants from nZVI-treated soils	Gil-Díaz et al. (2014a)
Edible rape (*Brassica campestris*) and Chinese cabbage (*Brassica pekinensis*)	Synthesized by reduction of steel pickling waste liquor with $NaBH_4$	Carboxymethyl cellulose	10–100 nm	5 mL nZVI suspension (0.3 g Fe^0 L^{-1}) per 1 g soil = 1.5 g kg^{-1} soil	Plant growth	Unpolluted and Cr-polluted soil	8 days, 1 month	Plant behavior depended on plant species, contamination and nZVI age. Uncontaminated soil: fresh nZVI favors Fe uptake, and aged nZVI (>72 h) has opposite effect. Different behavior in Cr-contaminated soil; fresh nZVI decreased germination and growth of plants; aged nZVI (>1 month) improvement of cultivation	Wang et al. (2014)
Lettuce (*Lactuca sativa*)	Synthesized by $NaBH_4$ method	Bare	50–60 nm	10, 20 mg L^{-1}	Plant growth	Hydroponic conditions	15 days	Chlorophyll content increased; no effect on length and water content of plants;	Trujillo-Reyes et al. (2014)

								no toxic effect due to the accumulation of iron observed for both nZVI and $FeSO_4$ at micrometer scale	
Thale cress (*Arabidopsis thaliana*)	Nanofer 25S (NANO IRON), RNIP-10DS (Toda Kogyo)	25S, polyacrylic acid; RNIP-10DS, data not shown	Data not shown	0.5 g L^{-1}	Cellular mechanics affected by nZVI	Hydroponic conditions	7, 14 days	nZVI enhanced root elongation by inducing OH radical-induced cell wall loosening (especially with RNIP)	Kim et al. (2014)
Thale cress (*Arabidopsis thaliana*)	RNIP-10DS (Toda Kogyo)	Data not shown	54 nm	0.1 g L^{-1}	Cellular mechanics affected by nZVI	Hydroponic conditions	10 days, 2 and 3 weeks	Decrease of apoplastic pH which was correlated with an increase in plasma membrane H^+-ATPase activity. Increase of leaf area	Kim et al. (2015)
Arabidopsis thaliana	RNIP-10DS (Toda Kogyo)	Data not shown	55 nm	0.5 g kg^{-1}	Cellular mechanics affected by nZVI	Soil + nZVI	3 weeks	Increase of H^+-ATPase activity which favored the increase of stomatal aperture; plants treated with nZVI showed similar drought sensitivity as untreated plants; thus, the former may have an increased CO_2 assimilation rate	Kim et al. (2015)

(continued)

Table 13.1 (continued)

Plant	Origin of nZVI	Surface stabilizer	Particle size	Dose of nZVI	Type of assay	Medium	Exposure time	Effects	References
Peanut (*Arachis hypogaea*)	Synthesized by $NaBH_4$ method	Polyvinylpyrrolidone	20–80 nm	10, 20, 40, 80, 160, 320 μmol L^{-1}	Germination test	Aqueous suspension	5 days	Any effects on the germination rates	Li et al. (2015)
Peanut (*Arachis hypogaea*)	Synthesized by $NaBH_4$ method	Polyvinylpyrrolidone	20–80 nm	10, 20, 40, 80, 160, 320 μmol L^{-1}	Growth assay	Quartz sand	18 days	Low concentrations (10–160 μmol L^{-1}) stimulated peanut growth. Higher concentrations (320 μmol L^{-1}) showed inhibitory effects on growth of peanuts. nZVI particles can penetrate plant seed coats	Li et al. (2015)
Barley (*Hordeum vulgare*), flax (*Linum usitatissimum*)	nZVI-B: synthesized by $NaBH_4$ method. nZVI-T: Nanofer 25S (NANO IRON)	nZVI-B: bare nZVI-T: polyacrylicacid	nZVI-B, 20–100 nm; nZVI-T <100 nm	1 g kg^{-1}	Germination test, seedling elongation	Soil + nZVI (soil slurry), soil + nZVI after leaching (column soil) and leachates	5 days	nZVI-T: positive effects or no effects. nZVI-B: strong inhibition of germination (>50%) in leachates and soil column, complete inhibition in soil slurry	El-Temsah et al. (2016)
Chinese cabbage (*Brassica pekinensis*)	Synthesized by $NaBH_4$ method followed by the addition of $NiCl_2$	Bimetallic Ni-nZVI, stabilized with polyvinylpyrrolidone	20–50 nm	0.03 g g^{-1}	Germination test	Soil	14 days	The phytotoxicity decreased if plants were placed 3 days after the remediation treatment	Wu et al. (2016)

Cress (*Lepidium sativum*), white mustard (*Sinapis alba*), and sorghum (*Sorghum saccharatum*)	American elements	nZVI	10–25 nm	4.8–33,560 mg L^{-1}	Germination test, seedling elongation	Aqueous suspension	72 h	No significant phytotoxicity effects; biostimulation at the highest concentration	Libralato et al. (2016)
Barley (*Hordeum vulgare*)	Nanofer 25S (NANO IRON)	Polyacrylic acid	60 nm (average)	5% (w:w)	Pot experiment in greenhouse	Cd-polluted soil, Cr-polluted soil, Zn-polluted soil	4 months	Cd: plants from nZVI-treated and untreated soil showed similar behavior. Cr: plants from untreated soils died, and those from nZVI-treated ones completed growing period and did not show symptoms of toxicity. Zn: plants from nZVI slightly improved growth. No overall increase of Fe uptake	Gil-Díaz et al. (2016b)
Barley (*Hordeum vulgare*)	Nanofer 25S (NANO IRON)	Polyacrylic acid	60 nm (average)	1%, 10% (w:w)	Pot experiment in growth chamber	As-polluted soil + nZVI	33 days	10% nZVI stimulated the growth of plants; Fe absorption was not favored	Gil-Díaz et al. (2016a)
Rice (*Oryza sativa*)	Synthesized by $NaBH_4$ method	Polyvinylpyrrolidone	20–60 nm	100, 250, 500, 750, 1000 mg kg^{-1}	Germination test and seedlings growth	Soil	14 days	Inhibition of rice seedlings growth at higher ZVI concentration (>500 mg kg^{-1}). Plants showed iron	Wang et al. (2016)

(continued)

Table 13.1 (continued)

Plant	Origin of nZVI	Surface stabilizer	Particle size	Dose of nZVI	Type of assay	Medium	Exposure time	Effects	References
								deficiency due to the transport of active iron from root to shoot was blocked, cortex tissue of root damaged	
Onion (*Allium cepa*)	Two types of synthesized nZVI	Data not shown	nZVI-1, 12.9 nm; nZVI-2, 57.3 nm diameter	125, 250, 500 mg L^{-1}	Plant growth	Sand	24 h	nZVI adsorption on root surface caused root tip, epidermal, and root hair damage. nZVI induced DNA damage, chromosome aberration, nuclear aberration, oxidative stress, and apoptosis. Different responses depending on the type of nZVI	Ghosh et al. (2017)
Vetch (*Vicia sativa*)	25S (Nanofer 25S, NANO IRON), RNIP-D and RNIP (Toda Kogyo)	25S, polyacrylic acid; RNIP, bare; RNIP-D, unknown organic dispersant	25S, 60 nm; RNIP, 70 nm (average)	1%, 5%, 10% (w:w)	Germination test	As- and Hg-polluted soils	4 days	The use of the three types of nZVI reduced soil phytotoxicity	Gil-Díaz et al. (2017a)

Barley (*Hordeum vulgare*), flax (*Linum usitatissimum*)	nZVI-B: synthesized by $NaBH_4$ method, nZVI-T: Nanofer 25P (NANO IRON)	nZVI-B: carboxymethyl cellulose; nZVI-T: bare	nZVI-B, 20–70 nm; nZVI-T, 30–150 nm	1 g L^{-1} for soil slurry and 4 g L^{-1} for soil column	Germination test	Soil + nZVI (soil slurry), soil + nZVI after leaching (column soil) and leachates	4 and 5 days for leachates and soil, respectively	Soil slurry, soil leachate, and soil column with nZVI-T exhibited no negative effect on seed germination, whereas nZVI-B showed a significant inhibition	Ševců et al. (2017)

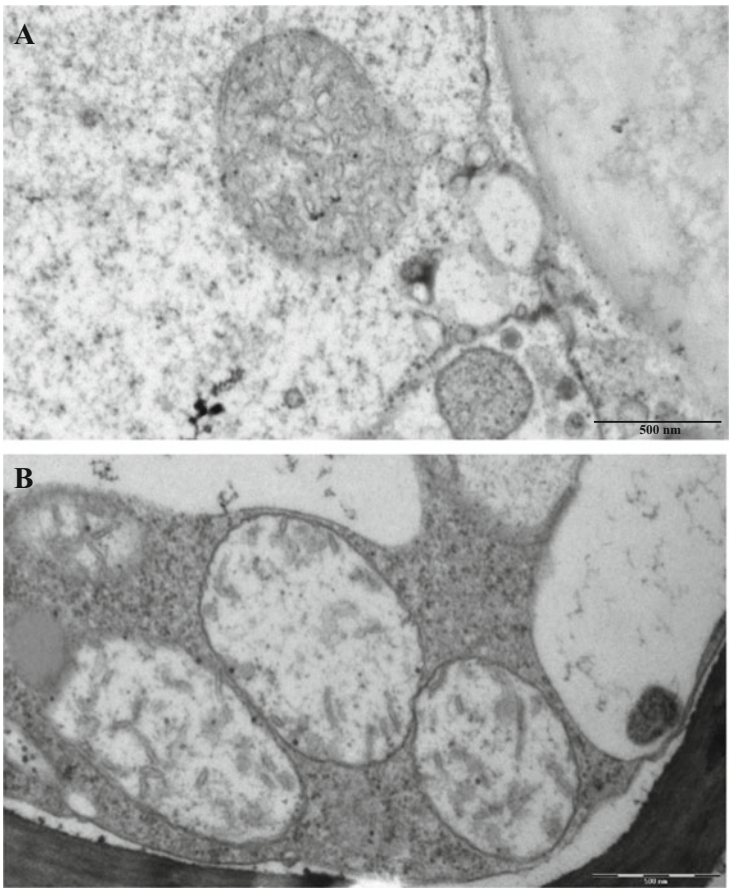

Fig. 13.1 TEM images of the root of barley plants obtained in a greenhouse experiment. (**a**) Plant grown in untreated soil (control) and (**b**) plant grown in soil treated with nZVI (Nanofer 25S, 5%)

the effect of nZVI on barley plants grown in a calcareous soil at greenhouse conditions (unpublished data). The plant development did not suffer significant changes due to the nZVI treatment, and the studies of transmission electron microscopy (TEM) revealed slight morphological alterations in the root structure. In this sense, Fig. 13.1 shows transmission electron micrographs of root cellules from nZVI-treated and untreated barley plants. The former showed damages of the cellular ultrastructure such as altered membranes, swollen mitochondria, and unstructured crests. These changes were not observed in leaves.

El-Temsah et al. 2013 compared the effect on plant development of the aqueous phase of an nZVI suspension (undiluted) on filter paper and on soil and found that the former showed much higher inhibition (near 90%). These results highlight the importance of the type of medium. Ma et al. (2013) performed a hydroponic experiment at

greenhouse for 4 weeks to evaluate the toxicity and absorption of bare nZVI by two plant species, cattail and hybrid poplars. Nanoscale zerovalent iron improved the growth of cattail plants at low concentrations (<50 mg L^{-1}), whereas at concentrations higher than 200 mg L^{-1}, these plants were strongly affected. In the same way, hybrid poplars were unaffected at low nZVI concentration (<50 mg L^{-1}), but the toxic effects increased with higher doses. Microscopic studies showed that large amount of nZVI coated on plant root surface as irregular aggregates and confirmed the internalization of nZVI by poplar root cells but not for cattail plants. The nZVI translocation to shoot was not observed in any case. These results again indicated the differential sensitivity among plant species. Libralato et al. (2017) studied the effect of nZVI on germination, seedling elongation, and biomass of three macrophytes, *Lepidium sativum*, *Sinapis alba*, and *Sorghum saccharatum*, and compared the effect of ionic and micro-sized iron at different concentrations in aqueous medium. Adverse effects were not detected neither for ionic iron nor for micro- or nano-sized iron particles on any of the studied plants; only a moderate biostimulation was observed at the highest doses for micro- and nano-sized iron. Microscopy analysis did not find nZVI in palisade cells or xylem, and nZVI aggregates were detected outside the cell walls, especially in *S. saccharatum*. Marsalek et al. (2012) also observed different responses to nZVI exposure from different plant species (*S. alba*, *L. minor*, and *D. subspicatus)* showing low toxicity in all cases.

Kim et al. (2014) studied the effect of nZVI on *Arabidopsis thaliana* root elongation after 7 and 14 days at hydroponic conditions. Dose of nZVI of 0.5 g L^{-1} from two different commercial producers, NANO IRON (Nanofer 25S) and Toda Kogyo (RNIP), induced an increase of root elongation by 150–200%, especially for the RNIP, whereas concentrations of Fe^{2+} ions of 0.05 and 0.5 g L^{-1} inhibited growth after germination. The authors explained the increase in root elongation as being due to the oxidation capacity of nZVI, leading to the release of H_2O_2, causing OH radical-induced cell wall loosening. Later, Kim et al. (2015) studied the same plants grown in soil treated with commercial nZVI (RNIP) at concentration of 0.5 g kg^{-1} for 3 weeks. As previously explained, the strong oxidizing capacity of nZVI led to the release of OH^-, consequently, producing an increase of pH which decreases the solubility of iron in the rhizosphere. The authors found that under the exposure of nZVI, *Arabidopsis* plants triggered high plasma membrane H^+-ATPase activity, which induced a decrease in the apoplastic pH, an increase in the leaf area and a wider stomatal aperture. The improvement in stomatal opening in *Arabidopsis* plants may lead to an increase of CO_2 uptake, which is very interesting from the point of view of CO_2 reduction and mitigating climate change. These results are very interesting and should be confirmed for other plants and other experimental conditions, as well as for plants grown in polluted soils.

Li et al. (2015) performed a germination study with peanut seeds at concentration of 10–320 μmol L^{-1}, and they did not find strong toxic effects on germination, obtaining the best germination indexes between 40 and 80 μmol L^{-1}. In fact, the nZVI treatment stimulated root growth, even more than the EDTA-Fe treatment. The study confirmed by TEM analyses that ZVI nanoparticles are able to penetrate seed coat while allowing water uptake into the seed. In a longer experiment with the same

concentration of nZVI in quartz sand, Li et al. (2015) observed that low concentrations of nZVI (10–160 μmol L^{-1}) promoted the growth of peanut plants probably due to the uptake of Fe from nZVI by the plants, whereas high concentrations of nZVI (320 μmol L^{-1}) had inhibitory effects on the growth of the plants. Studies carried out by Wang et al. (2014) evaluated the application of nZVI stabilized with CMC in unpolluted soils and observed that Fe absorption was favored by fresh CMC-nZVI, whereas 72 h-aged nZVI suppressed the Fe uptake.

In a study with rice plants grown in nZVI-treated soils at concentrations in the range 0–1000 mg kg^{-1}, Wang et al. (2016) also found negative effects at the highest doses of nZVI. They observed a decrease of iron in shoots but not in root, as well as visible symptoms of iron deficiency at the highest nZVI concentrations. Electron microscopy analysis showed that the cortex tissues of the plants were seriously damaged by nZVI which was transported from soil to root; thus the authors concluded that transportation of active iron from the root to the shoot was blocked. At concentration lower than 500 mg kg^{-1}, no negative effects on germination were observed.

Most of the studies evaluated the phytotoxicity of nZVI based on germination and growth assays, but little data are available regarding the genotoxicity and cytotoxic response of plants exposed to nZVI. In this sense, Ghosh et al. (2017) evaluated these effects in plants exposed to two different forms of nZVI with different surface chemistry. They studied the impact on uptake, root morphology, DNA damage, oxidative stress, and cell death in *Allium cepa* roots. The nZVI adsorption on root surface caused root tip, epidermal, and root hair damage. nZVI induced DNA damage, chromosome and nuclear aberration, oxidative stress, and apoptosis. Differential phytotoxicity was observed depending on the type of nZVI, which is in agreement with other studies (El-Temsah et al. 2016; Gil-Díaz et al. 2017a; Kim et al. 2014; Ševců et al. 2017). The nZVI which showed higher colloidal destabilization, smaller size, and higher uptake imparted enhanced DNA damage, chromosome/nuclear aberrations, and micronuclei formation compared to the another nanoparticle, which also induced cytotoxicity due to its higher dissolution, adsorption, and considerable uptake. In agreement with previous works, the authors concluded that the nZVI induced the formation of reactive oxygen species.

13.4.2 Phytotoxicity Evaluation in Contaminated Medium

Several studies have been performed using nZVI to remediate polluted soil, and the effects on different plants have been also evaluated (El-Temsah et al. 2013, 2016; Gil-Díaz et al. 2014a, 2016a, b, 2017a; Ševců et al. 2017; Wang et al. 2014; Wu et al. 2016). The obtained results are relevant before recommending a nanoremediation strategy in order to know its impact on the reduction of soil phytotoxicity that will contribute to restore soil functionality. In general, the soil phytotoxicity of polluted soils decreased after the nanoremediation treatment due to the decrease of the contaminant availability. In this sense, El-Temsah et al. (2013) performed a column experiment with soil artificially contaminated with DDT and studied toxicity of

leachates and soil by germination and plant growth studies, using flax and barley seeds. Untreated soil showed more toxicity than nZVI-treated soil. The authors found that the first leachates (especially second and third) had a negative effect on plant development, but after leaching four pore volumes, neither soil nor leachates showed negative effect on plants. Barley was found to be more sensitive than flax to nZVI, in the leaching experiment. In a posterior work, El-Temsah et al. (2016) compared the efficiency of two types of nZVI (nZVI-B made using precipitation with borohydride and nZVI-T produced by gas-phase reduction of iron oxides under H_2) for DDT degradation in water and soil samples, as well as the impact of the nanoparticle treatment on flax and barley plants. Differences in toxicity between the two types of nZVI were found. nZVI-B showed more negative effects, whereas nZVI-T showed mostly positive or no effects. Similar results regarding nanoparticle toxicity were found by Ševců et al. (2017) who studied the effectiveness of the same two types of nZVI (nZVI-B and nZVI-T) for PCB degradation in soil and their impact on plants and other soil organisms. Both types of nanoparticles effectively degraded PCB in water but not in soil samples. Lower toxicity was observed in soil column samples probably due to the rapid oxidation of nZVI and its interaction with soil organic matter and clay materials. nZVI-T had no negative effect on barley and flax germination in all the media tested (liquid and solid phase of soil slurry, leachates, and soil from the column experiment), whereas nZVI-B induced inhibition of germination and growth of both plants studied. This can be due to the fact that nZVI-B has a smaller size and hence a faster oxidation of Fe0, producing an excess release of Fe(II) which can induce the generation of harmful reactive oxygen species (ROS) (Ševců et al. 2011). In addition, the toxicity of nZVI-B can be due to the residual presence of boron as a by-product of the synthesis process. Another study comparing different types of nZVI was carried out by Gil-Díaz et al. (2017a). They evaluated the effectiveness of three types of commercial ZVI nanoparticles to immobilize As and Hg in two highly polluted soils and their impact in soil phytotoxicity according to the germination of common vetch seeds. In all cases, nZVI application reduced As and Hg availability as well as soil phytotoxicity, and differences were observed depending on the soil, type of nZVI, and dose. The seed germination index varied depending on the nZVI concentration and type of nanoparticles. In a previous study, Gil-Díaz et al. (2014a) applied nZVI to an As-polluted soil and found a significant reduction of As availability which led to a reduction of soil phytotoxicity increasing the germination of barley and vetch seeds.

In a longer study, Gil-Díaz et al. (2016a) evaluated the development of barley plants grown in As-polluted soil treated with two doses of nZVI (1 and 10%), and the best plant growth was observed at the higher dose, for which the As immobilization was the most effective (Fig. 13.2). Also, at this dose (10%), reductions of the transfer and the translocation factors were observed to be 67% and 92%, respectively, compared to untreated soils. Regarding the effect on Fe uptake, it was not favored at the experimental conditions. The available literature does not show an overall increase of Fe absorption; this depends on plant species, soil properties, grade of contamination, and type of nZVI. In this study, the soil had a neutral pH, and the addition of nZVI only produced a slight increase of Fe in the more available soil

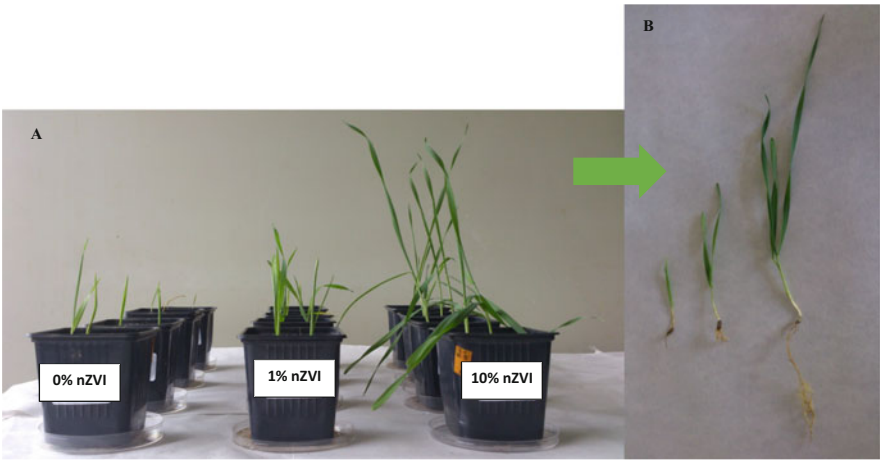

Fig. 13.2 (**a**) Barley plants grown in untreated As-polluted soil (0% nZVI) and treated with nZVI at 1 and 10% (Nanofer 25S). (**b**) Representative photograph of barley plants appearance after harvest

fractions (exchangeable and boned to carbonates) for soils treated at 10% of nZVI (Gil-Díaz et al. 2016a). In another study, Gil-Díaz et al. (2016b) evaluated the development of barley plants grown in artificially polluted soils with Cd, Cr, or Zn treated and untreated with nZVI until the end of their growing period (4 months). The highest toxicity symptoms were found in plants cultivated in Cr-polluted soils at the highest dose (230 mg kg^{-1}) which died within 1 week, and the application of nZVI significantly reduced the Cr availability in soils as well as the Cr uptake, and barley plants were able to complete their growing period (Fig. 13.3). The analysis of the total iron in the different parts of the plant (root, shoot, and grain) did not show an overall increase of Fe in barley plants from nZVI-treated soils. Neither was there an increase in the available iron in nZVI-treated soil samples collected at the end of the experiment. Wang et al. (2014) applied nZVI stabilized with sodium carboxymethyl cellulose (CMC) to Cr(VI)-spiked soil and studied the growth of rape and Chinese cabbage plants for 8 days. A significant reduction of Cr availability in soil and a concomitant reduction of Cr absorption by plants were observed. The CMC-nZVI negatively affected the germination and growth of these plants in the short term although in germination assays performed after 1 month of the addition of CMC-nZVI, an improvement of cultivation was observed for both plants. Different behaviors were observed between plants; rape was most seriously affected, and it took more time to recover. These results show that the toxicity of CMC-nZVI was time and plant species dependent. As previously commented, the decrease of nZVI phytotoxicity with time can be explained by the excess of Fe(II) produced by Fe0 oxidation. In addition, a decrease of iron availability in soil with time was detected. The availability of iron in nZVI-treated polluted soils depended on soil properties, mainly on soil pH. In this respect, Gil-Díaz et al. (2016a, b) in experiments with neutral and alkaline pH soils, respectively, did not observe a strong increase of Fe

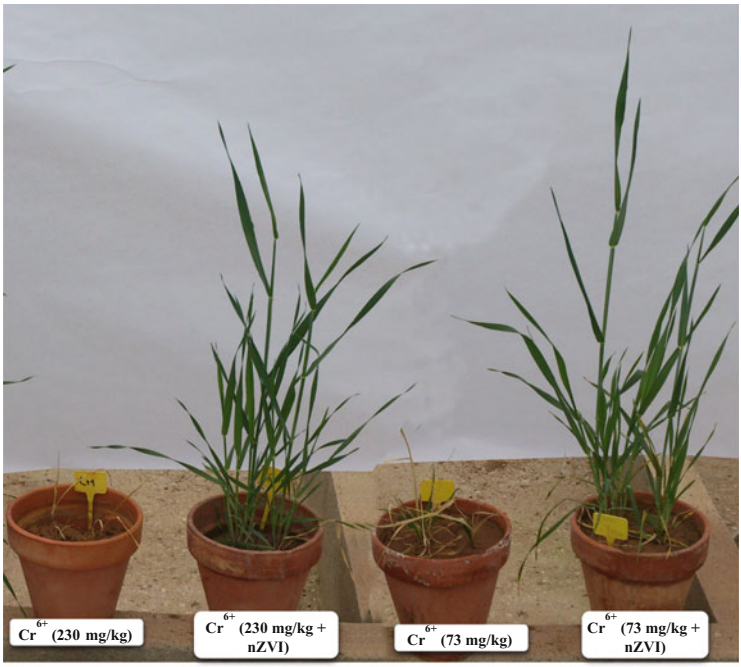

Fig. 13.3 Barley plants grown in Cr-polluted soils at two doses, treated with nZVI (Nanofer 25S) and untreated

availability in nZVI-treated soils. In contrast, acidic soils treated with nZVI showed a significant increase of iron availability (Gil-Díaz et al. 2014c). As previously explained, high concentrations of Fe(II) in plants can react with reduced form of oxygen producing free radical species such as $^{\bullet}OH$ which may negatively affect plants cells (Wang et al. 2014). Another potential reason which can explain the toxicity of high concentrations of iron is that iron precipitates on roots, forming an iron plaque that acts as a barrier against iron as well as the nutrient uptake due to damages in the epidermis surface of the roots (Jørgenson et al. 2013; Saaltink et al. 2017).

Studies carried out by Wu et al. (2016) evaluated the effect of Ni/Fe bimetallic nanoparticles on the phytotoxicity and translocation of PBDEs (polybrominated diphenyl ethers) by Chinese cabbage in polluted soil. These authors concluded that the phytotoxicity in treated soil was decreased after 3 days as well as the translocation of the pollutant to the crop.

13.5 Concluding Remarks

Analysis of the published studies to date shows that the phytotoxicity of nZVI strongly depends on the nZVI type, dose, plant species, time of exposure, and the medium of application. Most of the studies agree that the toxicity of nZVI is dose dependent; thus at low doses, no negative effects are observed, and nZVI can even promote the growth of the plants both in hydroponic conditions and in uncontaminated soil. Regarding the nature of nZVI, the available data confirm that nZVI synthetized by chemical reduction using sodium borohydride showed higher phytotoxic effects than those obtained by gas-phase reduction of iron oxides at high temperatures with a reducing gas. The latter is the preferred method by most of the enterprises manufacturing nZVI.

On the other hand, the use of particle-stabilizing coating agents and bimetallic or emulsified nanoparticles to improve nZVI efficiency supposes the introduction in the medium of other potentially contaminating substances. In this sense, consideration of these agents based on their lifetime is important to avoid the introduction of a new potential contamination source to the site.

In soil remediation assays, nZVI impact on plants is ameliorated due to the interactions with soil components. Depending on the soil characteristics, an increase in available iron concentration could be observed, and potentially it could be absorbed by the plant or mobilized through the soil profile toward groundwater. The published studies about the use of nZVI on polluted soils do not find negative effects; on the contrary the use of these nanomaterials leads to a decrease of the soil phytotoxicity due to the immobilization and/or degradation of the pollutants.

To date no study using nZVI in remediation assays has evaluated the presence of nZVI in plant tissues. Taking into account that nanoremediation is a promising strategy with potential application in contaminated sites, it is necessary to carry out studies on different contaminated soils with different plant species and different types and doses of nZVI, analyzing the effect on the growth of the plants and at cellular scale with the aim to avoid potential damage by the use of these nanoparticles. In addition, monitoring studies at long term are relevant due to the scarce data on the stability of the nZVI treatment.

Acknowledgments This work has been supported by the Projects REHABILITA CTM2016-78222-C2-1-R (MINECO, Spain) and FP-16-NANOREMED (IMIDRA, Comunidad de Madrid, Spain).

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Alumina Nanoparticles and Plants: Environmental Transformation, Bioaccumulation, and Phytotoxicity

14

Monika Asztemborska

14.1 Introduction

Nanotechnology is one of the fastest-growing fields of science and industry, and the largest areas of nano-based product application are coatings, paints, and pigments, followed by electronics and optics, cosmetics, and energy and the environment (Keller et al. 2013). Their exceptional properties make nanoparticles very attractive for industry but may also pose a threat for the environment. Assessing the risks imposed by the use of nanomaterials in commercial products and environmental applications requires a better understanding of their mobility, bioavailability, reactivity, ecotoxicity, and persistency.

One of the emerging areas of research is focused on studies of the impact of released nanoparticles on plants, which are an essential base component of all ecosystems and play a critical role in the fate and transport of NPs in the environment through plant uptake and bioaccumulation. The phytotoxicity of nanoparticles has become an important area of scientific interest.

Nanoparticles within the environment pose a potential risk to plants and therefore the functioning of ecosystems. Most investigations related to environmental release of NPs focus on questions of the implications of NP exposure for organism health. But, an additional approach to the subject is also necessary: Is the toxicity or bioavailability of NPs the same under laboratory and actual environmental conditions? Our biological and chemical knowledge allows us to assume that it is not. Consequently, besides the toxicity, potential nanoparticle transformation in the environment, which modifies their properties and alters their transport, fate, and toxicity, must be considered when assessing the potential environmental impact of NPs.

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_14

In the following chapter, the phytotoxicity of aluminum oxide (alumina, Al_2O_3) nanoparticles is discussed. In 2010, global nanoparticle production was dominated by metal oxides; one of those abundantly manufactured is alumina in particle size on a nanoscale (Keller et al. 2013). But knowledge about Al_2O_3 NP conversion under environmental conditions and interaction of nanosized alumina with plants is still incomplete. The discussion in the chapter, based on the results of selected research studies, presents the main information about possible alumina nanoparticle transformations in water and soil, bioaccumulation by plants of aluminum originating from nanoparticles, and the main aspects of Al_2O_3 NP phytotoxicity. The main goal is to present the direction of the undertaken research, possible suggested mechanisms of alumina phytotoxicity, together with appropriate results, which in some cases are contradictory.

14.2 Alumina Nanoparticles (Al_2O_3 NPs)

Aluminum oxide (Al_2O_3) is one of the dominant nanoparticles on the market in terms of mass flow through the global economy. Exceptional interest in alumina nanoparticles is an effect of their unique physicochemical properties: high melting point, good thermal stability, good wear resistance, high mechanical strength, good electrical insulation, excellent corrosion resistance, and other characteristics. The widest use of Al_2O_3 NPs is in the chemical industry for production of paints and coatings (Fig. 14.1). Catalyst production and electronics and optics are also important

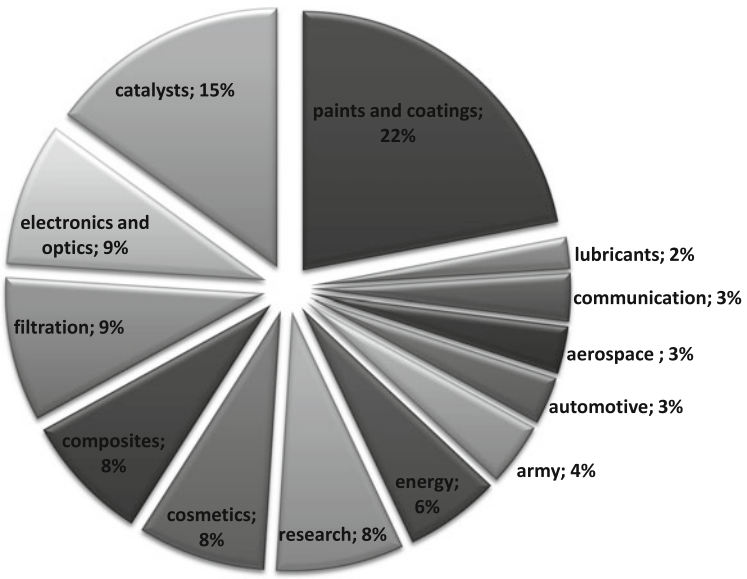

Fig. 14.1 Distribution of alumina nanoparticles across major application areas in 2012 (Future Markets Inc. 2013)

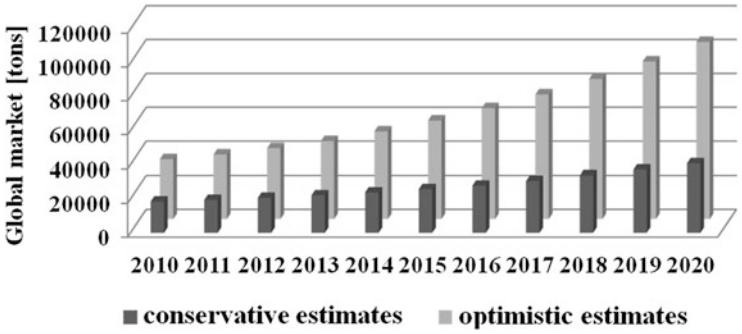

Fig. 14.2 Production of aluminum oxide nanoparticles (Future Markets Inc. 2013)

consumers of nanosized alumina. The price of alumina nanoparticles depends on the size, shape, purity, and synthesis method and varies within the range 12–1000 USD/kg (Future Markets Inc. 2013). The USA is the leading producer of nanoparticles, where 17 companies in 2012 were engaged in the manufacture of alumina. The annual world production of alumina nanoparticles in 2010 was 18,500 t, and this is constantly increasing (Fig. 14.2). It is estimated that in 2020 it may exceed 100,000 t. According to Keller et al. (2013), 63–91% of global nanoparticle production in 2010 ended up in landfills, with the balance released into soils (8–28%), water bodies (0.4–7%), and the atmosphere (0.1–1.5%).

14.3 Transformation of Al_2O_3 Nanoparticles in the Environment

In the natural environment, nanoparticles are subjected to various dynamic processes which have strong implications for their fate, transport, persistence, and, finally, bioavailability and toxicity (Amde et al. 2017). The kind of transformation that the nanoparticles undergo strongly depends on the chemistry of the environment and the physicochemical properties of the nanoparticles themselves. Commonly occurring processes in environmental and biological systems include:

1. Physical transformations: aggregation/agglomeration, adsorption, deposition
2. Chemical processes: dissolution, sulfidation, exchange of surfactants, mineralization, redox reactions
3. Interactions with macromolecules: hydrophobic or electrostatic interactions, ligand exchange, hydrogen bonding, flocculation
4. Biologically mediated transformation: distribution among organisms, bioturbation, ingestion-egestion dynamic-related transformations

Two of the most important factors impacting upon the bioavailability of alumina nanoparticles in aquatic and terrestrial environments are dissolution and aggregation. Partial dissolution of nanoparticles is very likely due to their high area to volume

ratio. Released ions are more mobile and bioavailable for plants. The chemical toxicity of aluminum ions released from alumina nanoparticles can be one of the main causes of Al_2O_3 NP toxicity. Dissolution is strongly affected by the size and morphology of the Al_2O_3 NPs—smaller NPs have higher specific surface areas than larger ones. Dissolution depends on the medium pH, natural organic matter (NOM), ionic strength (IS), temperature, and the presence of reactive compounds. The dissolution can be minimized by using stabilizing agents or various surface coatings.

The bioavailability and toxicity of alumina nanoparticles are also strongly affected by aggregation, resulting from the combined effect of van der Waals attraction, electrostatic repulsion, hydration force, and magnetic and hydrophobic interactions. Both homo-aggregation (formed due to the interaction between identical NPs) and hetero-aggregation (between NPs and other components in the environment) are possible; however, due to trace amount of NPs in the environment, hetero-aggregation is more probable (Schultz et al. 2015). Because of aggregation, the concentration of NPs in suspensions decreases together with increases in both particles and the aggregate size. This process favors the sedimentation and deposition of nanoparticles, resulting in reduced bioavailability of alumina for organisms and its toxicity. The research results obtained by Yoon et al. (2011), who investigated the cytotoxicity of alumina nanoparticles for a wide range of concentrations and incubation times using floating and adherent cells, showed that alumina NPs were gradually agglomerated over time, although a significant portion of sedimentation occurred at the early stage within 6 h. Particle agglomeration and sedimentation induce destabilization of alumina NPs in the culture medium and can affect cellular toxicity.

The physicochemical behavior of alumina nanoparticles is a function of, inter alia, pH and the presence of natural organic matter including humic acids commonly found in the natural environment. The NPs tend to aggregate as the pH of the suspension approaches the point of zero charge (ZPC), where van der Waals attraction forces dominate over electrostatic repulsion; however, NP colloidal suspensions are stable at pHs distant from ZPC (Ghosh et al. 2008). The stability of alumina nanoparticles was strongly enhanced in the presence of humic acids at the pH of ZPC (7.9) or above it, but in acidic conditions, NPs showed strong aggregation in the presence of humic acids. The presence of long-chain fractions in soil-extracted humic acids entangled with the NPs to form large aggregates. Therefore, the stability and mobility of NPs in the environment are affected not only by environmental factors such as pH but also by the structural properties of natural organic matter. The aggregation of alumina nanoparticles is influenced by the hydrophobic nature of the humic acid molecules; however, various organic matter samples will result in different colloidal behaviors of NPs and then their environmental fate and transport.

As the toxicity and bioavailability of alumina NPs depend on the primary particle size, the monodispersed state of NPs has to be achieved in the experimental medium. But, it is very complicated to achieve monodispersion of nanoparticles under laboratory conditions and is impossible in the natural environment because, among others, of natural organic matter, which comprises a heterogeneous mixture of different functional moieties derived from geochemical and microbial processes

affecting the physicochemical behavior of aluminum oxide nanoparticles. From this point of view, the agglomeration and sedimentation of alumina NPs become important and must be considered during bioavailability and toxicity investigations.

Transformation of alumina nanoparticles occurs not only in water but also in soil environments. Al_2O_3 NPs can undergo dissolution (increasing the concentration of aluminum ions in soil and nanoparticle mobility and bioavailability) and aggregation (reducing NP nano-associated toxicity), but they can also be strongly sorbed to soil surfaces and soil organic matter, which reduces their mobility and bioavailability (Dinesh et al. 2012). Additionally, as in aquatic systems, organic matter in soil may influence the surface speciation and charge of NPs and thus affects their aggregation/deposition properties. Studies of nanoparticle mobility in saturated sand (Rahman et al. 2013) have shown that aluminum oxide nanoparticles of a size smaller than 100 nm could be highly mobile. But, in more complex natural soils, different observations can be expected. Additionally, the presence of soil organisms may affect alumina nanoparticle mobility and bioavailability. Abundant in soils and crucial in the turnover of organic matter and in building soil structure are earthworms that process soil in the alimentary canal with digestive juices, improve organic matter degradation, and enhance the bioavailability of some soil nutrients. They influence the water solubility and bioavailability of alumina nanoparticles (Bystrzejewska-Piotrowska et al. 2012). Ten-day incubation of alumina nanoparticles in soil resulted in four times more aluminum extracted with water, indicating dissolution of alumina. The bioavailability was also increased. After 10 days incubation with earthworms, less aluminum was extracted with water. Thus, earthworms appeared to reduce aluminum present in the water-soluble fraction, indirectly proving that speciation of aluminum is changed in the gut of earthworms. Complexion of aluminum with organic compounds (products of earthworm activity) may reduce the aluminum phytoavailability and, consequently, aluminum toxicity for plants.

14.4 Accumulation of Al by Plants Exposed to Al_2O_3 Nanoparticles

Bioaccumulation of aluminum by plants exposed to nanosized particles of Al_2O_3 is important in terms of risk assessment and possible application in phytoremediation of contaminated sites. After nanoparticle uptake from water or soil, plants which produce organisms in the trophic chain can be a source of aluminum for higher organisms. The transfer of alumina nanoparticles or aluminum ions through the food chain can lead to bioaccumulation and biomagnification resulting in a long-term negative impact on the ecosystem.

Several plant species were tested to assess their suitability for accumulation of alumina nanoparticles (Table 14.1): onion (*Allium cepa* L.), corn (*Zea mays*), cress (*Lepidium sativum*), alligator plant (*Kalanchoe daigremontiana*) (Asztemborska et al. 2015), California red kidney bean (*Phaseolus vulgaris*) and rye grass (*Lolium perenne*) (Doshi et al. 2008), and common wheat *Triticum aestivum* (Riahi-Madvar et al. 2012).

Table 14.1 Bioaccumulation of aluminum in plants exposed to alumina nanoparticles

Plant species	Exposition conditions (Al_2O_3 NP size and concentration in medium; time and type of cultivation)	Concentration of Al (mg kg^{-1})		References
		Leaves	Roots	
Allium cepa	<50 nm; 0.1–10 g L^{-1}; 7 days; hydroponic cultivation	20.5–89.4	–	Asztemborska et al. (2015)
Zea mays	<50 nm; 0.1–10 g L^{-1}; 14 days; hydroponic cultivation	106.4–1107	5798–25,737	
Lepidium sativum	<50 nm; 1–100 g kg^{-1}; 7 days; soil cultivation	11.6–561	56.5–4077	
Kalanchoe daigremontiana	<50 nm; 2–10 g kg^{-1}; 3 months; soil cultivation	10.9–11.6	353.8–754.9	
Triticum aestivum	40 nm; 0.05–1 g L^{-1}; 5 days; agar cultivation	–	1633–3800	Riahi-Madvar et al. (2012)
Lolium perenne	100 nm[a]; 0.01–10 g kg^{-1}; 2 months; soil cultivation	2750–4525	–	Doshi et al. (2008)

[a]Nanosized aluminum particles with aluminum oxide coating

The efficiency of aluminum bioaccumulation depends on (Asztemborska et al. 2015, Riahi-Madvar et al. 2012):

- Particle size: the most effective uptake and transport of aluminum is observed for Al_2O_3 nanoparticles in comparison with microparticles.
- Particle concentration: aluminum content in plants was elevated by the increase in NP concentration in the growth media or soil, except for cases of extremely high concentrations, which favor aggregation and induce toxic effects.
- Type of medium: due to, inter alia, the alumina nanoparticle sorption on the soil surface and presence of natural matter, the amount of aluminum in plants cultivated in the hydroponics was higher in comparison with soil cultivations.
- Part of the plant: bioaccumulation is the highest in the roots of plants in comparison with aboveground organs, probably as an effect of nanoparticle adsorption on the roots, a process which raises the amount of aluminum in roots and limits transport of aluminum up the plants.
- Plant species: the highest efficiency of aluminum accumulation was determined in corn in comparison with onion (for comparable Al_2O_3 NP contamination and cultivation conditions).

Most research is based on determination of aluminum in plants exposed to alumina nanoparticles; however, plants are able to accumulate NPs from water and soil (Asztemborska et al. 2015), although the nanoparticles sediment easily and are still available for uptake by plants.

14.5 Influence of Alumina Nanoparticles on Plants

14.5.1 Root System

Roots are the primary target of phytotoxic aluminum, and the root apex was found to be the most Al-sensitive zone (Ryan et al. 1993). Therefore, most research is focused on toxic effects of aluminum nanoparticles on roots.

In Yang and Watts (2005), the phytotoxicity of alumina nanoparticles loaded with and without phenanthrene (Phen) was investigated by using root elongation for five plant species: *Zea mays* (corn), *Cucumis sativus* (cucumber), *Glycine max* (soybean), *Brassica oleracea* (cabbage), and *Daucus carota* (carrot). Nanoparticles reduce root elongation; however, when loaded with a monomolecular layer of Phen, the degree of the root elongation inhibition caused by the particles was reduced. This proves that the surface characteristics of the particles play an important role in the phytotoxicity of alumina nanoparticles.

Phytotoxicity of alumina nanoparticles is also time-dependent and dose-dependent (Yanik and Vardar 2015). Thirteen-nm-sized Al_2O_3 NPs reduced the root elongation by 40% in 5 mg mL^{-1} and 55% in 50 mg mL^{-1} after 96 h on plant wheat (*Triticum aestivum* L.) exposure.

Exposure of plant roots to Al_2O_3 NPs leads to phytotoxicity and results in morphological, cellular, and molecular alterations (Yanik and Vardar 2015). The effect of nanoparticles was also observed during histochemical analysis of plant roots. The studies revealed lignin accumulation, callose deposition, and cellular damage (epidermal and the cortex cells of maturation zone were damaged) in root cortex cells correlated with root elongation inhibition. Additionally, nanoparticle application decreased the total protein content and significantly enhanced the peroxidase activity and induced DNA fragmentation, which is one of the important markers of programmed cell death. The observed effects seem to be a response of the plant to stress related to alumina nanoparticle exposition. Lignin accumulation and callose formation were reported under metal stress such as Al toxicity in plants (Vardar et al. 2011). But, microscopic analysis revealed that the toxicity was related to NPs only, not Al^{3+} ions.

The latest scientific findings (Yanik et al. 2017) point even more strongly to programmed cell death as effects of aluminum oxide nanoparticles on plant roots. Programmed cell death is a functional process, which occurs as a defensive strategy to remove mutated, infected, or damaged cells during development or under environmental stress. Exposure of wheat (*Triticum aestivum* L.) to different concentrations of Al_2O_3 NPs decreased the mitotic indices, an important parameter which indicates the frequency of cell division, and caused chromosomal abnormalities such as c-mitosis, monopolar metaphase, and stickiness after 96 h. Loss of plasma membrane integrity, irregular microtubule aggregations, and nuclear deformations, which are advanced signs of programmed cell death, were determined at all concentrations.

Alumina nanoparticles have undoubted cytogenetic potential. Dose-dependent chromosomal aberrations, e.g., sticky, multipolar, and laggard chromosomes, chromosomal breaks, and the formation of binucleate cells, were found in root tip cells of *Allium cepa* because of plant exposition to alumina nanoparticles (Rajeshwari et al. 2015). There are reports that the production of reactive oxygen species (ROS) is responsible for inducing nanotoxicity, including cytotoxicity. Increased activity of antioxidant enzymes—superoxide dismutase (SOD) and catalase (CAT)— was found in *Triticum aestivum* exposed to alumina nanoparticles (Riahi-Madvar et al. 2012), while the activity of ascorbate peroxidase (APX) was relatively low. The high activity of CAT is probably attributed to the higher activity of SOD and determined low activity of APX.

In the scientific literature regarding alumina nanoparticle, phytotoxicity contradictory data can be found. According to Lin and Xing (2007), nano-Al_2O_3 suspension has no phytotoxicity toward *Brassica napus* (rape), *Raphanus sativus* (radish), *Lolium perenne* (ryegrass), *Lactuca sativa* (lettuce), and *Cucumis sativus* (cucumber), while only for *Zea mays* (corn) is root elongation reduced by 35%. Furthermore, a significant positive influence of nano-Al_2O_3 on root elongation of *Arabidopsis thaliana* (Lee et al. 2010; Jin et al. 2017) and *Triticum aestivum* (Riahi-Madvar et al. 2012) was observed. The mechanism for this effect is unclear. The increasing root length in cases with relatively high alumina nanoparticle concentration treatment can be explained by the presence of NPs in aggregate form causing changes in the color of the medium and a significant decrease in bioavailability of aluminum. Transcriptomic analyses of *Arabidopsis thaliana* exposed to a nanosized Al_2O_3 indicate that the beneficial effect of nano-Al_2O_3 is related to an increase in the transcription of several genes involved in root growth as well as in root nutrient uptake (e.g., upregulation of the root hair-specific gene family and root development genes) (Jin et al. 2017). Specifically, differentially transcribed genes in the NP treatment were mostly involved in cellular processes and single-organism processes (biological process category) and were located in cell parts and membranes (cellular component category) and involved in binding and catalytic reactions (molecular function category).

Tobacco (*Nicotiana tabacum*) plants showed extreme microRNA (miRNA) expression during exposure to Al_2O_3 nanoparticles (1%) as compared to other treatments and the control (Burklew et al. 2012). miRNA are a endogenous class of posttranscriptional gene regulators that function to alter gene expression by either targeting mRNAs for degradation or inhibiting mRNAs translating into proteins and may play a key role in mediating plant stress responses to nanoparticle stress in the environment.

14.5.2 Seed Germination and Plant Growth

Seed germination and plant growth are very common toxicity indicators, and these tests are very useful in investigating the phytotoxicity of various pollutants because of their sensitivity and simplicity. Alumina nanoparticles seem to not affect the

germination and growth of *Arabidopsis thaliana* (Lee et al. 2010), *Brassica napus*, *Raphanussativus*, *Loliumperenne*, *Lactuca sativa*, *Cucumis sativus* and *Zea mays* (Lin and Xing 2007), *Arabidopsis thaliana* (Riahi-Madvar et al. 2012). Selective permeability of seed coats which confronts roots with an excess in NPs and a low rate in transportation of this material to the shoot may provide an explanation for the lack of negative effects (Lin and Xing 2007).

Alumina nanoparticles may also have a positive effect on plants. Alumina nanoparticles substantially increase biomass accumulation of *L. minor* (Juhel et al. 2011). Simultaneously, morphological adjustments such as increased root length and number of fronds per colony and increased photosynthetic efficiency are observed. Importantly, the removal of alumina particles from the medium showed that the nanoparticles themselves are responsible for the biological effect and aluminum ions produced through particle dissolution are not involved in the observed phenomena—the growth enhancement by alumina nanoparticles is "nano-specific."

The effects of alumina nanoparticles and aluminum ions are different. Seedlings of cabbage (*Brassica oleracea* var. *capitata*) were exposed to Al^{3+} ions and alumina nanoparticles (Amist et al. 2017). Aluminum ions were phytotoxic and adversely affected seedling growth and biochemical parameters of the test crop with stunting of the stem growth, while lower doses of alumina NPs enhanced seedling growth, pigments, sugar, and protein contents of cabbage seedlings. Alumina NPs at higher concentrations adversely affected biochemical parameters and nitrate reductase activities of the treated seedlings. Alumina NPs induced activities of antioxidant enzymes: SOD, CAT, and POX. Antioxidant enzyme activities increased under all treatments with the maximum increase noted in those seedlings treated with aluminum ions and higher concentrations of alumina NPs. The lower amount of alumina NPs buttressed the metabolic processes of the test crop and appeared to mitigate the phytotoxic effects of aluminum ions.

14.5.3 Alumina Nanoparticles: Phytotoxic or Not?

Alumina nanoparticles may have contrary effects on plants. Toxicity, including inhibition of root elongation; morphological, cellular, and molecular alterations; and positive effects, including plant growth stimulation, are possible. Consequently, the unequivocal answer to the question of whether alumina nanoparticles are phytotoxic or not seems to be impossible at this stage. But, there are many factors affecting the results of toxicity studies: primarily the alumina nanoparticle size and concentration. Differences in cultivation and research methodology cannot remain without exerting any influence. There is no doubt that alumina nanoparticles affect plant growth, cell morphology, gene expression, and metabolic processes in a manner dependent on the physicochemical properties of Al_2O_3 NPs and environmental conditions.

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15 *Lantana aculeata* L.-Mediated Zinc Oxide Nanoparticle-Induced DNA Damage in *Sesamum indicum* and Their Cytotoxic Activity Against SiHa Cell Line

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15.1 Introduction

In recent years, advanced science and technology researchers have attempted to synthesize nanoparticles (NPs) within the size range of 1–100 nm, and this extensive research and concern on NPs is enlarging because of their wide application in areas of science and technology. Zinc oxide NPs belong to the class of metal oxides, which is characterized by photocatalytic capacity against chemical and biological species (Srivastava 2007). The progress of technology and life quality of mankind has been closely with the progress in material science. Most techniques applied in material processing are based on breaking up large mass of a material into preferred sizes and shapes in the processed material (Roco et al. 1999). Late improvements depend on the impact of different quantum size nanoscale particles, uncovering that the greater part of the novel work will be founded on properties of nanomaterials. The traditional processing techniques that provoke lattice defects and further imperfections will no longer be thinned for synthesis of nanoparticle by absolute number of atoms (Isobe et al. 2006). Moreover, the purposes of traditional draw near impart difficulties for synthesis of such small particles in an enviable size range.

Alternative artificial technique for NPs involves proscribed precipitation of NPs from precursors and dissolved in a solution (Warheit 2008). A micro suspension can also be formed using surfactants between two immiscible liquids, with the intransigent isolated inside a colloid, through hydrophobic in opposition to hydrophilic

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_15

forces (Masciangioli and Zhang 2003). The resultant NPs form a micro-colloidal suspension. The mechanism concerned in stabilization of NPs can be categorized as (Smith et al. 2007) electrostatic stabilization, concerning the creation of a double layer of adsorbed ions over the NPs resulting in a coulombic repulsion between approaching NPs, or steric hindrance, achieved by adsorption of polymer molecules over the NPs.

Nature has devised processes for the synthesis of nanoscaled inorganic materials which have contributed to the improvement of moderately new and largely uncultivated area of research based on the biosynthesis of nanomaterials (Papis et al. 2007). Synthesis using plant extract is congruent with the green chemistry principles. "Green synthesis" of NPs makes use of environmental friendly, harmless reagents and non-toxic.

15.1.1 Nanoparticle Concept and Production

Nanoparticles belong to the wider group of nanomaterial, where the prefix "nano" refers to infinitesimal physical dimension. Hence nanoparticles possess properties that are qualitatively or quantitatively distinctly different from their other physical forms (SCENIHR 2006), such as those of large size particles (bulk particles) made from the same material and their water-soluble form. Size-related differences in particle properties may be due to the large surface area per mass, resulting in increased ratio of surface to core atoms and increased number of corner and edge atoms. This results in increased reactivity or increased ion released (Elzey and Grassian 2010), which enables their use in novel applications.

Theoretically, engineered nanoparticles (ENPs) can be created from any substance; however normally the vast majority of the ENPs are integrated from transition metals, silicon, single-walled carbon annotates, fullerenes and metal oxide. Top-down and bottom-up creation are two specific methodologies for the era of nanoparticles. In top-down strategy, lithographic systems are utilized to cut vast bits of a material into NPs (Powell et al. 2008). The worldwide business sector for nanotechnology was esteemed at about $20.1 billion in 2011 and should reach $20.7 billion in 2012, and the production will probably increase sharply in the near future.

15.1.2 Plants as a Source of Nanomaterial Synthesis

Nanobiotechnology represents an economic alternative for chemical and physical methods of nanoparticle formation. It consolidates natural standards with physical and compound methods to create nanosized particles with particular capacities. Although chemical and physical methods may effectively create produce pure, well-defined nanoparticles, these strategies are very costly and possibly perilous to nature (Chandran et al. 2006). The preparation of nanoparticles utilizing green technologies is favourable over substance specialists because of their ecological

results. The biological method of synthesis of nanoparticles has proved to be a better method than other chemical methods due to the large amount of capital involved in the production of energy-intensive processes (Mukherjee et al. 2014).

Gardea-Torresdey et al. (2002) have demonstrated that gold and silver nanoparticles are formed within different parts of live alfalfa plant on accumulating the corresponding metal ions from solid media. In an attempt towards deliberate synthesis of metal nanoparticles, plant extracts like that of neem (*Azadirachta indica*) (Shankar et al. 2004), geranium (Shankar et al. 2003) and amla (*Emblica officinalis*) (Ankamwar et al. 2005) can be used for the size- and shape-directed biosynthesis of gold, silver (Gardea-Torresdey et al. 2002) and gold and silver bimetallic core-shell nanoparticles (Shankar et al. 2004).

Utilization of natural living beings, for example, microorganisms, plant extract or plant biomass, could be a distinct option for synthetic and physical methods for the generation of nanoparticles in an eco-accommodating way (Elumalai et al. 2010). The most important application of silver and silver NPs is in restorative industry such as topical ointments to prevent infection against burn and open injuries (Ismail and Bakar 2004).

15.1.3 Description of Plants

15.1.3.1 General Description of *Lantana aculeata*

The word *Lantana aculeata* is derived from Latin 'lento' which means to bend. The species was initially portrayed and given its binomial name by Linnaeus in 1753 (Kumarasamyraja et al. 2012). It is a member of the Verbenaceae family with 600 assortments existing around the world. *Lantana aculeata*, a native species of South and Central America and the Caribbean islands, has its presence recorded even in Brazil, Florida, Jamaica, Mexico and Trinidad. The species is spread over to a wide geological extent in neotropics, yet none is accounted for from the Old World (Day et al. 2003). Some species of *Lantana aculeata* (Fig. 15.1) are also believed to originate from Africa and one from India (Hiremath and Sundaram 2005).

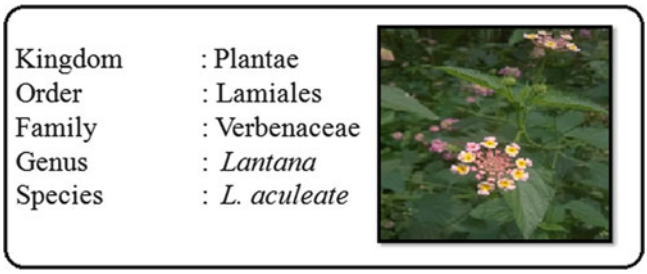

Fig. 15.1 Taxonomy of *Lantana aculeata*

Biology of *Lantana aculeata*

Lantana aculeata, also known as wild sage, is a thorny multi-stemmed, deciduous shrub with an average height of 2 m. The shrub's taxonomic position is characterized as fitting in with class Magnoliopsida, order Lamiales, family Verbenaceae and genus *Lantana* (Larson et al. 2001). Stems are square in blueprint, secured with bristly hairs when green, regularly furnished or with scattered little prickles. *Lantana aculeata* possesses a strong root system (Kumarasamyraja et al. 2012). The roots even after rehashed cuttings give new flush of shoots. Leaves are inverse, basic, with long petioles, oval cutting edges which are unpleasant, bristly and have gruff toothed edges. The leaves of *Lantana aculeata* have a solid smell. Its blossoms are little, multihued, in stalked, thick in level finished bunches with a corolla having slender tube with four short spreading projections. Their blossoms experience shading change consequent to anthesis. These blossoms happen in bunch which incorporates white-pink-lavender or yellow-orange-red blend (Hiremath and Sundaram 2005). The yellow shading of the blossom gives visual signal to pollinators, and change in shading is started on the demonstration of fertilization. Berries of *Lantana aculeata* are round, plump, two-seeded drupe with at first green in shading and turning purple lastly to blue-dark shading (Day et al. 2003). Be that as it may, the berries are extremely toxic in nature; however these are alluring to creepy crawlies and winged creatures. Seed germination is simple and speedier in *Lantana aculeata*.

Uses

Lantana aculeata though being a noxious weed has several minor uses, mainly in herbal medicine. There are series of research studies conducted on the exploitation of chemical constituents present in different parts of the plant species. The studies demonstrate that extracts from the leaves can be employed to combat antimicrobial, fungicidal, insecticidal and nematicidal problems. Its potential to serve as biocide has also been illustrated in several researches (Dobhal et al. 2011).

Impacts

Lantana aculeata has many negative impacts including potential to disrupt succession cycle, displacing native biota resulting in decreased biodiversity. Its infestations alter the structural and floral composition of native communities (Sharma and Raghubanshi 2010). As the density of *Lantana aculeata* in forest increases, allelopathic interactions increase, and hence there is decline in species richness (Day et al. 2003).

Lantana aculeata is a noteworthy issue in agricultural areas in most regions of India as it forms dense thickets, spreads gregariously, outcompetes pasture species and affects both flora and fauna. The field cases happen mostly in youthful creatures that have either been recently brought into a range where *Lantana aculeata* develops or are without access to other grubs. Children and adults in many countries often consume ripe fruits of *Lantana aculeata* without any ill effects. However, consumption of green fruit has proved to be fatal in some parts of India (Sharma et al. 2007). Apart from causing death of livestock, sublethal doses of *Lantana aculeata* toxin

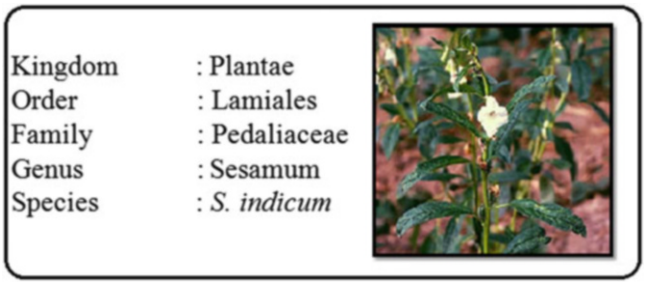

Fig. 15.2 Taxonomy of *Sesamum indicum*

cause reduction in potential production, manifested abortion, loss of milk production in dairy cows and chronic wasting in beef cattle.

15.1.3.2 *Sesamum indicum*

Sesamum is a flowering plant (Fig. 15.2). The wild variety of *Sesamum* occurs in Africa and small number in India. It is widely established in tropical region around the world and is cultivated mainly for its edible seeds, which grow in pods. *Sesamum* seed is one of the oldest seed crops, domesticated well over 3000 years ago. It is originated in India. The world's largest exporter of *Sesamum* seed is India, and the largest importer is Japan. Its annual plant grows in 50–100 cm (1.6–3.3 ft) tall, with opposite leaf 4 ft and 14 cm (1.6–5.5 in.) long with an enter margin; there are broad lanceolate, to 5 cm (2 in.) broad at the base of the plant.

Description of *Sesamum indicum*

Sesamum indicum is an annual broadleaf plant that grows 5–6 ft tall. It produces a 1–2-in.-long white, bell-shaped inflorescence growing from the leaf axils (where the leaf stalk joins the stem). The blooms do not open all at once, but gradually, from the base of the stem upwards to the top of the plant. The flowers are both male and female and will self-pollinate (Monalisa and Patra 2013). The seed is produced in a 1–1.5-in.-long, divided seed capsule that opens when the seeds are mature. There are eight rows of seed within each seed capsule, and seed may be yellow, white, brown or black. Due to the non-uniform, indeterminate nature of the bloom period, the reproductive, ripening and drying phases of the seed tend to overlap. Seed lowest on the plant will mature first, even as the upper part of the plant is still flowering or has just formed seed capsules.

Cultivation

Sesame is very drought-tolerant, in part due to its extensive root system. However, it requires adequate moisture for germination and early growth. While the crop survives drought, as well as presence of excess water, the yields are significantly lower in either condition. Moisture levels before planting and flowering impact yield most (Purakayastha and Bhatnagar 1997).

Most commercial cultivars of sesame are intolerant of waterlogging. Rainfall late in the season prolongs growth and increases high harvest-shattering losses. Wind can also cause shattering at harvest. Initiation of flowering is sensitive to photoperiod and to sesame variety (Rahman et al. 2013). The photoperiod also impacts the oil content in sesame seed; increased photoperiod increases oil content. The oil content of the seed is inversely proportional to its protein content. Sesame varieties have adapted too many soil types. The high-yielding crops thrive best on well-drained, fertile soils of medium texture and neutral pH. However, these have low tolerance for soils with high salt and waterlogged conditions. Commercial sesame crops require 90–120 frost-free days. Warm conditions above 23 °C (73 °F) favour growth and yields. While sesame crops can grow in poor soils, the best yields come from properly fertilized farms (Ismail 2012; Selvi and Gunaseeli 2004).

15.1.4 Green Synthesis of Nanoparticles from *Lantana aculeata*

Lantana aculeata Linn, family Verbenaceae, is a shrub available throughout central and south India. It is currently the major outlandish weed, spreading quickly in wastelands and rural fields (Raghubanshi and Tripathi 2009). This plant can be used for the synthesis of nanoparticles in eco-friendly manner because it has the capacity to extract heavy metals through its roots, stems and leaves and also due to its rapid propagation (Zhang et al. 2002).

Thirumurugan et al. (2011) demonstrate the AgNP synthesis using leaf extract of *Lantana aculeata*. The silver nanoparticles synthesized were distinguished using scanning electron microscopy (SEM) which showed the approximate size of nanoparticles about 39.60 nm. Kumarasamyraja and Jaganathan (2013) reported that the silver nanoparticles were synthesized using the aqueous extract of *Lantana aculeata* and assessed their antimicrobial activity. The synthesized silver nanoparticles were characterized by UV-visible spectrophotometer. The size and shape of silver nanoparticles was confirmed by particle size analyses and TEM. The particle size ranged 0.772 nm. *Lantana aculeata*-mediated silver NPs showed better antimicrobial activity.

15.1.5 Zinc Oxide Nanoparticles

Zinc oxide is an inorganic compound of white powder generally insoluble in water. Mechanical properties such as internal stress or adhesion are important in order to assure the patterning accuracy and durability for various types of commercial applications. The structure of zinc oxide is generally hexagonal wurtzite, spherical and zinc blende (Krishnan and Pradeep 2009).

15.1.5.1 Synthesis and Characterization of Zinc Oxide Nanoparticles

There are numerous physical and chemical methods for synthesis of zinc oxide nanoparticle in huge quantities in a short period of time. Simple solution-based

methods, chemical precipitation, sol gel, solvothermal, electrochemical and photochemical reduction methods, are the most preferable methods. Zinc oxide NPs can also be synthesized from plant, microorganisms (bacteria and fungi) and enzymes by using green amalgamation techniques. Green synthesis methods are eco-accommodating approach and perfect for pharmaceutical and other biomedical applications furthermore in horticulture in light of the fact that no poisonous chemicals are utilized as a part of these strategies.

Nanoparticles have increased expanding consideration on account of their novel properties, including an extensive particular surface region and high response activity (Babu and Narayanan 2013). Nanoparticles are atomic or molecular aggregates with no less than one measurement between 1 and 100 nm that can definitely change their physiological properties contrasted with the mass material (Nel et al. 2006; Roco 2003). The synthesis of nanoparticles by routine physical and chemical techniques has some unfavourable impacts like basic states of temperature and weight, costly and poisonous chemicals, long reflux time of response and harmful side effects (Vanaja et al. 2013; Iravani 2011). When compared to physical and chemical method, green synthesis of nanoparticles makes utilization of ecological cordial, non-dangerous and safe reagents (Mohanpuria and Yadav 2009). The effect of temperature on nanoparticle formation also has been investigated, and it has been reported that polydisperse particles with a size of 5–300 nm were obtained at lower temperature, while a higher temperature supported the formation of much smaller and spherical particles (Song and Kim 2009).

ZnO nanoparticles have been synthesized using the plant extracts of *Eichhornia crassipes*-mediated ZnO nanoparticles were biosynthesized and its effect were seen against antifungal activity (Vanathi et al. 2014). Vidhya et al. (2013) have portrayed *Calotropis gigantea*-mediated ZnO NPs. The particles obtained were spherical in shape and were agglomerates of nanocrystallites. The average crystallite size estimated from XRD analysis was in the range of 30–35 nm. ZnO NPs were green synthesized by Sangeetha et al. (2011) using aloe leaf broth extract. Their outcomes showed improved biocidal activity against different pathogens when compared to chemically synthesized ZnO NPs. They have also reported that the effectiveness of NPs increased with increased particle dose, treatment time and synthesis method. Nagarajan and Kuppusamy (2013) have reported the green route biosynthesis of ZnO NPs and their utility as catalyst. The nanoparticles are characterized by UV-Vis, FTIR, XRD, TGA, SEM-EDX TEM and GC-MS techniques. The obtained particles were in size range of 8–32 nm and also very stable even after a month.

15.1.5.2 Application of Zinc Oxide Nanoparticles

Once particles are synthesized, they significantly change their physical and chemical properties. The typical properties of the particle like heat treatment, mass exchange, synergist movement, etc. all change, but compared to non-metal nanoparticles, metal nanoparticles have more industrial applications. Nanoparticles offer much new development in the field of biosensors, biomedicine and bio-nanotechnology specifically in the areas such as drug delivery, medical diagnostic tools, cancer treatment agent (gold nanoparticles) and agriculture as bio-fertilizers to plants. Magnetic

nanoparticles are getting significant consideration due to their extensive variety of utilizations, such as the immobilization of the proteins and enzymes, bio-separation, immunoassays, drug delivery and biosensors. Nanoparticles and nanostructure are becoming a part in human medical applications, including imaging or the delivery of therapeutic drugs to cell, tissues and organs (Harter and Naidu 2001).

It is an important conventional band gap semiconductor with tremendous scientific and technological interest, having a direct wide gap (3.37 eV) (Huang et al. 2001). It is an exceedingly favoured multi-tasking metal oxide having an immeasurable rundown of appealing properties and has been generally utilized as a part of numerous modern ranges, for example, sun-oriented cells, UV-light-radiating gadget, gas sensor, photocatalysts, pharmaceutical and restorative commercial enterprises (Yang and Park 2008). It is non-harmful, self-purging (Yadav et al. 2006), perfect with skin, antimicrobial, dermatological, utilized as an UV blocker as a part of sunscreen and numerous biomedical applications (Krishnan and Pradeep 2009). The benefits of ZnO is bio-protected, biocompatible with extraordinary capacity like structure ward properties, electrical and warm transport properties, which could be changed as for molecule size, shape, morphology, introduction and perspective proportion, have brought about expanded enthusiasm for acquired this nano-metal oxide material (Dakhlaoui et al. 2009).

15.1.6 Cytotoxicity of Zinc Oxide Nanoparticles

ZnO nanoparticles show relatively high biocompatibility. Their bulkier form is generally recognized as safe (GRAS) by the FDA. Zinc is an important cofactor in various cellular mechanisms and plays an important role in maintaining cellular homeostasis; hence ZnO shows biocompatibility. The administered ZnO can be easily biodegraded or can take part in the active nutritional cycle of the body (Choudhury and Panda 2004). While extracellular ZnO shows biocompatibility, elevated levels of administered intracellular ZnO show enhanced cytotoxicity through zinc-mediated protein activity disequilibrium and oxidative stress (Kahru and Dubourguier 2010). ZnO nanoparticles have the unique ability to induce oxidative stress in cancer cells, which has been found to be one of the mechanisms of cytotoxicity of ZnO nanoparticles towards cancer cells. This property is due to the semiconductor nature of ZnO. ZnO induces ROS generation, leading to oxidative stress and eventually cell death when the anti-oxidative capacity of the cell is exceeded.

15.1.6.1 Mechanism of Cytotoxicity

The basic mechanism behind the cytotoxicity of ZnO NPs is the intracellular release of dissolved zinc ions, followed by ROS induction. This event causes zinc-mediated protein activity disequilibrium and oxidative stress, eventually killing the cell. Soluble extracellular zinc shows very little cytotoxicity. Recent research shows that extracellular soluble zinc, when exposed to cell culture and media, forms poorly soluble amorphous zinc-carbonate phosphate precipitates (phosphate from media).

This precipitate is supposed to protect the cell from the cytotoxicity of zinc (Kasemets et al. 2009). On the other hand, with the release of soluble zinc ions inside the cell, a cascade of pathways interrelated to each other takes place, which is responsible for the cytotoxic response of the ZnO nanoparticles.

Many in vitro studies have proved that ZnO NPs show selective cytotoxicity towards cancer cells. Jiang suggested that they show 28–35 times selective toxicity towards cancer cells compared with that of normal cells (Jiang et al. 1998). This selective cytotoxicity in cancer cells in in vitro condition can also be further exploited in the in vivo condition by selectively targeting ZnO nanoparticles towards cancer cells. ZnO NPs selectively kill cancer cells by inferring selective localization and selective cytotoxicity towards them.

15.1.7 Genotoxicity of Zinc Oxide Nanoparticles

DNA-damaging potential of ZnO nanoparticles in *Sesamum indicum* as representative of plant system could be confirmed in the comet assay and DNA laddering experiment. Comet test likewise called single-cell gel electrophoresis is a system for the location of DNA harm at the level of individual cells, which is a standout amongst the most exceptional methods acquainted with the agricultural sciences as of late. The assay test is the most popular tests of DNA damage detection by electrophoresis (Chakraborty et al. 2009). The assay is quick, simple to handle, non-obtrusive, visually and reasonably contrasted with most traditional procedure. Thus, it has rapidly gained importance in the field of genetic medicine, toxicology, agriculture and environmental studies (Srivastava 2007). The fundamental guideline of this test is to decide the DNA break by measuring the DNA harm which is evaluated by the extent of DNA, which moves out of the cores towards the anode when singular cell or isolated nuclei are embedded in a thin agarose layer. Diameter of nuclei of the studied species and the degree of DNA denaturation indicate the condition of DNA and are responsible for metabolic activities.

In previous study, a simple, rapid biological procedure has been evolved to synthesize ZnO nanoparticles from *Lantana aculeata* leaf broth extracted using Zn (NO3) as precursor. The synthesized nanoparticles have been characterized by various techniques which include UV-Vis, FTIR, XRD, EDX, FESEM and HRTEM (Figs. 15.3, 15.4, 15.5, 15.6, 15.7 and 15.8). The biological synthesis of ZnO nanoparticles was spherical in shape with an average size of 12–25 nm. These study indicate the benefits of using biological method in synthesizing ZnO nanoparticles that have antimicrobial activities, and also it could be effective in agricultural development (Narendhran et al. 2016). In the present study is the continuation to assess the genotoxicity and cytotoxicity activities of *Lantana aculeata*-mediated zinc oxide nanoparticles.

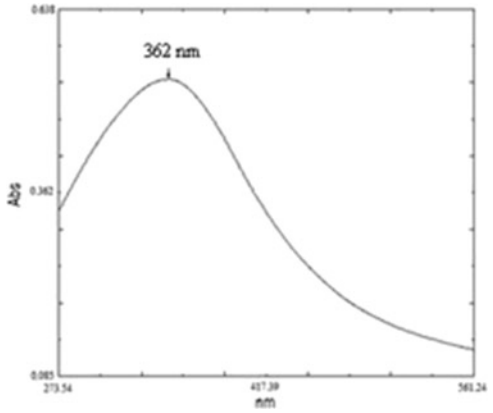

Fig. 15.3 UV spectrum of ZnO NPs

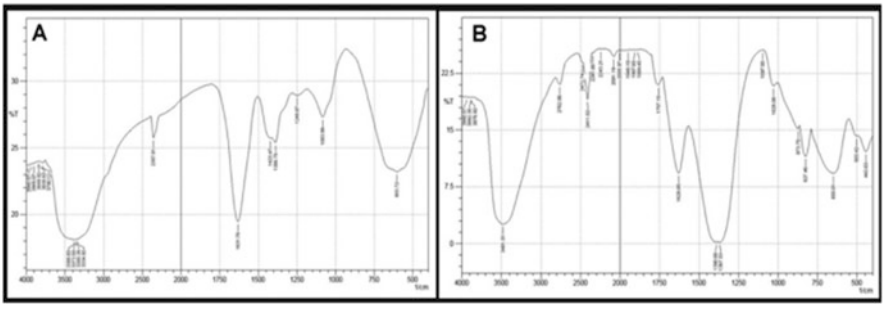

Fig. 15.4 FTIR spectrum of (**a**) *L. aculeata* leaf extract (**b**) *L. aculeata*-mediated ZnO nanoparticles

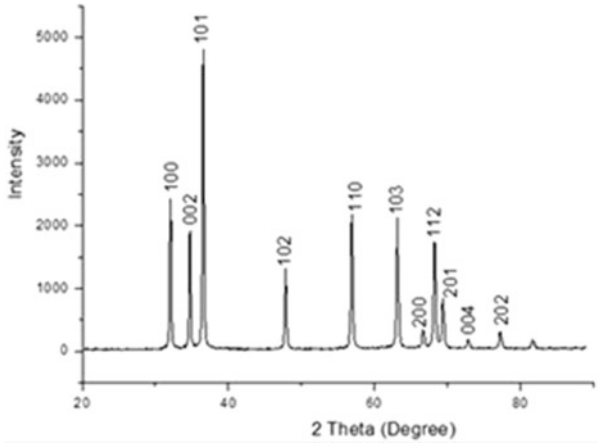

Fig. 15.5 XRD spectrum of ZnO nanoparticles

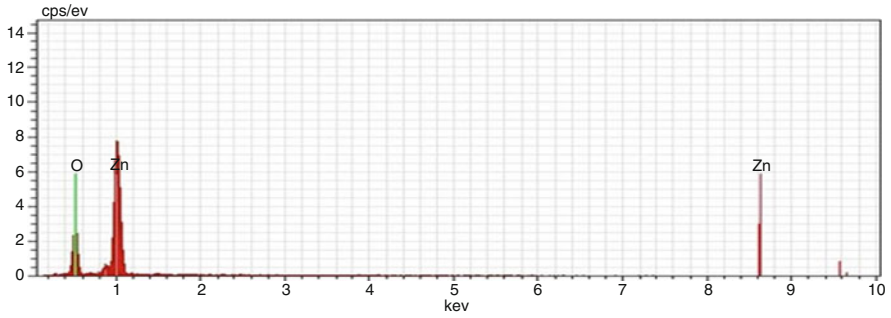

Fig. 15.6 EDX spectrum of *L. aculeata*-mediated ZnO nanoparticles

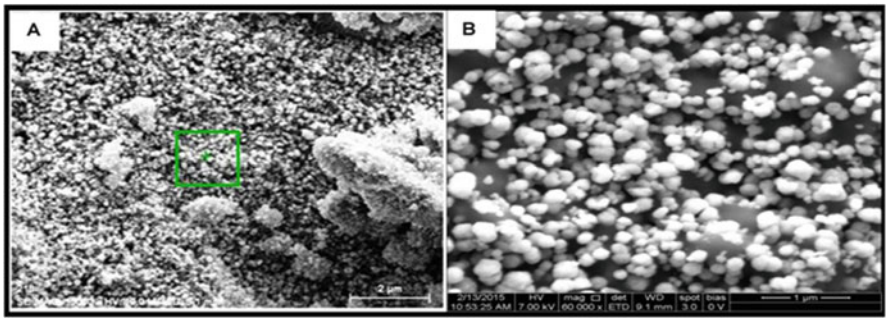

Fig. 15.7 (**a** and **b**) FESEM images of *L. aculeata*-mediated ZnO nanoparticles

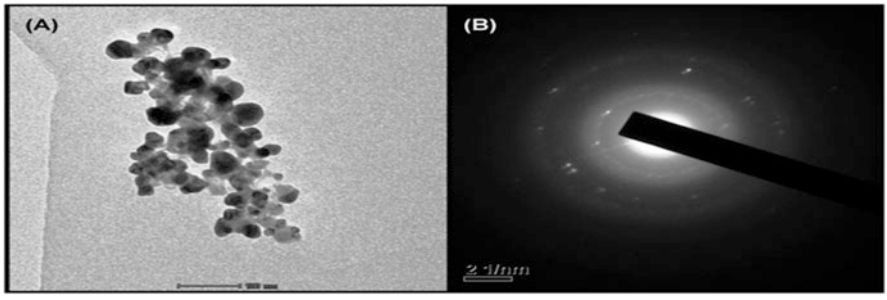

Fig. 15.8 (**a**) HRTEM images of *L. aculeata*-mediated ZnO nanoparticles (**b**) SAED pattern analysis of ZnO nanoparticles

15.2 Material

Fresh, healthy and young *L. aculeata* leaves were collected from Vadavalli region (11.0100° N, 76.9000° E), Coimbatore, Tamil Nadu, India. The sample was authenticated by Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2014-15/Tech/1418). *L. aculeata*-mediated ZnO nanoparticles synthesis with a particle size of 12–25 nm.

Zinc nanoparticles have been suspended using Milli-Q water and dispersed by ultrasonic vibration. For the present study, five concentrations, viz. 100, 250, 500, 1000 and 2000 mg L^{-1}, of ZnO NPs were used. *Sesamum indicum* (CO-1) seeds were immersed in a 2.5% sodium hypochlorite solution for 15 min sterilization and experimental consistency following Narendhran et al. (2016). After rinsing three instances with Milli-Q water, sesame has been soaked in ZnO suspensions at soaking duration of 1 day. Milli-Q water was used in the soaking method for a better control of the media. A pot experiment was conducted at Karpagam Academy of Higher Education Campus, Eachanari, Coimbatore, Tamil Nadu, India, during July 2015. Ten seeds had been sown in each pot (30 cm diameter and 25 cm deep) on zinc-deficient soil. After 60 DAS, zinc oxide nanoparticles treated and untreated leaf samples of *Sesamum indicum* were collected in brown paper covers and brought to the laboratory. Leaves were washed with tap water and air-dried. The leaves were sealed in plastic sacks, marked and stored at 4 °C for further studies.

15.3 Methods

15.3.1 DNA Damage Analysis Using Comet Assay in *Sesamum indicum* (Chakraborty et al. 2009)

The *Sesamum indicum* leaves were put for 2 min on ice to keep them turgid. For isolation of nuclei, leaf tissues were put in a petric plate containing Tris buffer (400 mM, pH 7.5). Using a fresh razor blade, leaves were finely and gently sliced allowing isolation of nuclei. The segregated nuclei were gathered in the buffer. Taking the nuclear suspension, slides were put in alkaline electrophoresis buffer (300 mM NaOH and 1 mM EDTA; pH $>$ 13) for 15 min to permit loosening up of the DNA in a horizontal gel tank took after by electrophoresis at 4 °C for 20 min at 26 V adjusted to 300 mA by changing support level in the tank. Slides were kept in 0.4 M Tris (pH 7.5) for 5 min lastly rinsed in water. Every trial was rehashed twice.

15.3.2 DNA Extraction and Laddering

DNA was isolated from leaf of *Sesamum indicum* using a modified CTAB method (Khan et al. 2007). The leaves were weighed and ground in extraction buffer (25 mM EDTA, 100 mM Tris buffer pH 8, 3% PVP, 2 M NaCl, 3% CTAB). The suspension was gently mixed and incubated at 65 °C for 20 min with infrequent blending. It is used converted to room temperature and an equivalent volume of chloroform: isoamyl alcohol (24:1) was included. The blend was rotated at 12,000 rpm for

5 min. The reasonable upper fluid stage after that exchanged to another tube, to which 2/3 volume ice-cold isopropanol was added and incubated at 20 °C for 30 min. The experimental resulting pellet was washed twice with the 75% ethanol. After that the pellet needs to be air-dried under a clean laminar hood and then the nuclei acid dissolved in TE (10 mM Tris buffer pH 8.1 and 1 mM EDTA) at room temperature itself and then kept stored at 4 °C until before start to use. RNA is eliminated by treating the sample with RNase (10 mg or ml) for 30 min at 37 °C. The DNA purity is determined through measuring the absorbance of diluted DNA solution at 260 and 280 nm. The isolated DNA from all the treated samples was determined on 2.5% agarose gel in TAE (Tris acetate EDTA) buffer at 100 V, at 4 °C. 100 bp ladder was loaded for proper reference. DNA was stained with aqueous solution of EtBr, photographed and visualized under a UV transilluminator.

15.3.3 Determination of In Vitro Antiproliferative Effect of *Lantana aculeata*-Mediated Zinc Oxide Nanoparticles on Cultured SiHa Cell Lines

SiHa cervical cancer cell lines that were purchased from NCCS Pune were maintained in Dulbecco's Modified Eagle Media (HiMedia) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37 °C in 5% CO_2 in a humidified atmosphere in a CO_2 incubator (NBS, Eppendorf, Germany). The cells were trypsinized [500 μl of 0.025% trypsin in PBS/0.5 mM EDTA solution (HiMedia)] for 2 min and passaged to T flasks in complete aseptic conditions. Extracts were added to grown cells at a final concentration of 6.25, 12.5, 25, 50 and 100 μg mL^{-1} from a stock of 1 mg mL^{-1} and incubated for 24 h.

The % difference in viability was determined by standard MTT assay after 24 h of incubation. MTT is a colorimetric assay that measures the reduction of yellow 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with an organic solvent dimethyl sulfoxide (HiMedia), and the released, solubilized formazan product was measured at 540 nm. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. The cells were washed with 1x PBS and then 30 μl of MTT solution added to the culture (MTT −5 mg mL^{-1} dissolved in PBS). It was then incubated at 37 °C for 3 h. MTT was removed by washing with 1x PBS, and 200 μL of DMSO was added to the culture. Incubation was done at room temperature for 30 min until the cell got lysed and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2 min to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank in a microplate reader (ELISASCAN, ERBA). The % viability was determined using following formula:

$$\%\text{Viability} = (\text{OD of Test}/\text{OD of Control}) \times 100$$

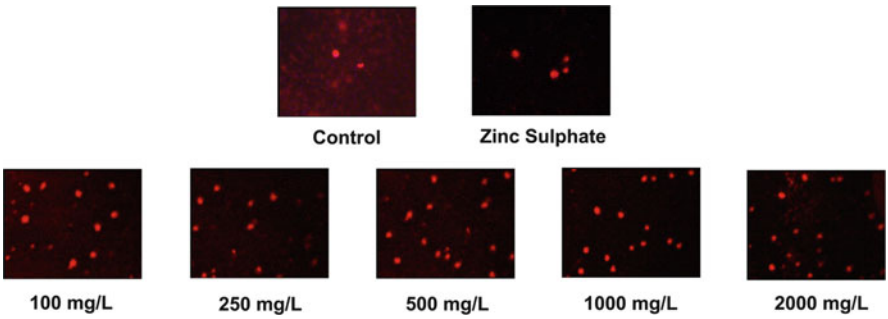

Fig. 15.9 Comet image of *Lantana aculeata*-mediated ZnO nanoparticle-treated *Sesamum indicum* leaf at different concentrations (mg L^{-1})

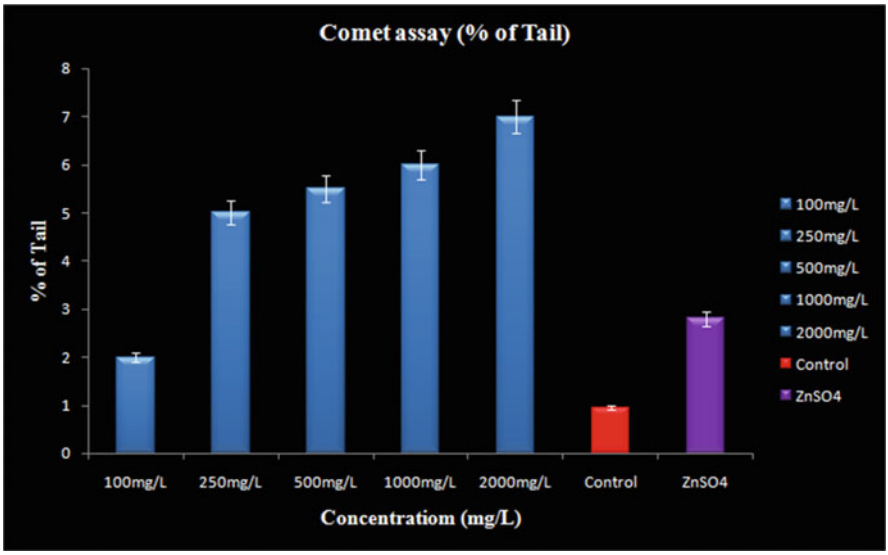

Fig 15.10 Graphical representation of ZnO nanoparticles (% tail DNA) at different concentrations

15.4 Result and Discussion

15.4.1 Comet Assay

The percentage (%) of the tail DNA in *Sesamum indicum* treated with *Lantana aculeata*-mediated ZnO is showed in Figs. 15.9 and 15.10. ZnO nanoparticles showed a sign of significant DNA damage at higher concentration and induced a dose-dependent increase in extent of DNA damage significantly at concentration above 1000 mg L^{-1}. It could be credited to a property of nanomaterials to frame agglomerates by goodness of which, with expansion in treatment focus the

nanoparticles tend to precipitate. The more prominent association of nanoparticles amongst themselves that could have expanded inferable from expansion in treatment fixation may have constrained the ZnO nanoparticles from communicating with the plant system (Zhang et al. 2005). It is evidenced that ZnO nanoparticles create large amount of hydroxyl free radical, thereby leading to DNA damage (Reeves et al. 2007). Abiotic stress (including heavy metal) results in DNA injury or damage to plant cell also straight or not directly (Kumari et al. 2009). Atha et al. (2012) reported that CuO NPs induced DNA harm or damage in agricultural and grassland plants. However, improved antioxidant enzyme (POD, SOD and CAT) activity in plant root tissues exposed to CuO and ZnO nanoparticle was observed.

15.4.2 Isolation of DNA

The DNA that was isolated from phytomediated ZnO nanoparticles in *Sesamum indicum* was further evaluated by DNA laddering. The outcome can be in all likelihood be related by means of that got as of comet test. While the negative control set indicated nearness of unharmed genomic DNA represented to by a thick band on the agarose gel, the most astounding degree of DNA harm was seen at ZnO nanoparticle treatment. The gel (Fig. 15.11) also clearly indicated an initial increase in DNA damage up to 1000 mg L^{-1} followed by subsequent decrease in extent of DNA injury/damage along with growing treatment attentions.

15.4.3 Cytotoxicity Study

The cytotoxicity of the zinc oxide nanoparticles was evaluated against SiHa cervical cancer cell lines at various concentrations (6.5–100 μg mL^{-1}). Figures 15.12 and 15.13 show the cytotoxic activity of zinc oxide nanoparticles, and IC_{50} value for zinc oxide nanoparticles was found to be 48.16 μg mL^{-1}. Maximum concentration of

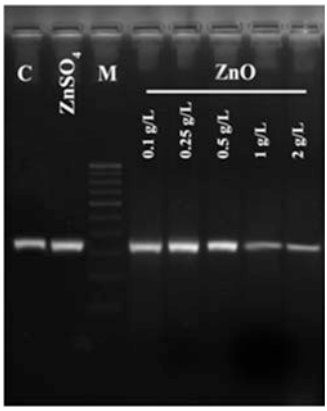

Fig. 15.11 DNA laddering of *Sesamum indicum* leaf DNA treated with different concentrations of ZnO nanoparticles. *C* control, *$ZnSO_4$* zinc sulphate, *M* marker and ZnO—*L. aculeata*-mediated zinc oxide nanoparticles

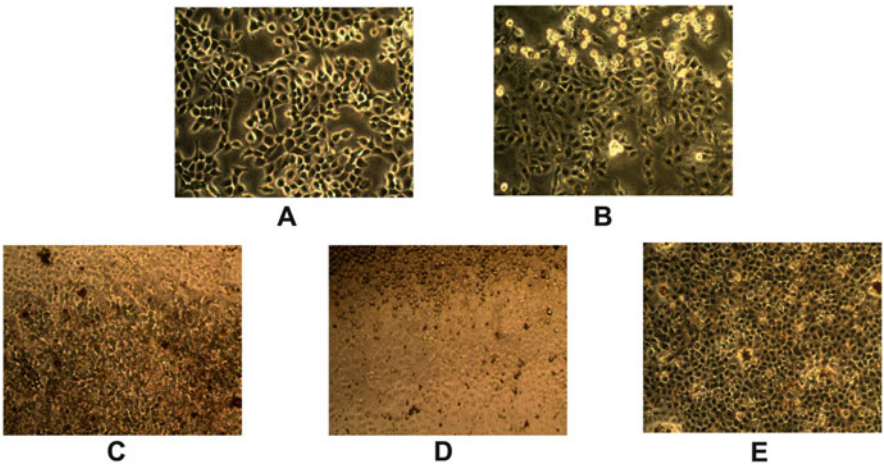

Fig. 15.12 Cytotoxicity effect of *Lantana aculeata*-mediated ZnO nanoparticles on SiHa cell lines. (**a**) 6.25 μg mL^{-1}, (**b**) 12.5 μg mL^{-1}, (**c**) 25 μg mL^{-1}, (**d**) 50 μg mL^{-1} and (**e**) 100 μg mL^{-1}

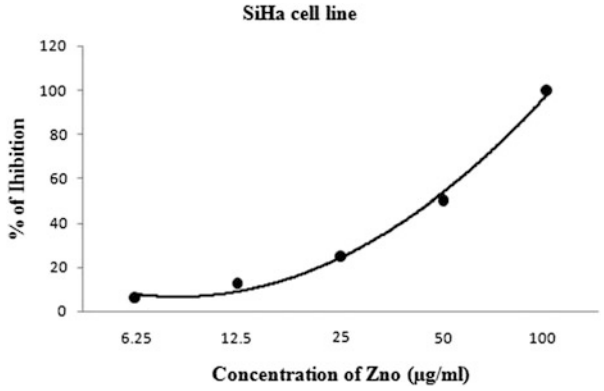

Fig. 15.13 Effect of *Lantana aculeata*-mediated ZnO nanoparticles on cell inhibition of SiHa cell line

zinc oxide nanoparticles (100 μg mL^{-1}) effectively inhibits the growth of cell by more than 98%. Sankar et al. (2013) reported the anticancer activity of *Origanum vulgare*-mediated silver nanoparticles and cytotoxic effects of green-synthesized *O. vulgare*-mediated silver nanoparticles against human lung cancer A549 cells.

15.5 Conclusion

The green synthesis method has prompted the improvement of biomimetic methodologies for the development of advanced nanomaterials. Biological synthesis of nanoparticle utilizing plants extract have been recommended as could reasonably be expected eco-friendly different option for chemical and physical methods. A simple, rapid biological procedure has been developed to synthesize ZnO nanoparticles using *Lantana aculeata* leaf extract. The synthesized nanoparticles were characterized by UV-Vis spectrophotometer, FTIR, XRD, EDX, FESEM, and HRTEM. The ZnO nanoparticles synthesized from biological method showed particles are spherical in shape with size range from 12 nm to 25 nm, respectively. *Lantana aculeata*-mediated zinc oxide nanoparticles were assessed in *Sesamum indicum* and SiHa cell line. ZnO used in this study were mainly nanosized but also showed a strong tendency to aggregate in spite of sonication of the suspension. DNA fragmentation as a marker for genotoxicity was determined by comet assay and DNA laddering. Results from this study demonstrate that ZnO nanoparticles were toxic to both plant and cancer cell lines.

Acknowledgement I thank the management of Sri Krishna Arts and Science College for providing necessary facilities to carry out the research work and management of Karpagam Academy of Higher Education.

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Uptake and Distribution of ^{14}C-Labeled Multi-walled Carbon Nanotubes by Wheat (*Triticum aestivum* L.)

16

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16.1 Introduction

Engineered nanoparticles (NPs) are currently being used in many commercial products with many additional applications expected in future years due to their unique properties. In particular, carbon nanotubes (CNTs) have been proposed to have potential applications in various fields such as medicine, hydrogen storage, sensors, and environmental applications (Mauter and Elimelech 2008; Shen et al. 2009; Shi et al. 2009) due to their unique one-dimensional hollow structure and extraordinary mechanical, electrical, thermal, and optical characteristics (Mauter and Elimelech 2008). As a result of these properties and usage in consumer products, production of CNTs is expected to increase their environmental release, either intentionally or inadvertently. Therefore, assessing the potential environmental implications of CNTs has become an emerging issue and has drawn extensive research interest (Ferguson et al. 2008; Khodakovskaya et al. 2011; Petersen and Henry 2012; Petersen et al. 2008a, b, 2009, 2010, 2011a, b, c).

Soil was found to be a major sink of engineered NPs released to the environment in a recent modeling study (Gottschalk et al. 2009), mainly from activated sludge application on soils. While some studies have been conducted on CNT toxicity to plants (Cañas et al. 2008; Khodakovskaya et al. 2009), bioaccumulation by plants remains largely unknown. This is in large part due to the difficulty associated with quantifying the carbon-based material in biological systems (Petersen and Henry 2012; Petersen et al. 2011c), which can be overcome with the ^{14}C-labeling technique. Recently, radioactively labeled carbon nanotubes have been used to assess CNT uptake and distribution in a number of terrestrial and aquatic organisms

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_16

(Ferguson et al. 2008; Petersen et al. 2008a, b, 2009, 2010, 2011a, b). While these studies have indicated minimal bioaccumulation in soil and sediment organisms and high concentrations in the gut tract in crustacean *Daphnia magna*, there is far less data available on MWCNT uptake by plants. As a result of the increasing number of potential applications of CNTs in the field of agriculture, there are growing concerns about the safety of this nanoparticle with crops (Nair et al. 2010). Raman spectroscopy and transmission electron microscopy indicated that the MWCNTs are able to penetrate the thick seed coat of tomato plants (Khodakovskaya et al. 2009); photoacoustic and photothermal methods have also indicated MWCNT uptake and distribution into the tomato plant leaves and crops (Khodakovskaya et al. 2011). However, these studies did not quantify the MWCNT uptake, and little is known about differences among plants with regard to CNT translocation. If plants do take up significant concentrations of CNTs, subsequent food chain transfer may represent an important CNT exposure pathway for larger organisms.

In this study, wheat (*Triticum aestivum*) was chosen as a model crop for uptake evaluation of MWCNTs, because it is mass produced worldwide and suggested as a model species by OECD (2006). The objective of this study was to quantify the uptake of ^{14}C-labeled MWCNTs by wheat seedlings which were cultured under hydroponic conditions.

16.2 Materials and Methods

16.2.1 Chemicals

The synthesis, purification, acid functionalization, and characterization for ^{14}C-labeled multi-walled carbon nanotubes (MWCNTs) used in this study were previously described (Zhang et al. 2011). These surface-modified MWCNTs have a specific radioactivity of 0.1 mCi g^{-1} determined by liquid scintillation counting after biological oxidation (OX 500; R. J. Harvey Instrument Co., Tappan, NY). The surface area of the acid-treated MWCNTs is 111 m^2 g^{-1} as measured by the standard Brunauer–Emmett–Teller (BET) method via nitrogen adsorption at 77 K (Micromeritics Gemini 2375, Norcross, GA). Microscopic investigations of MWCNTs were performed with a FEI Inspect F50 FEG scanning electron microscope (SEM) (Fig. 16.1). The diameters of MWCNTs ranged between 23 and 69 nm, and the average length is 353 nm ± 452 nm (n = 836; uncertainties here and hereafter indicate standard deviations) with a small number greater than 2000 nm (Zhang et al. 2011, 2012).

To prepare a stable stock solution, 150 mg of MWCNTs was dispersed in 1 L of deionized water by ultrasonication (34.7 W, Cole-Parmer CV33) for 6 h, and then the mixture was left at room temperature for 6 h. The stable supernatant with a concentration measured as 116.5 mg L^{-1} was collected and kept at room temperature as a stock solution for subsequent experiments.

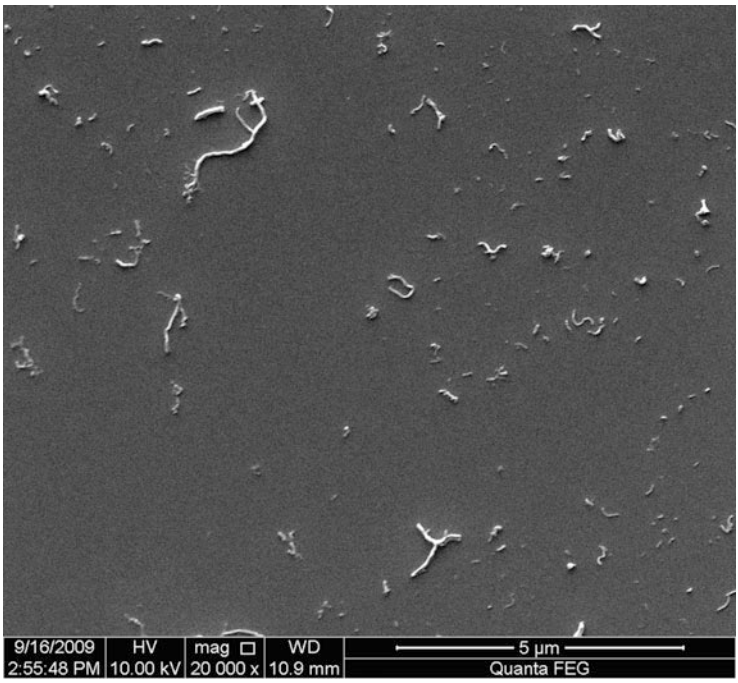

Fig. 16.1 Scanning electron micrograph of dispersed MWCNTs

16.2.2 Wheat Culture and Exposure Methods

Seeds of wheat (*Triticum aestivum* L.) were obtained from USDA. The seeds were surface-sterilized in 3% (v/v) H_2O_2 for 10 min, rinsed with deionized (DI) water, and then soaked in water for 24 h. After the radicle appeared, the seeds with uniform appearance were germinated in acrylic germination boxes on blotter paper saturated with DI water for 4 days. Seedlings of similar height (3 cm) were selected and transplanted to deep Petri dishes (125 mm × 25 mm), each containing 40 mL of a 1/10 dilution of Hoagland nutrient solution with suspended MWCNTs at 0, 11, 22, and 55 mg L^{-1}, respectively. The pH of the nutrient solution was adjusted to near neutral. Seedlings were cultured for 5 days at 25 ± 1 °C under a light irradiance of 210 µmol m^{-2} s (12 h: 12 h light/dark cycle). DI water was added to the Petri dishes to maintain the volume at 40 mL. Each Petri dish contained five seedlings, and six petri dishes were prepared for each dosage. Wheat seedlings were placed directly into the liquid medium without any supporters in order to avoid the loss of MWCNTs by adsorption to the supporters.

16.2.3 Uptake Assessment and Biomass Assay

Individual plant samples were collected after 5-day exposure. For each plant exposed to MWCNTs, the part which submerged in the medium were separated, put into a 20 mL vial, and washed at least five times using DI water until no radioactivity in the wash water could be detected. The rinsing water was collected for mass balance determination. Root and shoot length were measured using a ruler to the nearest mm. The plant samples were then dissected into root and shoot sections, dried at 70 °C for 48 h, and weighed. Root and shoot samples were combusted in an OX 500 biological oxidizer (R. J. Harvey Instrument) with the $^{14}CO_2$ released absorbed by scintillation cocktail and the radioactivity then determined using a Beckman LS 5801 liquid scintillation counter (LSC). Three plants were combusted per sample. The culture media left in the Petri dishes were sonicated continuously at 40% amplitude for 5 min. 1 mL of the media was mixed with 10 mL of scintillation cocktail (Insta-Gel Plus, PerkinElmer, MA), and the radioactivity was measured by LSC.

Uptake percentage and recover ratio were calculated according to Eqs. (16.1–16.3):

$$R_U\ (\%) = \frac{U}{I} \times 100\% \tag{16.1}$$

$$R_A\ (\%) = \frac{A}{I} \times 100\% \tag{16.2}$$

$$R_R\ (\%) = \frac{(C + A + U)}{I} \times 100\% \tag{16.3}$$

where R_U, R_A, and R_R are the uptake percentage, the adsorption percentage, and the recovery ratio of MWCNTs by the plants in each dish, respectively; U is the mass of MWCNTs accumulated in the plants, I is the initial mass of MWCNTs added, A is the amount of MWCNTs in the rinsing water (i.e., the nanoparticles adsorbed on plant surface) after the 5-day exposure, and C is the mass of MWCNTs determined in the culture medium after the 5-day exposure.

The correlations between root uptake and dosage or shoot uptake of MWCNTs were evaluated by Pearson's correlation coefficient (Ahlgren et al. 2003) (Eq. 16.4):

$$r = \frac{\sum_{i=1}^{n} XY - \frac{\sum_{i=1}^{n} X \sum_{i=1}^{n} Y}{n}}{\sqrt{\left(\sum_{i=1}^{n} X^2 - \frac{\left(\sum_{i=1}^{n} X\right)^2}{n}\right)\left(\sum_{i=1}^{n} Y^2 - \frac{\left(\sum_{i=1}^{n} Y\right)^2}{n}\right)}} \tag{16.4}$$

where r is the coefficient and X and Y denote the means of two variables. Significance of difference among treatments was analyzed by one-way analysis of variance (ANOVA),

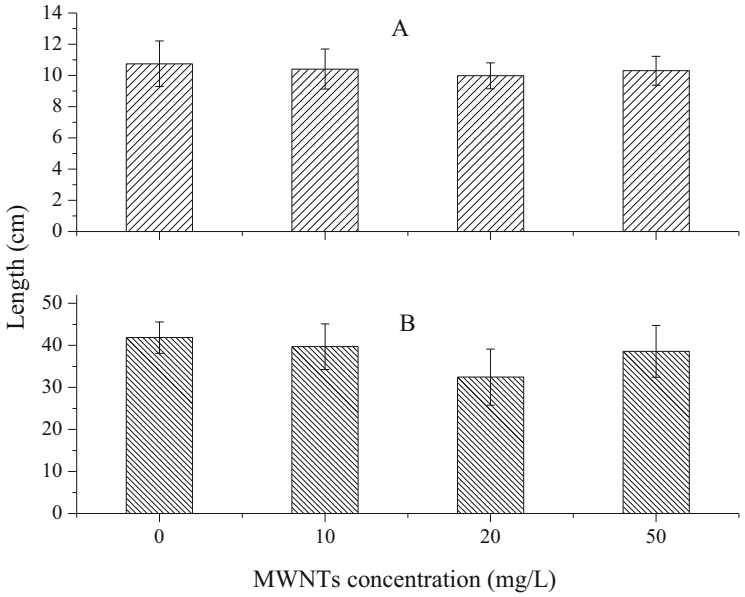

Fig. 16.2 MWCNT concentrations in shoots (**a**) and roots (**b**) of wheat seedlings after 5-day exposure. Values represent means of six samples, and error bars show standard deviations, ($n = 6$), *$p < 0.05$ and **$p < 0.01$, compared with the control

followed by Tukey's pairwise comparison at significance levels of $p < 0.05$ (*) and $p < 0.01$ (**).

16.3 Results and Discussion

After plant exposure to MWCNTs for 5 days, significant differences in root or shoot lengths were not detected between the treatments and control group (Fig. 16.2), and there were not visually apparent differences either (Fig. 16.3). Although the average values of the shoot dry weight in all treatment groups were lower than that of the control, these differences were not significant ($p > 0.05$) (Fig. 16.4). In contrast, the dry weight of the roots after treated with MWCNTs was significantly lower ($p < 0.05$) than that of the control. However, it was not possible from the experimental design of this study to conclude that the effects were due to the MWCNTs because the carbon-14 radioactivity may also have contributed to the observed effects.

The quantity of MWCNTs accumulated by wheat seedlings was shown in Fig. 16.5. A good correlation ($r = 0.968$) between the amount of MWCNTs in the root and the dosage and an even higher correlation ($r = 0.995$) between amount of MWCNTs in the root and the shoot were found. There were visible aggregates of MWCNTs adsorbed on both the roots and the part of shoots which were originally

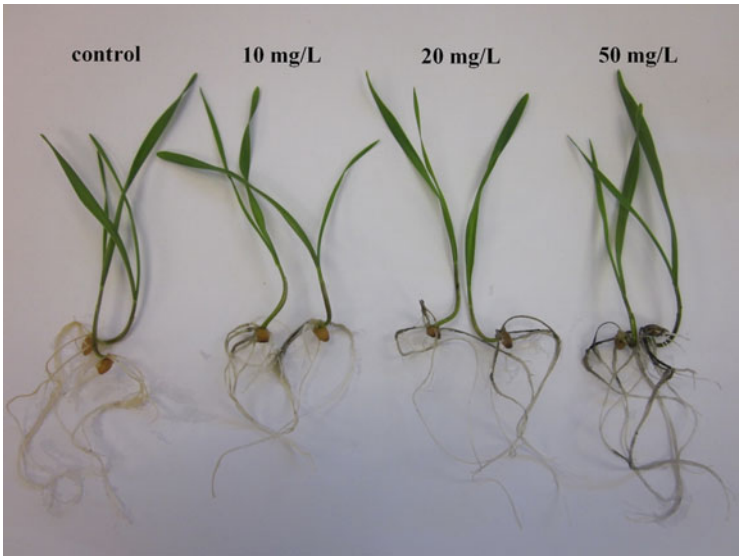

Fig. 16.3 Phenotype of wheat seeds grown in Hoagland media over 5 days without (control) or with (11, 22, and 55 mg L^{-1}) MWCNTs

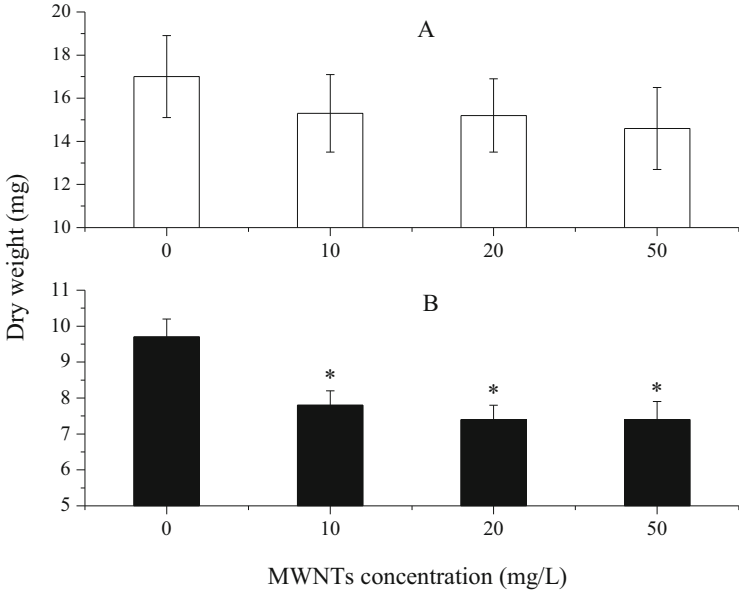

Fig. 16.4 Shoot (**a**) and root (**b**) lengths of wheat seedlings after 5-day exposure to MWCNTs. Values represent means of six samples, and error bars show standard deviations

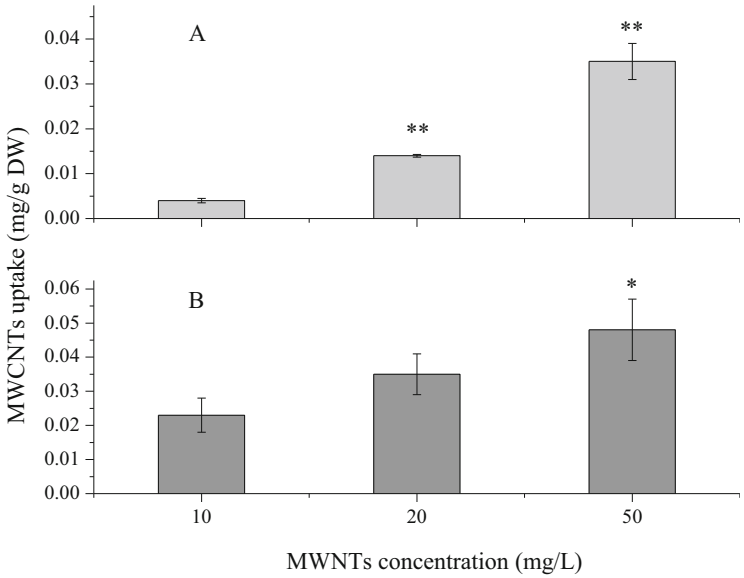

Fig. 16.5 Shoot (**a**) and root (**b**) dry weights of wheat seedlings after 5-day exposure to MWCNTs. Values represent means of six samples, and error bars show standard deviations

submerged in the culture medium for all exposure groups, especially for higher dosages, i.e., 22 and 55 mg L^{-1}. Based on observation during the 5-day exposure period, black aggregates on the plants were noted after 1 h of exposure and increased continuously with the growth of the wheat plants. However, these particles were removed by extensive washing of the plants prior to the determination of the MWCNT uptake.

Evident adsorption of MWCNTs on the root surface was observed when wheat seedlings were submerged in MWCNT suspension (see Fig. 16.3) and quantified by R_A. The R_A values decreased with dosage (Table 16.1). The uptake ratios (R_U) were 0.16% for both 11 and 22 mg L^{-1} dosage levels and decreased to 0.12% for the highest dosage. Good recovery ratios were measured indicated by R_R greater than 80% for the dosages of 11 and 22 mg L^{-1}. As for the dosage of 55 mg L^{-1}, particles were visually observed on the walls of the Petri dishes even after sonication; previous studies have indicated significant self-quenching when carbon nanotube aggregates are added directly to scintillation cocktail and then the radioactively determined by LSC (Petersen et al. 2008a, b), which likely explains the lower R_R value.

Detailed mechanisms of uptake and translocation of NPs by plants remain unknown at present. As described by Giesy and Kannan (2001), NPs can be taken up through natural nano- or micrometer-scale plant openings, and several pathways exist or are predicted for NP association and uptake in plants. NPs could enter the xylem via the cortex and the central cylinder at the site of lateral root formation. Recently, several reports were released with efforts to identify whether CNTs could

Table 16.1 MWCNT masses, biodistribution, and uptake

Exposure groups	11	22	55
Mass in culture medium (C, μg)	239.3 ± 30.5	470.8 ± 61.2	1085.5 ± 85.5
Mass adsorbed to plant roots (A, μg)	155.4 ± 23.2	232.9 ± 28.4	530.5 ± 40.7
MWCNT mass in plants (U, μg)	0.7 ± 0.0	1.4 ± 0.2	2.6 ± 0.3
Initial MWCNT mass added (I, mg)	0.44	0.88	2.20
Uptake ratio (R_U, %)	0.16 ± 0.0	0.16 ± 0.02	0.12 ± 0.01
Adsorption ratio (R_A, %)	35.3 ± 5.3	26.4 ± 3.2	24.1 ± 1.9
Recovery ratio (R_R, %)	89.9 ± 3.2	80.1 ± 2.9	73.6 ± 3.5

Values represent the mean and uncertainties represent the standard deviation, $n = 6$

enter the plant tissues. Wild and Jones (2009) used a two-photon excitation microscope to detect and visualize MWCNTs in living wheat tissues, and they demonstrated that MWCNTs primarily adsorbed to the root surface as individual and aggregated CNTs and can pierce through root cell walls. In this study, we observed substantial carbon nanotube uptake into the wheat plants, a result which agrees with qualitative findings previously reported for tomato plants (Khodakovskaya et al. 2009, 2011). Similarly, in a study with *Nicotiana tabacum* L.cv. Bright Yellow (BY-2) cells, single-walled carbon nanotubes (SWCNTs) were demonstrated to hold great promise as nanotransporters for walled plant cells and could deliver different cargoes into different plant cell organelles (Liu et al. 2009). These results in combination with the quantification results in this study indicate that uptake of CNTs by plant may vary among the plant species or different types of CNTs. The radioactive labeling method utilized in this study offers an analytical approach to accurately make this measurement.

Phytotoxicity caused by CNTs is probably not limited to the effects of themselves. Many chemicals used for agricultural production have high adsorption affinity with organic carbon. Thus, the use of CNTs in agriculture and their releasing into the environment are potential pathways to introduce these chemicals into the soil environment (Towell et al. 2011). Therefore, more work is needed to examine the potential uptake of engineered CNTs by various crop species and combined toxicity of CNTs and other chemicals.

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Plant Response Strategies to Engineered Metal Oxide Nanoparticles: A Review

17

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17.1 Introduction

Nanotechnology has got wide range of application in medicine, agriculture, targeted drug delivery, energy, electronics, sensor technology, and imaging. Due to wide range of application of nanoparticles (Nel et al. 2006), there is a great concern on the potential releases of nanoparticles into the environment. Plants constitute a major component of the ecosystem, and interaction of nanoparticles with plant system is an important factor to understand the fate of engineered nanoparticles in the environment and its associated risks. There were reports that the concentration of nanoparticles in the environment is much lower than the toxic concentration; however, it is important to evaluate the environmental effects of nanoparticles for its large-scale commercial application (Batley et al. 2013). Soil is an important source for the accumulation of nanoparticles in the environment, and the concentration of nanoparticles in soil was reported to be higher than in air and water (Gottschalk et al. 2015). There were several reports that plants provide a potential pathway for the transport of nanoparticles (Rico et al. 2011; Nair et al. 2010; Morales-Díaz et al. 2017; Raliya et al. 2016). The interaction of nanoparticles with plant system results in the uptake, transport, and accumulation of nanoparticles, and the response of plants to nanomaterials varies with the type of plants and nature of nanomaterials. There are different entry routes for nanoparticles into the plants, and uptake rate depends on the size, shape, concentration, and surface charge of nanoparticles (Tarafdar et al. 2012). The roots of plants are an important entry route as the soil constitutes one of the major medium for the accumulation of nanoparticles. Nanoparticles enter the root system through lateral root junctions and reach xylem through cortex and pericycle (Dietz and Herth 2011). However, entry of nanoparticles into plants is difficult due to the presence of cell wall, and the

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_17

entry rate is closely related to the morphology of nanoparticles and pore size of cell walls. The nanoparticles that could effectively cross the cell wall pores reach the plasma membrane and be translocated to different plant parts (Fleischer et al. 1999; Navarro et al. 2008). Larger nanoparticles can enter into plant system through aerial openings such as leaf stomata, hydathodes, and flower stigma. These aerial openings act as an important route for the entry of airborne nanoparticles which can be dispersed by wind, thus reaching the leaves and promote foliar uptake through aerial plant openings (Nair 2016). Other aerial transport pathways for nanoparticles include cuticle, bark surfaces, and trichomes.

Nanoparticles interact both physically and chemically with the plant system. They can be physically adsorbed to plant surface, resulting in physical damage to plant parts, or chemically interact with the system causing changes in different cell metabolic pathways. Chemical interactions result in the production of reactive oxygen species (ROS), oxidative damage to cells, and changes in ion-membrane transport activity (Auffan et al. 2008; Foley et al. 2002; Kamat et al. 2000). Nanoparticles can impart both positive and negative effects on plants (Yang et al. 2017; Nair et al. 2011, 2012), and the effects of different types of nanoparticles such as metal and metal oxide nanoparticles, carbon-based nanomaterials, magnetic nanoparticles, and polymeric nanoparticles were well studied. This chapter focuses on different response strategies by plants on interaction with metal and metal oxide nanomaterials. For the utilization of metal-based nanoparticles in agriculture for the development of smart fertilizers and nanopesticides, it is important to understand their impact on various morphological, physiological, and metabolic activities of plants.

17.2 Effects of Metal and Metal Oxide Nanoparticles on Morphological and Physiological Attributes in Plants

The major plant physiological parameters to be studied include germination efficiency, elongation of root, biomass, and leaf number. The impact of different nanoparticles on these physiological factors varies with the type of plants and the type of nanoparticles. The effects of green synthesized gold nanoparticles, without any capping and reducing agents, on the germination percentage of rice were studied (Ndeh et al. 2017). A very high germination percentage (95–98.38%), followed by a slight decrease in the root and shoot length compared to control, was reported. Increased hydrogen peroxide formation and lipid peroxidation in roots and shoots was observed, but not statistically significant which recommended the safe use of green synthesized gold nanoparticles as nanocarriers in plants. Studies on the effects of silver nanoparticles (AgNPs) on the germination and growth of 11 species of wetland plants reported both positive and negative effects depending upon the concentration of nanoparticles and coating agents. Root growth was found to be more affected than leaf growth on exposure to AgNPs (Yin et al. 2012). Zuverza-Mena et al. (2016) reported that nano-silver had null effects on the germination of radish even at a higher concentration (Zuverza-Mena et al. 2016). This can be

correlated to the presence of hard seed coat for radish that could prevent the entry of contaminants including nanoparticles (Koul et al. 2000). A reduction in water content and root and shoot length was observed at higher concentrations with respect to control plants. On exposing rice seedlings to different concentrations of AgNPs, it was investigated that there was significant reduction in root elongation, fresh weight of shoots and roots, and total carotenoid and chlorophyll contents. A dose-dependent increase in the amount of reactive oxygen species (ROS), lipid peroxidation, and hydrogen peroxide formation in roots and shoots was also reported along with increased proline and decreased sugar content (Nair and Chung 2014). A dose- and size-dependent decrease in the germination rate and further seedling growth of rice with AgNPs was also reported by Thuesombat et al. (2014). It was reported that large-sized nanoparticles caused more negative effects on seedling growth; however, smaller-sized nanoparticles were efficiently transported through the shoots, which highlighted the size effects. In peanut (*Arachis hypogaea* L.) plants, it was investigated that AgNPs caused severe damage to plant growth with respect to several physiological parameters such as plant biomass, height of the plants, grain weight, and yield. AgNPs were detected even in the edible plant parts in a dose-dependent fashion (Rui et al. 2017).

A significant reduction in seed germination was reported in response to nano-CuO stress in rice seedlings (Shaw and Hossain 2013) with stress-induced oxidative damage. Da Costa and Sharma reported that copper nanoparticles of size less than 50 nm showed inhibitory effects on rice seed germination rate, root and shoot length, and total biomass. Increased nanoparticles uptake was observed at higher concentration with more accumulation in chloroplasts which further led to decline in the amount of photosynthetic pigments, photosynthetic rate, and transpiration rate (Da Costa and Sharma 2016). Copper nanoparticles were also used to evaluate its effects on rice root growth, formation of ROS, and the expression of two genes associated with root growth. Reduced root growth with inhibited gene expression associated with root elongation and greater ROS production was reported on nanoparticle treatment (Wang et al. 2015). Moon et al. reported reduction in germination and inhibited root growth for cucumber on treatment with CuO NPs compared to bulk CuO (Moon et al. 2014). Studies on morphological, physiological, and molecular level effects of CuO NPs on Indian mustard reported shoot growth reduction, shortened primary and lateral root architecture, and reduced total chlorophyll and carotenoids contents. A significant increase in the amount of hydrogen peroxide, peroxidase enzyme activity, and lignification of shoots and roots was also observed (Nair and Chung 2015). Studies on soybean and chick pea with CuO NPs of size less than 50 nm reported a concentration-dependent change in growth of the selected plants. Effective growth was observed at certain optimal concentration; thereafter, an inhibited growth beyond this concentration was reported with adsorption and uptake of nanoparticles by roots (Adhikari et al. 2012). The effects of a range of CuO nanoparticles with different size and concentration on the germination and growth of *Phaseolus vulgaris* L. were investigated, and it was reported that seed germination was not affected by nanoparticles and again seedling weight was promoted by lower concentration and inhibited by higher concentration of 25 nm CuO. The high surface

area of 25 nm CuO at higher concentration might be the reason for its deleterious effects (Duran et al. 2017). This study highlighted the importance of nanoparticle structure for its physiological impacts. Altered root morphology was reported in wheat on treatment with CuO NPs due to Cu release from dissolution at root surface. An increase in Cu level modified the exogenous Indole Acetic Acid (IAA) distribution with inhibited root elongation and proliferated root hair formation (Adams et al. 2017).

Yang et al. studied the effects of nZnO on maize and rice plants, and null effect on seed germination was reported. However, at higher concentration of 2000 mg/L, root elongation was significantly inhibited (Yang et al. 2015). The effects of cobalt and ZnO nanoparticles on onion bulbs were investigated, and an inhibited root elongation with increase in concentration of nanoparticles was reported (Ghodake et al. 2011). Effects of different concentrations of engineered ZnO nanoparticles on the growth parameters, production of steviol glycosides, and antioxidant activities on *Stevia rebaudiana* were investigated, and a concentration-dependent favorable and adverse effects on physiology and glycoside production was reported (Javed et al. 2017). The effects of nano-CeO_2 and ZnO nanoparticles on the growth and yield of soybean were studied. It was reported that nano-CeO_2 caused a reduction in the growth and yield of plants. A negative impact on nitrogen fixation by soybean was also reported with high nano-CeO_2 concentration. An efficient uptake and distribution of nano-ZnO was also observed in soybean, and nanoparticles were detected in the edible plant tissues (Priester et al. 2012). Stress response and tolerance of *Zea mays* to CeO_2 NPs were studied by Zhao et al. (2012a, b). Nanoparticles triggered the increased production of several stress-related parameters which helped the plants to defend against oxidative injury caused by exposure to CeO_2 NPs. In radish (*Raphanus sativis* L.), it was reported that CeO_2 nanoparticles at a concentration of 10 mg/L had no effects on the growth of plants, whereas bulk CeO_2 enhanced plant biomass and ionic cerium (Ce^{3+}) had a negative effect on plant growth (Zhang et al. 2015). This study outlined that the effects on plant growth and physiological processes varied with the characteristics of the element. Rico et al. studied the impacts of cerium oxide nanoparticles on the physiology, productivity, and macromolecular composition of barley (*Hordeum vulgare* L.). Improved plant growth with increase in shoot biomass was observed with nano-CeO_2 at 125 mg/kg compared to the control plants. No grains were found in plants treated with 500 mg/kg of nano-CeO_2 (Rico et al. 2015). A positive effect on tomato plant growth and fruit production was determined on treatment with studied concentrations of CeO_2 NPs (Wang et al. 2012). A good level of cerium was detected in plant tissues upon treatment which suggested the uptake of nanoparticles by plant roots and further translocation to shoots and edible tissues. The growth cycle of barley plants treated with CeO_2 and TiO_2 nanoparticles was investigated, and it was observed that n-CeO_2-treated plants produced less number of tillers, reduced leaf area, and reduced number of spikes per plant whereas n-TiO_2 stimulated plant growth, which made clear that the plant response varies widely with the type of nanoparticles (Wang et al. 2012).

Engineered iron oxide nanoparticles have been extensively used for environmental remediation, and hence it is important to study the various effects of iron-based

nanoparticles on plant system. No negative effects were reported in maize seedlings grown under stress condition with different concentrations of hematite and ferrihydrite NPs. Surprisingly, an increased growth and chlorophyll content was observed with majority of the concentrations used (Marchiol et al. 2016). Similar results were observed in corn plants in which lower concentration of γ-Fe_2O_3 NPs had positive effects on seedling growth of corn (Pariona et al. 2017). The impacts of iron oxide nanoparticles and ferric ions on the growth of *Citrus maxima* were investigated by Hu et al. It was reported that γ-Fe_2O_3 NPs did not affect the biomass and root length. An upward translocation of nanoparticles were not observed which matched with the appearance of more γ-Fe_2O_3 NPs on the roots of corn (Li et al. 2016). The increase in the chlorophyll content due to treatment with γ-Fe_2O_3 NPs was reported to be concentration dependent.

Recent studies on effects of magnetite nanoparticles on oak trees reported improved germination and early growth (Hu et al. 2017). An increase in chlorophyll concentration was also observed due to increased iron supply from Fe_3O_4 NPs. This study potentially suggested the use of magnetite NPs to improve conservation and reforestation of threatened trees. The uptake of iron oxide nanoparticles by spinach plants grown hydroponically was studied. A dose- and time-dependent increase in the plant growth and biomass was reported due to the uptake of magnetic nanoparticles. This study provided new insights to application of nanoparticles in agriculture (Jeyasubramanian et al. 2016). Cobalt ferrite nanoparticles ($CoFe_2O_4$) have found several application in medical sciences for magnetic resonance imaging, drug delivery, and cell labeling (Liu et al. 2013; Park et al. 2015). However, their effects on plant system are least studied. The tolerance of tomato plants to $CoFe_2O_4$ NPs was studied, and it was reported that these nanoparticles did not affect germination and growth of plants. A concentration-dependent increase in the amount of Fe and Co in plant tissues was observed. An increased Mg and Ca uptake was noted on treatment with 125 mg/L $CoFe_2O_4$ whereas it decreased at higher nanoparticle concentration. A decreased catalase activity in tomato roots and leaves was also reported (López-Moreno et al. 2016). Toxicity and biotransformation of $Ni(OH)_2$ nanoparticles by mesquite plants (*Prosopis spp*.) were investigated, and it was reported that there was no reduction in plant size or chlorophyll production (Parsons et al. 2010).

Studies were carried out with TiO_2 NPs and Cd to understand the joint toxicity in rice seedlings (Ji et al. 2017). TiO_2 NPs did not cause any impact on rice seedling growth in terms of fresh and dry biomass whereas Cd toxicity to rice seedlings resulted in significant reduction of root length, plant height, fresh and dry biomass, and other physiological parameters. However, presence of TiO_2 NPs in the media reduced Cd toxicity to rice plants due to the adsorption of Cd by TiO_2 NPs, thus making Cd unavailable to plants. An investigation of early genotoxic and phytotoxic effects of cerium oxide nanoparticles (*n*CeO_2) and titanium dioxide nanoparticles (nTiO_2) in barley seedlings reported high oxidative stress with increased generation of ROS and ATP content. The nanoparticles did not cause any negative effect on caryopses germination; however, reduced root elongation was observed in seedlings treated with higher concentration of nanoparticles (Mattiello et al. 2015). Feizi et al.

reported that nano-TiO_2 at low concentration did not cause any changes in the germination rate of wheat whereas high concentrations had inhibitory effects (Feizi et al. 2012). Uptake and impact of TiO_2 nanoparticles on wheat and rapeseed reported that the germination, evapotranspiration, and plant biomass were not affected whereas increased root elongation was observed on exposure to nanoparticles (Larue et al. 2012). The developmental phytotoxicity of different metal oxide nanoparticles on *Arabidopsis thaliana* was investigated, and studies reported that direct exposure to nanoparticles caused significant phytotoxicity with reduced seed germination, root elongation, and leaf number (Lee et al. 2010). Rossi et al. reported that CeO_2 nanoparticles caused root anatomical changes in rapeseed (*Brassica napus* L.) that improved the salt stress tolerance in plants. The nanoparticles modified the formation of apoplastic barriers in plants that allowed the transport of more Na^+ ions to shoots and less Na^+ ion accumulation in roots. These changes in Na^+ ion flux resulted in better physiological response in plants which can be utilized for more nanotechnological applications in agriculture (Rossi et al. 2016). Priester et al. reported that soybean grown in soil amended with nano-CeO_2 or nano-ZnO experienced plant damage. Nano-CeO_2 caused oxidative stress in leaves due to reduced root nodule fixation, and nano-ZnO caused decrease in leaf chlorophyll concentration; however, such a decrease in leaf chlorophyll was not related to diminished plant growth, yield, or N_2 fixation potential (Priester et al. 2017).

The phytotoxicity of alumina nanoparticles was investigated, and it was reported that surface characteristics of nanoparticles play an important role in phytotoxicity. Studies were conducted on five different plant species, and inhibition of root elongation with nanoparticles got decreased with their surface modification (Yang and Watts 2005). Riahi-Madvar investigated the effects of alumina nanoparticles on the morphological properties of wheat seedlings and reported that root growth was affected by NPs but not the seed germination, shoot length, and dry biomass (Riahi-Madvar et al. 2012). Studies on the toxic effects of aluminum oxide nanoparticles on the root growth and development in wheat (*Triticum aestivum*) plants reported reduced root elongation with cellular damage in root cortex cells. Histochemical analysis revealed lignin accumulation and callose deposition (Yanık and Vardar 2015). Antisari et al. reported the effects of different engineered metal oxide (CeO_2, Fe_3O_4, SnO_2, TiO_2) nanoparticles and metallic (Ag, Co, and Ni) engineered nanoparticles on the morphological parameters of tomato plants. It was observed that root growth was promoted by Fe_3O_4 NPs and reduced by SnO_2 NPs. Accumulation of nanoparticles was mainly seen in tomato roots whereas engineered metal nanoparticles were observed both in above ground and below ground parts (Antisari et al. 2015).

17.3 Effects of Metal and Metal-Based Nanoparticles on Photosynthesis and Biochemical Characteristics in Plants

The interaction of plants with nanoparticles induces several biotic and abiotic stresses that accelerate the formation of reactive oxygen species (ROS). Several antioxidant enzymes such as catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR), and malondialdehyde (MDA) play a significant role to interrupt the cascades of uncontrolled oxidation which results in the alteration of ROS concentration (Santos et al. 2010; Gechev et al. 2006). The photosynthetic activities in plants can be altered by nanoparticles that result in the generation of ROS and activated the defense mechanisms in plants to combat oxidative stress damage (Du et al. 2017). The generation of ROS and antioxidant responses vary with the type of nanoparticles and plant species and exposure conditions.

In Indian mustard (*Brassica juncea*), on treatment with gold nanoparticles (GNPs), a regular increase in the antioxidant enzyme activities, H_2O_2, and proline content was recorded with increase in the concentration of GNPs. Results indicated that the production of ROS is highly dependent on the concentration of nanoparticles which imposed physiological and biochemical stress in mustard seeds (Gunjan et al. 2014). The toxicity of AgNPs and ionic silver, in mustard seedlings, at various concentrations was analyzed by investigating the root and shoot length, fresh mass, protein content, amount of photosynthetic pigments, cell viability, DNA damage, oxidative enzyme activities, etc. (Vishwakarma et al. 2017). Both nanoparticles and ionic silver reduced seedling growth with severe inhibition to photosynthesis and caused oxidative stress with DNA degradation and ultimate cell death. Antioxidant enzyme activities were inhibited by both forms of silver. These studies in toxicological research could help in designing novel strategies to reduce the adverse effects of nanoparticles on plants. In peanut plants, AgNPs did not change the predominant isozymes of each antioxidant enzyme; however, the amount of antioxidant isozymes got significantly increased in comparison to control plants (Rui et al. 2017). In a model aquatic plant *Spirodela polyrhiza*, it was reported that AgNPs affected photosynthesis and inhibited photosystem II maximum quantum yield and effective quantum yield (Jiang et al. 2017). AgNPs induced the formation of ROS, and the activity of Rubisco was found to be very sensitive to nanoparticles, thus slowing down CO_2 assimilation. This had resulted in decrease in solar energy consumption and promoted ROS generation in chloroplasts by excess excitation energy. Studies reported that AgNPs enhanced the growth of soybean plants under flood-stressed conditions (Mustafa et al. 2015, 2016). An increase in proteins related to amino acid synthesis and wax formation was observed in soybean plants on treatment with 15 nm AgNPs, which further improved the growth of plants under flood stress conditions (Mustafa et al. 2016). Tripathi et al. reported that nitric oxide protected the pea (*Pisum sativum*) seedlings from adverse effects of silver nanoparticles on growth and photosynthesis by regulating the accumulation of Ag and ROS and antioxidant defense system (Tripathi et al. 2017a).

Siddiqui et al. reported that nano-SiO_2 improved seed germination and growth characteristics by reducing malondialdehyde, H_2O_2 levels, and electrolyte leakage. Also, the application of nano-SiO_2 reduced chlorophyll degradation and enhanced net photosynthetic rate, stomatal conductance, rate of transpiration, and water use efficiency. An improved expression of several antioxidant enzymes resulted in reduced oxidative damage which resulted in an increased germination and growth characteristics (Siddiqui et al. 2014). Improved seed germination of soybean with nano-SiO_2 and nano-TiO_2 particles by increasing the amount of nitrate reductase was reported by Lu et al. (2002). Nanoparticles also enhanced the ability of plants to absorb and utilize water and fertilizer and also stimulate the antioxidant system with increased activities of SOD, POD, and CAT. This resulted the plants to thrive under adversities. The ability of silicon nanoparticles (SiNPs) in alleviating UV-B stress in wheat seedlings was investigated, and data indicated that SiNPs triggered the NO-mediated antioxidant defense system which neutralized the damage to photosynthesis that had occurred by ROS (Tripathi et al. 2017b).

In *Brassica rapa*, it was reported that treatment with bulk CeO_2 resulted in increased concentration of H_2O_2 in plant tissues at vegetative stage, and CeO_2 NPs increased the level of H_2O_2 at floral stage. A growth stage response was observed for SOD activity in response to different sized NPs and CAT activity was not at all affected with any sized NPs over the entire growth stages of plant (Ma et al. 2016). Hussain et al. reported that biologically synthesized cerium nanoparticles protected tomato seedlings against ferulic acid stress. The exogenous application of nanoceria resulted in reduced MAL and electrolyte leakage with an increase in the pigment content. As an antioxidant, nanoceria could protect the plants from autointoxication which is an important problem in monocropping (Hussain et al. 2017).

In corn plants (*Zea mays*), on treatment with CeO_2 NPs, it was investigated that the level of H_2O_2 increased in phloem, xylem bundle sheath cells, and shoot epidermal cells up to 15 days after germination. The CAT and APX activities also increased in corn shoots. At higher concentrations, nanoparticles triggered the upregulation of the HSP70 in roots which is an indication of stress response. Lipid peroxidation with increase in thiobarbituric acid and ion leakage was reported in this study. Nanoparticles did not affect leaf net photosynthetic rate, transpiration, and stomatal conductance. The antioxidant enzymes provided protection against the oxidative stress that might have occurred due to nanoparticle interaction (Zhao et al. 2012a, b). Rico et al. studied the impact of nano-CeO_2 on the oxidative stress and antioxidant defense system in germinating rice seeds. H_2O_2 generation in roots and shoots was found to be reduced in comparison to control plants at the studied two least concentrations. Concentration-dependent electrolyte leakage and lipid peroxidation were reported in seedling shoots. Enhanced membrane damage and photosynthetic stress due to the altered enzymatic activities with changes in the level of ascorbate and free thiols were observed in shoots. Modifications of antioxidant defense system with no consequential change in oxidative stress were observed in root system (Rico et al. 2013). In Bt-transgenic cotton, it was observed that the chloroplasts were swollen due to aggregation of CeO_2 NPs on the external surface of

chloroplasts which had led to its rupture. The vascular bundles were also got destroyed with CeO_2 NPs (Nhan et al. 2015).

The full life cycle of wheat (*Triticum aestivum* L.) plants was assessed on treatment with CeO_2 nanoparticles of low and high concentration. Decreased chlorophyll content and increased antioxidant enzyme activities were observed in plants treated with higher concentration of nano-CeO_2. Both low and high concentration delayed the flowering by one week and reduced the size of starch grain (Du et al. 2015). There were reports that catalase activity was significantly increased in shoots and ascorbate peroxidase in roots on growing cilantro plants in soil amended with CeO_2 NPs (Morales et al. 2013). Venkatachalam et al. reported that phycomolecules-coated ZnO NPs triggered heavy metal (Cd and Pb) tolerance in *L. leucocephala* by activating different biochemical pathways, thus avoiding cellular damage. An increase in the levels of MDA, photosynthetic pigments, and proteins was reported along with overexpression of antioxidant defense enzymes and favored genetic alterations (Venkatachalam et al. 2017). Zhao et al. studied the effects of nano-ZnO and nano-CeO_2 in corn plants, and it was reported that nano-ZnO at 800 mg/kg reduced the net photosynthesis in corn (Zea mays) plants by 12%, stomatal conductance by 15%, and relative chlorophyll content by 10% at day 20 of plant growth whereas these factors were not impacted with all studied concentrations of nano-CeO_2 (Zhao et al. 2015).

The biochemical and molecular response in *Arabidopsis thaliana* (L.) Heynh. plants to tetracycline (TC) and TiO_2 NPs was investigated, and it was reported that 1 mg/L TC reduced the plant biomass and the presence of nanoparticles alleviated TC toxicity. Higher antioxidant enzyme activity was observed in roots and shoots in the presence of TC which indicated the increased activity of ROS scavengers; however, TiO_2 NPs reduced the antioxidant enzyme activity during co-exposure treatments (Liu et al. 2017). The effects of $Cu(OH)_2$ nanopesticides of different concentrations to 3-week-old maize plants were studied to understand the gene expression of nine antioxidant-related enzymes, and this study provided important information on the responses of maize plants to $Cu(OH)_2$ nanopesticides at genetic, metabolic, and physiological levels (Zhao et al. 2017). Song et al. investigated the phytotoxicity of two differently synthesized nanoparticles, aerosol nano-TiO_2 and colloidal Ag NPs, on tomato (Song et al. 2013). No acute toxicity was observed on germination by either of nanoparticles, whereas root elongation was significantly reduced with Ag NPs at all studied concentrations due to its higher uptake. Ag NPs caused increased phytotoxicity which resulted in lower chlorophyll content, higher SOD activity, and less fruit productivity. Higher antioxidant enzyme activity was observed with nano-TiO_2 only at higher concentration.

To understand the effects of environmental conditions on the uptake and toxicity of ENPs, soil grown herbaceous annual plant (*Clarkia unguiculata*) was exposed to different nanoparticles such as TiO_2, CeO_2, and $Cu(OH)_2$ at different concentrations under distinct light and nutrient levels for 8 weeks. It was reported that during the maximum growth stage, the photosynthetic rate and CO_2 assimilation efficiency was decreased by TiO_2 and CeO_2 treatment under high light and nutrient growth conditions. $Cu(OH)_2$ nanoparticles disrupted photosynthesis in plants grown under

highly stressed conditions of high light and limited nutrients. The accumulation of nanoparticles was highly dependent on light and nutrient levels, and the results revealed the impact of abiotic conditions in mediating the uptake and further physiological effects in plants (Conway et al. 2015). Effect of alumina nanoparticles on miRNA expression profile in tobacco plants was studied. Plants were exposed to nanoparticle stress, and it was found that the root length, plant biomass, and leaf count were significantly decreased with increase in nanoparticle exposure. Also, an increase in expression of different type of miRNAs was observed with maximum expression for treatment with 1% Al_2O_3 NPs. This study suggested that miRNAs might play an important role in mediating the stress response in plants caused by nanoparticles in the environment (Burklew et al. 2012). Elevated activity of antioxidant enzymes such as superoxide dismutase and catalase was observed in wheat seedlings on treatment with 200 and 500 mg/L alumina NPs. This reduced the level of free radicals which helped to inhibit the phytotoxic effects of these nanoparticles on wheat seedlings (Riahi-Madvar et al. 2012). Yanik and Vardar reported increased peroxidase activity due to the application of nanoparticles to wheat seedlings with decreased total protein content with respect to control plants (Yanık and Vardar 2015). There were reports that foliar application of ZnO nanoparticles at low concentration of 10 ppm increased the chlorophyll, phosphorous, and total soluble leaf protein concentration in cluster bean (Raliya and Tarafdar 2013). Response of soybean mitochondrial proteins to aluminum oxide NPs of various sizes under flooding situation was studied. A large increase in voltage-dependent anion-channel protein on exposure to 135 nm Al_2O_3 NPs and increased isocitrate dehydrogenase upon exposure to 5 nm Al_2O_3 under flood-stressed condition were reported. This study suggested that Al_2O_3 NPs of different sizes had affected mitochondrial proteins under flood stress conditions by regulating membrane permeability and TCA (Tri carboxylic acid) cycle activity (Mustafa and Komatsu 2016). The effects of magnetite iron oxide nanoparticles (Fe_3O_4 NPs) of different size at a concentration of 200 mg/L were investigated on *Picochlorum* sp. (Trebouxiophyceae, Chlorophyta) during different phases of growth. Nanoparticles of size 20 nm at 200 mg/L reduced the viable cell concentration and chlorophyll a content during exponential growth phase compared to other sized nanoparticles (Hazeem et al. 2015).

17.4 Effects of Metal and Metal Oxide Nanoparticles on the Nutritional Quality of Crops

It is important to assess the effects of nanoparticle on the nutritional quality of plants. The effects of coated and uncoated nanoceria on the quality of tomato fruits were studied, and it was reported that citric acid-coated CeO_2 nanoparticles increased the B content and reduced the dry weight, total and reducing sugar content at different used concentrations (Barrios et al. 2017). B, Ca, Mg, and Mn amount were decreased at 500 mg/kg of $nCeO_2$ and bulk CeO_2 reduced the lycopene content at all the studied concentrations. It was observed that citric acid coated nanoceria affected the

fruit macromolecules and nutritional elements were affected by CeO_2 nanoparticles. Interaction of boron (B) with CeO_2 NPs and its responses in sunflower plants was studied, and it was reported that nano-CeO_2 reduced the B nutritional status of sunflower in original soil and B phytotoxicity in boron amended soil (Tassi et al. 2017).

Du et al. reported that there was no change in the starch and sugar content of wheat grains; however, an increase in the protein content of grains was observed in wheat plants on treatment with nano-CeO_2 (Du et al. 2015). Studies with nano-CeO_2 of 250 mg/Kg on barley plants reported remarkable increase in P, K, Ca, Mg, S, Fe, Zn, Cu, and Al in grains. An increase in methionine, aspartic acid, threonine, tyrosine, arginine, and linolenic acid contents in the grains was also reported (Rico et al. 2015). Morales et al. reported that CeO_2 nanoparticles could change the nutritional properties of cilantro by changing the chemical environment of carbohydrates in cilantro shoots (Morales et al. 2013). In cotton plants, it was reported that CeO_2 NPs significantly reduced the Zn, Mg, Fe, and P amounts in xylem sap compared to control plants. Also, a decrease in Indole-3-Acetic Acid (IAA) and abscisic acid (ABA) was also reported (Nhan et al. 2015).

The effects of CeO_2 and ZnO NPs on the nutritional value of soil cultivated soybean plants were investigated (Peralta-Videa et al. 2014). At higher concentration, nano-CeO_2 increased the amount of Cu and P and reduced the amount of Ca in pods. Low level of Na was detected in pods at all concentrations of nano-CeO_2, and high level of Zn was detected in pods at all concentrations of nano-ZnO. At medium concentration of nano-ZnO, the level of Mn and Cu in pods got increased. Hong et al. studied the impact of nanoscale and microscale CeO_2 and CuO on the fruit quality of cucumber (Hong et al. 2016). It was reported that fruit firmness was reduced with nano- and microscale CuO and nano-CeO_2 at 50 mg/L and bulk CeO_2 at 200 mg/L. The Zn and Mo levels of fruits were also impacted upon treatment with different concentrations of nano- and bulk CeO_2 and CuO. Change in the nutritional qualities of cucumber (*Cucumis sativus*) on treatment with CeO_2 and ZnO NPs was investigated (Zhao et al. 2014). Results showed that none of the ZnO nanoparticle concentrations affected fruit sugars, carbohydrate and protein, and antioxidant contents in comparison to control plants. An increase in starch and protein content was reported with 400 mg/kg of ZnO NPs which might increase the caloric value of fruit. A decrease in the concentration of micronutrients such as Cu and Mo was reported with ZnO nanoparticles. Several changes in fruit quality have been noted for CeO_2 treatment, such as changes in the amount of nonreducing sugars, phenolic content, and fractionation of proteins which further impacted fruit flavor and antioxidant ability. In corn plants (Zea mays), it was reported that nano-CeO_2 and n-ZnO reduced the yield of corn and altered the quality of corn. On treatment with nano-CeO_2, it was observed that Cu, K, Mn, and Zn were mainly localized at the insertion of kernels into cobs whereas Ca and Fe were distributed in other parts of the kernel (Zhao et al. 2015).

The effects of CuO NPs on conventional and Bt-transgenic cotton were studied, and it was reported that CuO NPs inhibited the plant growth and development, nutrient content, and also IAA and ABA concentrations in conventional and

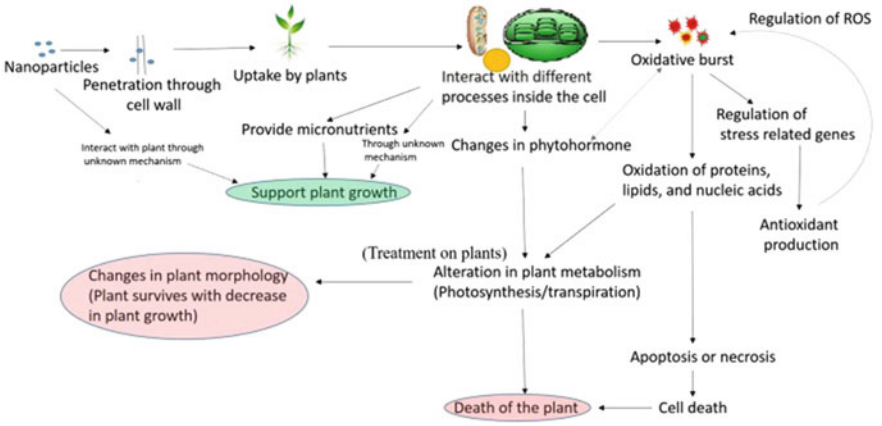

Fig. 17.1 General mechanism of interaction of nanoparticles with plant system resulting in various morphological, physiological, and biochemical effects (adopted with permission from Reference 100)

transgenic cotton plants. At low concentration, nanoparticles enhanced the expression of exogenous gene encoding Bt toxin protein in leaves and roots, thus providing added benefit for Bt cotton insect resistance (Van et al. 2016). Changes in the fatty acid content were observed in peanut grains on exposure to different doses on AgNPs (Rui et al. 2017), which indicated the effects of metal-based nanoparticles on crop yield and quality. Studies by Antisari et al. reported contamination of tomato fruits with Ag when the plants were treated with AgNPs (Antisari et al. 2015). The impact of CeO_2 NPs on the nutritional composition in wheat was investigated, and modifications in the storage of S and Mn in grains were reported. Changes in amino acid composition, increased linolenic acid, and decreased linoleic acid in grains were also reported on treatment with nano-CeO_2 at 125 mg/Kg. The study suggested the potential of nanoceria to modify the crop food quality that might cause unknown consequences for living organisms (Rico et al. 2014). Figure 17.1 shows the general mechanism of interaction of nanoparticles with plant system with various morphological, physiological, and biochemical effects [adopted with permission from Rastogi et al. (2017)].

17.5 Conclusion

Increased application of nanomaterials to the environment affects the growth of plants morphologically, physiologically, and biochemically. Metal-based nanomaterials have shown both beneficial and adverse effects on plant growth and production. Nanoparticles can be adsorbed on the plant surface or can be successfully absorbed and translocated to different plant parts including the edible portion of plants. Hence, it is high time to understand the risks associated with the interaction of nanomaterials with plant system. Reports suggested that nanoparticles at innocuous

concentration have not exhibited any adverse effects and seem to be beneficial to plants in many ways. However, toxicity at higher concentrations results in the production of antioxidant enzymes in plants to protect the cellular and subcellular system from cytotoxic effects. Nanomaterials at right concentration can be used for the smart delivery of agrochemicals and fertilizers that promote plant growth and production, thus reducing the use of conventional chemicals to prevent soil damage and to protect the environment.

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Nanobiotechnology in the Health Care: The Game and the Goal

18

Asra Parveen and Raghunandan Deshpande

18.1 Introduction

Nanotechnology deals with the production and modification of materials in nanoscale (10^{-9} m). Most countries around the world ensure rapid development of nanobiotechnology to improve the quality of life of human by creating jobs to promote the economic growth and enhance the security of our society. Nanotechnology has shown promising results in effective and efficient delivery of pharmaceutical compounds. Most of the biological molecules can be described by nanomechanics, while their biological activities are not affected (Hrapovic et al. 2004). The biological component is the enzyme in biosynthesis that selectively interacts with the substrate (Guilbault et al. 2004). Enzymes are the most vital catalysts of the stimulus, allowing analysis to be detected in different ways. Nanoparticles in mineral and inorganic nanoparticles show novel properties and functions that differ significantly from those mentioned in the bulk. The biodistribution mainly depends on size, shape, and charge of the nanoparticles (Baetke et al. 2015). Biomarkers are an unexploited application of nanoparticle technology and are likely to undergo significant growth. Nanoparticles can be used as functional polymers to quickly detect biomarkers and separate DNA (Jain 2007). Nanobiotechnology is active in finding the functional foods to improve the human health. It increases solubility and stability by facilitating a controlled release of biologically active micronutrients and compounds and protects during storage, processing, and distribution. Ultimately, understanding the targeted implementation mechanism will provide a basis for enabling food manufacturers to design intelligent food systems capable of ensuring optimal health for each individual (Moraru et al. 2003). Several nanomaterials have been used to analyze their characteristics and novel applications in biological sensors (Jianrong et al. 2004). Metal nanoparticles

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M. Faisal et al. (eds.), *Phytotoxicity of Nanoparticles*,
https://doi.org/10.1007/978-3-319-76708-6_18

are interesting materials with unique electrochemical and electrocatalytic properties depending on the size and morphology (Park et al. 2002; El-Deab and Ohsaka 2002). Metals nanoparticles have been studied in various studies on cancer cell lines, microbes, and plants (Parveen and Rao 2016). Small sized, improved solubility, larger space, and multifunctional nanomaterial has opened new possibilities of research for scientists (Rai et al. 2012). Nanobiotechnology is directed primarily to use the nanoscale zone to minimize and support biological processes. The aim is to develop nanomolecular components and analytical tools to investigate cell biology at cellular and molecular levels. For example, scientists are developing techniques to manipulate small sample sizes or even to examine individual molecules. This allows for further miniaturization of chip-based testing techniques to facilitate rapid and even targeted detection of smaller sample quantities. Based on these possibilities, experts hope to have a completely new vision in the functions of cellular biology. Development of effective medicine is important for improving the health care. New diagnostic tests in the laboratory at an early stage will help in taking preventive and curative measures. Nano-sized agents based in vivo imaging diagnostics become more sensitive and accurate (Buckway and GhandehariEm 2016). The targeted delivery of therapeutic agents to the diseased site can be improved in effectiveness than that of traditional delivery. While diseases vary in their pathways, they often require very different levels of maturity from the proposed technologies. Researchers are developing techniques to manipulate small sample sizes to examine individual molecules. The discovery of disease specific biomarkers will have the virtuous scope and should focus on pharmacological research and related fields (Rai 2007). Research on multidisciplinary agents should be supported for in vivo use of regenerative medicine that can offer wide applications in different diseases. Various nanodiagnostic studies have been reviewed to improve the sensitivity and to expand the current limitations of molecular diagnosis (Alharbi and Al-sheikh 2014). Other research programs aim to develop new remedial procedures involving the use of nanobiotechnological methods. Scientists often discussed on systems that move drugs to their target in a goal-oriented manner (Hobin et al. 2012). During treatment of cancer or inflammatory diseases such as ulcerative colitis and Crohn's disease, the tolerance of materials should be improved in order to increase therapeutic effectiveness. This includes high-throughput testing which uses nanoscale sensors to search new target structures and materials (Zhu et al. 2015). Nanoparticles in the form of lipid particles are being studied for cancer treatment, and many other drugs based on nanobiotechnological therapy are currently undergoing clinical trials (Charron et al. 2015). Keeping in view the diverse healthcare applications of nanoparticles, we tried to highlight some of major contributions of researchers in the field of nanobiotechnology

18.2 Application of Nanoparticles

Nanomedicine focuses on the diseases aiming to make meaningful improvements in areas that contain the most challenging healthcare issues in the future. The diseases if not treated on time will severely reduce the patient's quality of life and have a very high occurrence in the society. Nanotechnology is expected to have a significant impact on the cure of these diseases. The major applications of nanotechnology for cure and diagnosis has been mentioned accordingly.

18.2.1 Prophylaxis

Biofunctionalized noble metal nanoparticle with the surface modification with biomolecules were capable of specific molecular identification by introducing new strategies for molecular analysis and extended detection threshold for DNA and protein-based assays (Pedrosa and Baptista 2015). Beyitler and Kavukcu have used gold nanoparticles with Escherichia coli receptors to study prophylaxis of urinary tract infections in children (Beyitler and Kavukcu 2017). Efforts were being made to produce new, cost-effective, sensitive, and reliable biomarkers that can be made pre-assessed and risk-specific before symptoms emerge (Liesenfeld et al. 2014). Diseases with no secretion of vital indicators in blood or urine require high-specific imaging procedures for early detection.

18.2.2 Diagnosis

Medical examination is conducted to find signs or symptoms of the diseases. It is important that "false positives" are excluded by applying more specific diagnostic procedures. In this case, molecular imaging uses specific target factors which play a crucial role in localization and progression of disease and it is equally important to confirm the patient's health. The main advantage of using nanotechnology on quality of life and healthcare costs is early detection of disease resulting in lesser demands of costly therapeutic with improved diagnostic outcome (Caliendo et al. 2013). The use of nanotechnology in molecular diagnostics provides new options for clinical diagnostic procedures. However, once the disease is diagnosed, a therapeutic procedure is required. Diagnostic imaging procedures provide critical inputs for making advance clinical decision and treatment planning. Targeted delivery agents allow topical treatment that targets only diseased cells, thus increasing effectiveness with the reduction of unwanted side effects (Chavez et al. 2012). Biochemical imaging techniques monitor the release of drugs or to follow the progression of treatment. This therapeutic rationale will lead to the development of new disease modifying therapies that will significantly increase the quality of life of citizens by reducing the social and economic costs associated with the management of permanent disabilities (NanoMedicine Nanotechnology for Health 2006).

18.2.3 Nanobiosensors

Many biological sensors have been developed like environmental monitoring, food quality control, biological processes, agriculture, medical, and pharmaceutical (Li et al. 2009). Gold, silver, platinum, palladium, copper, cobalt, and other nanoparticles are also widely explored in the development of biological sensors (Sagadevan and Periasamy 2014). Nanobiosensors are nanosensors used to detect chemical or biological agents. Nanomaterials are superbly sensitive chemical and biological sensors (Jain 2003). These sensors will be inexpensive to manufacture and portable. It may be possible to develop detection and monitoring devices based on these detectors. The advances in nanotechnology, biochemistry, and information technology provide viable progress for designing the nanorobots (Liu and Shimohara 2007). Nanoproteomics is the study which enables the detection of one-molecule protein (Jain 2008). Due to the variety of nanoparticle techniques available, it is possible to design nanoparticle surfaces to selectively link a subset of biomarkers and to hold them for later study using high protein sensitivity tests (Swierczewska et al. 2012). Similarly, biobarcode assays aid in detection of trace amounts of proteins in body fluids that conventional methods cannot detect (Bao et al. 2006). The diagnosis and nanobiosensors reported for few of the important diseases as follows:

18.2.4 Microbial Diseases

The increasing number of infections all over the world is triggered by antibiotic-resistant microorganisms. Nanotechnology has focused on new treatment and rapid detection of the principal cause of the infection. Nanotechnology has facilitated the methods to detect single cells or a few molecules (Wang and Wang 2014) and development of novel and more effective drugs against microbial diseases (Zhu et al. 2014). Single-molecule hybridization has been detected through hybridization detection method using multicolor oligonucleotide-functionalized QDs as nanoprobes (Ho et al. 2005). Nanolaser confocal spectroscopy can determine the properties of cancer cells that separate them from interrelated nonpathogenic cells (Gourley et al. 2005). Most of the pathogenic bacterial diagnostic methods lack ultra-sensitivity and delays in attaining results. Hence, there is a need for quick and sensitive detection of pathogenic microbes. Gold nanoparticles have been assessed in bacterial susceptibility based on plasmon resonance shifts (Nath et al. 2008). A new biomedical diagnostics method had developed to detect salmonella from gold/silicon. The dye molecules were attached to silicon nanorods which produce fluorescence after contacting with Salmonella (Fu et al. 2008). Biofunctionalized AgNP has reported the highest antifungal and antibacterial activity against gram positive and negative bacteria (Fig. 18.1) (Parveen et al. 2012).

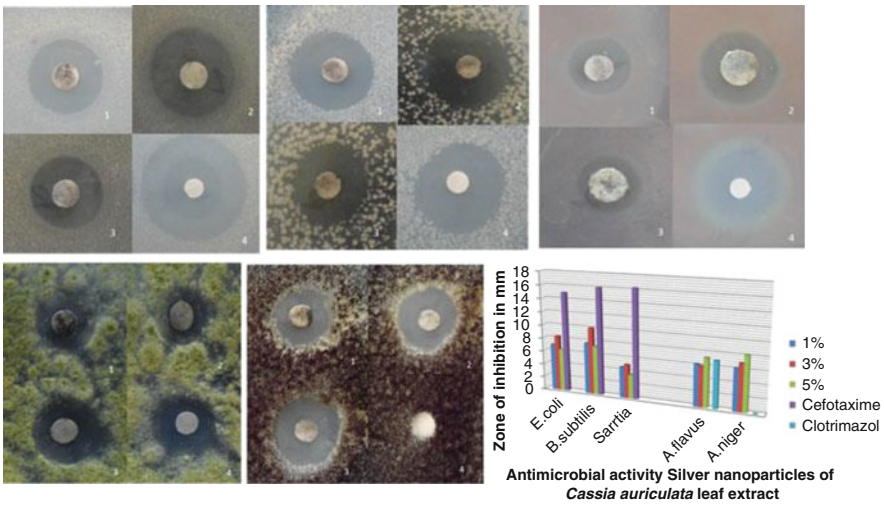

Fig. 18.1 Antimicrobial activity of AgNP against bacteria and fungi (Parveen et al. 2012)

18.2.5 Musculoskeletal Disorders

Nanotechnology has been extensively used for bone tissue engineering. It has been used to overcome some of the current limitations associated with bone regeneration. The methods include poor mechanical strength of scaffold, bone differentiation, ineffective cell growth, and insufficient production of growth factors required for bone cell growth (Peran et al. 2013; Kim and Fisher 2007). Musculoskeletal disorders cause long-term pain and physical disability by affecting people around the world. This problem accounts for half of the chronic cases in people. Back pain was the second leading cause of sick leave and fractures due to osteoporosis in the past decade (Woolf 2000). Clinical symptoms like pain and functional disability resulted in joint stiffness, and dysfunction has affected the daily life performance and work. Age, obesity, and joint distress are the key factors for arthritis. The use of biomarkers or imaging techniques could be challenging to diagnose osteoporosis symptoms at earlier stage (Kuo and Chen 2017). These techniques can visualize and monitor in vivo disease progression and treatment. Osteoporosis and osteochondrosis stimulate inflammation causing significant increase in degenerative processes (Ginaldi et al. 2005). Novel nano-regenerative medicine helps in osteoporosis disease with modified therapies using biologically active molecules as well as bionanoparticles (Barry et al. 2016). This treatment can repair the articular cartilage and restore intra-articular balance along with anti-inflammatory drugs. The nanoparticle-based treatment may also affect other inflammatory diseases like psoriasis and Crohn's disease (Kjems et al. 2008). On the other hand, new biocompatible and biodegradable materials should be developing for drug delivery and tissue engineering (Fig. 18.2) (Parveen et al. 2015). Nanoparticles research is under investigation to improve the quality and availability of these drugs for these diseases.

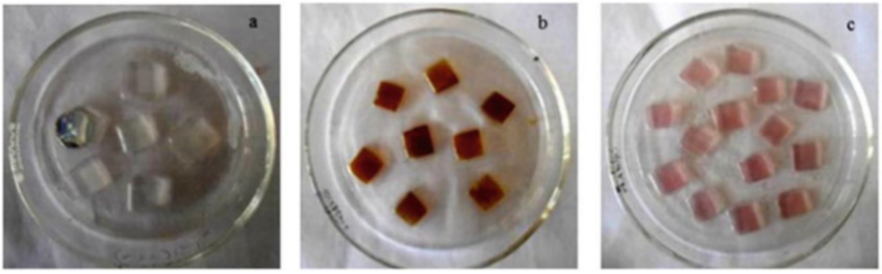

Fig. 18.2 Preparation of biodegrable Au-BNC and Ag-BNC films (Parveen et al. 2015)

18.2.6 Cancer

Cancer is currently the second leading cause of death in the world with highest clinical complications. The application of nanoparticles in identifying and destroying the cancer cells holds the ability to provide an effective answer to the complexity of this disease. Nanotechnology provides more therapeutic options and early diagnosis compared to the current conventional therapy for cancer (Jabir et al. 2012). Late stage metastatic cancer is difficult to cure and causing severe side effects, suffering, and costly. Diagnostic tests that allow the measurement of biomarkers are necessary to catch disease in a group (Rakowska and Ryadnov 2011). Nanobiotechnology can enable parallel measurements in the laboratory for many biomarkers at the same time, while keeping the test sensitive, simple, reliable, and low cost. Nanotechnology provides reliable diagnostic tool for detecting the biological agents. Radiotherapy is the standard form of treatment in more than 50% of cancer; nanotechnology can improve the treatment, monitoring, and diagnosis of cancer (Mi et al. 2016). Molecular imaging procedures will detect these inner sections using dedicated agents along with imaging systems and software. The imaging practice will contribute to radiotherapy with higher doses of radiation-resistant sections and fewer doses of radiation-sensitive sections, thereby reducing damage to the associated healthy cells. Target delivery schemes can be used to group the therapeutic agent specifically on diseased cells. The nano-carriers loaded with pharmaceutical and imaging agent are promising concepts under development (Panda et al. 2017). A combination of imaging with drug release allows for higher control over doses and a quantitative improvement of treatment. Today, tumor contraction is monitored by computed tomography (CT), which usually occurs after weeks of treatment. Molecular imaging allows faster assessment of patient's response to treatment which makes it possible to modify the previous method of the ontological treatment (Cormode et al. 2009). Regenerative medicine will bring the unique healing options against the side effects of usual chemotherapy like secondary immunodeficiency (NanoMedicine: Nanotechnology for Health 2006). Biosynthesis of nanoparticles and their effect on cancer cell lines have been studied (Fig. 18.3) (Raghunandan et al. 2011; Firdhouse and Lalitha 2013; Parveen and Rao 2014).

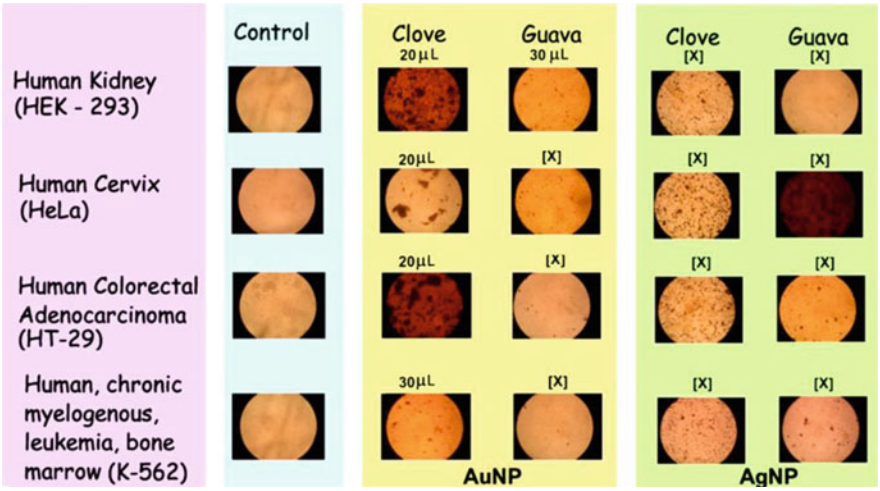

Fig. 18.3 Microscopic image showing the anticancer activity of biosynthesized AuNP and AgNP (Raghunandan et al. 2011)

18.2.7 Neurological and Psychiatric Diseases

Neurological conditions such as Alzheimer's disease and Parkinson's disease are age related and increasing significantly. Neurodegenerative disease decreases the quality of life and requires necessary care of the patients. Bioactive nanoparticles active regeneration carries a remarkable promise not only for treating symptoms but also for restoring nerve function (Sharma 2016). The disease-specific biomarkers affect the early diagnosis of degenerative conditions, preventing irreversible damage of nerve tissue. The brain's blood barrier usually prevents absorption of large molecules, which exclude many potential drugs for neurological or psychiatric conditions (Pardridge 2005). Biologically active nanoparticles can be easily transported through the blood brain barrier and increases the drug delivery to the brain (Alyautdin et al. 2014). Nanocarriers with surface functionalized properties carries a therapeutic drug through the blood–brain barrier (Masserini 2013). The MRI imaging using manganese oxide NPs has visualized the clear anatomical structures of the mouse brain as that of histological examination (Na et al. 2007). Nanoneuroscience is an exciting field to study the delivery of therapeutics to their targeted site of action inside the CNS for the treatment of various neurological and psychiatric disorders (Chhabra et al. 2015). Nanotechnology-based drug delivery methods can easily penetrate and facilitate the drug through the barrier by CNS therapeutic intervention due to its small and biofunctionalized characteristic (Soni et al. 2016).

18.2.8 Diabetes

Diabetes presents an increasingly serious problem which requires expensive medical care for the long period. Diabetes can lead to heart attacks and stroke, increase the risk of myocardial infarction in men, and increase the risk to quadruple in women. Many patients show no symptoms in the early stages of disease, which leads to diagnosis happening late or by accident.Early diagnosis and treatment of pre-diabetic type 2 offers both individual health and economic benefits (Liebl et al. 2015). People who suffer from insufficient glucose intolerance can reduce their relative risk of diabetes by changing lifestyle. Daily injections and blood measurements have worsen the acceptance in millions of patients. Nanotechnology is focusing on the ultimate goal in the treatment of diabetes free from the need to inject insulin (Veiseh et al. 2015). Diamond-like carbon metal nanocomposite films can be utilized in various medical applications and as medical prostheses. These nanocomposites of carbon minerals possess the softness of the atomic, chemical inertia, and properties of hardness close to those diamonds (Narayan 2005). The nanocomposite has an artificial glycoplastic surface that reduces adsorption and bioactivity with respect to fibrinogen as well as other blood proteins, ensuring its compatibility to immune system (Freitas 2005). The development of a glucose sensor that allows for noninvasive control of blood sugar level is one of the important clinical needs to improve disease diagnosis (Cash and Clark 2010). Diabetes patients lack fast wound healing activity as compared to nondiabetic; biofunctionalized AgNP has shown fastest healing activity in rats (Kumarasamyraja and Swamivelmanickam 2014). Various formulations of nanoparticles containing insulin designed to cross physiological barriers by releasing insulin into the blood-stream have been studied as novel therapeutic agents (Malathi et al. 2015; Sharma et al. 2015).

18.2.9 Cardiovascular Diseases

The primary cause of cardiovascular in most cases is the formation of a plate in the blood vessels. The formation of plaque resulted to stenosis of blood vessels followed by decreased tissue perfusion and hypoxia. In some critical cases, such as an infarction or stroke, the plaque becomes unstable and ruptures leading to a severe blockage in the blood vessels with death or disability as a result. Cardiovascular disease is often linked with risk factors such as small exercise and high cholesterol; however, recent research also indicates inherited causes. Bionanoparticles are expected to improve the diagnosis by acute intermediation and follow-up treatment (Baetke et al. 2015). Nanotechnology can be used for early diagnosis by achieving new trials in the diagnostic laboratory for atherosclerosis (Palekar et al. 2015). Research should develop a novel imaging technique that can describe plaque rupture because the imaging procedures available indicate stenosis. The effected patients should be provided a therapeutic dose that soothes the plaque and prevents rupturing. Nano-sized agents are being tested preclinical that makes the unstable plaque visible

in MRI by releasing the drug to stabilize the plaque (Chen et al. 2011). In the case of severe narrowing and vasodilation in the vascular system, ballooning and lubrication drugs are involved. In myocardial infarction, some of the heart tissue gets extremely damaged. Cardiopulmonary resuscitation is likely to repair limited or nonexistent tissues after cerebral injury. However, the recent scientific findings in regenerative medicine have opened the possibility of cell therapy and new pharmacological concepts for the treatment of cardiac insufficiency. The nanomaterials have the ability to attract local adult stem cells or cultured cells to the injury site (Pryzhkova 2013). The treatment provides cell therapy to improve cardiac function and reduce patient's mortality with acute heart failure. Cardiovascular nanoimaging is simple diagnosis that can help real-time tracking throughout the period of treatment and surgery (Deb et al. 2015).

18.3 Conclusion

Nanobiotechnology is an integration of physics, biology, chemistry, and biotechnology, which carries a great progress in health care and pharmaceuticals. Physical and chemical properties and high surface areas of nanoparticles make them ideal candidates for the development of biological labeling platforms. Various nanotechnology-related reports have introduced techniques and materials with some physical forms of energy like nanolasers. Nanobiotechnology application at the cellular level has prepared the stage for its role in health care such as in molecular diagnosis. In future, half of all pharmaceuticals may rely on nanotechnology over the next decade. Most of the nanobiotechnological methods are being started to use in the medical and pharmaceutical field. The nanobiotechnology projects in life sciences are currently undergoing for development of biochips, biosensors for earlier detection of diseases like cancer, Alzheimer's, rheumatoid arthritis, etc. The biofunctionalized nanoparticles have made great attention as potential cancer therapeutic agents. Nanoparticle capsules incorporated with chemotherapy drugs directly deliver to the tumor using laser pulse to protect healthy tissues. Nanoparticles can easily penetrate cell membranes because of their small size, thus facilitating targeted transport of to the target. Extensive in vivo work is needed before implementing the nanomaterials in daily life.

18.4 Acknowledgment

The authors highly appreciate financial support from SERB (PDF/2016/003910) and RGUHS Grant (P-007/2017-18).

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