www.amlett.com, www.vbripress.com/aml, DOI: 10.5185/amlett.2016.6048 Published online by the VBRI Press in 2016

Fate of inorganic nanoparticles in agriculture

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Received: 20 August 2015, Revised: 11 October 2015 and Accepted: 04 December 2015

ABSTRACT

In modern world, engineered nanoparticles (ENPs) are increasingly becoming an important component of daily life. They are becoming an integral part of a wide range of man-made products including electronics, paints, biomedical products, sunscreens, clothing, automobiles, etc. Rapid progress in the manufacturing of ENPs and the subsequent increase in its commercial applications always have had an impact on agriculture due to the exposure of living things to these ENPs. Also, human beings are directly dependent on the plants because of their nutritional values. Hence, the impact of nanoparticles on agricultural soil and plants is always of topical interest. It is imperative to be aware of the effects of nanoparticles on soil as well as on the soil ecosystem it supports especially the soil microbes and plants; or more specifically whether they have an influence on the agricultural yield and agri-economy. It is also important to study the effects of man-made nanomaterials on the properties of agricultural soil. This work reviews some of the key features of the impact of ENPs on the environment and the fate of ENPs in agriculture. Copyright © 2016 VBRI Press.

Keywords: Engineered nanoparticles (ENPs); agriculture; soil; risk assessment; environment.

Introduction

The surge in research in the field of nanotechnology has been attributed to the significant properties and qualities of the nanoparticles (NPs). These novel properties have thrown open avenues for new technologies and applications that were not achievable with bulk materials. In the natural world there exist many examples of structures that operate at nanometer dimensions including essential molecules within the human body and components of foods. Many technologies have inadvertently involved nanoscale structures for years. In modern world, nanotechnology uses the knowledge and techniques from different fields to develop products and services and apply them in diverse fields ranging from medicine to agriculture [**1-8**].

Although nanomaterials are currently being widely used in modern world, there is a serious lack of studies concerning the environmental implications of manufactured nanomaterials. The logical chain of events accounting for the fate of nanoparticles in environment depends on their routes of release and entrance into the ecosystem. It further travels down through the different food webs and consequently interacting with a variety of different environments. Due to the large surface area/volume ratio, NPs are more reactive in the biological system. Globally there is a concern on the human and environmental impact of nanotechnology based products as well as nanomaterials as they are being manufactured in enormous quantities. Due to the large scale use of nanomaterials, there is a possibility of unrestricted exposure of these nanomaterials to flora and fauna. In addition, the threats posed by these nanomaterials are enormous since the nanomaterials have the ability to cross the cellular barriers due to their extremely small sizes [**9, 10**].

Since the impact of these materials to humans as well as environment remains largely unknown, the safety aspect of this technology needs to be looked into with respect to health/environmental problems [**9, 10**]. Conclusively, such serious implication generates a need for the study of fate of NPs in the agricultural soil and environment. The potential benefits of nanotechnology have been widely reported but fate of nanomaterials on agriculture or environment is not well studied. The impact of nanomaterials on agriculture and the environment, in general, are discussed here.

Experimental

Nanomaterials

Before focusing on the main objectives of the review, a brief description of the nanomaterials is necessary. The nomenclature and exact definitions associated with nanomaterials as well as nanoscience is always a matter of debate. According to few recent papers regarding definition of nanomaterials, the NPs can be defined as the small particles having at least one dimension less than 100 nm. According to one other research group, the NPs are described as the particles having at least two dimensions in the range of 1 to 100 nm [**9, 11**]. These small NPs are present in nature, where they are synthesized by natural processes like biodegradation and biomineralization [**12**]. More precisely ENPs are composed of either metal, metal oxides, composite, carbon – based (C – based) nanomaterials, or semiconductors, including Quantum Dots (QDs) [**11-14**].

Classification and sources of nanomaterials: The NPs can be classified into different groups based on different criteria, like on the basis of their synthesis, the NPs can be divided into two classes (i) natural and (ii) man-made **[15]**. Hence, the main focus of this review is towards the man-made industrially synthesized NPs, for which the appropriate term is engineered nanoparticles (ENPs). The ENPs on the basis of their composition can be further classified into different groups, which are illustrated in **Fig. 1**. The detailed description on the classification of nanoparticles, their synthesis and applications in different industries as well as release into the environment is illustrated in further subsections of section.

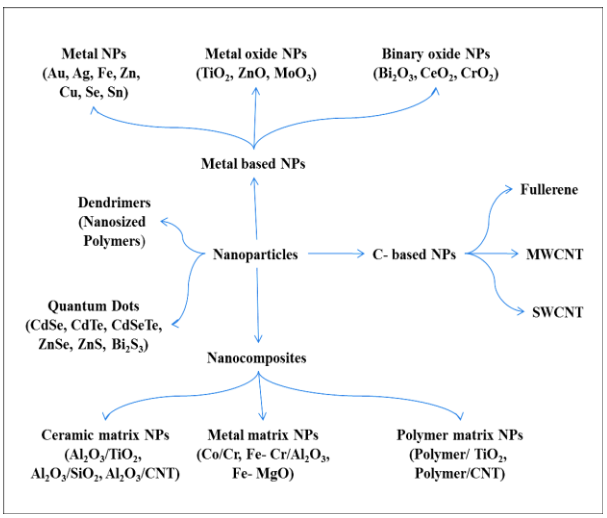

Fig. 1. Classification of engineered nanoparticles (ENPs) on the basis of their composition.

C-based nanomaterials: A 60C hollow sphere called fullerene was synthesized in 1985 at Rice University [**16, 17**]. After that, there was enormous growth in the synthesis of C - based materials. Later on, fullerene derivative MWCNT (multiwalled carbon nanotube) was synthesized under defined conditions to control the size and diameter of the tubes [**17-19**]. Later on, the single walled carbon nanotubes (SWCNT) were introduced in this world. These SWCNT have excellent thermal and electrical conductivity as well as excellent tensile strength, where the strength to weight ratio was 460 times that of steel [**17**]. The worldwide production of SWCNT is estimated to be 1000 tonnes in one year, while the overall production of CNT and fullerene is 1500 tonnes per year [**20**]. Due to their tensile strength, CNT and CNT derivatives have applications in plastics, automotive industries and aircrafts. On the other hand, due to their strong electrical and thermal conductivity, they are applied in electronics, battery, electrodes, super capacitors, sensors, conductive coating, adhesives and water purification system [**21**]. CNTs are released into the environment through burning, dry and wet drilling from polymer and through abrasion and sanding from epoxy [**22, 23**].

Metal based nanoparticles: This class includes zero-valent metal NPs and ionic NPs as well as metal oxides NPs. The zero-valent metal and ionic NPs are produced through the reduction of metal salts by a reducing agent [**20**]. These can be synthesized chemically as well as biologically, but at industrial level, chemical methods are mainly applied. In biological methods, there is still a need of further improvements like synthesizing the NPs in particular size range, removing the excess biological entities getting attached to NPs so that their interference during further industrial based applications can be avoided. On the other hand, these biological entities can be beneficial for the applications, whenever the need of enhanced biocompatibilities comes in the way [**24**]. These nanoparticles include a broad range of metal NPs, out of which most commonly applied are nanogold in Hyperthermia Cancer Therapy (HCT), diagnostics for heart and infectious diseases, sensors and electronics, nanosilver as antimicrobial agents in food packaging and fabrics like socks, zero-valent iron NPs are applied in soil, water and sediments remediation [**24- 26**]. In soil, iron NPs are used to remove nitrate by reduction [**20**]. Gold NPs are also applied in chemical industries- as a catalyst for certain oxidative reactions and fuel cell [**20, 27, 28**].

There are different form of metal oxides NPs are present which includes TiO_2, ZnO, MoO_3, Bi_2O_3, CeO_2, CrO_2, $BaTiO_3$ etc [**20**]. These metal oxide NPs are synthesized by top-down approach through several physical, chemical and biological methods [**12, 29**]. The metal oxide NPs like titanium dioxide (TiO_2), zinc oxide (ZnO), Silicon oxide (SiO_2), Aluminum oxide (Al_2O_3), along with metal nanoparticles like Silver (Ag) and Tin (Sn) have applications in paint industry. TiO_2 have applications in solar cells, sunscreens, cosmetics, bottle coatings due to their unique property to block ultra-violet radiations. Cosmetics and coating industries also utilize ZnO, SiO_2 and Ag nanoparticles [**20**]. ZnO NPs are part of fertilizer industry also. These metal and metal oxides nanoparticles are released into the environment through household wastes, waste water treatment wastes, waste water and industrial wastes.

Dendrimers: Dendrimers are multifunctional nanosized polymers which are built from branched units. These nanosized polymers can be tailored by controlling their size, flexibility, topology and molecular weight to perform different chemical functions in different fields like biology, catalysis, surface modifications. Due to their above mentioned unique characteristics, these are applied in chemical and physical fields including macrocapsules, nanolatex, colored glasses, chemical sensors, modified electrodes and in biological fields like DNA transfecting agents, hydrogels, DNA chips and in medicine as therapeutic agents for prion diseases, drug delivery. They are released into the waste water and industrial wastes [20].

Quantum dots: They display unique optical and electronic properties, such that they can absorb white or ultraviolet light and reemit it as a specific wavelength. In this conduction band electrons, valence band holes, or excitons are confined in all three spatial dimensions. QDs have important applications most especially in biomedical field like cellular imaging/labeling and it may be an excellent alternative to conventional fluorescent dyes used in imaging. They have important application in diagnostic tools and solar batteries. There are ranges of semiconductor nanoparticles like CdSe, CdTe, CdSeTe, InP, ZnSe, ZnS, Bi_2S_3 etc, which play a vital role in industrial sector. Quantum dots are mainly released into the environment through industrial wastes [**30, 31**].

Nanocomposites: Nanocomposites are hybrid materials made of distinctly dissimilar materials at the nanoregime to elucidate improved structures and properties. Nanocomposites are integral for several industrial sectors like automotive, electronics and biotechnology industries. This promises new applications in many fields such as mechanically reinforced lightweight components, non-linear optics, battery cathodes, nano-wires, sensors, batteries, bioceramics and energy conversion. Nanocomposites can be of different class: Ceramic Matrix Nanocomposites (Al_2O_3/TiO_2, Al_2O_3/SiO_2, Al_2O_3/SiC, Al_2O_3/CNT); Metal Matrix Nanocomposites (Co/Cr, Fe-Cr/ Al_2O_3, Fe-MgO); Polymer Matrix Nanocomposites (polyster/TiO_2, polymer/CNT). Nanocomposites are released into the environment through industrial wastes and wear and tear of polymers [**32**].

A brief view of the sources of the nanoparticles is represented in **Fig. 2** [**24, 33-36**]. Ultimately all these NPs which are released in the environment in different forms can reach to the soil or to the soil involved in agriculture work through runoff water, waste water, directly settling down in the soil from air suspensions at the site of agriculture [**9, 16, 19, 21-25**]. The detailed study about all these nanoparticles including their deposition, behavior and fate in agriculture is represented in sections.

Risk assessment associated with nanomaterials: In order to understand the impact, behavior, and the associated risks of NPs on the environment, it is essential to understand the different properties of nanomaterials, which help in design various methods of risk assessment. The risk assessment of any organism depends upon its response after exposure to the NPs and needs to be detected. It analyses the NPs in an environment [**20, 42**]. This includes various points like which materials should be selected as the reference materials for the ecotoxicological tests [**42, 43**]. As, many materials like silica quartz and C-black are also the part of the soil and sediments. Here, the focus can be that these materials and their NPs may not be harmful to the organisms like plants and soil microflora, as the previous grow in soil and the later remains present in the soil. Then the possibility of biotransformation also can't be rejected while developing the toxicological tests. There can occur the possibility that NPs will create transformations in organisms and will change their activity in negative or positive way [**23, 26, 27**]. While analyzing on the basis of the uptake level of a particular nanoparticle, ecological tests should use dispersed materials or not and should use aggregated material or not. There are some other points like response of the organism after exposure to the NPs [**20, 42, 43**]. To bring out the appropriate results, it is necessary that the biologists and chemists should work together in proper co-ordination. The role of chemists can't be ignored in designing the biological tests, but the biologists should not wait long for the development of all chemical points. They should keep on designing the new toxicological tests, by the time the relevant chemical characterization can be developed by the chemists which will be enough for biologists for the development of the toxicological tests.

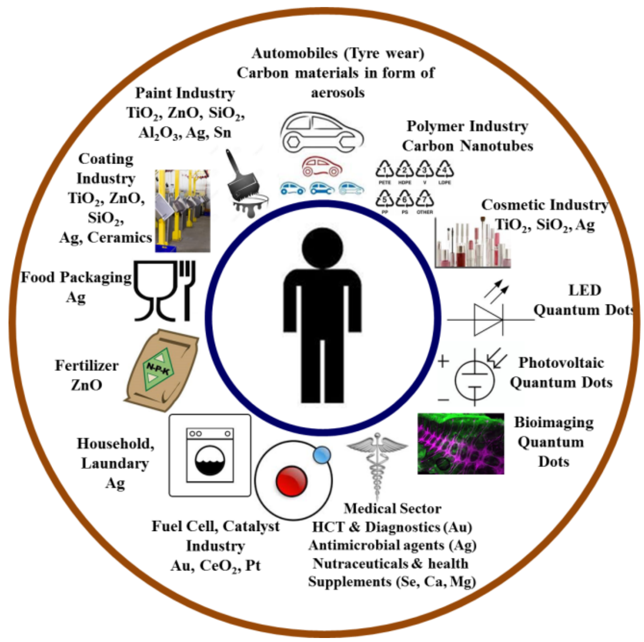

Fig. 2. Source of engineered nanoparticles (ENPs) in various industry.

Further, standard regarding quantity of nanoparticles commercially produced and the risk assessment will be designed accordingly. The proper rules should be made to properly manage that risk assessment [**42, 43**]. The risk assessment needs the ecotoxicity test strategies to be maintained, which can be designed in three tiered way – rapid tests, acute toxicity tests, long-term toxicity tests. First of all, ecotoxicity tests can be defined as the tools used to know the toxic levels created by the chemical substances released into the environment, ultimately answering the questions regarding the intrinsic dangers of these chemicals. After an analysis of the risk assessments with respect to the exposure assessments, the potential risk of the adverse effects of the chemicals can be characterized. While analyzing the risk assessment of nanoparticle using toxicological tests, the first requirement is the development of rapid tests. The rapid toxic tests for the initial screening of the nanoparticle should be developed significantly to examine that whether a nanoparticle is sufficiently different from the already existing bulk macroscaled chemical substance. This will create an alert for further toxicity testing. Further ecotoxic testing turns towards the acute ecotoxic tests. Although, the acute toxic effects occur for a smaller duration, but are very important to be analyzed before long-term tests. Acute tests are used with observation of organism survival. The short-term data can be used to examine the predicted no effect concentration. The longer term effects occur for a longer duration and lead to the severe effects. The longer- term tests are analyzed with the observation of sub-lethal effects of the chemical on the organism. During these ecotoxicity tests, the test substance is dosed into the test medium- soil or water can be applied for the ecotoxicity regarding agriculture and soil to provide the relevant medium. The nanoparticles should be used in the same form (agglomerated or dispersed) and in the same medium to provide the relevant environmental conditions for both exposure assessment as well as hazard assessment. Also, both assessments should be expressed in same unit. This will help in exact comparative analysis to compare both exposure and hazard assessments like by like. In last, it is very much necessary to know the end points regarding ecotoxicity testing, where the end points to be measured are Lethal Concentrations (LC), Effective Concentrations (EC) and No Observed Effect Concentrations (NOEC). Due to the rapid generation time, the end point considered for microbes is population growth while for other organisms the end points are survival, growth or reproduction [**20, 42, 43**].

Physico-chemistry of nanomaterials in soil and plants

The biosynthesis of metal nanoparticles using leaf extracts of *Azadirachta indica* [**44**] and Geranium has been reported. Shankar *et al.* (2004) reported the synthesis of triangular nanoparticles using lemongrass plant extract [**45**]. Similar work on the synthesis of gold nanotriangles was performed using Tamarind leaf extract as the reducing agent [**46**]. The formation of gold nanotriangles and silver nanoparticles using leaf extract of *Aloe vera* as the reducing agent was reported [**47**]. Huang *et al.* (2007) demonstrated the synthesis of silver and gold nanoparticles using sun-dried leaf extract of *Cinnamomum camphora*. They demonstrated that the sun-dried leaves were better than the aqueous leaf extract used for the biosynthesis. However, amla (*Emblica officinalis*) fruit extract, showed synthesis of extracellular gold and silver nanoparticles. The formed particles were highly stable [**48**]. The above plants described the synthesis of nanoparticles by using leaf extracts. In contrast to these, the sweet desert willow (*Chilopsis linearis*) has shown the ability of intracellular gold nanoparticle synthesis. This plant has the capability to take up gold (Au) from gold enriched media and synthesize nanoparticles. It has been shown that *Alfalfa* plants synthesize silver nanoparticles *in vitro*. The roots were capable of absorbing Ag (0) from the agar medium and transferring it to the shoot. The silver atoms arrange themselves by undergoing nucleation to form nanoparticles inside the plant. The nucleated nanoparticles further join to form larger particles [**49**]. Haverkamp *et al*. (**2007**) reported the synthesis of mixed nanoparticles suggesting the possibility to produce nanoparticle catalysts of specific composition [**50**].

In plant mediated synthesis of NPs, the plant material acts as the reducing agent as well as capping agent (surfactant) for stabilization of NPs. So, it provides the evidences of possibilities of affecting the oxidation reduction reactions inside the plants, soil and soil microflora. NPs released into the soil can alter the physical and chemical properties of soil like pH, BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand). The toxicity of nanoparticle depends on various physico-chemical properties of nanoparticle [**42, 51**]. The manufactured NPs are usually stabilized by certain surfactants, due to which they can form stable colloidal dispersions in the water and humid soil. The dispersion chemistry can be an important point to be recognized for these ENPs [**42**]. One other significant property which is important to study is their aggregation chemistry. It is well known that on long term storage, the ENPs form the aggregates. These aggregated NPs will either become more toxic or the toxicity in environment might get reduced. Phenrat *et al.* studied the aggregation and sedimentation of aqueous nanoscale zerovalent iron (NZVI) dispersions. They revealed that the limited mobility of NZVI is due to the aggregation. Their study confirmed that the rate of aggregation increased in case of NZVI magnetic, as compared to the nonmagnetic particles. It was mainly due to the magnetic forces between NZVI particles [**52**]. Once in the environment of soil and water, the NPs tend to interact with the other pollutants, non- nanopollutants and organic matter like fulvic acid and humic acid and also the metal ions like calcium ions (Ca^{2+}), which might act as another surfactant for the manufactured nanoparticles [**42, 51**]. In this case, there arise the two possibilities. First, these surfactants can provide increased stability. Second, these surfactants can further increase the aggregation and change the net charge on the nanoparticle aggregates. Illes *et al*. studied the humic acid (HA) coated magnetite particles form stable colloidal dispersion. They stated that the particle aggregation does not occur in a wide range of pH and salt tolerance is increased. They reported that even at low pH, in presence of increasing loading of HA, magnetite becomes negatively charged. In this situation, only a trace amount of HA is adsorbed on the magnetite surface as oppositely charged patches and the systems become unstable due to heterocoagulation. Also, above the adsorption saturation the NPs become stabilized due to the steric and electrostatic effects [**53**]. Iron oxide NPs are hydrated in aqueous systems. This is mainly because Fe-OH group distributed all over the iron oxide particles. The hydrous iron oxides have the amphoteric character [**53**]. Fang *et al*. studied the stability of TiO_2 NPs in soil suspensions. Their study reveals that the stability of TiO_2 contents in soil solutions are positively correlated with dissolved organic carbon and negatively with ionic strength, pH and zeta potential. Solution pH and surface charge mainly governs the stability of TiO_2 NPs in aqueous solution [**54**]. Guzman *et al*. studied that over 80 % of suspended TiO_2 NPs were mobile in micro channels over pH range of 1-12, except where the pH was close to the zero point charge of TiO_2. pH_{pzc} of TiO_2 is 6.2 [**55**].

The non-nanopollutant surfactant attached to the surface of NPs can also be a beneficial chemical present in the soil. In that case, both the particulate matter will be less available for its desirable uptake in the environment or the uptake of the particulate chemical by the soil microflora and organisms like plants will be enhanced. The next significant property of these NPs to be noticed is the effects of particle size, shape, surface area, and surface charge on ecotoxicity regarding the soil microflora and plants. In conclusion, we can say that all these physico-chemical properties can affect the uptake of NPs as well as their toxicological effects in the concerned environment of soil and agriculture.

Effect of inorganic nanoparticles on soil microflora

Large scale production of NPs and their use in different industries and further disposal will inevitably enter natural ecosystems, which affects the native soil microflora. Studies regarding the fate and interaction of nanomaterial in complex systems such as soil microflora open the window for risk assessments associated with interaction of nanoparticle with the environment. The quality of soil directly depends on the microorganisms present in the soil, which again is an integral component in the biogeochemical cycle, decomposition of soil organic matter, and plant growth. The release and accumulation of engineered NPs in soil and its interaction with soil microflora make undesirable effects on the microbial diversity. Interactions of these NPs with microbial community occur directly or in the form of an organic compound. NP's toxicity with microbes can be possible through disruption of membrane, generation of reactive oxygen species (ROS) or toxic constituents, which may further lead to protein damage, genotoxicity, and cell death [**20, 56, 57**].

NPs show unique fundamental properties, which include high surface area to volume ratio and high reactivity. As mentioned above, engineered nanoparticles (ENPs) include metal (Ag, Au, Fe etc.), metal oxides (TiO_2, ZnO, SiO_2, CuO etc.), quantum dots (CdSe, ZnS, CdS etc.) and carbon based (Fullerenes, CNT etc) that are formed as a by-product of human activity and that finally enter the soil. A few studies are available in the literature on the effect of ENPs on microbial communities in soil in the field conditions. Besides this, the effect of ENPs on beneficial microbes *in vitro* under controlled conditions and antimicrobial activity of these NPs is very well reported. Silver NPs exhibit antimicrobial activity by physical disruption of cell membrane and hence can impact the soil microflora [**58**]. In 1977, Cornfield (1977) reported a study on twelve soil microbial communities against Ag metal and found that Ag is the most toxic metal against soil microbial communities [**59**]. A detailed analysis by Johansson et al. (1998) suggests that the presence of soluble silver salts in the soil severely inhibits the activity of soil dehydrogenase and also inhibits the process of denitrification [**60**]. Further studies by other research group demonstrate that the addition of 100 mg of Ag/Kg of soil results in a significant decrease in the copy number of copper nitrite reductase, nirK. All these reports clearly demonstrate the toxicological effects of Ag on the nitrogen cycle. In the light of these studies, it can be understood that Ag in the nanoparticles form might be far more toxic than that is reported for microscaled Ag particles.

Inorganic nanoparticles – plants interactions

Commercially produced ENPs released into the environment from different sources closely interact with the surrounding environment. Plants play a significant role in an ecosystem. ENPs can easily interact with the plants in different way exerting positive, negative, and inconsequential impacts on plants. Here, our main focus is towards the impacts of ENPs on plants associated as a crop with agriculture. Certain factors are responsible for the impacts on plants. ENPs can interact with the plants chemically by adsorption on the roots as well as through physical interactions and induces phytotoxicity. During the phytotoxic effects some other factors like solvent factor, threshold level of toxicity as well as interaction of plants with growth substrate in the soil can have an effect on the phytotoxicity by NPs [**42, 61**]. Usually, the commercially available NPs contain stabilizers. In the absence of stabilizers, the life of NPs in solution is always very short. Barrena *et al.* (2009) reported the study of three NPs tested against lettuce and cucumber. The toxicity on cucumber and lettuce was zero to low. The effects were both positive and negative and were attributed to the stabilizers. The threshold level of toxicity depends on the particular plant species, and it was reported that higher concentrations of NPs were toxic against plants. The significance of the interaction of plants with growth substrates is that these interactions can inhibit or prevent the interaction of plant with the NPs. The physical interactions of NPs with the plants constitute the interactions between the plant cell pathways and NPs. Plant cell pathways are of two types-apoplastic and symplastic. NPs may inhibit the apoplastic movements, where the blockage of traffic through the intercellular spaces in the cell wall or cell wall pores occurs in the presence of NPs. The blockage of nanometer sized plasmodesmata - special types of connections between the cells, leads to the blockage of the symplastic movements. Plasmodesmata blockage was reported in maize by TiO_2 NPs leading to the inhibition of leaf growth and transpiration of maize seedlings. It was reported that it is primarily due to the reduction of hydraulic conductivities [**62**]. One other research reported that the diameter of maize root cell wall pores was reduced from 6.6 nm to 3 nm on pretreatment with NPs [**20, 61**]. Similarly, the NPs might interfere with several chemical reactions taking place in the plants like electron transport chain, cytochrome reduction, cyclic and non-cyclic photophosphorylation, etc.

Although there is a lack of extensive studies on the phytotoxic effects of NPs, the few studies available so far offer a conclusive remark on the phytotoxic effects of NPs. The effects of NPs on plants can either be positive, negative, or inconsequential. Lin and Xing (2007) analyzed the effects of Zn NPs on *Lilium multiforum* and that of ZnO on *Zea mays*. It was reported that the NPs lead to inhibition of seed germination and root growth. The inhibition was partially correlated to the concentration of NPs. The occurrence of inhibition during the seed incubation process rather than the seed soaking stage might lead to the conclusion that the NPs might be involved in interaction with the seedlings during the seed incubation process rather than the seed soaking stage [**63**]. Lin and Xing (2008) in one more experiment analyzed the effects of ZnO on *Lolium perenne* (ryegrass), where they studied the cell internalization and upward translocation of ZnO. Ryegrass biomass was reported as significantly reduced along with the shrunken root tip with highly vacuolated and collapsed cells in case of root epidermis and cortex. ZnO NPs were found strongly adhered to the root surface and were also present in the apoplast, protoplast of root endodermis, and stele. The combined effect of Zn and ZnO NPs was studied by giving a combined treatment for a comparative analysis of the NPs which revealed that the translocation factor of Zn was reduced in the presence of ZnO NPs. From the comparative study, the authors evidenced that the phytotoxicity of ZnO NPs was not directly correlated to their limited dissolution in bulk nutrient solution and rizosphere, which suggests that dissolution might not be the main factor regarding the phytotoxicity of the ZnO NPs. There might be some other factors involved which might play a bigger role regarding the phytotoxicity of ZnO NPs [**64**].

Hong *et al.* (2005) analyzed the effect of nano-TiO_2 on *Spinacia oleracea*, where they studied the effects of nano-TiO_2 on the photochemical reaction of chloroplasts. An increase in Hill reaction and chloroplast activity was reported, which further accelerated the cytochrome reduction and oxygen evolution processes. The photophosphorylation study reveals that in the presence of nano-TiO_2, the activity of noncyclic photophosphorylation increases in comparison to the cyclic photophosphorylation. For the reasons behind these effects, the authors explained that Nano-TiO_2 might enter the chloroplast and interferes with the oxidation-reduction reactions which further results in accelerated electron transport and oxygen evolution [**65, 66**]. Zhang *et al.* (2005) studied the comparative analysis of bulk TiO_2 and nano TiO_2 on naturally aged seeds of *Spinacia oleracea*. The points considered for the comparative analysis were germination rate, germination and vigor indexes, where an increase in indexes was reported after nano-TiO_2 treatments. The examination based on the observation during growth stage reveals an increase in plant dry weight and chlorophyll formation along with an increase in activity of ribulose bisphosphate carboxylase/oxygenase and photosynthesis rate. The results of bulk TiO_2 were found to be insignificant. So, a conclusion was drawn that the physiological effects were attributed to the nanoscaled particles [**67**]. In another experiment, Gao *et al.* (2006) examined the bulk-anatase TiO_2 treated *Spinacia oleracea*, where they observed that the activity of Rubisco was increased by 2.67 times in comparison to the control Rubisco, but the exact molecular mechanism behind the enhancement of Rubisco activity, leading to the promotion of C reaction was still not understood [**68**]. Xuming *et al.* and co-workers (2008) studied the mechanism by reverse transcription PCR and northern blotting methods and reported that the Rubisco small unit and large unit messenger RNAs (mRNAs) were promoted in NP treated plants, which resulted into the enhancement of the protein expression of Rubisco by 40 % in bulk- TiO_2 treated plants in comparison to the control [**69**].

Racuciu and Creanga (2007) analyzed the effect of iron based NPs coated with tetramethylammonium hydroxides on *Zea mays* (popcorn) plants on early ontogenetic stages. They found that iron based NPs influence the plants in two ways; magnetic influence as well as chemical influence. Magnetically, they have an impact on the structures of enzymes involved in photosynthesis, while chemically they had a stimulating impact on the growth of the plantlets on the condition that the NPs are added in small concentration in solution. The enhanced concentration of NPs had an opposite inhibitory effect instead of stimulation [**70**]. A brief description of effects of NPs on plants is represented in **Table 1**.

Table 1. Description of effects of engineered nanoparticles (ENPs) on plants.

Nanoparticle	Plant	Effects
TiO_2	*Mentha piperita* (Medicinal plant)	Toxic to seed germination, decreased shoot length, decreased shoot biomass [**71**]
Zn	*Lolium multiforum* (Rye grass)	Inhibits seed germination and root growth [**63**]
ZnO	*Zea mays*	Inhibits seed germination and root growth [**63**]
TiO_2	*Spinacea oleracea*	Increases hill reaction, chloroplast activity, non-cyclic photophosphorylation, and photosynthesis rate [**65,66**]
ZnO	*Lolium perenne*	Shrunken root tips, collapsed cells in root epidermis and cortex [**64**]
TiO_2	*Spinacia oleracea*	Increase in protein expression of Rubisco enzyme by 40% [**69**]
Fe	*Zea mays*	Impact on structure of photosynthetic enzymes, small concentration increases growth of plantlets, high concentration decreases growth of plantlets [**70**]
Ag	*Vicia faba*	Decreased rate of mitotic index, chromosomal aberrations, irreversible DNA damage [**72**]
Ag	*Allium cepa*	Generation of ROS, cell death, mitotoic index, micronucleus and mitotic aberrations, DNA damage [**73**]
TiO_2	*Allium cepa, Nicotiana tabacum*	Produce ROS (Reactive Oxygen Species), damage DNA [**74**]
CeO_2	*Spinacia oleracia*	Enhancement in SOD (Superoxide Dismutase) activity and chloroplast ROS – scavenging activity [**75**]
CuO	*Raphanus sativus, Lolium perenne, Lolium rigidum*	Damages DNA [**76**]

Biological uptake of metal and metal oxide nanoparticles in plants

The proliferation and release of NPs into the environment has raised important ecological and human health concerns worldwide [**15**]. The environmental contamination of the NPs profoundly affected the aquatic and terrestrial microbes, plants, and animals [**33, 39, 40, 61**]. Moreover, the detection of NPs in wastewater indicates potential human exposure and health concerns [**36, 41, 77, 78**]. The current and future impact of use of NPs on the environment and health should be understood before these problems become impossible to be resolved [**79, 80**]. Among all the NPs that have been used, Ag NPs are the most widely used. It is estimated to be approximately 800 megatons (1×10^6 tons) of global use of Ag NPs per year, among many different industries, due to their unique antimicrobial properties [**81**].

The biological uptake and accumulation of NPs by plants has drawn the attention of researcher in last few years. In order to transport and translocate NPs from root to shoot of plants, the NPs have to penetrate the cell walls and plasma membranes of the epidermal layers of roots to enter the vascular tissues (xylem). Cell walls of plants are a porous network of polysaccharide through which water molecules and other solutes pass. These pores are 3-8 nanometer (nm) in diameter, and function as natural sieves [**82**]. The NPs being smaller than the pore size are expected to pass through and reach the plasma membrane; however, the larger particle aggregates at the surface. However, Navarro *et al.*, (2008) proposed that the NPs may induce the formation of new and large size pores when NPs come in contact with cell walls which allow the internalization of large NPs [**51, 83**]. The first report on biological uptake of NPs by the plant was published by Zhu *et al.* (2008) [**84**].

They reported the translocation of iron oxide NPs (Fe_3O_4) in pumpkin (*Cucurbita maxima*) from the roots to the plant tissues. They showed that approximately 0.6 % of the fed NPs were translocated and detected in leaves, and about 45.5 % of fed NPs were accumulated in roots. However, when lima bean (*Phaseolus limensis*) was tested, the uptake and transport of iron oxide NPs were not observed by the same researchers [**84**]. Lin *et al.* (2009) reported the uptake and translocation of carbon NPs, fullerene C70 from root to shoot and from leaf to root by rice plants (*Oryza sativa*). They also used multi walled carbon nanotubes but similar results were not observed even at higher concentration (800 mg/L) because of larger size of MWCNTs than C70 [**85**]. Lin and Xing (2008) reported the uptake and accumulation of ZnO NPs at the root of rye-grass (*L. perenne*), but they did not observe the translocation of ZnO NPs from root to shoot [**64**]. Lee *et al.* (2008) reported the uptake and accumulation of Cu NPs in mungbean and wheat plant biomass. They presented a linear relationship of Cu NPs concentration in the growth media with uptake and accumulation of Cu NPs in plant tissues [**86**].

Hischemoller *et al.* (2009) reported the uptake and translocation of fluorescence nanocrystals, $NaYF_4$: Yb, Er from roots to leaves in moth orchid (*Phalaeonpsis spp*.) and *Arabidopsis thaliana*. They soaked the roots of orchid roots in the colloidal solution of $NaYF_4$: Yb, Er nanocrystals and the plant tissues were visualized using a confocal laser scanning microscope at different time interval and found that the NPs penetrated plant tissue through velamen radicum (a epidermis present in aerial roots) and passed through the cells in about ten minutes and reached the vascular tissues in few days [**87**]. Even though, much research has been in progress in this area of research, the investigation of plant uptake and accumulation of nanoparticles is still in its infant stage.

Toxicity and biological uptake of metal and metal oxide nanoparticles in plants and soil microflora

Nanomaterials exert their toxic effects by virtue of their characteristics properties such as size, surface area, morphology, and dissolution. Researchers have found that the carbon-based nanomaterials are more toxic at lower concentrations than their metal counterparts by performing screening studies using in vitro approaches [**88**]. Still the probable toxicity mechanisms for most of the nanomaterials have not been understood but the possible mechanisms include disruption of membranes or membrane potential, oxidation of proteins, genotoxicity, interruption of energy transduction, formation of reactive oxygen species, and release of toxic constituents [**89**]. Various characteristics features of nanomaterials such as high ratio of surface area to volume, surface charge, reactive hydrophobic and lipophilic groups which may interact with plant proteins and membranes, size of nanomaterials which may cause inhibition of important plant enzymes, bioaccumulation and chemical composition which increase their reactivity etc. may contribute to the enhanced toxic effects on plants [**90**]. However, there is some toxicity mechanisms which are difficult to observe and vary widely even within the same class of nanoparticle; for e.g., fullerenes (C60) or nanosilver. Fullerol (C60) [OH]x, (the hydroxylated form of C60) even after generating singlet oxygen is not significantly cytotoxic but still can behave as a potent oxidizing agent in biological systems [**91**]. After coating C60 with polyvinyl pyrrolidone, it produces a nanostructure which generates singlet oxygen that can cause lipid peroxidation and other cell damages [**92**]. Other studies have shown the antibacterial activity of fullerene water suspensions (nC60) in the absence of light or oxygen, which negates the exclusive influence of singlet oxygen [**93**]. Certain semiconducting core-shell nanomaterials such as QDs contain harmful and toxic metals in their cores (e.g. CdSe, CdTe, CdSeTe, ZnSe, InAs or PbSe) and shells (e.g. CdS or ZnS). These nanomaterials cause toxicity to bacterial cells via these toxic components. As far as the stability of quantum dots in the environment is concerned very less is known apart from the half-lives which are likely to be quite long, ranging from months to years and may vary with photolytic conditions [**94**]. It has been shown that the avid in conjugated CdSe containing QDs can be incorporated into the soil-dwelling amoeba *Dictyosteliumdiscoideum* by endocytotic pathways [**95**]. The fact that the QDs nanomaterials may enter a wide variety of cell types by endocytosis and may be retained within different tissues and organs for some time raise a potential concern for their long term effects [**96**]. The toxicological interactions between NPs and proteins mainly depend on two factors; (1) NPs physically interacting with proteins; (2) NPs producing ROS (Reactive Oxygen Species) or other damaging radicals. The generation of reactive oxygen species (ROS) is an important toxicity mechanism of many NPs. ROS include oxygen radicals having one or more unpaired electrons such as superoxide anion (O_2^-), peroxide ($O_2^{=}\cdot$), hydroxyl radical (·OH), and singlet oxygen ($1O_2$). All these radicals are formed in mitochondria as oxygen is reduced along the electron transport chain. Despite their beneficial effects, the presence of an unpaired electron makes these radicals highly reactive and can be toxic to cells and can cause damage cell membranes, cellular organelles, all macromolecules including lipids, proteins and nucleic acids such as DNA and RNA [**97, 98**]. Several *in vitro* studies have shown that nanomaterials such as TiO_2 and fullerenes can produce the toxic ROS [**99, 100**]. On the other hand, some researchers have reported that certain NPs such as fullerenes (C60) can even protect against oxidative stress [**101**]. This apparent contradiction underlines the need for research on nanoparticle-cell interactions and mechanistic aspects of metabolism of NPs in organisms and specific cells [**97**]. Iron–sulfur clusters that functions as cofactors in many enzymes can be damaged by NPs that generate ROS and ultimately leading to Fenton chemistry that catalyzes more ROS (reactive oxygen species) generation [**102**]. ROS can also disturb the structure and function of the protein by forming disulfide bonds between sulfur-containing amino acids [**103**].

The best studied NPs with respect to their microbial toxicity till date are Ag and TiO_2 which are established as antimicrobial agents, and their nanocrystalline forms may also act similarly [**57**]. Ag NPs may cause toxicity via multiple mechanisms but actual mechanism by which Ag NPs interfere with plants is yet to be understood.

Morones *et al.* also reported that the Ag NPs can adhere to the cell surface and alter the membrane properties, therefore affecting the permeability and the respiration of the cell; they can penetrate inside the cell and cause DNA damage by releasing toxic Ag ions [**57**]. Some researchers reported that Ag NPs damage bacterial/plant cells by destroying the enzymes that transport the cell nutrient and weakening the cell membrane or cell wall [**20**]. Other researchers suggested that nano-Ag destroys the ability of the plant DNA to replicate. It is believed that silver ions interact with thiol groups of proteins, resulting in inactivation of vital enzymes [**104**]. Ag NPs are also known to degrade lipopolysaccharide molecules, thus forming pits in the membrane, and changing the membrane permeability [**56**]. TiO_2 NPs, which are being used in sunscreen, can indirectly damage the DNA by producing ROS, which in turn leads to breaking and cross-linking of DNA strands [**105**]. CeO_2 NPs can switch between Ce^{3+} and Ce^{4+} oxidation states and when comes in contact with any living cell oxidize membrane components involved in the electron transport chain and cause cytotoxicity [**105**]. Photosensitive metallic and metal oxides that generate ROS as well as fullerenes which are used for photodynamic therapy are also known to cause damage to cells and DNA [**106**]. Fullerenes have also been reported to bind to DNA and cause deformation of the strand, adversely impacting the stability and function of the molecule [**107**]. Certain types of photosensitive fullerene derivatives are known to cleave double stranded DNA when they are exposed to light [**108**].

As far as the NPs induced genotoxicity in plants is concerned, still very less is known and most of the limited information is available from the last two years. Atha *et al.* (2012) reported for the first time that copper oxide NPs damage DNA in some agricultural and grassland plants (*Raphanussativus*, *Loliumperenne* and *Loliumrigidum*). They reported that the oxidatively modified compounds accumulate and lead to mutagenic DNA lesions which inhibit plant growth [**109**]. This isolated study on NPs genotoxicity in plants strongly supports the urgent need to evaluate the putative genotoxicity of the different classes of NPs in plants. Another important issue, which deserves attention, is the analysis of genotoxic end points for NPs genotoxicity. For example, Comets, FCM-HPCV, and micronuclei have provided similar information in metal genotoxicity in plants [**110**], but before jumping to any generalization, a careful and detailed study dedicated to NPs induced phytogenotoxicity should be done carefully.

These reports and literatures highlight the need for more information on the interaction of NPs with plants and soil components and more quantitative assessments of aggregation/dispersion, adsorption/desorption, precipitation/dissolution, decomposition, and mobility of manufactured NPs in the soil environment. This information will aid the interpretation of toxicity test data and will inform the correct protocols for the assessment of the toxicity of various NPs.

Conclusion

The field of Nanotechnology which holds promising potential to the agricultural sector may also cause a negative impact to the agricultural sector in the form of nanomaterial based pollutants. It depends on the nature and the fate of a particular nanoparticle and its interaction with the plants. A nanoparticle might possess a beneficial effect for the flora while it can have hazardous effects on the fauna. However, the fact that these NPs have the potential to enter the plant system as well as soil microflora depends on individual cases of the nanoparticles. The effect of different concentration of the nanoparticle cannot be ignored too. A particular nanoparticle can be beneficial to the plants and soil microflora at a particular concentration, while the same exhibits the opposite effects at very high concentration. Some of them are capable of affecting the plants and soil microflora badly. They can easily pass through the plant cell wall and get accumulated in the plants. Hence, unscrupulous use of NPs needs to be avoided. At the same time, the release of NPs in the environment through several industrial effluents brings different undesirable NPs to the soil too, which becomes a major concern. These not only affects the plants negatively, but also get passed to the animals and human beings through food chains. So, the designing of the better criteria for the detection and risk assessments as well as a complete study on the fate of nanoparticle in environment and agriculture is very necessary before their massive use, because some of the NPs like Ag NPs are extremely toxic in nature. They not only affect the plants negatively, but also can cause serious health ailments in animals and human beings.

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