Journal of Biomaterials
2019; 3(1): 1-6
http://www.sciencepublishinggroup.com/j/jb
doi: 10.11648/j.jb.20190301.11
ISSN: 2640-2602 (Print); ISSN: 2640-2629 (Online)

Review Article

Nanotechnology Is the Potential Cause of Phytotoxicity

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To cite this article:
Syeda Hafsa Ali, Syeda Ayesha Ali. Nanotechnology Is the Potential Cause of Phytotoxicity. *Journal of Biomaterials*. Vol. 3, No. 1, 2019, pp. 1-6. doi: 10.11648/j.jb.20190301.11

Received: April 12, 2019; **Accepted**: May 31, 2019; **Published**: June 12, 2019

Abstract: Nanoparticles due to its unique properties are a serious threat to the environment and health. Nanotechnology is an emerging industry with the use of nanoparticles in more than 800 products and this demand is expected to increase in the next few years. Usage of nano-technological products has spread nanoparticles into the environment during its manufacture, usage or disposal through water, air, and soil. The unintentional spread of nanoparticles have accelerated a robust debate among the scientific community and have drawn attention towards the potential impact of nanotoxicity in the environment. The physiochemical properties and reactivity of nanoparticles differ not only between nanoparticle with different chemical composition but also among identical nanoparticles with different shape, size, surface properties, and crystalline structure. Phytotoxicity occurs as nanoparticles are uptaken, translocated, or localized in a plant. Consequently, affecting germination rate, physiological processes that disrupt cell integrity at the molecular level and causes detrimental effects on plant growth and development of various crops. This toxicity produces Reactive Oxygen Species (ROS) that causes DNA damage and lipid peroxidation. However, these free radicals are actively scavenged by antioxidant enzymes to repair the damage and help the plant to withstand the stress. However, the continuous increase of nanoparticles can permanently damage the plant thereby reducing its ability to withstand. Therefore, cost-effective strategies are required to overcome the risk of nanoparticles.

Keywords: Nanoparticles, Phytotoxicity, Antioxidant System, Plant Physiology, Abiotic Stress

1. Introduction

Nanotechnology is among rapidly developing industry with a diverse use of engineered nanoparticles in industries, health care, commercial and consumer products. Nanoparticles are particles with one dimension smaller than 100nm Nanotechnology is improving the agricultural sector with a variety of applications in herbicides, insecticides, food, and agriculture waste to produce energy. Nanoparticles are particles with one dimension smaller than 100nm. The physiochemical properties and reactivity of nanoparticles differ not only between different nanoparticles but also within identical nanoparticles. Keeping the benefits aside, the risk posed by the use of nano-technological products provoke extensive debates on the effects of nanoparticles on the environment and human health [1]. Demand and usage of nano-technological products have been increased in recent years. Anthropogenic release of nanoparticles into the environment causes a potential impact on human health and the surrounding environment. Spread of nanoparticles into the environment has increased toxicity in plants [2]. Recently, the scientific community is drawing attention to analyze the fate of nanoparticles in plant and its risk for human health and the environment [3].

2. How Nanoparticles Enter the Plant System

Nanoparticles released into environment find their way into plant roots, where they are accumulated and translocated to other regions of the plant. Diverse nanoparticles have been tested on a variety of plants to investigate toxicity due to uptake, translocation, and localization in cells [4]. Nanoparticle uptake and translocation is multifactorial and depends on: characteristics of nanoparticle (its size, composition and surface properties), the dose of nanoparticle, delivery method and plant specie. Nanoparticle bioaccumulation occurs over time, leading to altered physiological processes that affects plant growth and development [5]. Highly studied nanoparticles and its impact in the environment are divided into carbon-based, metals, oxides and semi-conductor (Figure 1). Metal nanoparticles are highly toxic at low concentrations, yet rarely emitted in the environment (Cd, Zn, Al, Pb, Fe, Co). The metal oxides (SiO_2, ZnO, and TiO_2) are commonly used in the applied nanoparticle industry. Titanium dioxide (TiO_2) nanoparticles are widely used in industrial products, household items and in a variety of products. Approximately, 4 million metric tons of TiO_2 bulk is produced around the globe where the USA contributes 1.3 million metric tons annually. Computer modeling predicts 136 mg of TiO_2 nanoparticles per kg are present in sewage sludge and its intensive use is expected to increase the accumulation by 89µg TiO_2-NPs each year [6].

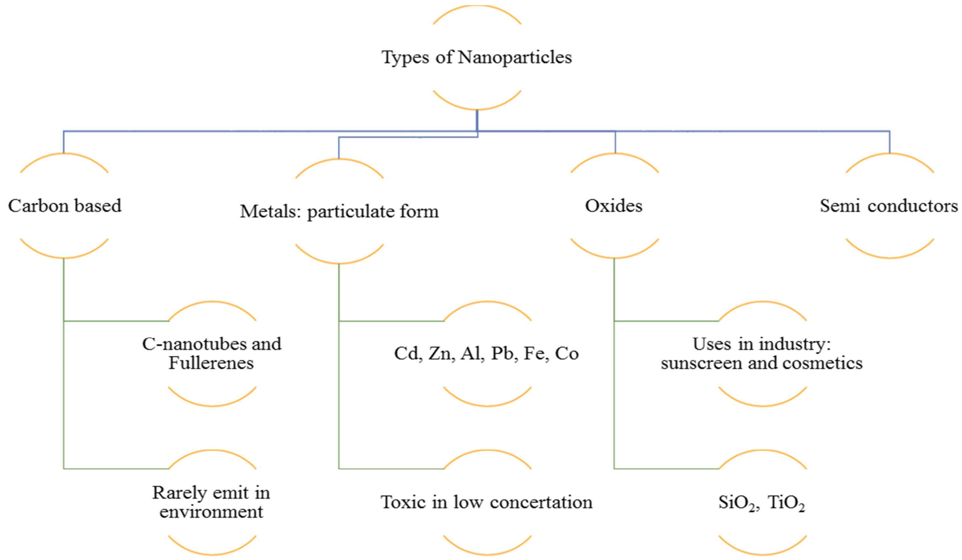

Figure 1. *Types of nanoparticles. Four types of nanoparticles can make their way into the environment and has the ability to cause possible toxicity in plants.*

Nanoparticles uptake in a plant varies with size and its surface properties to reach edible organs. Nanoparticles uptake is divided into two regions: aboveground regions that facilitate deposition of airborne nanoparticles onto shoots of the plant; and belowground regions that involve uptake via roots. The nanoparticle can penetrate and accumulate into leaves even if the cell wall diameter is smaller, although the efficiency of nanoparticle accumulation depends on plant species, and physiochemical parameters of nanoparticle [7]. Typically, shoot surface of plants are covered with cuticle waxes and are lipophilic in nature that increases deposition of nanoparticles. Large nanoparticles can penetrate into cuticle free regions. Belowground nanoparticles enter via lateral root through the cortex and central cylinder to enter xylem. Nanoparticle passes through cell wall pore and accumulates in plant roots. The pore size of the cell wall in root hair is 3.5-3.8 nm, where nanoparticles below 5 nm in diameter can transverse cell wall efficiently without damage [8]. The research aims to review the impact of nanoparticles on physiology, biochemical and molecular parameters of plants.

3. How Nanoparticles Impact Plant Physiology

Phyto-toxicity depends on size, hydrodynamic diameter, porosity, surface area, charge, the composition of nanoparticles. Plant surfaces exposed to air and soil environment face a high level of toxicity, resulting in adsorption via stromata or taken up by roots to different organs [9]. Nanoparticle effects plant physiology by mechanical, catalytic, chemical or surface impact. Reactive oxygen species (ROS) is a collective term used for oxygen-derived species specifically oxygen radicals: non-radicals like superoxide (O_2•), hydroxyl (OH-), hydrogen peroxide (H_2O_2), lipid peroxide (LOOH) that increases their reactivity—leading to oxidative stress commonly produced as a result on nano-toxicity. The mechanical effect of nanoparticle involves blockage of plant pores that inhibits water transport processes [10]. Plants exposed to metal nanoparticles causes oxidative stress due to interference in cellular processes (Figure 2). Catalytic effects of metallic nanoparticles involve distortion of metal-dependent mechanisms of plant cell such as modification of molecules and oxidation of proteins. Thus, metal ions (Fe, gold (Au), Co, Ag, Pt, Pd) even at low concentrations are compatible with the cell environment. Chemical effects of nanoparticles include nanoparticles such as Zn, Ni, mercury (Hg), Cd, and Cu—capable to attach with cellular components and modify their activities such as sulfhydryl, imidazole groups, carboxyl [11]. Metals like Fe, Cu produces H_2O_2 and O_2 by transferring an electron to O_2 acceptors that further produces OH radical by creating oxidative stress in affected cells. Metal oxides are toxic as they rapidly form ions. The concentration of nanoparticle in the soil environment is very low and is usually found associated with plant surfaces. The chemical effect in plant cells is caused by a high concentration of nanoparticle or massive intoxication at a specific site [9]. Certain nanoparticles also damage surfaces of the plant. The surface of metals comprises of OH layer, whereas, negatively charged surface attracts positive surfaces of cell components—usually proteins or side groups of proteins. Metal nanoparticles bounds with proteinaceous component of cell leading to complete or partial malfunctioning. Extreme toxicity occurs if metal ions covalently bind to the side chain of proteins, such binding is permanent in nature and causes complete malfunctioning of cellular processes [12].

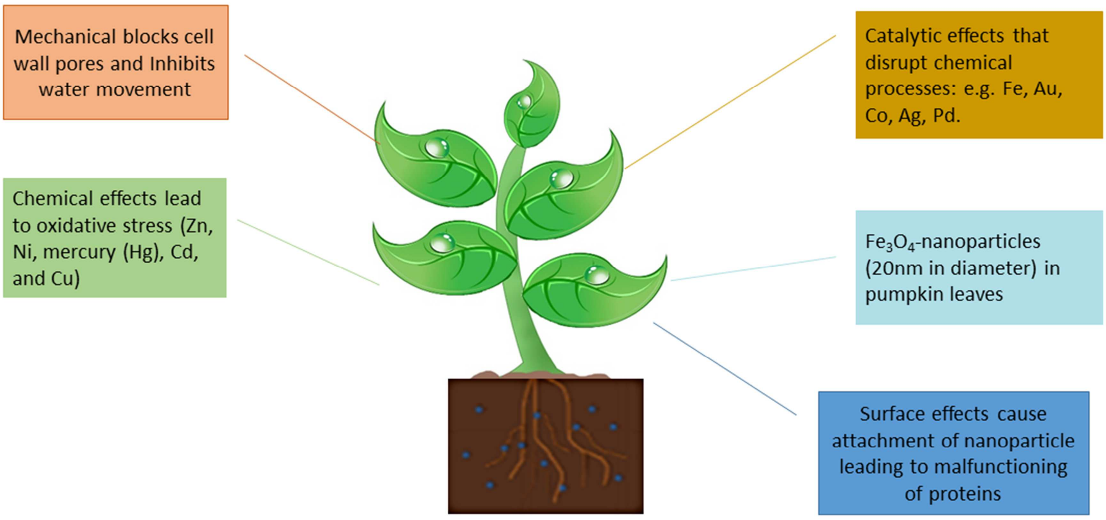

Figure 2. *Impact of nanoparticles on plant physiology and at the cellular level.*

Nanoparticles also affect plant physiology by mechanical clogging of cell structure. Seed germination occurs prior to the emergence of the radical form seed coat. Germinating seed comprises of a high content of amylase and protease that help to mobilize stored food in early seedling growth until it starts photosynthesis. The rate of water uptake and utilization of seed reserves are important physiological and biochemical processes in seed germination. During imbibition, seeds consume a high amount of oxygen to activate mitochondrial enzymes, to begin Krebs cycle, and electron transport chain. Enzymes act on stored substances and convert them into simpler substances to synthesize new substances. Conversion of molecules begins with amylase that converts starch and transfers the glucose molecules in germinating seeds, followed by transformation of protein into amino acid via proteases. Silver nanoparticles are taken up effectively than TiO_2 nanoparticle, yet both nanoparticles do not penetrate endosperm and seed coat, thereby show the limited effect on seed germination (Table 1). Environmental pollution due to TiO_2 nanoparticles affects the growth and development of plants [5].

Studies show lack of proper vessels in seed coat or embryo—acting as filters—macromolecules are stopped and allow movement of water molecules. Seed coat act as a barrier for the movement of nanoparticles, as nanoparticles do not ionize in water and agglomerate on treatment, hence germination is not affected [13]. However, Khodakovskaya [14] confirmed the penetration of multiwalled carbon nanotubes (MWCNTs) through seed coat of tomato after incubation as it punctures seed coat and allows movement of oxygen and water into the seed, thereby enhancing metabolic processes and accelerating seed germination (Table 1). Lu *et al.,* [15] demonstrated that SiO_2 and TiO_2 in combination enhance seed germination and biochemical activity of soybean. Ag nanoparticles are considered phytotoxic even at low concentration and effects the biomass and seedling growth, while 1000mg/L is a threshold after which seedlings do not survive to mature as adult plants [16].

Table 1. *Effect of different types of nanoparticles on plant seed germination, physiology, and biochemical level.*

S. No	Plant	Nanoparticle	Concentration	Impact	Reference
1	Triticum aestivum	Aluminum (Al) nanoparticles	0, 50, 75, 100 or 150µM	Root growth inhibition	[20]
2	Radish, Rape, Ryegrass, Lettuce, Corn, Cucumber	Nanoparticles (multi-walled carbon nanotube, aluminum (Al), alumina (Al_2O_3), zinc (Zn), and zinc oxide (ZnO)	20, 200, 2000 mg/L)	Terminated root elongation, inhibition on root growth	[26]
3	Alfalfa plants	Lead (Pb) nanoparticles	40 mg/L	Phyto-hormone toxicity	[25].
4	Pumpkin plants (cucurbita maxima)	Magnetite (Fe_3O_4) nanoparticles	0.5 g /L 20 nm,	Uptake and translocation to plant parts	[19].
5	Zea mays l	Titanium dioxide (TiO_2)	1g/L	Interfere with leaf growth and	[10].

S. No	Plant	Nanoparticle	Concentration	Impact	Reference
				transpiration	
6	Phalaenopsis and Arabidopsis plants	trifluoroacetate precursors ($NaYF4:Yb, Er$) nanoparticles	45 nm, 1% wt	Accumulation and translocation to plant parts.	[8].
7	Tomato seeds	Carbon nanotubes	10−40 µg/mL	Inhibiting germination and growth rates	[14].
8	Arabidopsis	Ultrasmall Titanium dioxide (TiO_2)		Accumulated in subcellular level	[4]
9	Zea mays l	Cerium dioxide (CeO_2) nanoparticles	50 µg (aerosols)	Adsorbed in leaves	[17].
10	Zea mays l. Vicia arbonensis l.	Titanium dioxide (TiO_2)	0.2 to 4.0 ppm	Disturbs mitotic activity, chromosomal aberrations, delayed germination	[1]
11	Arabidopsis thaliana	Ultra-small Titanium dioxide (TiO_2) nanoparticles	100 µM	Reorganization and elimination of microtubules	[5].
12	Lemna minor l.	Silver (Ag) nanoparticles	5 µg/L 20 nm to 100 nm	Inhibition of plant growth	[16].
13	Phaseolus radiates, Sorghum bicolor	Silver (Ag) nanoparticles	5 to 25 nm, 0–40 mg/L	Growth inhibition effect	[27].
14	Triticum aestivum	Titanium dioxide (TiO_2) nanoparticles	14 nm to 655 nm	Accumulated in plant cells	[7].
15	Tomatoes (lycopersicon esculentum)	Titanium dioxide (TiO_2) and Silver (Ag) nanoparticles	0, 50, 100, 1000, 2500 and 5000 mg/L	Lower chlorophyll contents, higher enzymatic activity, reduces fruit productivity	[13].
16	Onion seeds	Zinc oxide (ZnO) nanoparticles	(0.0, 10, 20, 30 and 40 g/mL	Chromosomal abnormalities	[22].
17	Maize (zea mays l.) And rice (oryza sativa l.)	Titanium Dioxide (TiO_2), Silica (SiO_2), Cerium dioxide (CeO_2), Magnetite (Fe_2O_4), Aluminum oxide (Al_2O_3), Zinc Oxide (ZnO), Copper oxide (CuO) nanoparticles	2000 mg/L	Inhibits root growth	[28].
18	Tomato (solanum lycopersicum l.)	Citric acid coated cerium oxide (CeO) nanoparticles	500 mg/kg,	Effect nutrients and enzymatic activity in roots, stems, and leaves	[29].
19	Maize plants inoculated with arbuscular mycorrhizae	Zinc oxide (ZnO) nanoparticle	800 mg/kg.	Affected chlorophyll content, enzymatic activity, nutrients content in leaves	[30].
20	Lycopersicon lycopersicum (tomato plants)	Cobalt ferrite ($CoFe_2O_4$)	0 to 1000 mg/L	Enzymatic activity decreased in roots and leaves	[31]
21	Wheat plants (Triticum aestivum l.)	Copper (Cu) nanoparticles	1-500 mg/L	Inhibited root and leaf growth, cell death in roots	[32].
22	Tomato plants	Zinc Oxide (ZnO) nanoparticles	0, 2, 4, 8, or 16 mg/L	Increases growth, photosynthesis, antioxidant enzymes	[33]
23	Basil (Ocimum basilicum)	Silica (SiO_2) nanoparticle under salinity stress	10mL	Growth and chlorophyll content was reduced	[34].
24	Solanum lycopersicum l.	Silver (Ag) nanoparticles	100 and 200 µg/mL	Increases content of proteins, carbohydrates, and phenolic compound, causes structural changes in plants	[35].
25	Phaseolus vulgaris l.	Cerium dioxide (CeO_2) nanoparticles	0, 250, 500, 1000, and 2000 mg/L	Effects photosynthesis, electron transport chain biochemical enzymes, induces membrane damage, and oxidative stress	[36].

Treating maize with Ag nanoparticle reduces root growth (Table 1). Trichrome covering the plant shoots also facilities deposition of CeO_2 nanoparticle aerosol in young maize (*Zea Mays*). Airborne CeO_2 nanoparticles penetrate the leaves through openings of stomata and deposited inside the leave—into the cell wall of a stomatal cavity or in neighboring cells [17]. In maize, nanoparticles with size 4.9 nm can cross cell wall but nanoparticle of 20 nm was obstructed due to the 3.5nm size limit of the primary cell wall. However, the size of pits in xylem vessels varies in mm range with respect to plant species such as *Eucalyptus regnans, Thuja plicata, Abies nordmanniana, Gingko biloba* [18]. The connectivity between adjacent cells depends on pit size that varies among species to regulate the movement of nanoparticle through xylem vessels. Studies by Zhu and his coworkers [19] showed the accumulation of 1.3% of Fe_3O_4-nanoparticles (20nm in diameter) in pumpkin leaves when exposed to 0.5g/L in hydroponic medium (Table 1). This nanoparticle was identified in pumpkin leaves via magnetometry as the samples vibrated in response to magnet [19]. Consequently, these nanoparticle induces alteration in the microtubule network and causes rearrangement or elimination of microtubules and proteasome-dependent degradation of tubulin [5].

However, various concentrations of TiO_2 nanoparticles showed no change in root growth [13]. Roots are in direct contact with nanoparticles and therefore level of ROS stress is high as compared to shoots [20]. However, the internalization of TiO_2 nanoparticles into the cell does not affect the viability and morphology of cells [4]. Yet, there is a possibility that nanoparticle causes cellular and molecular changes as a result of its uptake and distribution inside plant cells. Uptake of TiO_2 nanoparticles is reported to cause oxidative stress by creating ROS and lipid peroxidation products that cause DNA damage. Although the exact mechanism of nanoparticles causing damage is yet unexplored due to limited knowledge in this field. ROS are produced as a consequence of cellular toxicity caused by the nanoparticle.

Nanoparticle also causes point mutations or single or double strand break as a result of ROS [21]. Previous studies have reported chromosomal aberration in onion root tip as a result of ZnO nanoparticle treatment [22]. Plant stress tolerance is correlated to the level of stress exposed to them; this stress is usually controlled by antioxidant levels in plants. Plant tissues have an average concentration of H_2O_2 in leaves between 0.1 and 5 μmol/gFW. Fluctuations in the external or internal environment are sensed by abruptly leading to rapid turnover of H_2O_2 [23]. Thereby, a suitable quantity of H_2O_2 accumulation defines its function—to either cause cell toward death or tolerance in response to stresses. Therefore, plants have an active system to counter free radicals [24]. Heavy metals are studied to increase the activity of the anti-oxidant enzyme in plants mainly Ascorbate peroxidases and Catalases [25]. Treatment of Ag nanoparticle on Alfalfa decreases salt stress, by elevating the activity of Catalases, Peroxidases, and Superoxide dismutase [5]. However, activation of antioxidant enzymes requires the accumulation of high concentration of TiO_2 to cross the threshold, otherwise, low concentration fails to generate any antioxidant response [7].

4. Conclusion

Nanotechnology plays an important role in the industrial revolution. In the future, its importance can never be overlooked in exploiting new applications in the food and agricultural industry. Impact of nano-toxicity is size and concentration-dependent that causes its accumulation in plants, animals, and ecosystem. Moreover, determining the adverse impact of nanoparticles in edible plants is a rising concern for the scientific community. The studies indicate the toxic impact of nanoparticles on plant development, whereas an elevated level of antioxidants in plants combats to reduce the effect of phytotoxicity. Therefore, nanotechnologies cannot be claimed safe for health due to the risk associated with chronic exposure of nanomaterial. Although, it's long term effects in phytotoxicity and human health are yet to be identified and should be considered seriously. Cost-effective strategies are needed to design biodegradable and non-toxic nanoparticles to prevent prolonged adverse effects on the ecosystem.

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