International Journal of Plant and Environment
Vol. 4, No. 1 (January, 2018), 34-40

Nanotechnology: A New Approach for Reducing Heavy Metal Toxicity in Plants

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Publication Info

***Article history*:**
Received: 18.11.2017
Accepted: 01.01.2018
DOI: https://doi.org/10.18811/ijpen.v4i01.12419

***Key words*:**
Engineered nano-particles
Heavy metals
Plants
Translocation
Uptake

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Abstract

Nano-technology becomes a key technology of 21st century due to endow with immense and wide range applications in several interdisciplinary research fields. Ample literature is available on the individual effect of nanoparticles (NPs) and plant responses, whereas, they also interact with heavy metals (HMs) present in the environment. To date, very limited information is available on the systematic investigation regarding the probability of plant exposure to NPs under heavy metal stress conditions, particularly in context to the use of engineered NPs (ENPs) for reducing heavy metal toxicity. Furthermore, there is a need to explore the interactive mechanism of NPs-metals (NPs-HMs complex) including their uptake, localization, and fate in plants at physiological and molecular level. In this brief review, an attempt has been made to provide some aspects related to the possible mechanism of ENPs in the alleviation of metal toxicity in plants. Besides, an update on ENPs characterization, synthesis, uptake, and translocation was also discussed. This review will help to understand the nature of NPs-HMs complex and its potential to design innovative technologies over heavy metal free ecosystem development.

1. Introduction

Heavy metals (HMs) enter into the environment as a coupling consequence of geological changes, anthropogenic activities and population growth rate (Sahay and Gupta, 2017). Plants take up metals continuously from contaminated/polluted soil, water and air, and have a great impact on the ecosystem and human health through the food chain and food web (Gall *et al*., 2015). Although, some of the metals are beneficial at low concentration, always exerts harmful effects on plants if accumulated in excess. For instance, excessive/elevated uptake of heavy metals (viz., Cr, Pb, Ni, Cd, As, Se among others) by various crops such as mustard, rice etc., showed interruption in metabolic functions and imbalance homoeostasis of essential elements, resulted into reduced growth and productivity (Gupta and Ahmad, 2014; Shahid *et al*., 2014; Gupta and Gupta, 2015).

On the other hand, nanotechnology is a technological branch of science through which individual atoms, molecules or super molecules of certain materials undergo a manipulation by physical, chemical and biological processes to create specific properties for a particular application. Nanoparticles (NPs, also known as nanoscale particles, nanocrystals or nanopowders) evolved as fascinating device and have gained much importance since last few decades in the research field of nanoscience or nanotechnology, besides several interdisciplinary fields of physical science, chemical science, bioscience and biomedicine including agricultural sector (Campos *et al*., 2014; Handford *et al*., 2014; Jampilek and Kralova, 2015). As such, NPs endowed with wide-range applications such as in waste water treatment, cancer therapy, cosmetic and food industries, targeted drug delivery and biosensors. NPs are simply small dimension structured particles which is ultrafine (microscopic), covering a size ranging from 1 to 100 nm (strictly not more than 100 nm), and act as an entire unit while considering its transport and properties. Plants are primary producers and fundamental part of all ecosystems. Like heavy metals, NPs also interacted with plants and its environmental compartments such as soil, air and water sources (Ferry *et al*., 2009; Cornelis *et al*., 2014). Plants accumulated NPs may reach to living kingdom (human being and animals) through the food chain and thereby may affect entire biodiversity (Siddiqui *et al*., 2015). As such, the bio-accumulation of NPs in plants reported to induce both positive and negative effects from seed germination to genotoxic level by altering morpho-anatomical, physiological, biochemical, and genetic constituents of plants. However, the NPs toxicity in plants is assumed to depend upon chemical composition, chemical structure, surface structure, size, shape, surface area among others viz., plant species, plant age etc., as reviewed recently for plant responses to different metal/ metal oxide nanoparticles (Arruda *et al*., 2015; Tripathi *et al*., 2016, 2017; Du *et al*., 2017; Rastogi *et al*., 2017; Ruttkay-Nedecky *et al*., 2017; Siddiqi and Husen, 2017; Zuverza-Mena *et al*., 2017). Furthermore, the NPs phytotoxicity is also affected by the aggregation/agglomeration or individual nature of NPs, but in plant it needs to be explored. Although, plants possess detoxification strategies, the exact interaction of NPs-plants during phytotoxicity and plant defense mechanism is still unknown, and required additional research. A good understanding of the mechanism of NPs phytotoxicity is important for the targeted application of nanoparticles, with special reference to heavy metals.

2. Nano-Particles Occurrence and Applications

In environment, NPs are found mainly with two types of occurrence i.e., non-engineered and engineered. Non-engineered NPs (NENPs) are derived by natural incidents (weathering, terrestrial dust storms, erosion, volcanic eruption, wild fires, microbial activities among others) (Lead and Wilkinson, 2006), while engineered NPs (ENPs) are manufactured intentionally by artificial (man-made) mode of processes, using different organic (protein, polysaccharides etc) and inorganic materials (metals, carbon, aluminosilicates, alginates etc) (Iravani *et al*., 2014). These particles occur in a free state or as an aggregate or agglomerate. From the literature survey, we noticed that most of the studies reflected research on ENPs in the field of plant sciences. It might be due to that ENPs are increasingly manufactured and can be designed as magic bullets which enable to deliver agrochemicals to targeted tissues in a controlled manner (means “on demand” and “on command”), as well as simultaneous protection of agrochemicals from any type of damage by external agents. Thus, ENPs-encapsulated fertilizers (nano-fertilizers) have been proposed to functionalize ideally by releasing the nutrients in accordance to plant demands or as required by plants. Additionally, ENPs also offers to deliver target specific biomolecules (eg: nucleotide, proteins among others) and mediated successful genome editing into plant tissues, leading to the generation of precisely modified but not transgenic plants. Besides, antimicrobial, antiviral, antifungal, antipesticide, anticancer, antiparasitic activity and chemical sensing activity of some ENPs have made them as major ingredients in agricultural products, however, its role in reducing the toxic level of chemicals or metals in plants remains unexplored. The various applications of ENPs have been demonstrated in the recent publication by Duhan *et al*. (2017) and Prasad *et al.* (2017). In this review, we have discussed engineered nanoparticles of metals, non-metals, and metal oxide with respect to their characterization, synthesis, transport and their application in plants.

3. Characterization and Synthesis of ENPs

Till date, several techniques have been explored in the ENPs characterization (such as composition, size, shape including resistivity, electrical conductivity among others). The analytical techniques which have been used by several scientists in NPs including ENPs investigation included atomic force microscopy (AFM), transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction (XRD), dynamic light scattering (DLS), ultraviolet-visible (UV-Vis) spectroscopy, X-ray fluorescence microscopy (XFM), fourier transform infrared spectroscopy (FTIRS), high-performance liquid chromatography (HPLC), inductively coupled plasma mass spectrometry (ICP-MS), and other related techniques. All these techniques are based on separation, microscopic and spectroscopic methods and have different purposes and applications. Among these, spectroscopic techniques (viz., UV-Vis and FTIR) are the most efficient and frequent used techniques to characterize NPs in plants. A complete list of analytical techniques and their purposes, advantages and limitation associated with different NPs parameters has already been detailed in the recent publications (Zhao *et al*., 2014; Arruda *et al*., 2015; Wang *et al*., 2017).

4. Engineered Nanoparticle Delivery System in Plants

Plants growing in the presence of nanoparticles may absorb, translocate and accumulate the various type of ENPs in different plant tissues (Fig. 1A). It has been reported that ENPs have an ability to enter and accumulated in various plant tissues (Raliya *et al*., 2015) through above or below ground organ (shoot and root) tissues (root tips, rhizodermis, lateral root junction, epidermis, stomata, cuticles, trichomes, hydathodes, wounding among others) (Fig. 1A). Generally, the uptake and translocation of ENPs in plants are considered by the active transport mechanism by apoplastic (root epidermis) and symplastic (vascular bundle) movement of NPs (Fig. 1A), but it remains unclear that which one is more important. Zhu *et al*. (2008) carried the first study on uptake, translocation and accumulation of nanoparticles in cucumber. Uptake and translocation of ENPs by plants is recently reviewed by Wang *et al*. (2017). ENPs follow the two pathways for their uptake and translocation in plants (Ma *et al*., 2015). First pathway takes place from bottom to top (upwards) (Fig. 1A), where roots tissues takes up various sized ENPs, and subsequently translocate and accumulate them in aerial parts (viz., leaves, stem, fruits).

While, second pathway takes place from top to bottom (downwards) (Fig. 1A) where aerial plant parts i.e., leaves takes up the ENPs directly from air-environment and accumulate and transport them to others plant parts like stem or roots. However, plants may follow both the pathways simultaneously either grown hydroponically or in soil medium (Rico *et al*., 2015; Hernandez-Viezcas *et al*., 2016). In upward pathway, NPs comes in contact to pores on cell wall and cell membrane of root hairs and move through a complex series (vascular bundle and stele), and then accumulated into leaves tissues (unidirectional distribution) (Zhao *et al*., 2014). The cell wall and cell membrane due to its semi-permeable nature allow only specific NPs to cross through specific sized and shaped-pores/barriers (Zhang *et al*., 2015). For instance, in apoplastic transportation, cell wall pores/barriers restrict the movement of NPs having size >5-20nm, as suggested by Eichert *et al*. (2008) and Dietz and Herth (2011), while in symplastic transportation, plasmodesmata controls particles up to the size typically 3-50 nm able to enter through it (Dietz and Herth, 2011; Zhai *et al*., 2014). Further, Wang *et al*. (2016) demonstrated that only 20-40 nm sized Cu-NPs were distributed throughout the root and shoot tissues. Deng *et al*. (2014) reviewed the pores of cell wall with their characteristics that restrict the entry of agglomerate NPs. Contrary to above results, it has also been reported that NPs up to 30-60 nm size can also be able to enter into plant cells (Eichert *et al.,* 2008; Larue *et al.,* 2012). One possible reason was assumed that like environmental conditions (such as drought condition), NPs might also be insisting to pores/barriers of different cell parts to change their properties. For instance, ENPs such as nTiO_2, ZnO and nAg have been reported to influenced the cell wall, plasmodesmata and cuticle to induce the formation of additional new and larger pores/barriers accordance to NPs size and shape, and causes structural changes (Kurepa *et al*., 2010; Eckhardt *et al*., 2013; Larue *et al*., 2014). However, it is poorly understood that whether larger sized NPs interact and induce novel pores/barriers in the positive or negative way to facilitate the transfer of NPs in across the plant cell parts. A meter scale defining the size exclusive limits of pores/barrier in different cell parts (viz., cell wall, extracellular space of cell wall, cuticle, stomata, casparian strip, lenticle, wounding, among others) for ENPs uptake (Wang *et al*., 2017). In case of downward pathway, ENPs enter through cuticle, stomata, stigma, trichomes, hydathodes among other routes of leaves and finally redistribute to stem and eventually to roots via phloem (bi-directional distribution) (Larue *et al*., 2014). It is important to know whether intact NPs can be taken up by plants and transported to different plant tissues. The uptake and translocation of various metal, non-metal and metal oxide NPs by different plant parts have been reviewed earlier (Rico *et al*., 2013; Anjum *et al*., 2016).

5. Role of NPs in Reducing Metal Toxicity

It is well-known fact that both NPs and HMs are present in the environment and always ready to accumulate in plants. From the results of several studies, it may be suggested out that the mechanism for ENPs-alleviation of heavy metal toxicity in plants includes the reduction in heavy metal accumulation leads to the inhibition in ROS generation and up-regulation of antioxidant defense and uptake of mineral nutrients which overall provides protection from heavy metal induced oxidative stress. However, it is not vivid and remains to explore that how ENPs interact with HMs and reduces their toxicity into a plant cell. As such, most of the studies have been carried out on the role of ENPs in the removal of heavy metals especially from contaminated soil or water (waste water). Studies showed the characterized ENPs are as a potential tool in phytoremediation i.e., use of nanoparticles in reducing heavy metals induced toxicity by absorbing heavy metal ions pollutant from soil solutions, waste water and in plant tissues at some extent (Dickinson and Scott, 2010; Wang *et al*., 2012; Stietiya and Wang, 2014). For instance, iron/ironoxide nanoparticles (Fe, Fe_3O_4, Fe_2O_3, Fe_2O_3 NPs) has been used as nanoabsorbents and removed As, Cu, Pb, Hg, and Cd from water/wastewater (Dave and Chopda, 2014). Only a few studies have been explored and reported that only some ENPs able to reduce the toxicity but for specific metal(s). A recent publication on iron oxide ENPs has been reported to reduce the toxicity of Pb, Zn, Cd, and Cu in wheat and Indian mustard plants (Konate *et al*., 2017; Praveen *et al*., 2017). The treatment of TiO_2 NPs in rice has been found in reducing the Cd toxicity by restricting its uptake and distribution in rice leaves and roots (Ji *et al*.,

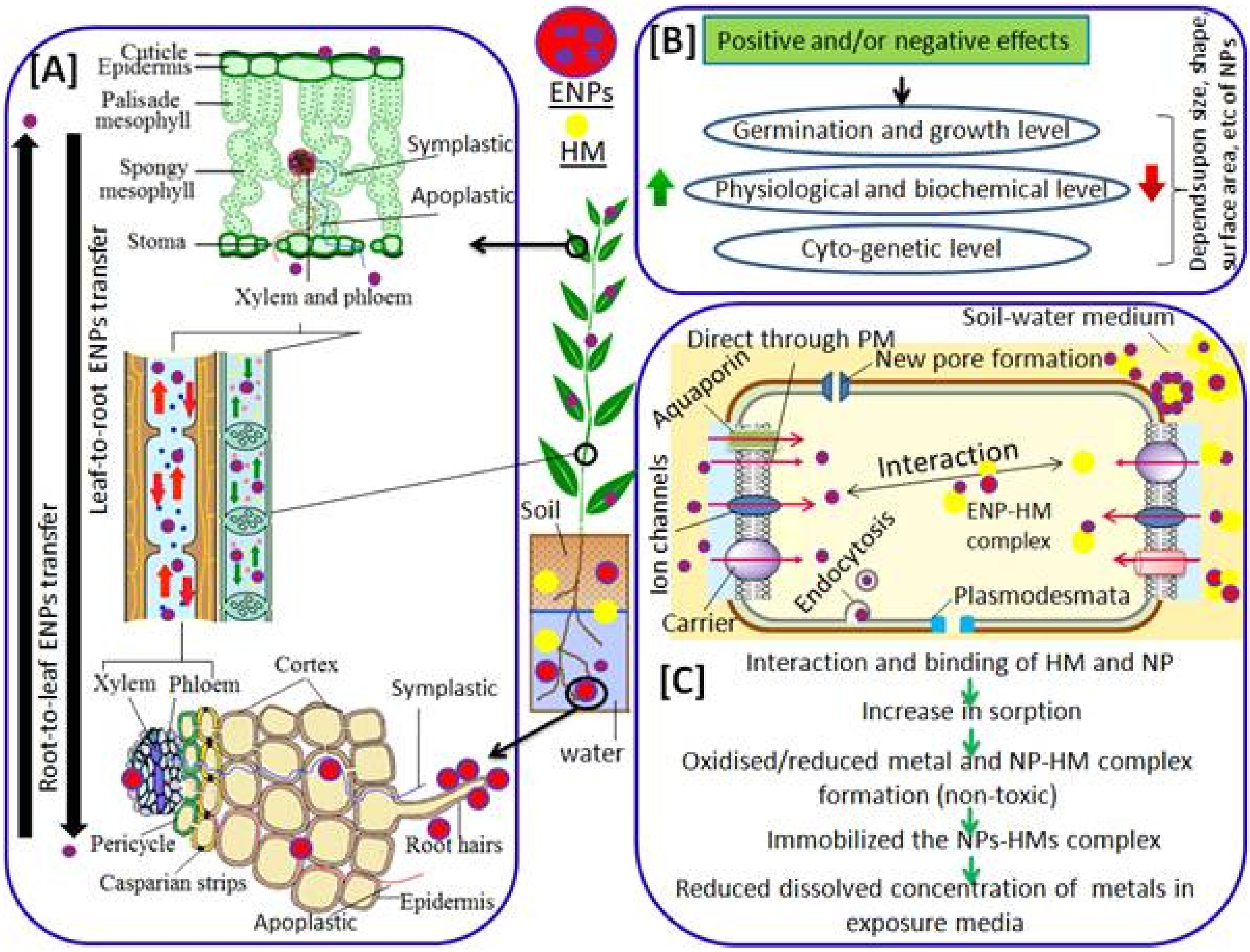

Fig. 1A-C: Schematic representation of ENPs uptake, translocation and accumulation process with upwards (root-to-leaf) and downwards (leaf-to-root) pathways (A), positive or consequential and/or negative effects of NPs (B), and possible mechanism of NPs interaction with heavy metals in order to reduce their toxicity along with possible modes of entrance in a plant cell (C).

2017). Additionally, Venkatachalam *et al.* (2017) reported that ZnO NPs has potential to reverse the oxidative stress induced by Cd and Pb treatment in *Leucaena leucocephala* seedlings.

The expected mechanism of interaction of ENPs with heavy metals to reduce possible metal toxicity in plants is showed in Figure 1C. In contaminated soil or water, ENPs reduces the heavy metal concentration by their adsorption process, where ENPs taken up or hold the HMs and produces ENP-HM complex. The resulted ENP-HM complex might be expected to less toxic than free-state metal ions release either from metal treatment or NPs itself. Increase in adsorption leads to increase in their surface areas and make ENP-HM complex enable to enter through various types of barriers/pores of the plasma membrane and cell wall, thus reduce the dissolved concentration of free heavy metals ions in exposure media. Use of ENPs is the way to lower the chance for metals accumulation in plants growing in contaminated environment. As such, it might be possible that some concentration of heavy metals, as well as ENPs, may become enter into plants prior to their binding together. In this condition, it is interesting to mention that ENPs may also be interacting with HMs in the similar way in a plant cell and producing NPs-HMs complex (less toxic form), thus ENPs decreases the intracellular content of heavy metals. Further, it is also possible that some of ENPs-

HMs complex may success to enter into a plant cell, which depends upon the type of interaction of ENPs with heavy metals. Thus, ENPs interact with heavy metals with two possible phases: single and agglomerate phase. Obviously, when single NP took up or binds heavy metals yields ENP-HM complex with less surface area as compared to resulted from the binding of agglomerate NPs with heavy metals. Finally, uptake, transport and toxicity of NPs-HMs interactive product is very complex process into plant cells and still not well understood in plants, thereby making a current challenge to the scientists to reveal it.

6. Conclusion

Earlier, NPs were considered only as their ability to cross through a membrane (0.45 micron) of plant cell but NPs research is currently an area of intense scientific interest due to increasing number of impressive commercial advantages of these particles in several interdisciplinary fields. Over last two decades, scientists have created interest to focus on the interaction of ENPs-metals and their effect on physiological and molecular mechanism associated with ENPs-alleviation of metal stress, which is limited or still not well known in plants. Furthermore, there is a need to explore the uptake, transportation and localization of ENPs-HMs complexes, and simultaneously most important to know whether the binding complexes are really non-toxic or may also cause toxicity to plants. Therefore, understanding the interactive nature of NPs and heavy metals may have great potential to offers innovative technologies over heavy metal free ecosystem development.

Acknowledgement

Author SS thanks to University Grant Commission (UGC) for financial assistance in the form of Post-Doctoral Fellowship under Grant No. F./PDFSS-2015-17-UTT-12296.

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