



Enthralling the impact of engineered nanoparticles on soil microbiome: A concentric approach towards environmental risks and cogitation

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ARTICLE INFO

Edited by: Professor Bing Yan

Keywords:

Nanoparticles
Toxicity mechanism
Rhizosphere activities
Soil-ecosystem
Micro-organisms
Risk assessment
Mitigation approaches

ABSTRACT

Nanotechnology is an avant-garde field of scientific research that revolutionizes technological advancements in the present world. It is a cutting-edge scientific approach that has undoubtedly a plethora of functions in controlling environmental pollutants for the welfare of the ecosystem. However, their unprecedented utilization and hysterical release led to a huge threat to the soil microbiome. Nanoparticles (NPs) hamper physicochemical properties of soil along with microbial metabolic activities within rhizospheric soils. Here in this review shed light on concentric aspects of NP-biosynthesis, types, toxicity mechanisms, accumulation within the ecosystem. However, the accrual of tiny NPs into the soil system has dramatically influenced rhizospheric activities in terms of soil properties and biogeochemical cycles. We have focussed on mechanistic pathways engrossed by microbes to deal with NPs. Also, we have elaborated the fate and behavior of NPs within soils. Besides, a piece of very scarce information on NPs-toxicity towards environment and rhizosphere communities is available. Therefore, the present review highlights ecological perspectives of nanotechnology and solutions to such implications. We have comprehend certain strategies such as avant-garde engineering methods, sustainable procedures for NP synthesis along with various regulatory actions to manage NP within environment. Moreover, we have devised risk management sustainable and novel strategies to utilize it in a rationalized and integrated manner. With this background, we can develop a comprehensive plan about NPs with novel insights to understand the resistance and toxicity mechanisms of NPs towards microbes. Henceforth, the orientation towards these issues would enhance the understanding of researchers for proper recommendation and promotion of nanotechnology in an optimized and sustainable manner.

1. Introduction

The exploration of engineered nanoparticles (NPs) in different realms has observed sky-rocketed growth owing to its unique characteristics. Nanotechnology has gained attention due to its multifaceted applications in environmental, agricultural, and related sectors such as

industries and medical applications (Biswas and Wu, 2005; Raffi and Husen, 2019). Nanoparticles are classified into inorganic metal-based NPs, metal oxides, quantum dots, carbon-based and organic NPs (Rajput et al., 2018a, 2018b). The extensive usage of NPs is observed because of their potential roles in remediating soil contamination in the ecosystem (Zhou et al., 2021). All these factors led researchers to

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<https://doi.org/10.1016/j.ecoenv.2021.112459>

Received 8 April 2021; Received in revised form 6 June 2021; Accepted 23 June 2021

Available online 30 June 2021

0147-6513/© 2021 The Authors.

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develop NPs for improving agricultural yield, control pollution, and biotechnological perspectives (Tripathi et al., 2018a, 2018b). The supplementation of NPs accompanies this into soils for remediation purposes that eventually ends up raising concerns against microbial communities. As a result of recalcitrant nature, they accumulate in the environment, ultimately hampering the physiological and metabolic activities of living organisms (Tripathi et al., 2017). This is mainly accompanied by generation of reactive oxygen species and oxidative stress markers within the cells to attack the functional activities of living organisms (Ahmad et al., 2010, 2019; Kohli et al., 2019). It has been observed that NPs directly attack the cell membranes causing lipid peroxidation along with stimulating the levels of free radicals. Therefore, the main mechanism behind the NP toxicity lies in the generation of oxidative stress that hinders the cell activities abruptly (Buffet, 2014; Buzea et al., 2007).

Moreover, due to their beneficial aspects towards controlling industrial and heavy metal pollution, and improving agricultural activities and other medical applications on massive scale, ignorance was observed in their constant accumulation in soils. Besides finding suitable solutions to such problems, we have surfaced major concerns with NPs regarding toxicity, stress, and accumulation that impede the environment and its inhabitants (Singh et al., 2016). Therefore, this promising advancement must be balanced to rule out unforeseen adversities towards soil ecosystem and rhizospheric communities. The potential hazards on microbial activities are relatively emerging due to NPs deposition within soils required to be understood. For instance, the potential hazards caused by NPs towards microbes includes their clearance from the rhizosphere and hindering their biological activities such as nitrogen fixation, phosphate solubilization, potassium uptake, nodulation and hormone synthesis. It has been found that NPs impair the outer cell wall of microbes and degrade it to further invade the cell and disrupt the cellular and biochemical functions inside the cells. Furthermore, they also impede the mitochondrial machineries, cell signaling mechanisms and cause cell apoptosis (Ameen et al., 2021). It is a matter of concern that the fertility of soils in terms of productivity has continuously deteriorated. However, there is an urgent need for risk assessment of soils polluted with NPs to conserve their fertility.

Soil comprises a set of services that work in accordance with ecological engineers, microbes and they also work as indicators to suspect soil perturbations (Holden et al., 2014). The critical factor is the availability and adversity of NPs towards rhizospheric activities, including biota residing within this zone. All biological processes (nitrogen fixation, phosphate solubilization, symbiotic associations, potassium uptake, nodulation, ionic homeostasis, mineral acquisition etc.) within the rhizosphere, including biogeochemical cycling, are worse affected by NPs deposition (Huang et al., 2005). Furthermore, they can penetrate deeper regions of soils and may enter the food chain (Nair and Chung, 2014). They penetrate food chain from plants towards animals and other living organisms. Through absorption from soil, they enter the plants via vascular tissues and translocate towards different organs. Later, from plants they enter to animals and humans through consumption in the form of food (Nair and Chung, 2014). Therefore, it is of paramount importance to acquire about NPs behavior within soil ecosystem for evaluating the risk associated with different communities. As soil serves to be a sink of NPs, many tiny NPs are sorbed onto soil particles, translocated to underground water sources, or may cause leaching or transformation into toxic forms (Boxall et al., 2007). The NP-toxicity is often depicted in terms of oxidative stress generated after the contact of NPs with microbes, followed by the membrane disruptions, oxidation, lipid peroxidation, and impeded energy mediated pathways (Dinesh et al., 2012; Abbas et al., 2020a). Alongside, the reactive oxygen species (ROS)-generation disrupts the mitochondrial apparatus, cell membrane, ATP-synthesis, and DNA replication within organisms (Moreno-Garrido et al., 2015). This ROS-mediated oxidative stress is primarily cause of nano-toxicity towards physiological, biochemical and molecular alterations (Abbas et al., 2020a). However,

there are an array of defense-related mechanisms possessed by microbes to combat NP-toxicity.

To wisely use nanotechnology in different fields, it is substantial to understand its phytotoxicity, cytotoxicity, ecotoxicity and genotoxicity, and interactions with soil and their residents. Within soils, the easiest target for NPs is to affect soil physicochemical properties, fertility, and microbes. Microbes play a synergistic relationship in the soil in maintaining soil functioning, soil structure, organic matter, degradation of toxins, and nutrient cycling. Also, they are remarkably effective in inhibiting soil-borne pathogens from mediating plant growth (Karimi and Fard, 2017). Any disruption in soil composition and soil microbes due to NPs could limit their well-designed soil integrity. To illustrate nitrogen fixation, phosphate and potassium solubilization, siderophore production, and related processes are induced by microbes and can be hampered due to NPs. Henceforth, it is essential to protect the environment and growth-promoting microbes from NPs (Siddiqi and Husen, 2017). And for this, scientists should play an active role in examining NPs repercussions towards microbes. It is pertinent to mention that the entire NP-cycle from production to synthesis generates massive ecological risks throughout the process. Currently, it is pretty intricate to examine the NP-levels in the ecosystem because of some drawbacks. Therefore, a model should be developed to monitor NPs fate within the environment (Patil et al., 2016). As the plethora of studies prevail with both positive and negative outcomes, we should find a possible way to overcome their negative aspects for their judicious use. A limited information related to NP-toxicity and environmental risks, is available hence, it is a need of the hour to shed light on this aspect for safeguarding the environment. The ecotoxicological assessment allows us to assess the role of NPs towards microbial communities for better risk analysis. There are numerous studies that have been conducted so far regarding the positive impact of NPs in the environment but this substantial issue has not been given so much importance. In the present review, we have mainly focused on NPs, and its adverse effects towards soil-ecosystem along with the aspects of dealing with this issue in a sustainable manner. We have also explained the mechanisms underlying NP-toxicity and their impact on rhizospheric activities and micro-organisms. Apart from this, the major concern with NPs related to mitigation of NP-toxicity from the environment has also been discussed in this review. So, present information will enhance the understanding with this regard to pave a new path for establishing differential strategies with this issue.

2. Sources and types of nanoparticles in environment

NPs are ubiquitous entities, and their origin is categorized as either natural or anthropogenic. These are created by numerous physical processes ranging from erosion to fuel combustion. The NPs present in the air are referred to as ultrafine particles, whereas those present in water and soil are colloids, possessing a slightly variable size range (Klaine et al., 2008). Naturally occurring NPs are present in the environment since planet earth was created and are extensively distributed in the atmosphere, oceans, soil, ground and surface water, and living organisms (Bundschuh et al., 2018). Volcanic eruptions, forest fires, photochemical reactions, soil erosion, dust storms, radioactive decay, and atmospheric nucleation are the major natural processes resulting in the release of NPs into the environment (Bundschuh et al., 2018; Sharma et al., 2019). Frequently occurring hair and skin shedding in insects and animals also contributes to nanomaterials in nature (Jeevanandam et al., 2018). In urban areas, anthropogenic emissions, mainly industrial and vehicular along with occupational procedures, are the primary source of environmental NPs (Griffin et al., 2018). An estimate suggests that naturally occurring NPs, formed during biogeochemical processes, are present in the range upto several thousand teragrams/year compared to the mass of engineered NPs ranging between several hundred to thousand teragram/year (1 Tg = 1 million metric tons) (Sharma et al., 2015). Because of natural abundance and

relatively easy culturing methods, microbial resources have emerged as a new horizon for NP-synthesis. From simple prokaryotic organisms to complex eukaryotes like bacteria, fungi, algae, yeasts, actinomycetes, and viruses, all can be easily employed and harvested for fabricating NPs (Prasad et al., 2016; Sharma et al., 2019). The NPs synthesized in such ways possess the high catalytic activity and can sustain varying environmental conditions. Furthermore, NPs can be designed in various shapes and sizes in comparison to other sources. There are numerous types of NPs that have been synthesized by different processes due to their relevance in each and every field. For example, Cu-NPs, ZnO-NPs, Ag-NPs, Au-NPs, Se-NPs, Pt-NPs, Te-NPs, Pd-NPs are most commonly used NPs in different sectors. The different types of NPs, their production, size/shape and their sources are summarized in Table 1.

3. Hazardous effects of NPs on environment

NPs, since their discovery, have been identified as remarkable material with unique size. Specifically, engineered NPs have seen a tremendous elevation in their intended designing, manufacturing, and most imperatively integration into plants owing to their novel physiological features (Buchman et al., 2019). In recent decades, considerable advancement enhanced understanding about underlying mechanisms of influence of NPs towards the environment. In the initial years of research, studies were aimed at elucidating the impact of nanomaterial on model organisms. Presently, there has been a shift of interest towards establishing an understanding of mechanisms involved in toxicity associated with NPs to more environmentally pertinent organisms (Qiu et al., 2018; Ke et al., 2018; Hao et al., 2020). The most imperative aspect in regard to NP toxicity is its concentration and persistence within the environment. The different types of NPs present in the environment are quite specific in their value of causing toxicity. Like, the concentration of Ag-NPs from 6×10^{-8} to 6×10^{-4} mgL⁻¹ are lethal that are generated from industries (Lee et al., 2012). The waste management practices also emit NPs within a range of 0.1–1.3 mgL⁻¹. An abundant amount of NP accumulation is observed in the environment, for example TiO₂ accumulate through effluents within the range of 75–100 µg L⁻¹ respectively (Mitrano et al., 2014). The usage of personal care products also emit NPs in some range, such as TiO₂-NPs ($0.87-1 \times 10^3$ metric tons/year) and ZnO-NPs ($1.8-2.1 \times 10^3$ metric tons/year) that

represents nearly 94% of total discharge within the environment. Moreover, this discharge is mostly observed in landfills (80–90%), in air (0.7–0.8%) and in water bodies (24–36%) (Keller et al., 2012). It has been documented that ZnO-NPs within the range of 76–760 µg L⁻¹ in water and 3.1–31 µg L⁻¹ causes adversities and risk to living population (Ghosh et al., 2016).

It was revealed by Kuhlbusch et al. (2011) that the most significant shortcoming of current monitoring approaches is the lack of delineation of background particles present along with NPs. The knowledge regarding aggregation rate and nanomaterial deposits is relatively scanty due to the multifaceted nature of the system and deficient instrumentation for quantifying characteristics. Additionally, to comprehend hazards associated with NPs is directly related to dose-dependent response to nanomaterial and duration of exposure, consequently determining the entry of NPs in model organisms (Kuhlbusch et al., 2011). Fig. 1 elucidates the impact of NPs on microorganisms and plants.

Conventional researches revealed that NPs harmed pure cultures of *E. coli* (Auffan et al., 2008), *Bacillus subtilis* (Diao and Yao, 2009) and *Pseudomonas fluorescens* (Lee et al., 2008). It was affirmed by research groups that oxidation of nZVI (nano-zerovalent iron) resulted in ROS-production in living cells. Moreover, Lee et al. (2008) further suggested that Fe⁺-ions enter cells which subsequently cause redox imbalance resulting in: i) disruption of membrane structure, ii) intracellular materials leakage, and iii) impairment of the biochemical process that ultimately causes cell death. It was revealed by Ma et al. (2013) that nZVI showed phytotoxicity in *Typha latifolia* and hybrid poplars (*Populus deltoids* × *Populus nigra*). nZVI notably lowered transpiration rates, growth, and development of hybrid poplar plants. Moreover, at lower levels, the growth and development of phytoplanktons, zooplanktons, and earthworms were significantly down-regulated by nZVI-exposure (El-Temsah and Joner, 2012). Few studies affirmed toxic influences of nanomaterial on microorganisms, CuO and Fe₃O₄ presence altered soil microbial population in the vicinity of exposed soil (Ben-Moshe et al., 2013). Contrastingly, Fajardo et al. (2012) and Tong et al. (2007) suggested the diminutive impact of nZVI and C₆₀ on microbial community and cellular viability of microbial cells. They further revealed that NP-toxicity is directly related to solubility and bioavailability of specific nanomaterial. Garner and Keller (2014) recommended

Table 1
Various types of nanoparticles and their synthesis.

NP-type	Microbial source	Production mode	Substrate employed	Size (nm)	Shape	Reference
Cadmium sulfide	<i>Shewanella oneidensis</i>	Extracellular	Sodium sulfide+cadmium chloride	2–6	Spherical	Zhuravliova et al. (2018)
Copper	<i>Pseudomonas fluorescens</i>	Extracellular	Copper sulfate	49	Hexagonal, spherical	Shantkriti and Rani (2014)
Copper	<i>Salmonella typhimurium</i>	Extracellular	Copper nitrate	40–60	Not identified	Ghorbani et al. (2015)
Gold	<i>Streptomyces griseoruber</i>	Extracellular	Chloroauric acid	5–50	Spherical, triangular, hexagonal	Ranjitha and Rai (2017)
Gold	<i>Stephanopyxisturris</i>	Intracellular	Chloroauric acid	10–30	Triangular, spherical	Pytlik et al. (2017)
Gold	<i>Tricholomacrassum</i>	Extracellular	Chloroauric acid	5–25	Hexagonal, cuboidal, triangular,	Basu et al. (2018)
Lead sulfide	<i>Rhodospiridium diobovatum</i>	Intracellular	Lead nitrate	2–5	Spherical, cubical	Seshadri et al. (2015)
Platinum	<i>Saccharomyces boulardii</i>	Intracellular	Chloroplatinic acid	80–150	Spherical	Borse et al. (2015)
Palladium	<i>Desulfovibrio desulfuricans</i>	Intracellular	Sodium tetrachloropalladate	0.2–8	Icosahedral	Omajali et al. (2015)
Selenium	<i>Streptomyces bikiniensis</i>	Extracellular	Selenium dioxide	17	Nanorods	Ahmad et al. (2015)
Selenium	<i>Vibrio natriegens</i>	Intracellular	Selenite	100–400	Spherical	Fernández-Llamas et al. (2017)
Silver	<i>Oscillatoria limnetica</i>	Extracellular	Silver nitrate	3.3–17.93	Quasi-spherical	Hamouda et al. (2019)
Silver	<i>Aspergillus fumigatus</i>	Extracellular	Silver nitrate	0.681	Cubical	Shahzad et al. (2019)
Silver	<i>Bacillus licheniformis</i>	Extracellular	Silver nitrate	7–31	Spherical	Deljou and Goudarzi (2016)
Tellurium	<i>Aspergillus welwitschiae</i>	Extracellular	Potassium tellurite	60.80	Oval, spherical	Elsoud et al. (2018)
Zinc sulfide	<i>Shewanella oneidensis</i>	Extracellular	Sodium sulfide+zinc chloride	2–6	Spherical	Zhuravliova et al. (2018)
Zinc oxide	<i>Rhodococcus pyridinivorans</i>	Extracellular	Zinc sulfate	100–120	Hexagonal	Kundu et al. (2014)
Zinc oxide	<i>Lactobacillus plantarum</i>	Extracellular	Zinc sulfate	124.2	Not identified	Yusof et al. (2020)

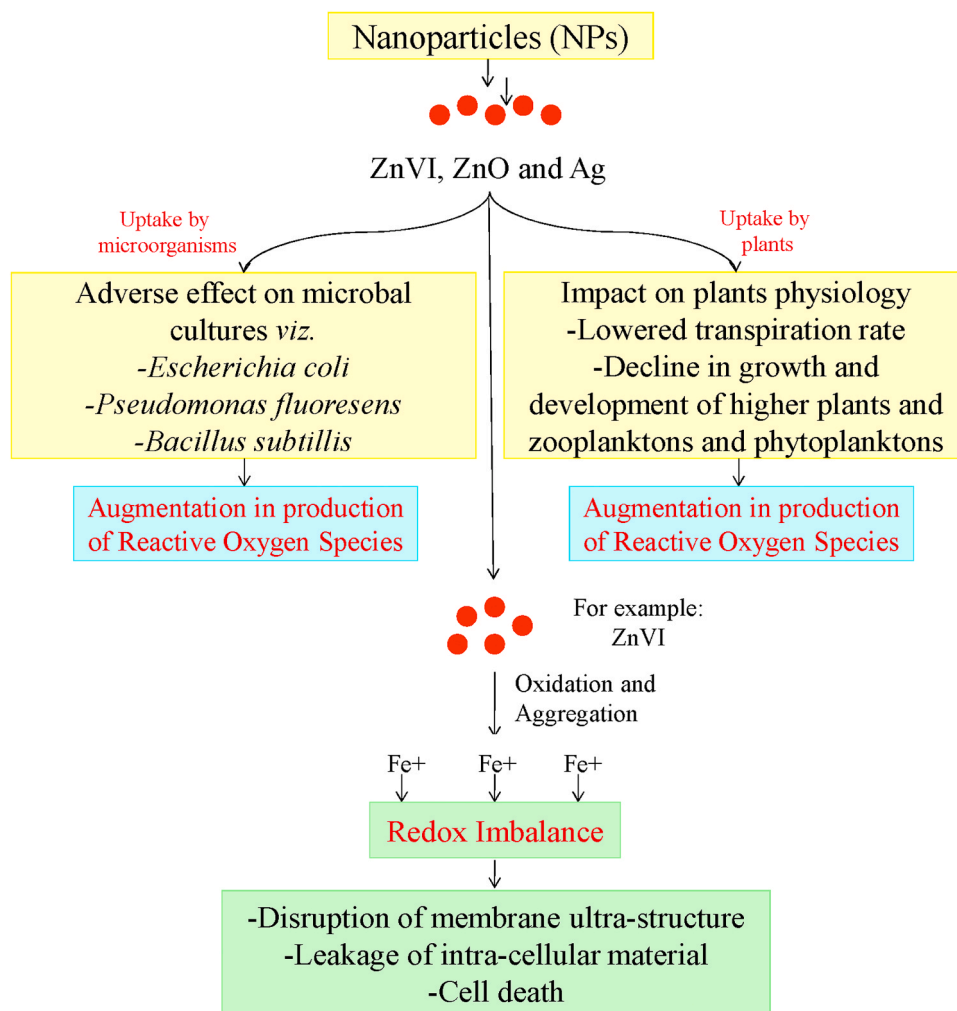


Fig. 1. Impact of nanoparticles (NPs) on microorganisms and plants. Uptake of NPs occurs in microbes (*E. coli*, *P. aeruginosa*, *B. subtilis*) and plants that negatively affected physiological characteristics, transpiration, growth and development followed by the generation of reactive oxygen species to impair redox homeostasis. These disturbances causes membrane disruption, leakage of cellular contents and cell death.

that if existing production and discharge of NPs was elucidated to augment to 100-folds, then nZVI, ZnO, and silver (Ag) are of prime concern.

In addition, plants have also been noticed to be adversely affected by NPs depending upon their size, shape and chemical nature they are translocated through xylem to different plant organs (Rajput et al., 2018a, 2018b). They further cause toxicity in plants through dissolution inside the cell followed by ROS generation. The threshold levels to cause phytotoxicity range between 200 and 500 mg kg⁻¹ approximately, and this accumulation reduce crop productivity and enter food chain to harm human health (Rajput et al., 2018a, 2018b). Reduction in the seed germination, root elongation are affected the most, due to the fact that NPs may enter the root tissues followed by aboveground parts (Rajput et al., 2020). Plants accumulate NPs in metal ionic form and tend to reduce shoot/root length, biomass, nitrogen fixation and cause necrosis in plants (Cota-Ruiz et al., 2018; Wang et al., 2015). They also affect the root architecture, cortical cells, epidermal tissues, vascular cylinder and ultrastructure of cell organelles (plastids, mitochondria, protoplasm, chloroplast, cell wall, plasma membrane etc.) (Rajput et al., 2018a, 2018b). Therefore, all these indications limit the plant performance by affecting the substantial plant processes like photosynthesis, and plant-water relations. Furthermore, NPs also negatively affects the human by penetrating into their cells via cell wall or membrane (Anreddy, 2018). NP accumulation causes accrual of mitochondrial superoxide anions, leading to its dysfunction and they also gets deposited

in lysosomes followed by the activation of caspase-independent cell death mechanism to cause NP-mediated phytotoxicity (Zhang et al., 2018). Alongside, they also enhance the ROS levels within cells to mediate oxidative stress. It has also been noticed that NP contamination in soil can directly enter the human gastrointestinal tract through food chain and further impact the cell viability, morphology, integrity and production of immune cells respectively (Ude et al., 2017). Enhanced levels of ROS also mediate epigenetic changes in cells that primarily affect DNA methylation affecting the entire genome (Lu et al., 2016). ROS generation is also associated with membrane blebbing, reduced cell viability, cytotoxicity, reduced inflammatory response etc. (Umar et al., 2019; Wang et al., 2018).

Therefore, it is imperative to monitor the discharge and impact of NPs on the environment with a specific focus on nZVI present in the groundwater and soil (El-Temsah and Joner, 2012).

3.1. Mechanism associated with NP-toxicity

Important knowledge obtained regarding the NPs-toxicity mechanism will aid in the intended re-modeling of nanomaterials to lessen the influence on the environment. The chief mechanisms associated with nanotoxicity include stressing cells by directly binding onto the exterior membranal surface, dissolution of toxic ions, and stimulating oxidative stress (Djurišić et al., 2015). After elaborating the nanotoxicity mechanism, we have elucidated the effect of NPs on rhizosphere activities,

NPs-associations with micro-organisms, and mitigation approaches for NP-toxicity in the environment, thus providing a better understanding of reducing toxicity and endorsing sustainable employment of NPs in the future.

3.1.1. Cell binding mechanism

Amongst several pathways, direct binding of NPs with cell-surface is a major toxicity-inducing mechanism. Interestingly, NPs when present in close vicinity of cells or organisms, the interaction between them is dependent upon electrostatic attractions. Usually, a bacterial cell is observed to exhibit a negative charge on the surface (Dickson and Koochmarai, 1989). It was evident from the above observation that positively charged-NPs are directly associated with bacteria rather than negatively charged-NPs. In the case of multicellular model organisms, such interplay might result in NP-uptake by a specific set of cells (Fabrega et al., 2011). In bacterial culture, NPs stay on the cell surface, causing damage to the membrane due to removal or disruption of membrane lipids (Mensch et al., 2017). Such an alteration in physical features of membrane subsequently leads to stimulation of internal signaling cascade that disrupts cells (Hussain et al., 2014). The NPs are then dissolved to discharge cell-permeable ions (toxic in nature) on the surface of cells via toxic pathways (von Moos and Slaveykova, 2014; Ameen et al., 2021).

In a study by Feng et al. (2015), it was revealed that AuNPs (gold NPs) were chelated with compounds with positive charge, viz. mercaptopropylamine or poly(allylamine hydrochloride), i.e., PAH or with compounds functionalized with mercaptopropionic acid carrying a negative charge. It was demonstrated that AuNPs are bound to the cell surface of bacteria as observed by transmission electron microscopy. Results further revealed that in certain images, the lipid-bilayer of the membrane was entirely extracted and bound to the AuNPs. Flow-cytometric analysis of bacterial culture also revealed that binding with negatively charged AuNPs was minimal or negligible compared to positively charged. Additional experimental evidence was provided by Jacobson et al. (2015), which suggested that the association of NP with gram-negative bacteria was probably due to the binding of negatively charged moieties on the cell surface with lipopolysaccharides. The NPs bound to the cell surface caused disruption and injuries to the membrane, subsequently resulting in cell death. Moreover, another study revealed that poly-(diallyldimethylammonium chloride) coated with cadmium-selenide (CdSe) quantum dots (colloidal semi-conductor nanocrystals) were found embedded in lipid-bilayer was demonstrated by employing atomic force microscopy and quartz crystal microbalance instrumentation (Mensch et al., 2018). Attachment of NPs to cells results in disruption of cell-membranes by collapsing hydrophilic domains of lipid-bilayer. These liquid domains are imperative for signaling and membrane transportation in eukaryotic as well as prokaryotic cells (Abbas et al., 2020a, 2020b; Ameen et al., 2021).

3.1.2. Activation of dissolution of ions mechanism

The most imperative mechanism of toxicity caused by NPs is the dissolution of toxic elements from NPs and elicit oxidative burst in affected organisms. A wide array of ways exist by which toxic ions are released from NPs and are indirectly dependent on the identity of ions released. Few ions bind to critical enzymes and proteins thereafter altering their metabolism and subsequently result in suppression of major cellular processes (Williams et al., 2018). The toxic ions are steadily released from metal oxides and absorbed by membranes resulting in direct association with functional groups of nucleic acids and proteins such as amino(-NH), mercapto(-SH), and carboxyl (-COOH). These associations also have huge effects on cellular structure and enzymatic activities, eventually disturbing the entire physiology of the exposed organism. Another way is by associating toxic ions directly with the phospholipid bilayer of the affected organism or even its genetic material (Dupont et al., 2011). Consequently, metal-ions trigger oxidative burst by elevating ROS in organisms (Abbas et al.,

2020a). In the case of AgNPs, the dominant mode of dissolution of toxic ions is one of the prime causes of toxicity in organisms (Hudson-Smith et al., 2016). Palladium nanolayers, size ranging from 0.4 to 22.4 nm and nanowire, 18–20 nm was prepared on polyethylene naphthalate medium, affirmed to have antibacterial bioactivity attributed to silver and palladium ions released in the solution (Polivkova et al., 2015, 2017). In contrast, Zhang et al. (2010) revealed that only feeble antibacterial activity was shown when metal oxides were present in suspension and added to the culture, suggesting that metal-ion dissolution might not be accountable for the antibacterial activity of NPs.

Dissolution of ions was affirmed to be a key mechanism to cause toxicity of few complex oxides, namely, lithium, nickel, manganese, and cobalt oxides, to bacterial cells of *Shewanella oneidensis* MR-1 (Hang et al., 2016). The researchers employed optical density analysis to determine bacterial proliferation and respirometer to quantify bacterial respiration. This study revealed that complex oxides of nickel, manganese, and cobalt were dissolved to release Li, Ni, Mn and Co ions. However, toxicity observed from these nanomaterials was directly dependent upon continuous dosing of nanomaterials and their release. To further emphasize the significance of dissolution to nickel, manganese and cobalt (NMC) oxides-toxicity, an equistoichiometric NMC was created in varied morphologies and toxicity towards bacterium *Shewanella oneidensis* (Hang et al., 2018). These were selected as they depict various crystal faces, revealing that dissolution depends on variation in crystal faces that affected the transition of metal coordination. As it was affirmed earlier, dissolution is related to materials exposed surface area. The toxicity of NMC of the varied morphologies was also dosed on surface area rather than the mass of NMC. With a surface area as the basis of dose, morphologies showed similar toxicity in bacterial strain with no specific effect of the crystal face on toxicity. Hence, from the above observations, it was affirmed that crystal faces and surface area have no significant role in altering NMC-toxicity to *Shewanellaoneidensis*.

3.1.3. Induction of oxidative burst

The plethora of reviews have been documented in recent times affirming ROS-generation and oxidative burst as an imperative mechanism related to NP-toxicity (Imlay, 2013; Jiang et al., 2014). The four major ROS types are superoxide anion, hydrogen peroxide, hydroxyl ion, and singlet oxygen, formed from short-term stress-induced reactions (Symonds et al., 2008). Singlet oxygen has been identified to be responsible for physiological injuries. Under normal environmental cues, a balance is maintained between ROS-production and scavenging in exposed organisms. Conversely, when an excess of ROS is generated, intercellular redox balance is disturbed, which wires for oxidation (Peng et al., 2013). The prime consequence of ROS is lipid-peroxidation and disruption of major enzymes such as mononuclear iron proteins (Anjem and Imlay, 2012). Moreover, ROS-generation results in oxidizing bases and deoxyribose of DNA, resulting in mutations and DNA damage (Imlay, 2008). A correlation is being established between electronic structures of NPs, ROS, and toxicity. Likewise, Li et al. (2012) demonstrated similar phenomena in *Escherichia coli*, where Li and coworkers employed 7-metal oxides of NPs with their band edges near the redox potential of reactive redox couples. It was revealed that the amount of abiotic-ROS generated against NPs-exposure was directly related to the extent of toxicity within the organism.

Furthermore, in another experimental study toxicity of 24-different metal oxides of nanoparticles was elucidated in *Escherichia coli* (Li et al., 2012). Out of 24-metal oxides of NPs only seven were toxic and caused augment in intercellular ROS levels. Employing nanostructure activity association analysis, it was confirmed that toxicity of NPs was directed related to: i) conduction band energy of nanomaterials ii) hydration enthalpy determining its capability to dissolve. Furthermore, it was suggested that if conduction bands of biomolecules and nanomaterials overlap, then these materials dissolve quickly and are relatively more toxic. The NPs have also been revealed to stimulate signaling cascade within organisms leading to oxidative burst. Another

observation made by Domínguez et al. (2018) in *Daphnia magna* guts revealed that when guts were exposed to negatively or positively charged nanodiamond particles (5 and 15 nm), the larger particle was found to trigger ROS-production in a dose-associated manner in comparison to lower sized NPs. It was further affirmed that there was an inclined expression of oxidative burst-related genes, signifying that cells were countering ROS. Fig. 2 elucidates underlying mechanisms associated with NPs-toxicity.

4. Effect of NPs on rhizosphere activities

The rhizosphere, an area enclosed to plant roots, is inhabited with rhizospheric organisms, root-exudates, metabolites, and specific chemical agents. They mediate interactions among plants, pathogens, microbes, and soil. Rhizospheric organisms also serve as ecosystem engineers influencing soil health, productivity, and yield of crops. The NPs possess positive and negative associations with plants and rhizospheric communities. It is quite evident that NPs within the soil can go belowground to hamper soil properties and cause hazardous effect on the environment (Eduok et al., 2013). They have been known to cause colossal hindrances towards soil microflora either through toxicities or altering the bioavailability of toxins. Indirectly, they also hamper organic compound synthesis and build antagonistic relations (Haris and Ahmad, 2017). Moreover, the impact of NPs on microbes also depends on factors such as physicochemistry, concentration, time, growth medium, etc. (Aruguete and Hochella, 2010). Strikingly, NPs are colloidal due to which microbes face intricacy in their uptake. Therefore, they are alleged to exert toxicity by solubilizing ions followed by invasion through membrane disruption (Kloepfer et al., 2005). Above all, free radicals generated by NPs also impede microbes by cell-wall and nuclear

damage, exopolysaccharides suppression, biofilm biosynthesis, and lipid peroxidation (Pelletier et al., 2010). Further, membrane leakage of soluble sugars, lipids, proteins, enzyme denaturation, cell lysis, disrupted vesicles are also observed thereby, inhibiting cell respiration (Li et al., 2010).

4.1. NPs behavior and sorption in soil

The soil, and in particular, agricultural soils, when infested with harmful NPs can pose a severe risk to plant life, and in further extension, to human life. The entry sources can be direct in applying NP formulated fertilizers, pesticides, and herbicides added to the soil. Indirect entry chiefly includes sewage-sludge that has NPs from several anthropogenic sources (Chen, 2018). The organic matter and clay minerals present in soil have charged surfaces that have a significant impact on NP-interactions with soil (Tourinho et al., 2012). Furthermore, the soils fit for plant growth are complex system consisting of gaseous, solution and solid phases and thus, also influences NP-sorption process and behavior (Chen, 2018). The organic matter present in solid-phase exists in particles or coatings on surfaces of minerals. In the solution phase, it is known as dissolved organic matter and a most reactive fraction originated from root exudates in the rhizosphere, plant litter, humus, and plant biomass (Santiago-Martín et al., 2015; Chen, 2018). Therefore, it chiefly comprises a variety of organic compounds such as humic acid, amino acids, hydrophilic acid, fulvic acid, fatty acids, etc., which influence the behavior of NPs in soil (Nebbioso and Piccolo, 2013). The pH, redox condition, and nature of solid and solution phase of organic matter govern their proportion in soil. The dissolved organic matter tends to adsorb on the surface of NPs and, thus, play an essential role in regulating physico-chemical properties. Therefore, their behavior in

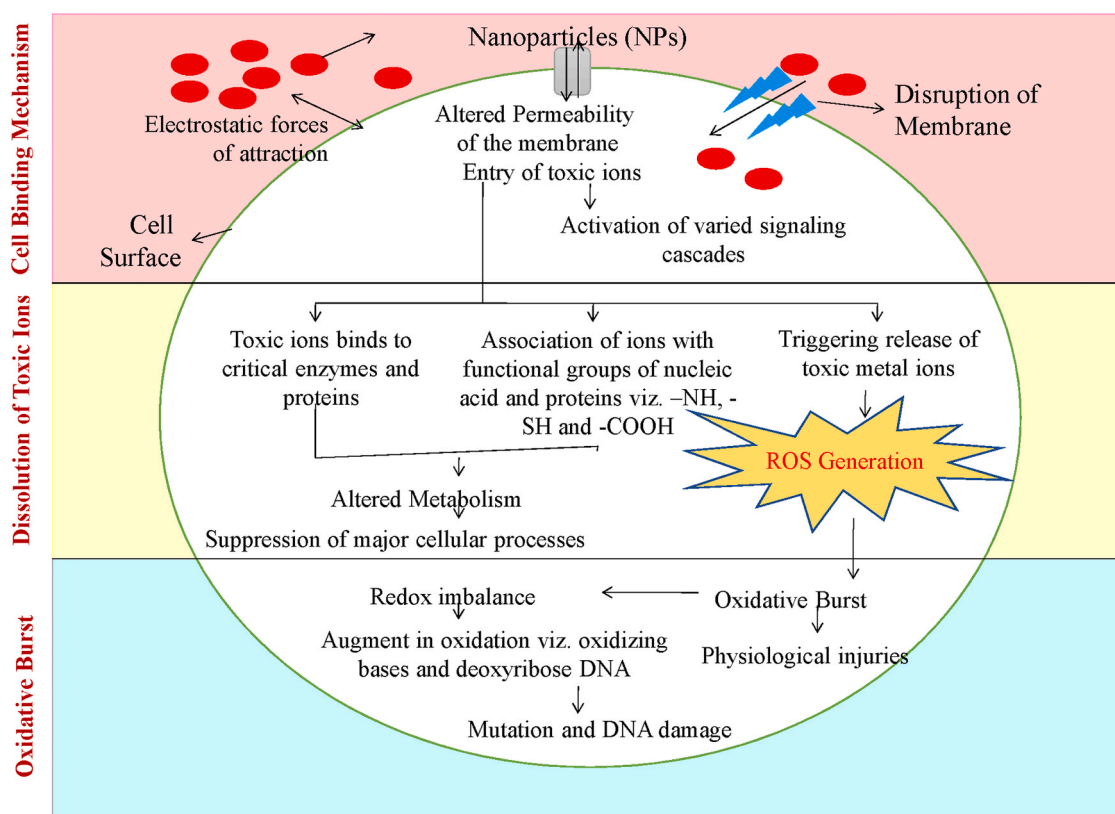


Fig. 2. Underlying Mechanisms Associated with NPs-Toxicity. (a) Cell binding mechanism: NPs bind cell surfaces and alter membrane permeability and disrupt the membranes through electrostatic forces and enter into the cell. Initiation of signaling cascade takes place (b) Dissolution of toxic ions: After the activation of signaling cascade, NPs hinder enzymes, proteins, functional groups (-NH, -SH and -COOH), trigger toxic ions in plants followed by ROS production to suppress metabolic activities and various cellular processes (c) Oxidative burst: ROS-mediate oxidative burst impairs redox homeostasis and induce the oxidation (oxidizing bases and deoxyribose DNA) to cause physiological disruptions, DNA damage and mutations.

soil-ecosystem further affects soil micro-and macro biota(Santiago-Martín et al., 2015).

Apart from the soil system, NPs size also directly influences its behavior and fate in soil (Santiago-Martín et al., 2015). It has been postulated by Buffet (2012) that NPs with smaller sizes have high mobility and bioavailability. The NPs also have the tendency to form aggregates or clusters, leading to change in surface properties (Hotze et al., 2010). Size of NPs, their chemical composition, and surface charges have ability to control the aggregation process (Abbas et al., 2020b). These aggregates can become more stable if they get fixed with the soil matrix (Hotze et al., 2010). Hence, the level of aggregation regulates their mobility and reactivity in the soil environment. The NPs within the soil solution, in nanoscale-form, are controlled by Brownian diffusion. The high diffusion rate enhances collision rate leading to several interactions among NPs and immobile soil particles (Lin et al., 2010). These interactions include Van der Waals forces, electrical double-layer interactions, hydration forces, hydrophobic interactions, and steric interactions (Navarro et al., 2008). In the case of aggregates, strong chemical bonds come into play. Also, weak bonds such as Van der Waals forces lead to the formation of agglomerates (Jiang et al., 2009). These forces greatly influence the bioavailability of the nanoparticles. Aggregate formation hampers the dissolution process of NPs in soil by reducing the diffusion of free ions. The level of dissolution of NPs is directly related to their thermodynamic stability. In thermodynamically unstable NPs, ions are released from a double layer of the core to soil solution (Borm et al., 2006). Aggregate formation is also influenced by soil properties. The pH of the soil has been regarded as one of the critical factors governing the NP-behavior (Dimkpa, 2018). The acidic-pH stimulates the formation of ionic species from the NPs, while the alkaline-pH favors aggregate formation (Molleman and Hiemstra, 2017). Hence, in acidic conditions, NPs become ion sources and release them continuously increasing reactive ionic species (Wang et al., 2013a, 2013b). In other words, NPs-dissolution gets enhanced, leading to ion-complexation by soil chemicals (Wang et al., 2013a, 2013b). Alkaline-pH, on the other hand, reduces the dissolution process due to enhanced aggregation. Therefore, in both cases, specific properties of NPs are attenuated (Dimkpa, 2018). pH of soil also has a key role in regulating surface charges NPs, that governs the aggregation process as explained earlier (Dimkpa, 2018). For instance, titanium oxide-NPs at higher pH have a negative surface charge and show more stability in their nano-form.

In comparison, at lower pH, the surface charge becomes positive, and the size of NPs becomes smaller. Such a change, although, is reversible with change in pH. Still, aggregation due to pH can disrupt the mobility and bioavailability of NPs (Loosli and Stoll, 2017). However, it cannot be denied that NPs can show variable behavior with variable pH. The main mechanism underlying NP size is pH that changes with the alteration of surface charges and dissolution mechanism of ions. Therefore, they directly effect their sizes as well as bioavailability within the soil. Thus, studies are required to determine specific pH for specific engineered-NPs to have a maximum outcome.

Similarly, another soil property, which is salt concentration, also has a significant effect on NPs present in the soil. Salt levels indicate the ionic strength of soil, and it has been postulated by Legg et al. (2014) that high ionic strength leads to higher aggregation among NPs. In addition to pH and ionic concentration, organic compounds, which are function of soil properties, also play a major role in the aggregation and disaggregation of NPs. Therefore, a combination of soil properties and NP-properties together affect their mobility, sorption, and bioavailability in the soil-system.

4.2. NPs impact on soil enzymes

Out of all ecosystems(soil, water and air),soil ecosystem is believed to receive the highest levels of NPs from all sources (Chai et al., 2015). Therefore,it becomes imperative to gain an insight into the effect of NPs

on soil enzymatic activities and its impact on the environment. The soil enzymes can broadly be categorized into intracellular and extracellular enzymes. The intracellular enzymes are present inside the cells of soil-micro-and macro-biota, while these organisms release extracellular enzymes into the soil. Extracellular enzymes, therefore, can exist both in soil solution and in-bound form with soil components (Peyrot et al., 2014). The expression of soil enzymes also indicates the biological diversity of soil, which signifies the biogeochemical and metabolic pathways going on (Peyrot et al., 2014).

Additionally, such monitoring of enzymatic activities also helps in analyzing soil health and biochemistry (Asadishad et al., 2017). The enzymes responsible for cellulose and lignin degradation are chiefly studied for soil assessment as these are abundantly found in plant litter (Allison et al., 2007). Soil is also a major site for carbon, nitrogen and phosphorus cycles and thus, enzymes of these cycles also become imperative indicators of soil quality (Sinsabaugh et al., 2008; Baldrian, 2009). Apart from these, enzymes for nitrogen-containing compounds (proteins, peptidoglycans, chitin, etc.) in soil and phosphorus-rich compounds(nucleic acids, phospholipids) are also assayed (Caldwell, 2005). The studies have shown both positive and negative impacts of NPs on soil enzymes (Table 2). The studies of soil enzymes with respect to NPs can, therefore, aid to detect contamination and anthropogenic disturbances at early stages.

4.3. NPs impact on biogeochemical dynamics

Biogeochemical cycles are an inherent part of fertile soils as they support plants, microflora and microfauna. The nitrogen, carbon and phosphorous cycles have high environmental significance and are reported to be affected by NPs interference (Raffi and Husen, 2019). In particular, nitrogen fixation is an imperative part of the nitrogen cycle, which is a basic requirement for the biosynthesis of essential macromolecules that are building blocks of life. Soil is a major source of nitrogen supply to the plants that utilize it for their own growth and development and for heterotrophs that are directly or indirectly dependent on them. It has been reported that enzymes and microorganisms involved in it are affected by NPs (Raffi and Husen, 2019). Urease, especially, is involved in the conversion of urea into carbon dioxide and ammonia, an essential step in nitrogen fixation. Several reports have cited the adverse effects of NPs on this enzyme activity (Table 2). It was reported by Cao et al. (2017) that root-exudates, as well as urease-producing microorganisms in the rhizosphere,have been negatively affected by AgNPs, thereby leading to the inhibitory effect on urease.

Conversely, assessment of urease activity and its reduction in response to NPs also indicates reduced rhizospheric microbes (Raffi and Husen, 2019). The direct effect of NPs has also been reported in the roots of nodular plants. Like, cerium oxide (CeO₂) NPs hampered the process of nitrogen fixation in root nodules of soybean plants which further led to reduced plant growth and development (Priester et al., 2012). Similarly, it was reported that tungsten NPs(WO₃)were damaging to nitrogen-fixing bacteria (Allard et al., 2013). This effect was particularly noticed in molybdenum (Mo) deficiency which further was caused due to the release of WO₃ ions from NPs, and was taken up by catechol-type siderophores. Interestingly, the production of these siderophores was also reported to be stimulated by these NPs (Allard et al., 2013). Apart from the effect on urea to ammonia conversion, NPs have also been found to alter the nitrification process, where ammonium ions are converted to nitrates. It was demonstrated in a study conducted by Choi and Hu (2008), where AgNPs caused inhibited growth of nitrifying bacteria. The AgNPs were observed to affect enzyme alkaline phosphatase present in the rhizosphere of wetland plants, there by affecting the phosphorus cycle of the wetland ecosystem (Cao et al., 2017).

The effect on biogeochemical cycles through exoenzymes and microorganisms in the soil go hand in hand. Many soil bacteria and fungi are directly or indirectly involved in the completion of various

Table 2
Effect of NPs on Soil Enzymes.

NPs	Conc.	Soil Enzymes Tested	Effect	References
CuO	10, 100, 500 mg/kg	Nitrate reductase, nitrite reductase, nitric oxide reductase, nitrous oxide reductase	Nitrate reductase and nitric oxide reductase activities inhibited. Nitrite reductase enhanced at 100 mg/kg, nitrous oxide reductase remained unaffected. Enzyme activities remained unaffected.	Zhao et al. (2020)
AgNPs	1.5 ⁻⁵ , 0.00015, 0.0015 mg/kg	β -glucosidase, urease, phosphatase		Montes de Oca-Vasquez et al. (2020)
Black phosphorus nanosheets	10, 50 mg/kg each in black soil and burozem	Catalase, urease, acid phosphatase	No significant effects on enzymes in black soil, while an inhibition in catalase and urease activities in burozem, recovered on prolonged exposure.	Zhang et al. (2020)
ZnNPs	50, 100, 200, 400 mg/kg	Soil catalase, urease, invertase, peroxidase, polyphenol oxidase	Catalase, urease activities showed initial increase while a decline at higher concentrations. Increased invertase activity at 50 and 100 mg/kg and declined at higher levels of NPs. Peroxidase activity increased, while polyphenol oxidase decreased.	Galaktionova et al. (2019)
CuNPs	50, 100, 200, 400 mg/kg	Soil catalase, urease, invertase, peroxidase, polyphenol oxidase	Catalase, polyphenol oxidase activity declined, while urease showed enhancement. Increased invertase and peroxidase at 50 and 100 mg/kg while declined at higher levels.	Galaktionova et al. (2019)
Nano-Cu, nano-CuO	10 mg/kg	Dehydrogenase	Decreased dehydrogenase activity with nano-Cu, while increased with nano-CuO	Josko et al. (2019)
Fe ₂ O ₃ , ZnO	10, 40 mg/Kg	Dehydrogenase, alkaline phosphatase, acid phosphatase	Soil resilience towards heat stress enhanced.	Kumar et al. (2019)
Li ₂ O	4, 51, 474 mg/kg	β -glucosidase and urease	Increased β -glucosidase activity and decreased urease activity at highest concentration.	Avila-Arias et al. (2019)
MoO ₃	2, 35, 173 mg/kg	β -glucosidase and urease	Decreased β -glucosidase activity.	Avila-Arias et al. (2019)
NiO	11, 211, 1018 mg/kg	β -glucosidase and urease	Lowered β -glucosidase activity.	Avila-Arias et al. (2019)
Polystyrene NPs	0.1, 1 mg/kg	Dehydrogenase, cellobiohydrolase, β -glucosidase, alkaline phosphatase, leucine-aminopeptidase	Dehydrogenase, leucine-aminopeptidase and alkaline phosphatase activities declined at all concentrations. Cellobiohydrolase and β -glucosidase showed decreased activities at lower concentrations. Enhanced activities at higher concentrations of NPs declined with prolonged exposure.	Awet et al. (2018)
AgNPs	1.600, 3.2 mg/kg	Soil acid phosphatase, β -glucosaminidase, β -glucosidase, arylsulfatase activities	Enzymes showed decreased activities at 1-hour and 1-week-exposure period. Longer exposure showed mixed activities signifying temporary effects of AgNPs.	Eivazi et al. (2018)
ZnO	500, 1000, 2000 mg/Kg each in black and saline alkali soil	Invertase, urease, catalase, and phosphatase	Decreased catalase activity and increased urease activity. Invertase activity enhanced in black soil and declined in saline alkaline soil. Phosphatase declined only in black soil.	You et al. (2018)
CeO ₂	500, 1000, 2000 mg/kg each in black soil and saline alkali soil	Invertase, urease, catalase, and phosphatase	Urease activity decreased, while no significant difference in other enzymes.	You et al. (2018)
Fe ₃ O ₄ , TiO ₂	500, 1000, 2000 mg/kg each in black soil and saline alkali soil	Invertase, urease, catalase, and phosphatase	Catalase and invertase activities declined in saline alkali soil.	You et al. (2018)
Citrate-coated nAu	0.1, 100 mg/kg	Cellobiohydrolase, β -1,4-xylosidase, β -1,4-glucosidase, β -1,4-N167	Increased activities of soil enzymes.	Asadishad et al. (2017)
Polyvinylpyrrolidone (PVP)-coated nAu	0.1, 100 mg/kg	Acetylglucosaminidase, acid phosphatase Cellobiohydrolase, β -1,4-xylosidase, β -1,4-glucosidase, β -1,4-N167	Decreased NPs size led to enhanced activities of soil enzymes at lower concentrations.	Asadishad et al. (2017)
CeO ₂	0, 100, 500, 1000 mg/kg	Urease, phosphatase, β -glucosidase	Concentration more than 100 mg/kg inhibit activities of urease and β -glucosidase, while stimulated phosphatase activity.	Li et al. (2017)
AgNPs	0.00125, 0.0125, 0.125, 1.25, 6.25, 31.25 mg/Kg	phosphomonoesterase, arylsulfatase, β -D-glucosidase, leucine-aminopeptidase	Activities of soil enzymes negatively affected.	Peyrot et al. (2014)
AgNPs	1, 10, 100, 1000 mg/kg	Urease, acid phosphatase, arylsulfatase, β -glucosidase	Enzymatic activities inhibited.	Shin et al. (2012)

ecological processes and NPs have been shown to have significant effects on them. Studies have shown dose-dependant impact of NPs on bacterial communities leading to reduced diversity in the rhizosphere, and detrimental impact on flora (Raffi and Husen, 2019). Many nitrogen-fixing bacteria such as *Rhizobiales* and *Bradyrhizobiaceae*, methane oxidizing bacteria like *Methylobacteriaceae* are affected by NPs (Raffi and Husen, 2019). Such an effect has been speculated to result from either direct toxicity towards microbial community or indirect impact through variations in abiotic factors such as water and soil nutrients (Raffi and Husen, 2019). It was established in a study conducted by Xu et al. (2015) that CuONPs introduced in water-logged paddy soils caused a significant

decline in the microbial biomass. A similar study by You et al. (2018) also showed substantial alterations in soil bacteria in two soil types viz. saline-alkali and black soils in response to ZnO, TiO₂, CeO₂ and Fe₃O₄NPs, which indicated changes in nitrogen fixation. Apart from this, ZnO and CeO₂ NPs were also reported to reduce the abundance of *Azotobacter*, P-solubilizing and K-solubilizing bacteria and negatively affect their enzyme activities (Chai et al., 2015). The toxic effects of ZnONPs were also demonstrated on the ammonification process in the soil where both time-dependant and dose-dependent responses to NPs were observed (Shen et al., 2015). The same study also showed the dependence of NPs-toxicity on soil-type, where acidic soil was reported

to show the highest toxicity and alkaline soil showed the least.

4.4. NPs impact on soil microbiota

Soil fit for the growth of plants harbors a wide range of microbiota consisting of prokaryotic and eukaryotic microorganisms. These microorganisms not only contribute in maintaining the soil ecosystem, but many also have imperative roles in the healthy growth of plants, thus becoming a vital factor for crop growth and yields. NPs from various sources have a direct impact on microbiota (Table 3) and further on plants. Therefore, they function as a sensitive indicator for changes in soil due to NPs (Rajput et al., 2018a, 2018b). PGPRs, in particular, are vital for plant health and thus, become crucial subjects to study the effects of engineered NPs. Apart from PGPRs, mycorrhizal fungi and other rhizobial bacteria harbor the rhizosphere, which can exist in a symbiotic relationship with plant roots.

It has been assessed by Tian et al. (2019) that symbiosis highly depends upon properties of NPs, concentration, species of rhizospheric organisms and characteristics of substratum, mainly soil. For instance, the surface properties of NPs, coating, charge and size, play significant roles in determining their effects. It was shown in a study that AgNPs coated with polyvinyl pyrrolidone reduced colonization by arbuscular mycorrhizal fungi in tomato roots, while non-coated Ag₂SNPs had no impact (Judy et al., 2015). Similarly, positive and negative surface charges carried by amine-Fe₃O₄ and carboxylic acid Fe₃O₄, respectively, had different effects on the nodulation process of soybean. The positively charged-NPs aided in enhancing the process as compared to negatively charged (Burke et al., 2015). Furthermore, concentration levels of NPs have a critical role in microbial interactions. It has been speculated that NP-toxicity on soil microbes is directly proportional to their low concentration levels as assessed on mycorrhizal fungi, tomato and maize plant-interactions in response to ZnONPs (Wang et al., 2016a,

2016b). Both studies showed successful colonization by fungi at higher levels of NPs. Similar observations have also been documented for interactions between rhizobia and NPs, wherein higher concentrations have adverse effects on nodulation (Tian et al., 2019). Effects of NPs with regard to microbial species, however, are highly variable and no clear conclusions can be drawn. This might be because soil microbiota remained a neglected topic concerning plant growth and NP-interactions. This has led to a deficiency of elucidation of mechanisms underlying the behavioral patterns of microorganisms in NPs presence with variable factors of soil conditions and plant types. Therefore, studies can be conducted to unravel NP-microbe-plant interactions, keeping in mind types of NPs, soil, plant and microbial species, which could aid in crop improvement, yields, and NP-toxicities and methods for its amelioration.

5. Association of NPs with micro-organisms

Soil microorganisms have imperative participation in nurturing soil ecosystem, soil health and crop yield. NPs have been affirmed to have both negative and positive influences on the root system and rhizospheric microorganisms. It is known that engineered NPs are released into the environment, especially soil. Hence the impact of these NPs on varied soil phenomena and microorganisms is of prime importance. The NPs have an impact on soil microorganism through i) relatively direct toxicity effects, ii) alterations in bio-accessibility of toxins or essential elements, iii) interface between toxic organic molecules (antagonistic or synergistic impact), and iv) indirect influence on associations with organic compounds (Haris and Ahmad, 2017). Engineered NPs are added to the soil via sewage, faulty agricultural practices and other sources of human origin (Frenk et al., 2013). As a result of ever enhancing commercial usage, there is an increase in their discharge into the marine and terrestrial ecosystems through various routes with

Table 3
Effects of some NPs on soil microbes.

NPs	Microorganism affected	Effect	References
Nano-CuO, nano-Cu	Fungi	Decreased fungi population with nano-Cu, while increased with nano-CuO	Josko et al. (2019)
Ag	Arbuscular mycorrhizal fungi	Negative effects on colonization with tomato plants.	Noori et al. (2017)
Ag, ZnO	<i>Sinorhizobium meliloti</i>	Rhizobial symbiosis negatively affected with alfalfa.	Mohaddam et al. (2017)
TiO ₂	Arbuscular mycorrhizal fungi	Negative effects on colonization with rice plants.	Priyanka et al. (2017)
Ag	<i>Acidobacteria</i> , <i>Actinobacteria</i> , <i>Cyanobacteria</i> , <i>Nitrospirae</i> , <i>Proteobacteria</i> and <i>Planctomycetes</i>	<i>Acidobacteria</i> , <i>Actinobacteria</i> , <i>Cyanobacteria</i> and <i>Nitrospirae</i> declined in number, while <i>Proteobacteria</i> and <i>Planctomycetes</i> increased in number.	Wang et al. (2017)
Ag	<i>Glomus aggregatum</i>	Negative effect on mycorrhizal symbiosis with faba bean plants.	Abd-Alla et al. (2016)
ZnO	<i>Funneliformis mosseae</i>	Negative effect on mycorrhizal symbiosis with soybean plants.	Jing et al. (2016)
ZnO	<i>Rhizobium leguminosarum</i>	Rhizobial symbiosis negatively affected with pea.	Sarabia-Castillo and Fernández-Luqueño (2016)
Mo	Microbial diversity in chickpea rhizosphere	Diversity and symbiotic effectiveness of the microbial community enhanced.	Shcherbakova et al. (2017)
ZnO	<i>Pseudomonas chlororaphis</i> O6	Caused disruption in cell-signaling. Reduced phenazine production in dose-dependent manner which resulted in reduced quorum sensing molecules, acyl-homoserine lactones.	Goodman et al. (2016)
TiO ₂ , CuO	Microbial community of paddy soil	Toxic effects on microbial community.	Xu et al. (2015)
CuO	Native soil bacteria from agricultural soil	Toxicity revealed due to membrane degradation, cellular collapse, lysis in bacterial isolates.	Concha-Guerrero et al. (2014)
Capped and uncapped NPs	<i>Nitrosomonas europaea</i> , <i>Nitrospiramultiformis</i> , <i>Nitrosococcus oceani</i> , <i>Escherichia coli</i> and <i>Bacillus subtilis</i>	Inhibited activities of ammonia-oxidizing bacteria. <i>E. coli</i> and <i>B. subtilis</i> showed higher inhibitory effects of capped AgNPs than uncapped.	Beddow et al. (2014)
Ag, Cu	Rhizobacteria in <i>Vignaradiata</i> and <i>Brassicajuncea</i> rhizosphere	Reduced population of rhizobacteria in rhizosphere.	Akhilesh et al. (2013)
Ag	<i>Bacillus</i> , <i>Pseudomonas fluorescens</i>	Bacterial growth inhibited.	Dhas et al. (2014)
Ag	Arbuscular mycorrhizal fungi	Enhanced infection rate of fungi.	Feng et al. (2013)
Ag	<i>Phytophthora infestans</i>	Inhibition in fungal growth.	Giannousi et al. (2013)
TiO ₂ , ZrO ₂	<i>Bacillus megaterium</i> , <i>Azotobacter vinelandii</i> , <i>Pseudomonas fluorescens</i> , <i>Bacillus brevis</i>	TiO ₂ showed toxicity towards PGPRs and <i>B. brevis</i> showed highest susceptibility followed by <i>B. megaterium</i> , <i>P. fluorescens</i> and <i>A. vinelandii</i> respectively.	Karunakaran et al. (2013)
Ag	<i>Bacillus</i>	Bacterial transformation in chloroplasts due to cell-damage, thereby, causing leakage of proteins and sugars.	Mirzajani et al. (2013)

environmental concerns (Singh et al., 2016). It has a detrimental impact on plants, including suppression of growth and development and germination of seedlings (Nair and Chung, 2014). These NPs from the aquatic and terrestrial sources enter into food crops and subsequently enter higher trophic levels via—food chain. Various studies have revealed that AgNPs showed toxic activities against mitochondrial metabolism and elevated ROS-production (Kim et al., 2012). In algae and microbes, the AgNPs stimulates ROS and consequently oxidative damage. Plants and microorganisms develop various approaches to combat stress-induced by NPs.

5.1. Transport and agglomeration mechanism

The intake, transportation and sequestration of NPs in cells are directly related to the ultra-structure of the cell, membrane permeability, dimensions of NPs and cellular attributes (Li et al., 2015). The cell-wall operates as a hindrance for the intake of AgNPs into cells from the environment. The cell-wall is composed of proteins, lipids, carbohydrates and glycoproteins, which form a blocking point and a rigid subtle network (Navarro et al., 2008). It acts as a selectively permeable strainer, which sieves out larger-sized NPs and permits entry of smaller-sized NPs (Navarro et al., 2008). The AgNPs have been reported to have small dimensions and large surface area allowing their entry through the cell-wall and subsequently to the plasma membrane (Samberg et al., 2011). Due to the entry of AgNPs into cells, larger pores are created in the cell membrane, which further triggers the entry of AgNPs inside (Navarro et al., 2008). The probable mode of access into the plasma membrane is via lipid bilayer by employing endocytic processes (Siddhanta et al., 2015). Beside this, another imperative route is through ion channels present in the membrane (Mueller and Nowack, 2008). Subsequently, NPs get attached to organelles viz. Golgi bodies, lysosomes and endoplasmic reticulum (Miao et al., 2010). As soon as NPs reach the cell interior, they initiate disruption of metabolic processes by ROS-generation and consequently biochemical processes of cell (Miao et al., 2010).

5.2. Toxicity mechanisms

The NPs reflect toxicity towards microflora, for example, AgNPs create a toxic environment for microbes and inhibit microbial proliferation by creating a toxic ambiance (Choi et al., 2008). It has been often found that NPs react to the -SH functional site of proteins to in active entire bacterial cell machinery. Not only this, the Ag-ions of AgNPs interfere with the microbial respiration process in terms of electron uncoupling and disrupted phosphorylation process followed by impeded membrane stability (Feng et al., 2000). Apparently, NPs-interactions with microbes disturb membrane integrity owing to the greater surface area to volume ratio of NPs as well as size. Studies have found that both gram-positive and harmful bacteria are adversely affected by NPs. Moreover, they also impair the nucleic-acid synthesis and DNA-replication process (Feng et al., 2000).

Furthermore, proteomic studies depicted that NPs hinder heat-shock proteins, membrane disruption and leakage of intracellular contents from cells. Consequently, membrane structures become leaky, ATP and cell viability are also impaired (Lok et al., 2006). The membrane leaked contents are also released in glucose and trehalose (Siddhanta et al., 2016). The anti-microbial action of NPs initiates if these particles are bound onto microbial cells through adhesive forces, which suppress their functioning (Reidy et al., 2013). Apart from this, NPs also bind to proteins and alter the functioning of thiol groups to de-stabilize the membrane (Reidy et al., 2013).

5.3. Resistance mechanisms

The microbes possess defense machinery to combat the noxious effects of NPs. To illustrate, they contain a peptidoglycan layer that

provides them thickness as the first line of defense against all the odds and evens of NP-toxicity (Sedlak et al., 2012). Moreover, they protect cell structures by heat-shock proteins (Sedlak et al., 2012). Also, they have very efficient efflux-pumps that impart resistance against NPs. Interestingly, this efflux-pump is encoded by plasmid-borne cassettes that may get transferred horizontally to other bacteria as well. Alongside, the synthesis of periplasmic proteins also mediates the efflux-mechanism through P-type-ATPases, ion-exchangers, or transcriptional proteins (Kvitek et al., 2009). A study reported by Khan et al. (2011), revealed that *Bacillus pumilus* is resistant against AgNPs due to the putative role of extracellular polymeric substances. Apart from this, it has also been observed that microbial species also accumulate organelles within central compartments of cells as a defense response (Jung et al., 2008). This region is densely populated with nuclear material, thereby possessing a defensive strategy. Furthermore, the defense strategy is also mediated at the transcriptional level, when genes encoding plasmid and chromosomes are upregulated in the presence of NPs for resistance (Tripathi et al., 2017).

6. Mitigation approaches for NP-toxicity in environment

It is evident that NPs end up within the environment to harm different organisms with respect to its dispersal, ecotoxicity, persistence and accumulation within the environment. Considering all the ecological concerns concerning NPs, various investigations are required to be undertaken to assess their risks and mitigation practices. In the subsequent sections, we have reviewed some of the valuable strategies and approaches concerning NP toxicity and their mitigation in terms of avant-grade engineering practices, sustainable and regulatory measures.

6.1. Avant-garde engineering strategies

Being highly progressive, NPs usage is prevailing in the present era, though there are scarcity in understanding their risks and assessment tools. There are minimal, highly expensive and challenging analytical methods available for measuring NP-toxicity in the environment (Montaño et al., 2014). However, it is intricate to deduce the toxicity of NPs within the ecosystem, subsequently, appropriate data should be gathered to develop avant-garde techniques. Due to adequate detection and monitoring devices, NP-interaction within the environment was neglected, but now various tools elucidating the fate of NPs in the environment are being focused. Different biomarkers are useful for tracking NPs within the environment. Therefore, serve as the most crucial biological assessment tool. In addition, an idea of permeable-ion barriers has also been formulated to arrest NPs (Patil et al., 2016). The permeable-ion barriers are designed as single or multiple on the basis of containment zones to reduce the NPs and these are installed in a flow path to immobilize or accumulate the particles. Following this decontamination process, the tracking of NPs in situ is done, while accumulated NPs are eradicated to impede their further spread.

Furthermore, the latest engineering technology based on complicated electron conductivity imaging technique, a type of geophysical protocol has also been developed for monitoring and assessing NPs in groundwaters (Flores Orozco et al., 2015). Apart from this, another practical approach that has been widely observed is designing or synthesizing modern and smart NPs by taking into account the environmental perspective. These NPs are sophisticatedly designed with coatings with functional groups to trigger their stability, mobility and function (Thatai et al., 2014). This process enables better engineering practices to develop efficient NPs for the remediation of numerous contaminants without harming the ecosystem.

6.2. Regulatory actions

Apart from eco-friendly and contemporary engineering strategies, various legal, as well as regulatory methods are required to be developed

that monitor the spread and management of NPs within the ecosystem (Wang et al., 2013a, 2013b). A pressing challenge is the extended usage of NPs, followed by regulation of employment and deployment of these strategies to make their maximum outcomes. Different nations around the world have considered NPs as new pollutants and have been governed under various regulatory bodies (Bowman and Hodge, 2006). On the contrary, few developing nations have stressed nanotechnology regulations. Like in the case of India, it has become quite intricate to handle due to lack of expertise and resources (Barpujari, 2011). The major limitations lie in current legislative measures on NPs that prove a serious threat during the entire process of production, distribution and discard. A complete form of advanced and comprehensive technology comprising of public awareness and integrated legislation is a substantial and proactive approach to regulate the intricacy of NPs to prevent their toxicity (Wang et al., 2013a, 2013b). Further, to consider ecotoxicology and NP fate within the environment, different researchers have come forward with the view that the development of various regulatory actions and environmental testing and monitoring would provide validated results (Kühnel and Nickel, 2014). Therefore, these assessments are required to be modulated with contemporary approaches to give accurate results to avert NP-mediated environmental damage.

6.3. Sustainable strategies

The biosynthesis of NPs from organic material, specifically plant organs, have been prevailing for some time as a green technology. The synthesis of NPs is done from natural plant products such as leaves, stems, fruits, and medicinal plants with higher antioxidant abilities (Kharisova et al., 2013). This green strategy is highly advantageous as it is eco-friendly, cost-effective, efficient and lowers toxicity. Moreover, the raw material is readily available in plant residues with higher nutrients (Hoag et al., 2009). Researchers have reported that the NPs synthesized can very well remediate soils with excessively hazardous wastes like heavy metals, organic and inorganic substances (Machado et al., 2013). They have also determined that the green chemistry of NPs enables us to synthesize various NPs (silver, iron, cobalt etc.) from plants like tea, banana, grapes, coffee, etc. (Kharisova et al., 2013). A study conducted by Shahwan et al. (2011), revealed that Fe-NPs synthesized from tea leaves lead to decolorization of dyes like methylene blue and methyl orange. Therefore, the usage of green NPs is 'one-pot process with cost-effective approach. However, it also showcases contaminant degradation with a sustainable approach declining the risk of toxicity. In addition, NPs synthesized from microbes, bio-NPs are non-toxic and serve the purpose of biodegradation (Johnson et al., 2012). One more eco-friendly and sustainable technique is the utilization of zero-valent emulsified Fe-NPs. Fe are encapsulated within a membrane made of oil in this process so that contaminants get adsorbed and degraded into non-toxic particles (Quinn et al., 2005). Sidewise, the usage of coaters also acts as stabilizers (Grieger et al., 2010). By implementing these strategies, NPs were nearly non-detectable in the environment, thereby indicating the best sustainable application to combat NP-toxicity within a limited time frame.

6.3.1. Microbial-mediated synthesis of NPs

Currently, the most imperative area of nanoscience research deals with the production of nanometer-sized particles exhibiting different sizes, morphologies and monodispersity (Iravani, 2014). The NPs can be easily synthesized by employing different physical, chemical and aerosol techniques. Nevertheless, these techniques are capital and -intensive. They involve the use of toxic chemicals, nonpolar solvents, and synthetic additives/capping agents, thereby impeding their implementation in biomedical, clinical and agricultural fields. Using natural alternatives such as plant and microbial enzymes, polysaccharides, vitamins, biodegradable polymers and biological systems to synthesize NPs is gaining importance (Iravani, 2014). Recently, in nanotechnology, microbial synthesis of NPs has surfaced a promising deal. The most

preferred microorganisms or potential biofactories for NP-synthesis include bacteria, actinobacteria, fungi and yeasts, attributed to their rapid growth rate, simple cultivation and capability to proliferate at ambient temperature, pressure and pH (Fariq et al., 2017). The biological synthesis of NPs is a bottom-up approach in which atoms assemble and grow from more minor to larger particles, atom by atom (Musarrat et al., 2011). In general, NP-biosynthesis using green biotechnology includes three main steps, i.e., selection of reaction medium, biological reducing agent and non-toxic substances for NP-stabilization. Biologically generated NPs possess a larger surface area, elevated catalytic reactivity and better contact between metal-salt and enzyme due to microbial carrier matrix (Li et al., 2011).

However, the exact mechanism for NP-synthesis employing microbial agents is not clearly understood because different microbes react differently with metal ions. Following intracellular and extracellular reduction mechanisms, microorganisms owe an inherent potential to fabricate metal and metalloid NPs from various inorganic resources. They entrap metal ions present in the environment and convert them into elemental forms by enzymatic activities. The extracellular formation of NPs involves the entrapment of metal ions on the cell surface and its reduction by microbial enzymes, proteins and organic molecules located on the microbial cell membrane or released into the growth medium. The NPs thus, synthesized are either adsorbed onto cell membrane or may suspend in medium (Fariq et al., 2017). The intracellular NP-synthesis involves the transportation of metal ions inside the microbial cell and its enzymatic conversion into NP (Li et al., 2011). In the intracellular process, the microbial cell-wall plays a critical role. The process involves an electrostatic interaction between positively charged metal ions and negatively charged components of the cell-wall. The ions then interact with enzymes, proteins and co-factors present within the cell-wall and get reduced to NPs, diffused through the cell-wall (Fariq et al., 2017). The mechanism for extra and intracellular NP-synthesis varies depending upon the microbe used. The extracellular microbial reduction is however, preferred over intracellular reduction due to higher efficiency, low cost and simple extraction or easy downstream processing (Fang et al., 2019). The intracellular method involves supplementary procedures like using appropriate detergents or ultrasonic treatment to release the produced NPs, making laborious and costly procedure (Deljou and Goudarzi, 2016). Fig. 3 depicts intracellular and extracellular methods employed by different microbes for the production of NPs.

Bacterial biomass or cellular extracts have been reported by various researchers for the production of NPs such as gold, silver, titanium, palladium, platinum, magnetite, cadmium sulfide, etc. S-layer and magnetotactic bacteria are commonly employed for the synthesis of inorganic materials. Besides the living bacteria, even the dead entities of certain bacteria have been reported to synthesize NPs. Usually, in living bacteria, the metabolic processes are responsible for the reduction of metal ions into NPs, while in dead bacterial entities, the ions get bound to bacterial cells, thus providing nucleation sites for NP-synthesis. Different functional groups like -OH, -NH₂, -COOH and -SH of proteins released by bacteria play an imperative role not only in reduction but also stabilization of NPs by providing binding sites for adherence of ions followed by their reduction either on the surface of cell wall outside the cells or in the periplasm (Shahzad et al., 2019). A plethora of bacterial species, including *B. subtilis*, *E. coli*, *Lactobacillus* sp., *Rhodospirillum rubrum*, *Cornebacterium* sp., *P. stutzeri*, *Streptomyces albidoflavus* and *Klebsiella pneumonia* have been reported to synthesize NPs (Hulkoti and Taranath, 2014; Purohit et al., 2019).

In the fungal-based synthesis of NPs, extracellular enzymes are often produced, which reduce macro/micro-scale metal-salt into nanoscale dimensions through catalytic activity. Secretion of enzymes extracellularly provides an advantage for obtaining pure and monodispersed-NPs free from biological components (Tarafdar and Rathore, 2016). Because of comparatively greater biomass, the yield of NPs is more in fungi. Fungi are easy to handle and possess different enzymes, to synthesize

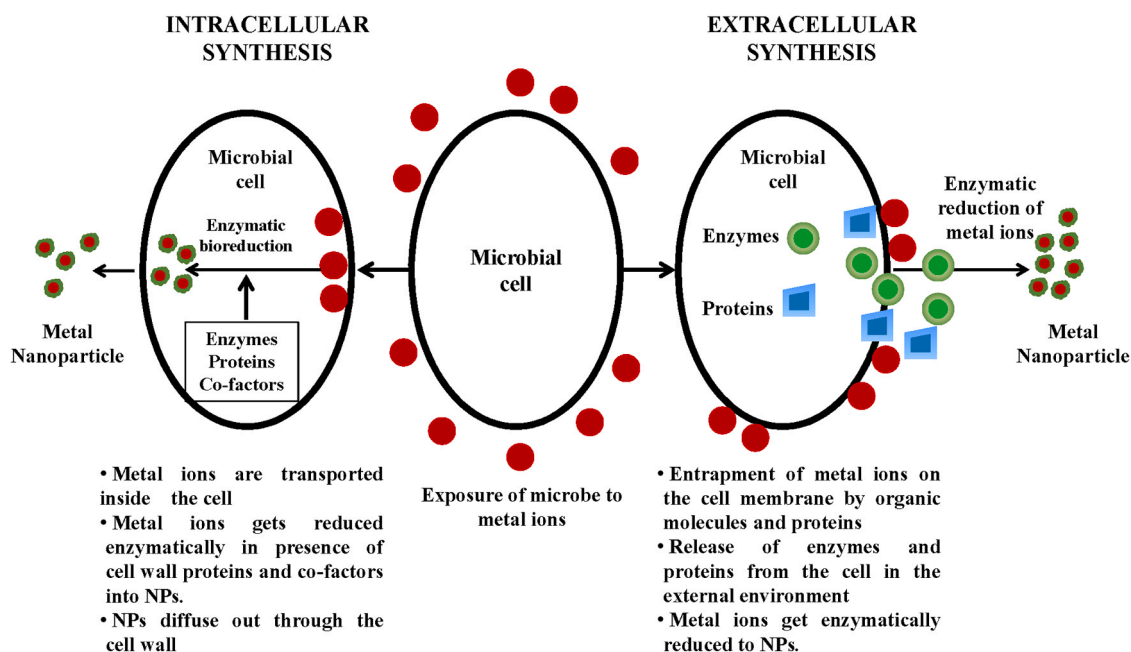


Fig. 3. Schematic Presentation of Nanoparticle Biosynthetic Mechanism in Microbes. Intracellular synthesis, metal ions get transported within the cells and undergo enzymatic reduction with the aid of cell wall proteins and other co-factors to form NPs followed by diffusion outside the cell. Extracellular synthesis, Metal ions are entrapped onto cell membrane through organic molecules and proteins. Enzymes and proteins are synthesized within the cell to outside environment followed by enzymatic reaction to synthesize NPs.

NPs of varying shapes and sizes. In comparison to bacteria, fungi can secrete more quantity of proteins, causing higher productivity of NPs. Different fungal species such as *Verticillium luteoalbum*, *Trichoderma viride*, *Fusarium oxysporum*, *Aspergillusoryzae*, *Colletotrichum* sp. etc., are reported to generate NPs with varied shapes and sizes (Shahzad et al., 2019). To overcome metal toxicity, yeast has developed different mechanisms such as enzymatic oxidation/reduction, surface sorption, chelation with extracellular polysaccharides/peptides, etc. Detoxification employed by yeast also results in the formation of NPs exhibiting variations in size, monodispersity, location and properties. Glutathione and ligands, phytochelatins and metallothioneins, are responsible for detoxification that determines NP-formation and stabilization (Hulkoti and Taranath, 2014).

Algal species depending upon their characteristics, can also be employed for the fabrication of metallic NPs (Siddiqi and Husen, 2016). Biomolecules such as proteins, sugars and various secondary metabolites released by algae play a fundamental role in NP-biosynthesis. Algal membrane-proteins template metals ions and extracellular polysaccharides to reduce variable metal ions and stabilizes metal-NPs. Flavonoids and terpenoids have been found as effective capping and stabilizing agents for producing metal-NPs, thus, fabricating the size, shape and design of the NPs (Purohit et al., 2019). Algal species, *Chlorella vulgaris* fabricates AgNPs, *Phaeodactylum tricorutum* generate CdSNPs and *Stephanopyxis turris* readily forms AuNPs (Jeevanandam et al., 2018). In addition, screening actinomycetes for their inherent ability for the production of NPs has opened an area for further exploration. *Thermomonospora* sp., extremophilic actinomycete, can extracellularly synthesize monodispersed, spherically shaped AuNPs with an average size of 8 nm (Golinska et al., 2014). Different genera of actinomycetes viz. *Streptomyces*, *Thermomonospora*, *Nocardia* and *Rhodococcus* have been studied extensively for biosynthesis and structural characterization of NPs (Ranjitha and Rai, 2017).

Owing to nanoscale sizes and shapes, viruses are generally considered natural nanoarchitectures. The three-dimensional viral particle structure is profusely covered by proteinaceous subunits, which are further built up of different amino acids containing amino, thiol and carboxylate group side chains representing an extremely reactive layer

with a high affinity for metal ions. These side chains help in the nucleation of the metal ions resulting in metallization at the outer surface of the virus (Shah et al., 2015). The virus particles also possess a hollow internal cavity within the viral capsid, where metals can readily diffuse and interact with internal amino acid side chains. Thus, the internal cavity of the virus serves as a natural nano-reactor that fabricates metal NPs of varied shapes and sizes (Purohit et al., 2019). Despite being a stable, reliable and green approach to synthesis, the rate of formation of NP is not equivalent to non-biological production methods. The microbial synthesis will have more considerable commercial recognition only if this biological approach can synthesize NPs more speedily and cost-effectively on a large scale. Nonetheless, for achieving shape, size, composition, stability, and the production rate of NPs, biological approaches can be optimized (Shahverdi et al., 2011). Bacterial and fungal NP-synthesis has gained more significance compared to actinomycetes and yeast, mainly due to the availability of more mature technology. Moreover, due to their non-hazardous nature and improved efficiency, the microbial resource is considered as one of the best resources for the synthesis of NPs.

7. Conclusion and future perspectives

It is quite a fact that technological advancements in nanotechnology have gained immense importance. However, it has caused some dilapidating effects on the ecosystem. Researchers revealed that NPs stimulates plant growth and controls pollution, but these tiny particles pose serious threat to the environment and rhizospheric populations owing to their accumulation potential. Due to their dual actions, it is necessary to attain useful information regarding NPs and their impact on biological interactions among soil. The abundant production of nano-goods and their discharge and persistence within soil ecosystem has disrupted beneficial microflora and soil composites. Owing to characteristic properties of surface charges, area, size and reactivity the hinder the positive interactions among soil-plant-microbes. Like, few of them adsorb onto microbial cells or get invaded to cause further destruction. However, the lethal impact of NPs on rhizosphere needs to be explored further in detail by state-of-art techniques. Besides, there are few

knowledge gaps in relation to NP interactions, NP-mediated damage to microbes and soil, and NP-stress within environment. In the present review, it has been well documented that NPs possess an adverse impact on soil health, enzymes and microbial proliferation by their toxicity mechanisms. Moreover, interactions and mechanistic overview of NPs with soil-ecosystem and rhizospheric community have been well presented. Here, we have comprehensively presented the NP-synthesis and types along with their hazardous impact. The direct impact of NP-accumulation is deposition affects on soil health, therefore, a regulatory network is also devised to combat NP-toxicity. An explosion of NPs in soil could hamper microflora, thereby, a very close association among the two has been explained in the form of toxicity, accumulation, and resistance mechanisms. Together, we have indicated the adverse effects of NPs and their behavior within the ecosystem, along with suggested mitigation strategies. We have also elucidated several approaches for NP-engineering and regulation based on sustainability. By implementing these methods, it could be quite feasible to focus on the fate of NPs in the environment and methods safeguarding the ecosystem and their engineers. Presently, due to the paucity of knowledge, we can anticipate that this review could extrapolate the comprehensive framework of NPs impact on soil and ecosystem engineers, mainly rhizosphere communities.

A depth-in knowledge by genomics and proteomics can further aids the comprehensive understanding. Furthermore, the future perspectives include the aspects encompassing bioavailable levels of NPs and metal ion interactions with soil and microbes. This would provide us with a wholesome approach regarding negative aspects of NPs. Taking into account the structural and functional toxicity of NPs towards environment, techniques for safe disposal in soil agroecosystem are prevalent and safe and should be developed so as to minimize their contact with soil microflora. In other words, the products should be designed as target-specific for their application. Henceforth, by conducting microcosm research based on this regard, many elusive results may be established to pave new paths in this field. We strongly need these studies to effectively understanding this subject to protect the ecosystem.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Kanika Khanna, Sukhmeen Kaur Kohli, Neha Handa, Harsimran Kaur, Puja Ohri, Renu Bhardwaj designed and wrote the first draft of the manuscript. **Balal Yousaf, Jörg Rinklebe and Parvaiz Ahmad** revised the manuscript to the present form.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

None.

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