



A Concise Overview of Effect of Nanomaterials in Soil and Associated Microbiota

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ABSTRACT

Engineered nanomaterials are a cause of concern for environment in entirety due to their unregulated release, which in turn is due to their unregulated synthesis/ production, disposal and continuously rising applications in multiple fields. Although a complete account of impact of these particles in environment is absent, it has already drawn much attention of scientists and crucial work is being done in this field. Current report intends to deliberate on issues related to the harmful impacts of waste containing nanoparticles, particularly on soil and organisms therein. Particularly, we focus on the toxicity of nanoparticles and how they affect soil habitats. In addition, this paper discusses major prevention and risk management approaches. The increased use of nanoparticles (NPs) resulted in their accumulation in the environment, such as soil, generating concern about their hazards. Industrial nanoparticles contain heavy metals, which may cause problems due to toxicity and bioaccumulation. Nanowaste treatment requires understanding of chemical, physical, and biological properties of NPs. Waste management also requires thorough risk assessment of new materials.

Keywords: Engineered nanomaterials; Soil; Toxicity; Risk assessment.

1. INTRODUCTION

The field of nanotechnology developed as a result of the innovative, exploitable characteristics that these materials have in the nano size range (Malakar *et al.* 2021; Yu *et al.* 2021). These NPs exhibit distinct physical and chemical behaviour due to their large surface to volume ratio, incredibly small size, and size-dependent optical characteristics (Sajid *et al.* 2015). The types of nanomaterials (NMs) have diversified with time. Besides this, other factors like routes of synthesis and the range of fields in which they are used and their final disposal may add additional stress on soil system (Fig. 1). Nanomaterials are mainly divided into four categories, such as inorganic-based nanomaterials, carbon-based nanomaterials, organic-based nanomaterials and composite-based nanomaterials. Apart from their material, nanoparticles vary in terms of dimensions, shapes, and sizes. Nanoparticles can be zero dimensional, where their length, breadth, and height are all fixed at one point as in nano dots; one dimensional, where they can have only one parameter as in graphene; two dimensional, as in carbon nanotubes; or three dimensional, as in gold nanoparticles, where they have all three dimensions (Fig. 2). Broadly, metals and metal-oxides come under inorganic nanomaterials (Ealias *et al.* 2017). Silver, gold, aluminium, copper, zinc are some of the examples of metal-based nanomaterials, whereas zinc oxide, copper oxide, cerium oxide, iron oxide fall under metal-oxide nanomaterials (Sharma *et al.* 2015). As per the illustration in Fig. 3, considering organic

nanomaterials, they are formed from the organic substances eliminating carbon materials such as dendrimers, liposome, micelle and cyclodextrin (Bhatia *et al.* 2016). Composite based nanomaterials are the combination of metal based, metal oxide, carbon based and organic/inorganic nanomaterials (Zhao *et al.* 2019).

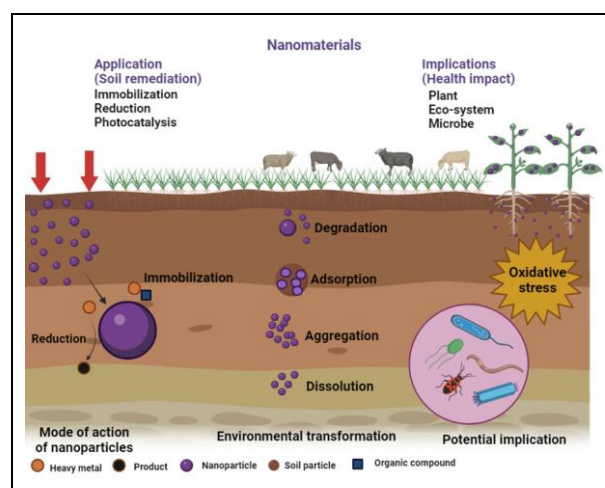


Fig. 1: A depiction of effect of nanomaterials on soil habitat and environment

With the widespread usage of nanotechnology, nanomaterials are released into the environment. Since industry uses nanotechnology, wastes with residual nanomaterials are produced (Shafique *et al.* 2019). The effects of nanoparticles on the receiving body depend on

their size, mass, chemical composition, surface, and how they aggregate. Exposure assessment, toxicology, toxicological databases, environmental and biological fate, transport, persistence, and transformation of nanoparticles, and recyclability and overall sustainability of nanomaterials can be used to assess nanotoxicity.

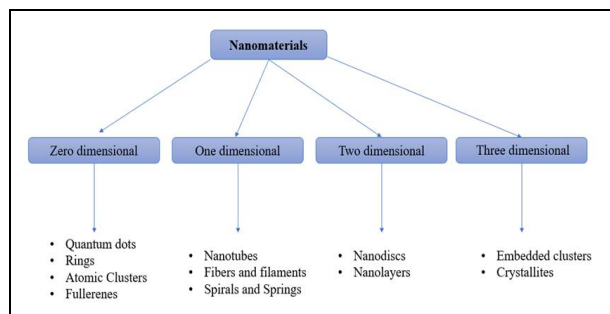


Fig. 2: Types of nanomaterials as per their dimension

New nanotoxicology research programmes are needed because nanoproducts are spreading rapidly in the environment. The fact that nanoparticles are so small is what makes them so valuable in industry and medicine, but it is also one of the key things that could make them potentially harmful to human health. Recent research works demonstrate that smaller the particles, greater the reactivity and the more harmful the impacts. This is due to the likelihood that any intrinsic features of particles will be highlighted by the increase in surface area per unit mass (Liao *et al.* 2023). It is acknowledged that there are inherent risks associated with new technologies. However, one particular concern with nanotechnology is the type of toxicity that results from surface modification. It has been shown that increased endocytosis, which may potentially have a tendency towards inflammatory and prooxidant activity, is mostly caused by surface chemistry (coating) of nanoparticles and in vivo surface modifications. The Organisation for Economic Co-operation and Development (OECD) recently began testing the nanomaterials and their effects on health and environment (Kühnel *et al.* 2014).

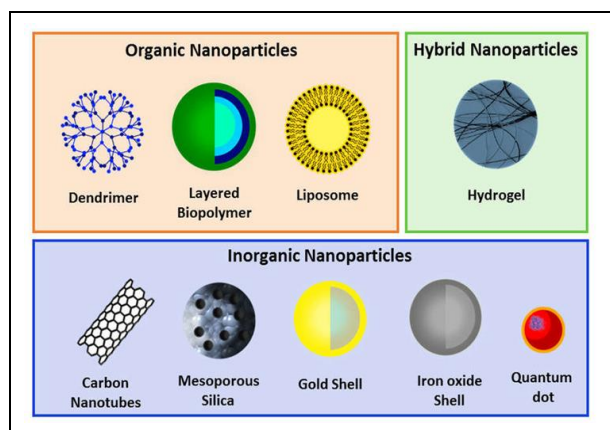


Fig. 3: Types of nanomaterials as per their shape and size (Silva *et al.* 2019)

2. OBJECTIVES

The aim of this article is to bring together a concise understanding of impact of both nanomaterials and their engineered forms on soil and associated organisms and the current status of measures to tackle the same. We conducted a detailed review of literature (2010 to 2023) regarding the sources of nanomaterials affecting the soil enzymes, microbiota and their effects on variety of plants, their prevention and risk management.

3. ENVIRONMENTAL FACTORS AFFECTING THE TOXICITY OF NANOPARTICLES

Nanoparticles are employed in a wide variety of goods for a variety of uses, increasing the likelihood of contamination of the environment. Nanomaterials can be released inadvertently or on purpose. Nanoparticles represent a concern when discharged into the air, soil, and water because they are very small particles that can float into the air or be transported to another location via water. Soil contamination causes a long-term build up and pollution of groundwater. Nanoparticle toxicity may be influenced by environmental conditions (Sajid *et al.* 2015). Weather variables like humidity, temperature, wind speed, latitude, and kind of light may have an impact on several NP qualities that contribute to their toxicity (Rajput *et al.* 2018). At higher temperatures, NPs can scatter more quickly than at lower or normal temperatures. It is well known that NPs react differently to different types of light, including visible and ultraviolet light. With the help of wind speed, these tiny fragments can penetrate the tissues of both plants and animals (Zhang *et al.* 2018). It will be necessary to take into account that the following factors in order to predict the effects of nanomaterial in the ecosystem: (i) the form, route, and mass of nanomaterial entering the environment (characterization and risk assessment); (ii) the fate and transport of NMs in environmental media (bioavailability); (iii) response of organism to nanomaterial exposure (ecotoxicology); and (iv) the effects of nanomaterial inputs on ecological communities and biogeochemical processes. It may be impossible to distinguish between the direct effects of the nanoparticles and the indirect effects caused by components released from the original engineered nanoparticles (ENP) in experiments where ecological impacts are quantifiable if the fate, transport, and transformation of the initially added ENPs cannot be determined. In the absence of measurements, it is impossible to identify which environmental mechanisms 'protect' the biota from ENP toxicity. These mechanisms could include simple ENP aggregation into less reactive large particles, absorption of ENPs onto organic matter or soil minerals, or dissolution of ENPs into constituent solutes. Therefore, the analytical constraints that exist now significantly limit our ability to create mechanistic explanations for the effects of ENP during ecological study (Bernhardt *et al.* 2010).

4. SOURCES OF NANOPARTICLES IN SOIL AND THEIR EFFECTS

Models indicate that soil, more so than air or water, is a significant receiver of NPs, which is causing increasing worry. These days agricultural practices are introducing the use of excessive amounts of various NMs, making soil a potential sink (Rajput *et al.* 2020). Currently, the primary way of NP deposition onto land is the dumping of sewage sludge from wastewater treatment plants (WWTPs), where NPs released from consumer products into wastewaters may partition into sewage sludge during the wastewater treatment process (Fig. 4 and Fig. 5). As an example, depending on the washing agents employed and how the particles are mixed into the textile, different amounts of Ag NPs may be released during the washing of textile products. According to the experimental data, up to 99% of the TiO₂ NPs that enter WWTPs are retained in the sludge phase and end up in the terrestrial region rather than aquatic environment. During transportation, nanoparticles are dispersed into the soil environment. When the NPs interact with the organic and inorganic components of the soil matrix, it becomes more complicated leading to altered toxicity. Therefore, it is crucial to understand the link between their real exposure concentrations and biological impacts on crops and symbiotic organisms.

Nanoparticles can enter the soil through industrial spills, landfill sites or during the application of sewage sludge as a fertilizer (Mukherjee *et al.* 2018). Different entry points for the metal and other NPs into soils are possible. Metal-based nanoparticles have a variety of distinctive properties that are thought to have a significant role in regulating their ecotoxicity, environmental behaviour, and fate (Dubey *et al.* 2016). These include physical characteristics, particularly size and shape, and chemical characteristics such as the acid–base character of the surface and the aqueous solubility of the metal (Gottschalk *et al.* 2011). Understanding how particular species are exposed to nanoparticles present in various phases (soil, soil water) as well as how the presentation of the NPs within these phases further affects exposure, is crucial in the context of ecotoxicity (Zahoor *et al.* 2021). The growth and productivity of significant crops may be hampered by the widespread usage of metal- and metal oxide NPs and their potential introduction into the food chain through plants. Reviewing earlier works on the possible toxicity of NPs in crops is therefore useful to regulate the disposal of NPs (Table 1). Plant health is indicated by morphological metrics such as leaf area, shoot and root lengths, and shoot and root weights. Negative impacts of metal-based nanoparticles such as ZnO, Fe₂O₃, aluminium dioxide (Al₂O₃), and CuO on shoot/root growth and elongation have been found in several crop species including rice, wheat, maize, tomato, and barley.

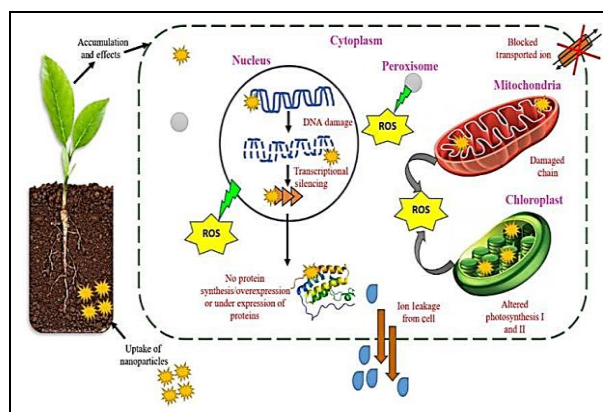


Fig. 4: Accumulation of nanoparticles by plants and their effects (Bakht *et al.* 2020)

According to recent studies, toxicity may be caused by increased metal ion release from NPs (Rizwan *et al.* 2017). Many researchers have reported that many crops, including wheat, rice, sorghum, and tomato were hindered in terms of root growth and biomass by Ag NPs (1000 ppm, size: 25 nm, 12 days). They damaged rice root cell walls and vacuoles, possibly due to substantial NP penetration through small cell wall pores (Dimkpa *et al.* 2012; Vannini *et al.* 2013). It has been found that treatment with lower concentrations of Ag NPs (up to 30 mg L⁻¹) stimulated rice root growth, whereas at higher concentrations (above 60 mg L⁻¹), the root growth was slow leading to root mortality. Shoot development was more vulnerable to NP stress (Thuesombat *et al.* 2014). It has been found that CuO NPs (20 ppm for 15 days) reduced root length by 49.5% and 47.6% in lettuce and lucerne, respectively, and the roots turned brown when compared to control plants (Hong *et al.* 2015). Decreased root growth in soybean and chickpea by using CuO NPs over 500 ppm has been reported (Adhikari *et al.* 2012). Several studies have found that ZnO NPs are hazardous to the growth of several crops grown in a wide range of habitats. ZnO NPs (500 and 750 mg kg⁻¹ of soil in pot) decreased lucerne root and shoot biomass by 80% and 25%, respectively (Bandyopadhyay *et al.* 2015). Mousavi Kouhi *et al.* 2015 investigated the anatomical and ultrastructural changes in rapeseed roots and leaves treated with ZnO NPs (100 mg L⁻¹, size: for 2 months). ZnO NPs reduced the diameter of the root tip as well as the size of epidermal and pericyclic cells. The NPs also reduced the number of chloroplasts, mitochondria, and plastoglobuli in the leaves while increasing the size of starch grains and the quantity of plastoglobuli (Mousavi Kouhi *et al.* 2015). Although NPs have both positive and negative impacts on plant development and morphology, the response varied with dose, plant species, experimental settings, and exposure period. In Fig.5, one can observe that in soils contaminated with metal- and metal oxide NPs, the first step that influences the success of crop growth is seed germination. Metal nanoparticles have recently been widely shown to have harmful effects on the germination of food crop seeds. In general, the

toxicity of NPs decreased seed germination rates and spread out the germination events of many varieties of crops (Moon *et al.* 2014). The findings demonstrated that Ag NPs reduced faba bean and turnip germination in a dose-dependent manner. Silver NPs reduced the germination of barley seeds by 10% to 20% in comparison to the control. With increasing Ag NP dose and size, germination of rice seeds decreased. Similarly, by adhering to seed surfaces and releasing free metal ions close to the seeds, metal oxide nanoparticles (CuO, NiO, TiO₂, iron oxide (Fe₂O₃), and CO₃O₄) inhibited the germination of lettuce, radish (*Raphanus sativus* L.), and cucumber seeds (Joško *et al.* 2013). According to the researchers, the adverse effects of NPs on seed germination differ depending on the crop (lettuce > cucumber > radish) and seed size. A research team from University of Oklahoma, Kibbey *et al.* 2019 have studied the effect of three different types of nanomaterials, i.e TiO₂ nanopowder, fluorescent red polystyrene nanoparticles and groundwater nanomaterial suspension at different concentrations on lettuce seedlings. They have observed the TiO₂ might have attached to the surface of rhizospheric bacteria ultimately influencing

the plant-microbe interaction by changing the surface activity of the microorganism (Kibbey *et al.* 2019). Additionally, seed germination differed in various soils; for example, it was less evident in clayey soil than in sandy soil (Damodaran *et al.* 2019). The impact of cerium oxide (CeO₂) NPs on the seeds of tomato, cucumber, maize and lucerne (*Medicago sativa* L.) at a concentration of 0–4000 mg L⁻¹ has been studied. The result showed that the seed germination of tomato, maize and cucumber reduced by 20–30% by the application of 2000 mg L⁻¹ CeO₂ (López-Moreno *et al.* 2010). According to several studies, NPs may partially dissolve and release harmful metal ions or cause surface modifications in the exposure solution or plant tissues, which might hinder NP-mediated seed germination (Ma *et al.* 2011). However, scientists claimed that NP concentrations above a certain point were harmful to plants and inhibited seed germination. TiO₂ NPs improved the germination of wheat seeds at concentrations between 2 and 10 ppm while decreasing it at higher concentrations (100 and 500 ppm) (Feizi *et al.* 2012).

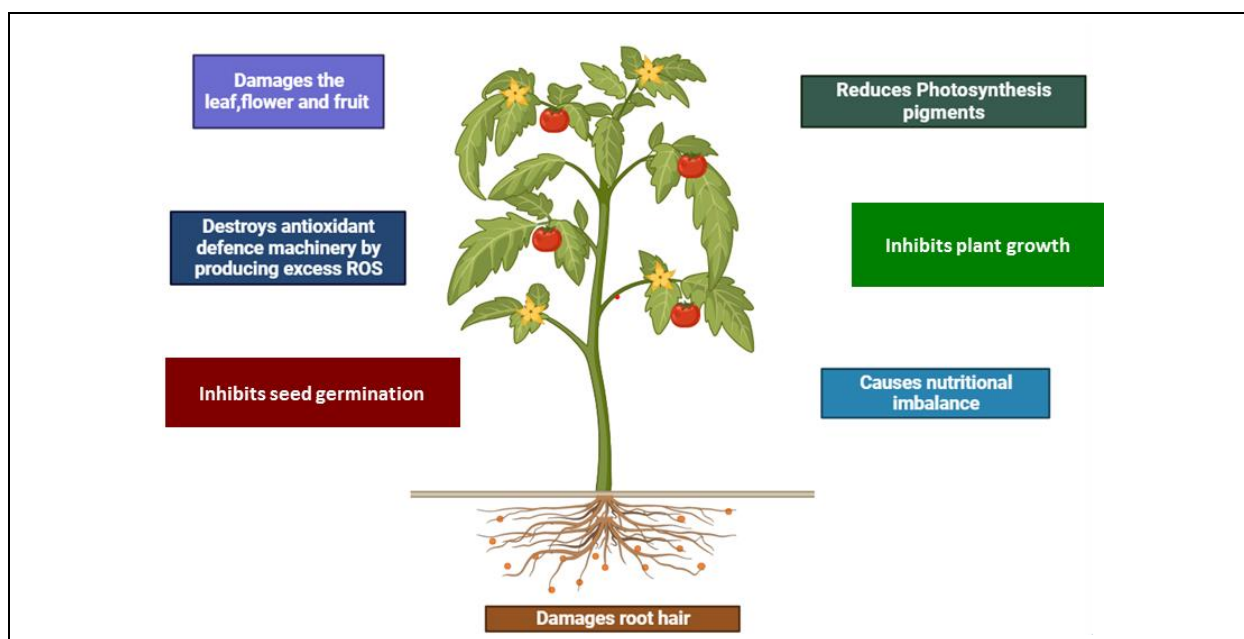


Fig. 5: Effect of nanoparticle on plants via soil (Kumar *et al.* 2023)

The synthesis of NPs has expanded quickly, and they are widely used in agriculture, increasing the probability that they may reach the soil. Many NPs accumulate in plant tissues, including the edible portion of plant tissues, and it is now widely acknowledged that some of them have an adverse impact on crop development and output (Rajput *et al.* 2018). After uptake, NPs might be poisonous to plants on their own, or it might be because NPs release toxic ions after breaking down. The negative biological effects of NPs include an increase in ROS generation due to the

presence of additional electrons or as a result of NPs interacting with biomolecules and generating oxidative stress as well as affecting the water uptake in plants along with the metabolic pathways and photosynthesis (Kumar *et al.* 2019). Inhibition of germination, reduced shoot and root growth, toxicity, and drop in photosynthetic rate and chlorophyll concentrations have all been observed due to the effects of NPs on crops like onion, spinach, coriander, wheat, rice, soybean, mung bean, radish, lettuce, barley, cucumber, and tobacco (Tripathi *et al.* 2012; Singh *et al.* 2016) (Fig. 4). Hong *et al.* (2015) have found that a

significant change in photosynthetic characteristics of cucumber were seen after treatment with 200 mg L⁻¹ of CeO₂ and CuO₂ NPs. Alteration in transcriptome in response to NPs and DNA degradation has been reported (Shen *et al.* 2010; Kumari *et al.* 2011). Ma *et al.* (2011) have studied that CeO₂ nanoparticles are hazardous to cotton plants causing the death of vascular bundles and chloroplasts as well as affect the nutrients intake. According to existing knowledge, these engineered nanomaterials have an impact on the environment and interact with plants, soil, and plant-soil microbiomes (Fig. 6). The microbiomes of the soil and plants are deeply impacted by these interactions, which in turn impacts plant health. The size, shape, chemical makeup, surface charge, hydrophobicity, and other characteristics affects the activity of engineered nanoparticles (Khan *et al.* 2022).

Microorganism secretes beneficial organic acids, enzymes and hormones which get affected by the accumulation of nanoparticles in soil. To summarise, metal- and metal oxide nanoparticles decreased or enhanced seed germination in a variety of plants. The plant response varied considerably between NPs and was inversely linked with dose and size of NPs. Since soil, soil microbiome, and plant microbiome differ from plant to plant and soil to soil, the relationships of nanomaterials with the soil ecosystem cannot be broadly generalized. Although NPs either positively or negatively impact the

seed germination of the investigated plants, the mechanisms underlying germination, particularly in soil, are still in a stage of discovery.

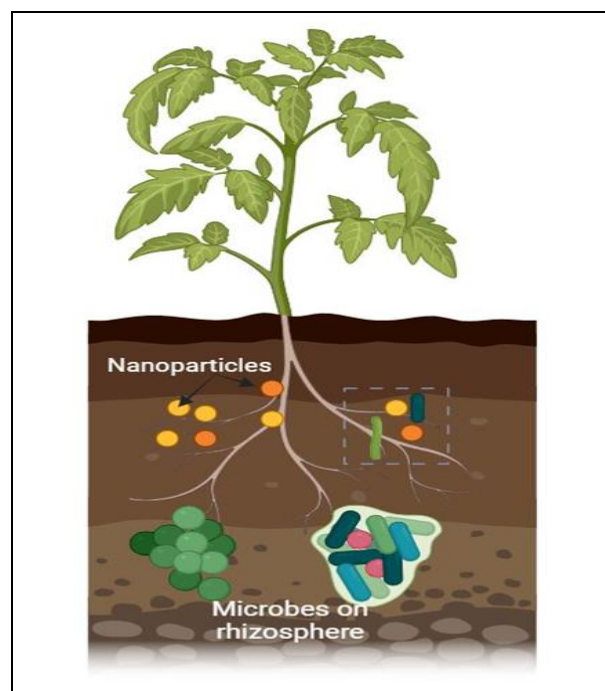


Fig. 6: Illustration of accumulation of nanoparticles with soil microbiota (Ameen *et al.* 2021)

Table 1. Effects of ZnO nanoparticles on plant performances

Plant species	Concentration mg L ⁻¹	Toxic effect of ZnO nanoparticles on different plant species	References
Alfalfa	500-750	Reduced root and shoot biomass by 80%	(Bandyopadhyay <i>et al.</i> 2015)
Wheat	500	Reduced root growth, increased lipid peroxidation and oxidized glutathione in roots, decreased chlorophyll content in shoots, increased ROS production	(Dimkpa <i>et al.</i> 2012)
Spinach	1000	Reduced root and shoot length, total weight, chlorophyll and carotenoid content	(Singh and Kumar, 2016)
Soybean	0.05-0.5	Reduced root and shoot growth, affected seed formation	(Priester <i>et al.</i> 2017)
Pea	25-500	Reduced chlorophyll and catalase content in leaves and ascorbate peroxidase in roots and leaves	(Mukherjee <i>et al.</i> 2013)
Maize	20	Aggregates penetrated the root epidermis, cortex and accumulated in xylem vessels	(Zhao <i>et al.</i> 2012)
Mustard	500-1500	Affected seed germination and seedling growth	(Zafar <i>et al.</i> 2016)
Bean	100-500	Inhibited growth, imbalanced nutrient in shoots, Na increased, Fe, Mn, Zn and Ca decreased	(Dimkpa <i>et al.</i> 2014)

Table 2. Effects of various nanoparticles on soil microbial communities

Nanoparticles	Concentration in soil (mg kg ⁻¹)	Toxic effects	Reference
ZnO Fe ₃ O ₄	500-1000 2000	Affected enzymatic activities (invertase, urease, catalase, and phosphatase) and bacterial communities of saline-alkali and black soils	(You <i>et al.</i> 2018)
Fe ₃ O ₄	0.1-10.0	Significantly decreased the content of bacteria in soil	(Cao <i>et al.</i> 2020)
ZnO	1000	Affected plate counts of <i>Azotobacter</i> , <i>P-solubilizing</i> and <i>K-solubilizing</i> and inhibited enzymatic activities	(Chai <i>et al.</i> 2015)
CuO	0-1000	Decreased soil microbial biomass, enzymatic activities, disturbed community structure	(Xu <i>et al.</i> 2015)
Fe	550	Individual analysis showed effect on bacterial group	(Shah <i>et al.</i> 2014)
CuO	10	Affected soil microbial community	(Ben-moshe <i>et al.</i> 2013)
Cu	220	Reduced C and N biomass, disturbed microbial community structure	(Kumar <i>et al.</i> 2012)

5. INFLUENCE OF NANOPARTICLES ON SOIL ENZYMES AND ORGANISMS

An estimated 11 million tonnes of metal- and metal oxide NPs are produced worldwide each year, with soil resources ultimately receiving them (Zhao *et al.* 2019). Eventually, the nanoparticle gets accumulated to the roots of different plants and goes deep down by penetrating the layers of soil and disturbs the soil pH as well as microbe present in the rhizospheric region (Fig.6). Soil extracellular enzymes are important in physiological soil activities such as breakdown of organic compounds, mineralization, and nutrient recycling. Moreover, their ability to respond to environmental stimuli makes them a possible indication of soil microbial quality (Colman *et al.* 2013). Thus, determining soil enzymatic activity is one technique to evaluate the effects of engineered nanoparticles as an external disturbance on soil microbial processes (Table 2) (Baldrian *et al.* 2012). After being exposed to nano Ag, extracellular soil enzymes such as leucine amino peptidase and phosphatase exhibited 52% and 27% reduced activity, respectively. It has also been demonstrated that soil protease, catalase, and peroxidase activities are suppressed in the presence of 100 nm nano ZnO and nano TiO₂ (Du *et al.* 2011). At 300 – 1000 mg kg⁻¹ soil, single-walled carbon nanotubes significantly decreased microbial biomass and the activity of soil extracellular enzymes involved in phosphorous, nitrogen, and carbon cycling (Jin *et al.* 2013). Peyrot *et al.* (2014) discovered that 210 nm polyacrylate-coated nano Ag inhibited the activity of soil hydrolases involved in the hydrolysis of P, S, C, and N. A recent study found that exposure to 50 nm citrate-

coated gold nanoparticles (nAu) resulted in significant increases in the activity of five extracellular soil enzymes after 30 days, whereas exposure to 50 nm PVP-coated nAu resulted in an initial decrease in enzyme activity but a recovery or increase after 30 days (Asadishad *et al.* 2017). The impact of ENP amendments on the soil microbial community must also be considered. For example, if changes in the composition of the soil microbial community modify the soil nutrient cycling capacity or overall bacterial diversity, such changes may have an impact on crop productivity. Nanoparticles may penetrate bacterial cell walls by endocytosis, and many of them, particularly silver, copper, and zinc, have antibacterial characteristics (Sun *et al.* 2014). In the cells of two soil bacteria, *Bacillus cereus* and *Pseudomonas stutzeri*, Ag NPs had bactericidal effects and altered their morphology, while Al₂O₃ NPs exhibited no detectable toxicity at all of the tested doses or time points (De Volder *et al.* 2013). Factors including functionalization, concentration, exposure time, and soil texture have a big impact on how Ag NPs affect the soil microbial community. Also, it has been noted that Ag NPs inhibit enzymatic activities, while Cu and Zn-based NPs inhibit bacterial growth and biomass.

5.1. Impact of nanopesticides

When nanopesticides are introduced to farmland, the interactions between biological systems and nanoparticles are found to be complex. Fojtová *et al.* (2019) have tested four model nanopesticides which includes chlorpyrifos and tebuconazole loaded on polymeric and lipid nanocarriers. The fate and bioaccumulation of the nanopesticides in soil microcosms harbouring earthworms (*Eisenia fetida*) and

lettuce (*Lactuca sativa*) were studied. The higher bioavailability of active chemicals in nanopesticides was detected in comparison to pure active ingredients, indicating a larger bioaccumulation of active ingredients in *Eisenia fetida*. More importantly, the intense transfer of the tebuconazole nanopesticides from the roots to the edible sections of the lettuce may endanger human health via the food chain. As reported by Keller *et al.* 2018; Kah *et al.* 2019 copper oxide nanopesticides were applied to edible plants such as lettuce, kale, and collard green. Furthermore, a certain fraction of copper oxide nanopesticides was taken up by plant leaves, resulting in copper oxide nanoparticle bioaccumulation, posing a risk to humans who consume these edible plants.

6. PREVENTION AND RISK MANAGEMENT RUNOFF

A significant number of nanoparticles, including those in the ambient air and water, have entered the environment as a result of the growth of nanotechnology. Information from several fields, such as source characterisation, fate and transport, modelling, exposure assessment, and dose-response characteristics, must be integrated in the complicated process of risk assessment (Rana *et al.* 2013). An early risk assessment entails following the Environment, Health, and Safety (EHS) standards, which are frequently neglected in various workplaces and the environment and hence restrict the assessments of exposure to hazardous substances. The potential for the nanoparticle to be harmful is assessed during the hazard evaluation (Bakand *et al.* 2016). Endpoints such as physiological, genetic, or functional consequences, either acute or chronic, can be used to quantify hazards such as toxicity and ecotoxicity. But under a risk assessment paradigm tailored to nanoparticles, there may also be additional potentially harmful environmental impacts on atmospheric/stratospheric processes, soil stability, and the bioavailability of mineral nutrients.

In the process of risk assessment, dose-response evaluations come after hazard identification (Auría-Soro *et al.* 2019). It may be necessary to conduct laboratory studies or use mathematical models in order to determine dose-response connections (Stone *et al.* 2010). However, dose-response relationships may not be straightforward for nanoparticles because the method of synthesis may lead to changes in surface reactivity and hence toxicity (Solano *et al.* 2021).

7. CONCLUSIONS

The pH of soil is not significantly impacted by nanoparticles unless they are put in high quantities, but they may have a specific impact on the geochemical cycle of the elements in the soil, influencing the amount of organic matter, nutritional elements, and even microelements. There is a possibility that nanoparticles

can easily penetrate to the cell wall to enter the interior of the plant. Similar to conventional pollutants, nanoparticles have a hormesis effect on plant growth and development. There are just a few of the ways that nanoparticles can promote plant growth and development at low concentrations. Nonetheless, at elevated levels, nanoparticles may cause plants to produce reactive oxygen species, hinder the assimilation of macro- or microelements, interfere with regular physiological processes, and impact the composition and functionality of the plant's symbiotic microbiome. Plant growth and development would be inhibited as a result of them. Further study is therefore needed to assess the toxicity and transformation of ENPs upon human exposure, utilizing biosensor and computationally assisted analytical approaches in addition to the already used in vitro and in vivo methodologies. The widespread use of nanotechnology and nanomaterials in products will generate large amounts of new-generation waste in the near future. Future research should focus on nanotoxicology, nanobiomonitoring, and environmental effects of nanoparticles.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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