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REVIEW

Current understandings of toxicity, risks and regulations of engineered nanoparticles with respect to environmental microorganisms

Krishnamoorthy Hegde[1] · **Satinder Kaur Brar**[1] · **Mausam Verma**[1,2] · **Rao Y. Surampalli**[3]

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Abstract Engineered nanomaterials offer exciting application opportunities in diverse fields ranging from biomedical, agriculture, environmental, cosmetics to household commodities. Increasing use of nanomaterials in day-to-day applications has also increased their exposure to environment and ecosystems significantly, which has raised the concern for environmental safety due to their potential adverse and toxicological effects on microbial community. Although several risk and safety assessment studies to evaluate the fate of nanoparticle in the environment and their effect on living organisms are being carried out in the recent years, still the current knowledge on impact of these nanomaterials on microorganisms is limited. The present review comprehensively summarizes and interprets the impact of nanomaterials on microbial community. Further, the technical challenges associated with the nanotoxicity evaluation, data interpretation and environmental regulations with respect to engineered nanomaterials are discussed.

Keywords Nanotoxicity · Ecotoxicity · Risk assessment · Engineered nanomaterials

Introduction

The unique properties of the engineered nanomaterials (ENMs) offer great promise to provide innovative technological solutions in diverse field of applications. Today, ENMs are increasingly being introduced into array of everyday products, such as health care, cosmetics, household appliances, broad spectrum antimicrobial agent and many other applications [44, 59]. The widespread use of nanoproducts also resulted in its rapid incorporation into the environment via various process routes, such as manufacturing, product use and waste disposal [39]. Several reports indicate that concentration of some of the ENMs, such as TiO_2, fullerenes, nanosilver, ZnO, carbon nanotubes (CNT), is significantly higher in the environment, and the evidence on potential toxic effects of ENMs on living organisms is constantly being accumulated. Exposure of hazards associated with ENMs could potentially impact the function of soil, sediment and aquatic microbial communities [14, 16]. The mechanism of uptake of ENMs by microbes, their environmental fate and persistence, adverse effects on metabolism and bioaccumulation in living organisms is still not completely understood. Moreover, our knowledge on the environmental and ecological impact of ENMs is limited and it significantly lags the pace of industrial growth of nanotechnology.

Risk assessment and toxicological studies of nanowastes on biological organisms is a very important segment of ecotoxicology and nanotechnology, which is essential to predict the effect of potential non-toxicity on living organisms so that the most efficient and effective action to prevent or remediate any detrimental effect of ENMs can be identified [35]. Unfortunately, due to the limited predictive power of ENMs, and unavailability of standard test

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✉ Satinder Kaur Brar
satinder.brar@ete.inrs.ca

[1] INRS-ETE, Université du Québec, 490 Rue de la Couronne, Quebec, QC G1K 9A9, Canada

[2] CO2 Solutions Inc., 2300, rue Jean-Perrin, Quebec, QC G2C 1T9, Canada

[3] Department of Civil Engineering, University of Nebraska–Lincoln, N104 SEC P.O. Box 886105, Lincoln, NE 68588-6105, USA

methods to identify adverse effects of nanomaterials, understanding of the mechanism of interaction of ENMs with an organism at molecular or cellular level is largely unknown. In the recent years, a number of efforts have been made to assess the risk factors associated with ENMs in the environment to calculate environmental concentrations of nanoparticles along with some experimental approaches to study the fate of ENMs under natural conditions [57]. The reports on several such toxicological studies of ENMs on microorganisms summons the critical need to establish a competent evaluation process to predict the environmental behavior of ENMs and their impact on soil microbial communities.

The microbial communities in the soil play a pivotal role in ecological and agricultural perspective. They are directly related to several balanced processes in the environment, such as decaying of organic matter in the soil, nutrient recycling, bioremediation of pollutants and symbiotically associated with several terrestrial plants [60]. Thus, the knowledge on the interaction of nanomaterials with the ecologically important microbial communities is very important for the balanced harmony of the ecosystem. Nevertheless, limited data are available on the interaction of ENMs with the environmentally important microorganisms. This review mainly focuses on the impact of ENMs on ecologically relevant microorganisms. Further, the present review summarizes and interprets the current knowledge status of the impact of nanomaterials on microbial community and the technical challenges associated with the nanotoxicity evaluation and data interpretation.

Toxicity of ENMs to microbial community

Route and fate of ENMs in environment

The technical advantages of the ENMs are attributed to their unique properties, such as small size, shape, chemical composition, solubility, surface structure and aggregation, which significantly differ from the properties of the bulk materials of same composition ([4, 44]). However, the same properties also contribute to their catastrophic fate in the environment and negative impact on microorganisms [35, 36]. Several studies focusing on the exposure modeling of the ENMs suggest that the ENMs concentration is higher in soil than in water or air, indicating the soil to be the major sink for the ENMs released into environment [16, 31, 47, 84]. For example, discharged nanowastes may undergo sorption with organic matter and biomass, aggregate or interact with other compounds or even undergo microbial biotransformation [9, 19, 46]. Further, the transport of ENMs with particle size <100 nm through porous media has higher mobility efficiency in the environment and is influenced by environmental conditions and Brownian diffusion [19, 65, 67, 70]. It is also true that the natural organic matter in the aqueous environment plays a crucial role in transport of ENMs due to their inclination toward colloidal absorption and aggregation via hydrophobic interactions. Thus, ENMs in the environment are influenced by several factors listed earlier, which may change its properties leading to altered bioavailability. On the contrary, it has also been reported that sorption of ENMs to biosolids in wastewater might be hindered in the presence of surfactants in effluent discharge [19, 40, 46].

Mechanism of ENM toxicity to microbial communities

Scientific knowledge gathered on the interaction of ENMs with biological systems in the environment indicates that they can bind and interact with biological matter and change their surface characteristics. Studies have also shown that biological cells can readily take up nanoparticles via active or passive mechanisms. Figure 1 shows various transformations of ENMs in the environment.

Microbial communities are exposed to ENMs at various levels in the environment, such as water, sewage, soil and sediments. The ENMs may have impact on microbial communities by various mechanisms, such as: (1) direct toxic effects, (2) indirect effects as a result of their interaction with natural organic compounds, (3) enhancing the toxicity of persistent organic pollutants in soil and water by interacting with them and (4) by changing the bioavailability of toxins or nutrients [16, 78]. Although the exact mode of toxicity of ENMs on microbial communities is still not completely understood, some of the possible mechanisms include: (1) damage of cell membrane; (2) oxidation of proteins; (3) genotoxicity; (4) interaction with respiratory chain and local proton depletion and; (5) reactive oxygen species (ROS) production and/or apoptosis [16, 36]. The mechanisms of antimicrobial action of ENMs are summarized in Fig. 1.

In most of the studies reported so far, the concept of ENM toxicity to the microorganisms is largely centered on cellular level mechanisms as discussed earlier, which describes the cellular function of the affected microorganisms. However, there are very little data available on the nanomaterial interaction at molecular level. For instance, the interaction of ENMs with Gram positive and Gram negative bacteria differs as the phospholipid bilayer, lipopolysaccharides and peptidoglycan composition of the cell wall in these organisms are different, thus, leading to different interactions and varied level of toxicity. This is also supported in recent reviews. Tegou et al. [82] and Zou et al. [95] reviewed that cell wall composition and charges on the bacterial cell wall largely attribute to the adhesion of

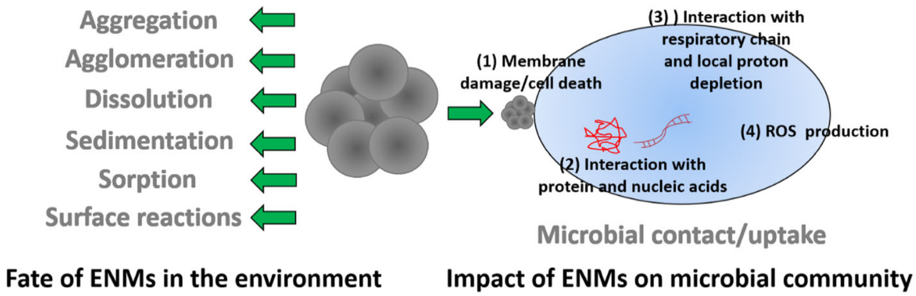

Fig. 1 Illustration of the dynamic transformations of ENMs in the environment and their impact on microorganisms (modified from [19, 57])

graphene nanomaterials and subsequent phenomenon of bacterial toxicity.

Though the toxicological effects of ENMs on pathogenic and ecologically relevant microorganisms are similar, the issue that raises severe concern is their toxicity to microorganisms that are beneficial to plant and those involved in the nutrient mineralization, nitrogen cycling and organic carbon degradation in soil. For example, ENMs such as TiO_2, ZnO, CuO, Ag, carbon nanotubes (CNTs) and fullerenes have been shown to reduce soil microbial communities and plant growth promoting microbial consortium, such as rhizobacteria and mycorrhiza. In some cases, the ENMs dissolve in the soil solution (Cuo, ZnO) or seawater (Ag) and affect the microbial community by a well-known mechanism of metal ion toxicity to cells [29, 39, 73, 85].

Rapid, reliable and cost-effective analytical methods for detection of ENM-microbial interaction are also very important for understanding the mechanism of interaction and the safe use and monitoring of nanomaterials in the environment. Several sophisticated spectroscopic methods, such as Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (NMR), mass spectrometry, Raman spectroscopy are being used for studying the microbial interaction and changes in the intercellular composition of the microorganisms [23]. The methods, such as FTIR, are highly reliable to assess the microbial interactions with nanomaterials at molecular level. However, most of these methods are time-consuming and not cost-effective. Further, these methods also need special instrumental infrastructure and skilled manpower for the analysis, which might not be available in most of the research laboratories. Further, the real-time monitoring of the microbial interaction in the soil and water might not be possible. In a recent study, Suppi et al. [81] proposed a simple and effective ‘spot test’ for the evaluation of biocidal effect of ENMs under the same test conditions for various organisms, such as bacteria, fungi and algae. In this test assay, the organisms were exposed to ENMs in deionized water to reduce the speciation-related effects on toxicity results and further cultured on agarized medium to study the lethal effect of ENMs. The study involved environmental and clinical specific bacterial strains, namely *E. coli* MG1655, *S. aureus* RN4220, *P. fluorescens* OS8, *P. aeruginosa* DS10–129, *Janthinobacterium* sp, *Microbacterium testaceum* PCSB7 and eukaryotic microorganism, *Saccharomyces cerevisiae* BY4741 and microalga *Pseudokirchneriella subcapitata*. The biocidal potency of different ENMs of various compositions, such as Ag, CuO and TiO_2 and MWCNTs, revealed that the mechanism of toxicity is similar among the organisms, irrespective of type of microorganisms. The facts indicate that in the environment, where the microorganisms are not protected from toxic chemicals, the level of tolerance to ENMs is rather chemical dependent than the type of organisms. Though the test method is reliable for ‘quick’ evaluation of toxicity of ENMs on various microorganisms under laboratory conditions, the method might not be accurate for real-time monitoring of the toxicity in the environment due to the fact that the bioavailability of the ENMs is influenced by several factors in the environment, which may change the properties of ENMs.

The following section briefs the recent toxicological studies relevant to some class of ENMs, which are abundantly used in various consumer products and other applications. Table 1 further summarizes the effect of some of the engineered nanoparticles on microorganisms.

Toxicity of silver nanoparticles (Ag-NPs)

Ag-NPs are one of the most commonly used antimicrobial nanomaterials in range of consumer products and for medical applications. The release of Ag-NPs into the environment may have negative impact on the environmental microorganisms responsible for the various biogeochemical cycle in the environment. Various studies indicate that Ag-NPs interfere with cell membrane integrity of microorganisms [1, 33]. Toxicological effect of Ag-NPs on ecologically important bacteria, such as nitrifying bacteria (*Nitrosomonas* and *Nitrobacte*r), *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*, has been extensively studied for pure culture media [5, 7, 11, 49, 66, 80]. For instance, Beddow et al. [7] studied the effect of capped and uncapped

Table 1 Effect of some of the engineered nanoparticles on the microorganisms

Nanoparticle	Microorganism	Effects	References
Ag-NPs biosynthesized by fungi	Fungus (*Aspergillus niger*) and bacterial strains (*Staphylococcus* sp., *Bacillus* sp. and *E. coli*)	Toxicity	Rajkishore et al. [71]
Ag0	*Escherichia coli*	MIC 100 µg/ml	Chudasama et al. [12]
AgNPs	*Escherichia coli*	33–45 % damaged *E. coli* cells	Beddow et al. [7]
AgNPs	*Chlamydomonas reinhardtii*	Toxicity	Navarro et al. [62, 63]
AgNPs	Heterotrophic (*rhizobacteria*), chemolithotrophic and soil formation bacteria	Toxicity	Throback et al. [83]
Aqueous graphene oxide/reduced graphene oxide dispersions	*Enterococcus faecalis*	ROS production	Tegou et al. [82]
Aqueous graphene oxide/reduced graphene oxide dispersions	*Bacillus subtilis*	Cell membrane disruption	Tegou et al. [82]
Aqueous graphene oxide/reduced graphene oxide dispersions	*Escherichia coli*	Growth inhibition	Tegou et al. [82]
C60 nanoparticle aggregates (nC60)	*Escherichia coli*	Toxicity	Lyon and Alvarez [55]
CdSe QDs (quantum dot)	*Escherichia coli*	Toxicity	Pokhrel et al. [68]
CdSe/ZnS QDs	*Nitrosomonas europaea*	Toxicity	Yang et al. [91]
CdSe/ZnS QDs	*Pseudomonas aeruginosa*	Toxicity	Yang et al. [93]
CeO_2	*Escherichia coli*	Toxicity	Pelletier et al. [66]
CeO_2	*Shewanella oneidensis*	Toxicity	Pelletier et al. [66]
Cu-doped TiO_2	*Shewanella oneidensi*	Toxicity	Wu et al. [90]
CuO	Plant growth promoting strains (*Klebsiella pneumonia*, *P. aeruginosa*, *Salmonella paratyphi* and *Shigella*)	Antibacterial activity	Rajkishore et al. [71]
CuO	*Bacillus subtilis*	Toxicity	Baek and An [6]
Graphene	*Escherichia coli*	Antimicrobial	Zou et al. [95]
Graphene oxide conjugated with silver nanoparticle	*Escherichia coli*	Cell membrane disruption	Tegou et al. [82]
Lithium Nickel Manganese Cobalt Oxide	*Shewanella oneidensis* MR-1	Significant impair in cell growth and respiration at conc. of 5 mg/L	Hang et al. [34]
Multiwalled carbon nanotubes (MWCNT)	*Escherichia coli*	Toxicity	Simon-Deckers et al. [77]
Nanozerovalent iron particles (nZVI)	*Escherichia coli*	Bactericidal effect	Lee et al. [48]
Nanosilverparticles(Ag0)	*Escherichia coli*	Minimum inhibitory concentration (MIC) of 1 µg/ml	Vertelov et al. [89]
nC60	*Bacillus subtilis*	Toxicity	Lyon and Alvarez [55]
Polymer nanocomposites with carbon nanotubes	*Pseudomonas aeruginosa*	Cytotoxicity	Goodwin et al. (30)
Reduced graphene oxide conjugated with silver nanoparticle	*Pseudomonas aeruginosa*	Growth inhibition	Tegou et al. [82]
Sb_2O_3	*Bacillus subtilis*	Toxicity	Baek and An [6]
Silver nanoparticles (AgNPs)	Ammonia-oxidizing bacteria (*Nitrosomonas europaea, Nitrosospira multiformis* and *Nitrosococcus oceani*)	Significant inhibition to the nitrification potential rates	Beddow et al. [7]

Table 1 continued

Nanoparticle	Microorganism	Effects	References
SiO_2	*Escherichia coli*	Toxicity	Li et al. [50]
SiO_2 and CeO_2	*Pseudokirchneriella subcapitata*	Toxicity and decreased photosynthetic activity	Van Hoecke et al. [87, 88]
TiO_2	*Anabaena variabilis*	Disruption of cellular structure and components; generation of ROS; impact on ecological food web	Cherchi et al. [10]
TiO_2	*Vibrio fischeri*	Toxicity	Heinlaan et al. [37]
TiO_2	*Pseudomonas aeruginosa*	Toxicity	Hessler et al. [38]
ZnO	*Escherichia coli* 1313	0.1 mg/ml for 1 h; 92 % inhibition	Applerot et al. [3]
ZnO	*Escherichia coli*	100 % mortality at 20 mg/l ZnO	Jiang et al. [42]
ZnO	*Escherichia coli* O157:H7	960 mg/l; 100 % inhibition	Liu et al. [52]

nanosilver, and Ag_2SO_4 on the activities of various ammonia-oxidizing (viz., *Nitrosomonas europaea*, *Nitrosospira multiformis* and *Nitrosococcus oceani*) and other bacteria (*Escherichia coli* and *Bacillus subtilis*) and found that all Ag-NPs treatments caused significant inhibition to the nitrification potential rates and growth of the bacteria. The inhibitory effect on the growth of all the bacteria was found to be in the order of $Ag_2SO_4 >$ capped nanosilver > uncapped nanosilver. However, data obtained by such laboratory experiments from pure microbial culture are often challenging to extrapolate to the natural environments. An exposure to natural environment, such as soil and sediment systems may provide additional sink to ENMs to reduce or enhance their bioavailability. Thus, it would be more appropriate to validate the toxicological assessment in the adsorbent system, such as soils and sediments [56].

Studies with bacterial enrichment cultures reproduced from surface sediments suggest that Ag-NPs interfere with the nitrogen cycle in the aquatic environment and reduce the ammoxidation due to their toxic effect on ammonia-oxidizing bacteria [54]. Apparently, Ag-NPs inhibit the growth of ammonia-oxidizing bacterial biodiversity due to their antibacterial property which leads to the reduction in ammoxidation. On the contrary, similar studies with Au-NPs showed no notable reduction in ammoxidation [54]. Similar concentration-dependent inhibitory effect of Ag-NPs on soil nitrification process mediated by nitrifying bacteria in an agricultural soil has also been reported by Masrahi et al. [56]. Several mechanisms have been reported for the toxicity of Ag-NPs on microorganisms. Number of studies have shown that toxicity of Ag-NPs is caused by the release of Ag^+ which interfere with functional proteins, such as ATP synthase, monooxygenase and hydroxylamine oxidoreductase [56, 92]. However, there are also reports on other modes of action, such as destabilization of the outer membrane, decrease in intracellular adenosine triphosphate (ATP) levels [43, 53] and generation of ROS [11].

Toxicity of carbonaceous nanomaterials (CNMs) and quantum dots (QD)

There are limited data available on the removal of CNMs, such as CNTs, graphene and QDs from the wastewater treatment plants (WWTPs), which ultimately end up in the environmental matrices. Moreover, the present WWTPs are not efficient enough to remove these ENMs to satisfactory level due to the technical challenges and barriers in quantification of them in the wastewater treatment process [94]. However, there are sufficient data to state that these materials are released to the environment in various stages of the synthesis lifecycle and usage to cause adverse effect on the environmental microorganisms. For instance, the interaction and toxicity of the graphene is influenced by various factors, which can be categorized into intrinsic or physiochemical properties of the graphene and surrounding/environmental factors. Firstly, the intrinsic properties, such as lateral size, number of layers, size, surface modifications and agglomeration/Dispersion properties, greatly determine the antimicrobial properties of graphene [95]. In addition, various parameters of the surrounding environment, such as aerobic and anaerobic conditions, state of the medium (liquid or solid) and mode of experiment (in vivo and in vitro), also influence the antimicrobial intensity on particular microorganism. Similarly, CNMs may also be directly toxic to soil microorganisms or they may interfere with the nutrient bioavailability or alter the toxicity of the organic compounds in the soil. There are also possibilities of indirect impact of ENMs on symbiotic microorganisms when they are toxic to plants [60]. For instance, C_{60} have been found to inhibit the growth of commonly occurring soil and water bacteria. The hydroxylated forms of C_{60} or C_{60} coated polyvinyl pyrrolidone ENMs can act as potent oxidizing agents in biological systems due to generation of singlet oxygen that can cause lipid peroxidation and cell damage [41]. Effects of C_{60} and CdSe quantum dots (QD) on microbial catalyzed oxidation of organic matter in freshwater sediments focusing on their effect on acetate oxidation by nitrate-reducing bacteria revealed that C_{60} at concentration of 140 µg per liter completely inhibited the microbial oxidation of acetate and CdSe QD at concentration of 200 µg per liter negatively affected the rate of acetate oxidation in the sediment slurries [28]. Some of the studies indicate that the toxicity of QDs to bacteria is primarily due to the release of harmful components, such as heavy metals or ions, which they possess in their core or shells [28]. However, detailed reports available on the stability and dissociation of quantum dots in the environment and their potent toxic effects on microbial communities are sparse.

CNTs are generally considered to be toxic to soil microorganisms regardless of the composition and functionalization of the CNTs [30, 60]. Studies suggest that direct contact of microorganisms with highly purified CNT aggregates leads to decrease in cell viability and cell death [13, 45, 77]. Physical piercing and oxidative stress are the general mechanism associated with cytotoxicity of CNTs [45]. Studies with antimicrobial effect of CNTs on pure culture of microorganisms also suggest that they negatively affect the bacterial growth [13]. Chung et al. [13] investigated the effect of multiwall carbon nanotubes (MWCNTs) on the activity and biomass of soil microorganisms. The study revealed that at a concentration of 500 µg MWCNT per gram soil, most of the enzyme activities, such as 1,4-β-glucosidase, cellobiohydrolase, xylosidase, 1,4-b-N-acetylglucosaminidase, and phosphatase, were repressed and at concentration of 500 µg MWCNT per gram soil all enzymatic activities as well as microbial biomass was significantly lowered [13] indicating the toxic effect of MWCNTs on microorganisms and their metabolic process. Similar antimicrobial effect of MWCNTs on soil microorganisms has also been reported for culture studies [45, 77]. Studies on effect of single-wall carbon nanotubes (SWCNTs) on pure *E. coli* and soil with low or high organic contents showed that it can affect the microbial community and induce changes in soil metabolic activity in the low organic matter systems [85]. Toxicology study consisting of SWCNTs either as raw or functionalized with polyethyleneglycol or m-polyaminobenzene sulfonic acid exposed to soil microbial community for 6 weeks showed that repeated exposure of raw SWCNTs is toxic to the metabolic activity of bacteria [85]. Similarly, polymer nanocomposites of carbon nanotubes (PNC) are proven to be toxic to *Pseudomonas aeruginosa* [30].

Toxicity of metal oxide-based nanoparticles

Metal oxide-based nanoparticles have been proven to be toxic to soil microorganisms. Numerous reports and reviews are available on the toxicity of Zn-, Cu-, Ti-based metal oxide nanoparticles, which are most common and largely used metal oxide nanoparticles in various commodities. In addition, various lanthanide oxide-based nanoparticles (LnONps), such as dysprosium oxide nanoparticle (nDy2O3), which are increasingly being used in the biomedical fields have adverse effect on natural biological systems and interfere with the metabolic activity and structural integrity of the microorganisms, such as *E. coli* [2]. Rousk et al. [74] studied the ecotoxicity of Zn- and Cu oxide-based nanoparticles on soil bacterial consortium using soil samples. In addition to Zn-and Cu oxide-based nanoparticles, the study also used two referral compounds, namely bulk oxide of non-nanoparticulate form and highly soluble sulfate forms of the metals to elucidate if any observed toxicity was due to its nanoparticulate form, or due to metal ion solubilization in soil solution. Further, the study revealed that $CuSO_4$ is highly toxic to soil bacteria in comparison with its oxide forms and the bulk (macroparticulate) form of CuO was non-toxic. In contrast, all forms of Zn were toxic to soil bacteria with highest toxicity for bulk-ZnO form compared to nano-ZnO. The study also showed a strong correlation between dissolved concentration of metals in solution and the bacterial growth, and the principle mechanism of toxicity was due to dissociation of metal oxides and sulfides into their corresponding metal ion form, which is toxic to the microorganisms. A comparative analysis of ecotoxicity of TiO_2, SiO_2 and ZnO nanoparticles to *Bacillus subtilis* and *Escherichia coli* showed that the antibacterial activity generally increased from SiO_2 to TiO_2 to ZnO, and *B. subtilis* was highly susceptible to such toxic effects [72]. Further, the effect of nanoparticle may be different at in vivo and in vitro level in microorganisms. Recent study on effect of TiO_2 nanoparticle (10–100 nm size) on the intestinal commensal bacteria in *Drosophila* showed that it could inhibit the growth of intestinal bacteria in the dosage and particle size-dependent manner in vitro in the cultured bacteria [51]. However, same dosage and particle size of the TiO_2 had no antibacterial effect on the gut bacteria of the *Drosophila* (in vivo). The study also showed another surprising phenomenon that the inhibition was independent of photocatalytic activation of TiO_2. Commonly, TiO_2 inhibits the bacterial growth by photocatalytic activation, which results in ROS and H_2O_2 formation due to UV radiation leading to cell death. Similar parameters also influence the metal oxide-based ENMs in the environment. The toxicity governing factors in the environment include concentration and form of organic matter in soil (which increase the tendency of ENMs to form aggregates and interaction with biomolecules), effective cation exchange capacity of ENMs, which is influenced by soil pH, [74]. However, it is also true that different bacterial orders are affected differently in the soil [15, 26].

Nickel manganese cobalt oxide nanoparticle (NMC) is a new class of nanomaterial used in the battery components. Due to its higher performance and lower cost, it is being widely considered for the applications in the batteries of the electric vehicles. However, the recent toxicological studies showed that as low as 5 mg/L of NMC is lethal for the bacterial growth and respiration for soil bacteria, *Shewanella oneidensis* MR-1 [34]. The toxicological study with NMC sheet-like structure revealed that the toxicity is largely due to the partial incongruent dissolution of NMC rather than NMC itself, which release Li^+, Ni^{2+} and Co^{2+}, responsible for toxicity of globally distributed soil microorganisms, such as *Shewanella oneidensis* MR-1. The mechanism of the toxicity showed that the released metal ions, such as Co^{2+} and Ni^{2+} are the essential trace elements for the microorganisms, and they enter the cells by specific uptake pathways meant to bring them inside the cells. However, large concentration of these metal ions in the cell leads to several impaired activities in the cell, such as binding to protein and compete with the metal cofactors of metalloenzymes and even the damage to DNA, which causes the cell death [34]. This fact is also evident from various other in vitro and in vivo studies that showed nickel disrupting the activity of metalloenzymes, such as dioxygenase (Fe-dependent enzyme) by replacing the iron ion by nickel(II) ion to inhibit the catalytic activity. Nickel exposure also interfered with the activity of the superoxide dismutase (SOD) in microorganisms, such as *B. vietnamiensis*, *P. putida* and *E. coli* by inducing oxidative stress. It is evident from numerous studies that the microbial toxicity in the environment due to ENMs need not be due to their mere exposure as nanoparticle, but also could be due to the series of transformation cycles of the nanomaterials, which could lead to devastating fate of the environmental microorganisms. This is also the reason that makes developing ‘universal’ method for risk assessment of any type of nanomaterials so far manufactured, highly challenging.

In contrast to the studies revealing toxic effects on microbial communities as discussed in the earlier sections, there are several reports indicating the beneficial effects of ENMs on bacterial growth, probably due to the very same properties which cause toxicity, such as large specific surface area and capacity to release electrons. It has also been reported that electrons generated by nanoparticles may enhance enzymatic function of external membrane proteins, may speed up electron transport chain function, and facilitate cell metabolism. Some of the studies have also shown no noticeable influence of nanoparticles on bacteria [25, 58].

Safety and risk assessment aspects for ENMs

Challenges in the risk assessment of ENMs

Risk assessment is a task of characterizing the level of risk, typically in terms of a relative ranking. The prime objective of carrying out a risk assessment is to deliver information that will be helpful to evaluate alternatives [20, 35]. The steps in the risk assessment of ENMs (i.e., hazard identification, hazard characterization, exposure assessment and risk characterization) remain so far the same as that of the methods used for the risk assessment of hazardous chemicals. In brief, the steps of this process involve identification and characterization of the hazards, establishing the link between dose and response for various end points, and then predict the probability of exposure. However, this process becomes complicated when it comes to nanomaterials. First of all, ENMs are not a uniform group of materials [8], secondly, the elemental composition of different nanoparticles and their properties, such as surface area, characteristics of the surface, tendency to aggregate and surface charge, are completely different from one form of nanomaterial to other. Thirdly, nanomaterials are manufactured from various materials in different forms and sizes and often with different coatings. Thus, the risk assessment of such diverse materials requires standard validation methods for both final product and their bulk samples which makes it difficult to set a standard protocol and significantly complicates the risk assessment process of ENMs [75].

Another important issue that needs thorough attention is related to possible exposure of ENMs to the environment during the entire production life cycle. Though it is true that most of the products are not likely to cause exposure as long as they are embedded in the matrices or polymers [75], the scenario may reverse during the processing and usage. Studies have shown that ENMs may undergo agglomeration, aggregation, adhesion, diffusion, dissociation, degradation, adsorption upon release into the environment finally leading to bioaccumulation and biomagnification in trophic pyramids [27]. The tendency of nanoparticles to undergo above-said processes depends on their properties as well as the local environmental and cellular conditions [27, 64]. Thus, it is necessary that the toxicological evaluation process of ENMs includes all the factors, such as physical (size, shape, surface area and agglomeration state), chemical (chemical composition, charge and chemical reactivity), biological (route of administration, metabolism, excretion, adduction to biological molecules) and environmental (the presence of microbes, temperature, pH, salinity, acidity, viscosity) factors [27].

Further, the lack of available data on persistence, mobility, bioavailability and ecotoxicity of ENMs makes it difficult to establish a standard protocol for testing their ecotoxicity. Recent studies have revealed that the existing theoretical methods and/or experimental protocols to detect and quantify the concentrations of nanoparticles in the environment are still at the very early stage of their development and effective methodologies are yet to be developed though several attempts are being done [27, 32, 76]. Most of the current data available on the microbial nanotoxicity have been drawn by traditional bacterial and microbiological assay to assess the toxicity, or by study of morphological changes and ROS generation using electron microscopy and fluorescent probes, respectively [57]. This indicates that very little data are available on behavior of ENMs in environmental matrices, such as water, air and soil, which makes it difficult to predict the interaction of nanoscale materials with microorganisms and the ultimate impact on ecosystem [27]. Furthermore, certain nanoparticles, such as Ag-NPs and TiO_2-NPs, are meant to exert toxic effects on pathogenic microorganisms as they are used as antimicrobial agents. Thus, their release into environment may inevitably and adversely affect the beneficial microbial communities such as nitrifying bacteria until and unless they are removed from the early stage of their release into environment. Thus, it is very important that the toxicity assessment studies focus on, characterization of ENMs in a complex, natural ecosystem at functional level and account for natural conditions that cause a known change to the ENMs characteristics, such as a natural occurring gradient of ionic strength or pH [57].

Approaches for effective risk assessment of ENMs with respect to microorganisms

The accumulating data on toxic effects of various nanomaterials on microorganisms and the poor state of knowledge related to the risk assessment of the ENMs warrant for the urgent need to develop alternative assessment procedures which are more efficient and stringent in evaluation.

One possible way to simplify the process is to focus on the main and relevant components of the large pool of data deriving exposure levels of ENPs through exposure assessment [69]. For instance, dividing ENPs to definite groups and types based on the size distribution consisting of finite size classes. This would help to address the issue of infinite numbers of ENM particle sizes discharging into environment. This also helps in representing the only one average particle size (such as average or medium particle size) in a better way during assessment [69]. In the similar way, ENMs coatings can be divided into different classes according to their properties, such as steric, electrostatic and electrostatic repulsion properties and mode of action [21, 79]. Another possible categorization is related to interaction of ENMs with the environment. For instance, once in the environment, ENMs encounter a wide range of components, such as suspended particulate matter, dissolved natural organic matter, biota, surfactants and metals, which can potentially interact and affect the ENMs' surface properties. Thus, categorizing the type of interactions, such as surface transformations, sorption, agglomeration, deposition, and then identifying the dominant process would help in prioritizing the research focus [69].

It is also crucial that the hazard assessment not only focuses on the concentration of the ENMs in the environment but also its properties and characteristics, such as size, shape and degree of aggregation. An array of sophisticated analytical techniques, such as microscopy, dynamic light scattering and size separation approaches combined with the detection methods like inductively coupled plasma MS, would be helpful in faster and efficient assessment. However, owing to the drawbacks of their own in each techniques, a combination of these techniques would be more helpful [84].

Another way of approach is to use computational methods to predict properties, reactivity and mechanisms of actions for various molecular systems. Methods such as Quantum chemical calculations and molecular dynamics (MD) simulations, Quantitative Structure–Activity Relationships (nano-QSAR) can aid to address the potential risks associated with nanomaterials [27]. Results from such theoretical data combined with experimental work might help in deciphering the complexity of ENM assessment [69].

Environmental regulations/legislations for nanomaterials

The current evaluation methods for the regulation of nanomaterials are not sufficient to deal with its unique issues and potential risks to the environment and health. The limited availability of exposure and hazard data is the prime hindrance in stringent law enforcement for nanomaterials. However, the situation is improving and the government agencies in many countries are actively involved in sorting out the issue. For instance, the US government proposed a budget of $2.1 billion (which is $201 million increase from the 2010 endorsed budget) for the multiagency national nanotechnology initiatives to understand the potential benefits and risks of nanomaterials [17].

In fact, countries, such as USA and Europe, are changing their approach toward regulation of nanomaterials. In USA, the nanomaterials are mainly regulated by Environmental Protection Agency (EPA) under the Toxic Substances Control Act (TSCA) and also Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Other federal government agencies, like Food and Drug Administration (FDA) also regulates nanomaterials, sometimes in collaboration with EPA to monitor nanomaterials that appear in various categories of products, such as cosmetics, medical devices, drugs and food. The EPA has modified its approach toward regulation of nanomaterials. Earlier, the nanomaterial manufacturers were encouraged to provide volunteer information on nanomaterials through Nanoscale Materials Stewardship Program (NMSP); however, at present EPA has shifted toward mandatory approaches to collect detailed information to set standards on production, usage and safe disposal of nanomaterials, which includes Pre-manufacture notifications for new nanomaterials and stringent rule on gathering of information on new and existing nanomaterials [17, 86]. *Under Pre-manufacture notification rule*, TSCA seek specific information on new chemical substances for review on risk to environment and human health, prior to manufacture. Since 2015, EPA reviewed more than 160 new chemical substances under nanoscale materials, such as carbon nanotubes and agency, has taken several actions to control/limit such nanomaterials, like limiting the usage, use of appropriate personal protective equipment (PPE), limiting environmental release and need of testing to generate environmental and health effect data. Several nanoscale materials are limited to manufacture under consent order or Significant New Use Rules (SNUR) under TSCA [86]. *Under information gathering rule*, EPA is trying to gather more comprehensive information on nanoscale materials by urging the manufacturers to notify details of materials for one-time reporting and recordkeeping. The details, such as manufacturing volume, manufacture and processing method, details on exposure and environmental release as well as available health and safety data are expected to be furnished to EPA under TSCA rule before manufacturing such nanoscale materials [86]. EPA is also addressing several nanoscale pesticides under FIFRA. Nanomaterials intended to work on mitigation or prevention of pests and microorganisms are regulated under FIFA, which is working on modification of pesticide registration guidelines to know the nanomaterial ingredients in pesticides. Similar to EPA, FDA is also regulating nanomaterials that occur in various categories of cosmetics, food and medical products [24]. FDA has published various industrial guidelines for use of nanomaterials in cosmetics, food ingredients and food contact substances and color additives in food [24].

In European Union, the chemical management is mainly regulated under 'Registration, Evaluation, Authorization and Restriction of Chemicals' (REACH) regulations and Classification, Labeling and Packaging of Substances and Mixtures (CLP). At present, there are no particular regulations in European legislations targeting nanomaterials and the regulations of REACH on chemical substance do not clearly differentiate the nanomaterials with other chemicals. However, there has been a growing development in the nanotechnology regulations in Europe. In 2011, European Commission released specific recommendations to consider nanomaterials in different European regulations including REACH and CLP [18]. Further, the European Chemicals Agency (ECHA) in collaboration with other EU Member States published various guidance documents for accounting nanomaterials by industries, which would also facilitate registering the nanomaterials in REACH [22]. Similarly, several mandatory reporting and tracking systems are introduced in France under the Grenelle II Act and in Austria under Nanotechnology Action Plan for the safety of nanomaterials.

In Canada, there are no specific legislation on regulation of nanomaterials and they are regulated under various existing legislations, including Canadian Environmental Protection Act, 1999 (CEP), Pest Control Products Act (PCPA), Fertilizers, Feeds, Food and Drugs Act. However, the Canadian government is actively working on bringing changes in the nanomaterial regulations by funding and encouraging health and safety research on nanomaterial [61].

Asian nations are also actively participating and spending substantial resources in nanotechnology promotion and regulation schemes. The effort on conducting several survey reports on nanomaterial safety research is underway by the Ministry of Health, Labour, and Welfare (MHLW) and Ministry of the Environment (MOE) in Japan. The National Institute of Advanced Industrial Science and Technology (AIST) has also published important risk assessment data and reports on various nanomaterials, such as fullerenes, CNTs and TiO_2 in effort to make regulations on nanomaterials [17]. The countries like Australia, Thailand and Korea are among others to pursue several regulatory strategies and policy initiatives to regulate nanomaterials.

Concluding remarks

Nanotechnology has furnished our life with wide range of products to make our life more comfortable. However, the increasing use of ENPs in consumer products has introduced several toxic group of compounds into the ecosystem, leaving a toxicological challenge to deal with. Although numerous reports available on the deleterious effect of ENMs on the ecologically important microbial communities, our research and current understanding of the interactions of ecological microorganisms at molecular level are limited by the cost-effective analytical methods. The present analytical and risk assessment methods and stringent regulations to tackle the problems of ENMs in the perspective of environmentally important microbial communities lag with the pace of nanotechnological industrial growth. A multidisciplinary approach combining experimental, computational and theoretical approaches would help in finding advanced effective risk assessment methods to tackle the ecotoxicological problems associated with engineered nanoparticles. Further, the research also needs to focus on sustainable analytical methods for real-time monitoring of the microbial interactions in the environment to understand and prevent the inadvertent negative impact of ENMs on microbial communities.

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