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Review

Engineered nanoparticles in the soil and their potential implications to microbial activity

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ABSTRACT

Among the factors influencing soil quality, biological indicators are reported as critically important because soil organisms directly influence soil ecosystem processes, especially the decomposition of soil organic matter and the cycling of nutrients. Hence, any factor that affects soil microbial biomass, activity and populations would necessarily affect soil quality and sustainability. Presently, a staggering number of engineered nanoparticles (ENPs) employed for industrial and environmental applications or formed as by-products of human activity are finding their way into soils. Common ENPs include the metal ENPs (elemental Ag, Au, Fe etc.), fullerenes (grouping Buckminster fullerenes, CNTs, nanocones etc.), metal oxides (TiO$_2$, CuO, FeO$_2$, ZnO, Al$_2$O$_3$, SiO$_2$ etc.), complex compounds (Co–Zn–Fe oxide), quantum dots often coated with a polymer e.g. cadmium–selenide (CdSe) and organic polymers (dendrimers, polystyrene, etc.). The antimicrobial activity of these ENPs has been extensively studied with human pathogenic bacteria. Similarly, studies also exist on the affect of ENPs on beneficial microbes *in vitro* under controlled conditions. But very little information is available on how these ENPs affect microbial communities in soil under field conditions. The few published literature does suggest that among the ENPs, fullerenes and their derivatives are less toxic, while small size metal and metal oxide ENPs are detrimental to soil microbial communities. However, under field conditions, soil organic matter and related components like humic and fulvic acids could possibly negate the toxic effects of these ENPs through various mechanisms. Also, the resistance and resilience of soil microbial communities to such perturbations cannot be discounted. The paper also stresses the need for more information on interaction of ENPs with soil microorganisms under field conditions.

Keywords:
Fullerenes
Metal and metal oxide nanoparticles
Soil microbial activity
Soil organic matter

Contents

1. Introduction

Soil quality is defined as the capacity of a soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation (Karlen et al., 1997). Among the

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factors influencing soil quality, biological indicators are reported as critically important (Doran and Zeiss, 2000) because soil organisms directly influence soil ecosystem processes, especially the decomposition of soil organic matter and the cycling of nutrients (Kennedy and Smith, 1995). Therefore, protection of soil microbial biomass and diversity is one of the major challenges for sustainable resource use because greater levels of microbial biomass and diversity mean greater nutrient turnover (Torsvik and Øvreås, 2002) and disease suppressiveness of the soil (Janvier et al., 2007). The opposite being true for a sick soil with low nutrient and carbon reserves and greater levels of contaminants caused by the presence of xenobiotic (human-made) chemicals or other alterations in the soil environment. Among the xenobiotics are the staggering numbers of new nanoparticles engineered for industrial and environmental applications or formed as by-products of human activity, which are already finding their way into soils (Maurice and Hochella, 2008). While the concentrations of most ENPs in the environments still remain unknown, exposure modeling suggests that soil could be a major sink of ENPs released into the environment and that ENP concentrations in soil are higher than in water or air (Gottschalk et al., 2009; Klaine et al., 2008; Tiede et al., 2009).

2. Engineered nanoparticles and their toxicity to microorganisms

Engineered nanoparticles (ENPs) are particles that are produced by man because they have specific nanotechnological properties in terms of size, properties, behavior etc. The main reasons why materials built of ENPs have different optical, electrical, magnetic, chemical and mechanical properties from their bulk counterparts are that in this size-range (between 1 and 100 nm) quantum effects start to predominate and the surface-area-to-volume ratio (sa/vol) becomes very large. The sa/vol of most materials increases gradually as their particles become smaller, which results in increased adsorption of the surrounding atoms and subsequently change their properties and behavior. Once particles become small enough, they start to obey the quantum mechanical laws. Materials reduced to the nano-scale can suddenly show very different properties, compared to what they exhibit on the macro-scale, which enables unique applications. For example, opaque substances become transparent (copper); stable materials become combustible (aluminum); inert materials become catalysts (platinum); insulators become conductors (silicon); solids turn into liquids at room temperature (gold) (Hristozov and Malsch, 2009).

ENPs can be made of single elements like carbon (C) or silver (Ag) or a mixture of elements/molecules. ENPs are often classified based on their chemical composition, occasionally supplemented with size or morphology characteristics. A crude division is between carbon-based ENPs and mineral ENPs. Several ENPs are described (Norwegian Pollution Control Authority, 2008) viz., Fullerenes [(grouping Buckminster fullerenes, carbon nanotubes (CNTs), nano-cones etc.)], metal ENPs (elemental Ag, Au, Fe etc.), oxides (TiO_2, CuO, FeO_2, ZnO, Al_2O_3, SiO_2, CeO_2 etc.), complex compounds like Co–Zn–Fe oxide, and quantum dots often coated with a polymer e.g. cadmium–selenide (CdSe) and organic polymers (dendrimers, polystyrene, etc.). The increasing entry of these ENPs will inevitably lead to their accumulation in soil, which has raised concerns about their potential adverse effects on soil microbial activity and diversity.

Currently very little information is available on how these ENPs affect the soil microbial community. They may have an impact on soil microorganisms via (1) a direct effect (toxicity), (2) changes in the bioavailability of toxins or nutrients, (3) indirect effects resulting from their interaction with natural organic compounds and (4) interaction with toxic organic compounds which would amplify or alleviate their toxicity (Simonet and Valcárcel, 2009). While toxicity mechanisms have not yet been completely elucidated for most ENPs, possible mechanisms include disruption of membranes or membrane potential, oxidation of proteins, genotoxicity, interruption of energy transduction, formation of reactive oxygen species (ROS), and release of toxic constituents (Klaine et al., 2008). However, close contact is necessary for membrane disruption to occur, and it is unlikely that NPs cross into the cytoplasm although accumulation within the cytoplasm, probably after membrane disruption, is often observed (Neal, 2008). Raghupathi et al. (2011) reported that the antibacterial activity of NPs might involve both the production of ROS and the accumulation of NPs in the cytoplasm or on the outer membranes. The ENPs also appear to cause structural changes to the microbial cell surface that may eventually lead to cell death (Suresh et al., 2010). It is, therefore, apparent that ENPs stimulate the production of ROS in organisms and cause damage in possibly every cell component (Bhatt and Tripathi, 2011).

2.1. Antimicrobial activity of carbon based ENPs

The antimicrobial activity of ENPs has been extensively studied with human pathogenic bacteria. Among the ENPs, fullerenes are an ideal model material for environmental studies and earlier work has shown that C_{60} is either harmful (Lyon et al., 2005) or has neutral biological consequences (Jia et al., 2005). The study by Fortner et al. (2005) on the effects of C_{60} aggregates on two common soil bacteria Escherichia coli and Bacillus subtilis suggested that prokaryotic exposure to nano-C_{60} at relatively low concentrations is inhibitory, indicated by lack of growth (≥ 0.4 ppm) and decreased aerobic respiration rates (4 ppm). Besides, fullerene water suspensions (FWS) whose properties differ from those of bulk solid C_{60} exhibited relatively strong antibacterial activity and fractions containing smaller aggregates were more toxic to B. subtilis at minimum inhibitory concentrations of C_{60} of 1.5–3.90 mg L^{-1} (Lyon et al., 2006). Studies on the antibacterial activity by aqueous suspensions of fullerenes (Sayes et al., 2005) suggested that the degree of toxicity to E. coli was linked to production of ROS. Contrarily, it has also been suggested that the FWS of fullerenes, nC_{60} exerts ROS-independent oxidative stress in bacteria, with evidence of protein oxidation, changes in cell membrane potential, and interruption of cellular respiration (Lyon et al., 2008). Though pristine C_{60} is non-toxic to the cells (Levi et al., 2006), FWS (polyvinylpyrrolidone (PVP)/C_{60}, gamma-cyclodextrin (gamma-CD)/C_{60}, and nano-C_{60}) and fullerenols (C_{60} (OH) 12, C_{60} (OH) 36.8H_2O, and C_{60} (OH) 44.8H_2O) have been found to be toxic to six kinds of bacteria and two kinds of fungi (Aoshima et al., 2009). The toxicity of C_{60} has been attributed to its ability to bind and deform the DNA stands, thereby interfering with DNA repair mechanisms (Zhou et al., 2005). However, how much of this effect was due to facilitated exposure or experimental artifacts such as solvent effects remains unclear (Nyberg et al., 2008). It is well-known that fullerenes interact strongly with cell membranes, but this property has no inherent relationship to toxicity (Colvin, 2003). One possible mechanism of aqueous fullerene toxicity to eukaryotic cells has been shown to be lipid peroxidation (Sayes et al., 2005). Carbon based ENPs like CNTs have also been found to inactivate E. coli, Staphylococcus epidermis, beneficial soil microbes like P. aeruginosa, B. subtilis as well as diverse microbial communities of river and waste water effluent (Kang et al., 2009).

2.2. Antimicrobial activity of metal and metal oxide ENPs

Microbial toxicity has been reported for metal NPs, like elemental Ag, Au, Fe; oxides of Ti, Fe, Co–Zn–Fe etc. These NPs raise serious environmental concerns because of their unique dissolution properties and electronic charges, in addition to their small sizes and large surface-to-mass ratios (Wang et al., 2010). For instance, Ag NP is toxic to E. coli and Staphylococcus aureus (Rai et al., 2009) and B. subtilis (Suresh et al., 2010). Even Ag NP biosynthesized by

fungi showed potent activity against fungal and bacterial strains like *Aspergillus niger*, *Staphylococcus* sp., *Bacillus* sp. and *E. coli* (Jaidev and Narasimha, 2010). *E. coli* and *S. aureus* (Jones et al., 2008) and *Pseudomonas putida* (Gajjar et al., 2009) were inhibited by ENPs of Ag, CuO and ZnO. However, reports on the relative toxicities of these metal and metal oxide NPs are contradictory. For instance, Jiang et al. (2009) found that among ENPs of ZnO, Al_2O_3, SiO_2 and TiO_2, the most toxic was ZnO causing 100% mortality of *B. subtilis*, *E. coli* and *P. fluorescens*. Conversely, Dimkpa et al. (2011) reported that CuO NP was more toxic to beneficial rhizosphere isolate *P. chlororaphis* O6 than ZnO NP. Similarly, among NPs of CuO, NiO, ZnO, and Sb_2O_3, Baek and An (2011) found that CuO NP was the most toxic to *E. coli*, *B. subtilis*, and *Streptococcus aureus*.

Even water suspensions of nanosized TiO_2, SiO_2, and ZnO were found to be harmful to varying degrees, with antibacterial activity increasing with particle concentration (Adams et al., 2006). They also found that the antibacterial activity generally increased from SiO_2 to TiO_2 to ZnO, and *B. subtilis* was most susceptible to their effects. Likewise, oxides of Zn, Cu and Ti NPs have been reported to be toxic to the microalgae *Pseudokirchneriella subcapitata* (Aruoja et al., 2009). Wang et al. (2010) found that electrospraying of NPs of NiO, CuO, or ZnO (20 nm, 20 μg, in 10 min) reduced the total number of living *E. coli* by more than 88%, 77% and 71%, respectively. However, TiO_2, Co_3O_4, and Fe_2O_3 showed no significant antibacterial activities in either the aqueous exposure mode or the aerosol exposure mode.

Though the bactericide mechanism of Ag NPs is still not clear (Pal et al., 2007), Ag NP interactions with bacteria have been found to be dependent on the size and shape of the NPs. Ag NPs have spherical (7 and 29 nm) and pseudospherical shape (89 nm) with a narrow size distribution. Among these, Martínez-Castañón et al. (2008) found that the 7 nm Ag NPs presented the best activity against *E. coli* and *S. aureus*. Because of their size, 7 nm Ag NPs can easily reach the nuclear content of bacteria and they present the greatest surface area; therefore the contact with bacteria is the greatest (Lok et al., 2006). This could be the reason why they present the best antibacterial activity. Similar to Ag NPs, the antibacterial activity of the ZnO NPs was also found to be inversely proportional to their size in *S. aureus* (Raghupathi et al., 2011). Basically, the smaller size they are, the greater their surface area to volume ratio and the higher their microbial contacting efficiency (Jeong et al., 2005; Lok et al., 2007; Thiel et al., 2007; Wong et al., 2010). While the size-dependent interaction of Ag NPs with gram-negative bacteria has been reported (Elechiguerra et al., 2005; Morones et al., 2005), it has also been demonstrated that Ag NPs undergo a shape-dependent interaction with the gram-negative organism *E. coli* (Pal et al., 2007). They found that truncated triangular silver nanoplates with a {111} lattice plane as the basal plane displayed the strongest biocidal action, compared with spherical- and rod-shaped nanoparticles and with Ag (in the form of $AgNO_3$). Although the toxicity of Ag NPs is reported to be dependent on various factors such as particle size, shape and capping agent, the results of El Badawy et al. (2011) suggested that surface charge is one of the most important factors that govern the toxicity of Ag NPs. They demonstrated that four Ag NPs representing various surface charging scenarios ranging from highly negative to highly positive viz., uncoated H_2–Ag NPs, citrate coated Ag NPs, polyvinylpyrrolidone coated Ag NPs, and branched polyethyleneimine coated Ag NPs exhibited surface charge-dependent toxicity on the *bacillus* species investigated.

Possible effects of Ag NP include interaction with the bacterial membrane, causing pitting of the cell wall, dissipation of the proton motive force, and finally cell death (Choi et al., 2008). Ag NP would also bind with bacterial DNA, and this might compromise the DNAs replication fidelity (Rai et al., 2009; Yang et al., 2009). More interesting is the fact that these metal oxide NPs may act as 'Trojan-Horses', entering cells and releasing ions intracellularly (Limbach et al.,

2007). In a study to model the quantities of ENPs released into the environment, Mueller and Nowack (2008) found that the predicted environmental concentrations (PEC) values for nano-TiO_2 in water are $0.7 - 16\ \mu g\ L^{-1}$ and close to or higher than the predicted no effect concentrations (PNEC) value for nano-TiO_2 ($<1\ \mu g\ L^{-1}$). They found that the risk quotients (PEC/PNEC) for CNT and Ag NP were much smaller than 1.0, and suggested that there was no reason to expect adverse effects from these particles. Their study, however, does not necessarily absolve these ENPs from toxic effects on soil microorganisms. In a review done by Kahru and Dubourguier (2010) to evaluate the currently existing data on toxicity (L(E)C50 values) of synthetic NPs on organism groups representing main food-chain levels (bacteria, algae, crustaceans, ciliates, fish, yeasts and nematodes), the most harmful were NPs of Ag and ZnO that were classified "extremely toxic", (L(E)C50 $<0.1\ mg\ L^{-1}$), followed by C60 fullerenes and CuO NP that were classified "very toxic" (L(E)C50 $0.1–1\ mg\ L^{-1}$). SWCNTs and MWCNTs were classified "toxic" (L(E)C50 $1–10\ mg\ L^{-1}$) and TiO_2 NP was classified as "harmful", (L(E)C50 $10–100\ mg\ L^{-1}$). Clear evidence of the antibacterial activity of Ag NPs and their toxicity to fungi, virii and algae in studies done in vitro is also provided in the review by Marambio-Jones and Hoek (2010).

3. Effects on soil microorganisms

While there is little doubt on the toxicity of ENPs to microorganisms, the issue that raises serious concern is their toxicity to microorganisms that promote plant growth and those that benefit nutrient cycling in soils. Plant growth promoting rhizobacteria (PGPR) like *P. aeruginosa*, *P. putida*, *P. fluorescens*, *B. subtilis* and soil N cycle bacteria *viz.*, nitrifying bacteria and denitrifying bacteria have shown varying degrees of inhibition when exposed to ENPs in pure culture conditions or aqueous suspensions (Mishra and Kumar, 2009). Metal oxide NPs of Cu (80 to 160 nm) were tested for antibacterial activity against plant growth promoting *Klebsiella pneumoniae*, *P. aeruginosa*, *Salmonella paratyphi* and *Shigella* strains (Mahapatra et al., 2008). Iron and copper based NPs are presumed to react with peroxides present in the environment generating free radicals known to be highly toxic to microorganisms like *P. aeruginosa* (Saliba et al., 2006). The few published studies that have dealt with the effects of ENPs on soil microorganisms are summarized in Table 1.

3.1. Toxicity of fullerenes and CNTs

The studies conducted to date found limited impact of C_{60} fullerenes on soil respiration and microbial biomass. For instance, Tong et al. (2007) treated soils with either $1.0\ \mu g\ C_{60}\ g^{-1}$ soil in aqueous suspension (nC_{60}) or $1000\ \mu g\ C_{60}\ g^{-1}$ soil in granular form and incubated the soils for 180 days. The soil was a silty clay loam with 4% organic matter and near neutral pH (6.9). They did not find any inhibition in the activity of dehydrogenase (an enzyme that indicates oxidative microbial activity in soils) and activities of enzymes involved in N (urease), P (acid-phosphatase) and C (β-glucosidase) cycles in the soil, suggesting that C_{60} did not cause any stress to soil microbial activity.

Johansen et al. (2008) applied agglomerates of pristine C_{60} fullerenes (50 nm to μm-size) to soil at 0, 5, 25, and $50\ mg\ kg^{-1}$ dry soil (clay loam texture, 2.5% organic matter, pH 6.7) and measured total respiration, biomass, number, and diversity of bacteria and protozoans during 14 days. They found that the number of fast-growing bacteria decreased by three-to four folds immediately after incorporation of the C_{60} and protozoan number decreased only slightly in the beginning of the experiment. The initial decrease in fast growing bacterial population was attributed to the direct effect of C_{60} by forming ROS which has been found to disrupt membrane lipids and DNA and indirect effect due to adsorption by C_{60} of substrates and nutrients essential for bacterial growth in soils. However, such direct and

Table 1
Published literature on the effects of engineered nanoparticles on microorganisms in the soil.

Nanoparticle	Effects	Nature of study	Source
Carbon containing fullerenes/CNTs	No inhibition in the activity of dehydrogenase and activities of enzymes involved in N (urease), P (acid-phosphatase) and C (β-glucosidase) cycles in the soil. A slight shift in bacterial DNA, indicating a minor change in the community structure measured using PCR-DGGE.	Incubation study	Tong et al. (2007)
	Number of fast-growing bacteria decreased by three-to four folds immediately after incorporation of the C60 and protozoan number decreased only slightly in the beginning of the experiment. A slight shift in bacterial DNA, indicating a minor change in the community structure measured using PCR-DGGE.	Incubation study	Johansen et al. (2008)
	No significant effect on the anaerobic community of biosolids from anaerobic wastewater treatment sludge over an exposure period of a few months. No change in methanogenesis and no evidence of substantial microbial community shifts due to treatment with C_{60}.	Microcosm study	Nyberg et al. (2008)
	Multi-walled CNT significantly inhibited the activities of 1,4-β-glucosidase, cellobiohydrolase, xylosidase, 1,4-β-N-acetylglucosaminidase, phosphatase and microbial biomass-C and -N in soils.	Incubation study	Chung et al. (2011)
Metal and metal oxide ENPs	Effect of Ag-NP on soil dehydrogenase activity was severe and bacterial colony growth was inhibited at levels between 0.1 and 0.5 mg Ag kg^{-1} soil.	Incubation study	Murata et al. (2005)
	Ag-NP inhibited soil denitrifying bacteria when Ag was added to soils in amounts ranging from 0.003 to 100 mg kg^{-1} dry weight.	Incubation study	Throbäck et al. (2007)
	Soil respiration studies show that there were no statistical differences between the time and sizes of peaks in CO_2 production and the total mineralization of glucose due to addition of nano-Al.	Column study using silica-sand mixture	Doshi et al. (2008)
	The influence of Si-, Pd-, Au- and Cu-NPs on microbial communities was insignificant.	Microcosm study	Shah and Belozerova (2009)
	Ag-NP did not influence microbial biomass-N, enzyme activities (Leucine-aminopeptidase, β-cellobiohydrolase, acid phosphatase, β-glucosidase, chitinase and xylosidase), soil pH and organic C. Microbial biomass was significantly decreased while basal respiration and metabolic quotient (basal respiration per unit of microbial biomass) was increased with increasing Ag-NP application rate.	Incubation study	Hänsch and Emmerling (2010)
	TiO$_2$- and ZnO-NPs reduced both microbial biomass and bacterial diversity and composition indicating that nanoparticulate metal oxides may measurably and negatively impact soil bacterial communities.	Microcosm study	Ge et al. (2011)
	TiO$_2$- and ZnO-NPs significantly inhibited soil protease, catalase, and peroxidase activities; urease activity was unaffected	Field study	Du et al. (2011)
	Zn- and ZnO-NPs inhibited the activities of dehydrogenase, β-glucosidase and acid phosphatase in soils	Pot study	Kim et al. (2011)
	Ag-, Cu- and Si-NPs impacted arctic soil bacterial community; Ag-NPs were highly toxic to a plant beneficial bacterium, *Bradyrhizobium canariense*.	Incubation study	Kumar et al. (2011)
Nano-scale zero valent iron (nZVI)	Inhibited ammonia oxidizing potential during the first 7 d, stimulated dehydrogenase activity, minimal influence on hydrolase activity	Incubation study	Cullen et al. (2011)

indirect effects of C_{60} on microbes could not be confirmed because of the complex nature of soils environment. Nevertheless, both the studies (Johansen et al., 2008; Tong et al., 2007) have reported that C_{60} application to soils caused only a slight shift in bacterial DNA, indicating a minor change in the community structure measured using denaturing gradient gel electrophoresis (DGGE). Findings by Nyberg et al. (2008) also suggested that C_{60} fullerenes have no significant effect on the anaerobic community of biosolids from anaerobic wastewater treatment sludge over an exposure period of a few months. This conclusion is based on the absence of toxicity indicated by no change in methanogenesis relative to untreated reference samples. DGGE results also showed no evidence of substantial community shifts due to treatment with C_{60}, in any subset of the microbial community. In an short-term incubation study, Chung et al. (2011) found that application of up to 5000 μg MWCNT g^{-1} soil significantly inhibited the activities of enzyme involved in C (1,4-β-glucosidase, cellobiohydrolase, xylosidase), N (1,4-β-N-acetylglucosaminidase) and P (phosphatase) cycling and lower microbial biomass-C and -N in soils, which suggested that introduction of large quantities of MWCNTs could act as stressors to soil microorganisms and have a broad impact on nutrient cycling mediated by soil microorganisms.

3.2. Toxicity of metal and metal oxide ENPs

Unlike C_{60} which has been found to be less toxic or neutral, metal NPs like Ag have been proven to be toxic to soil microorganisms. It has been demonstrated that the effect of Ag on soil dehydrogenase activity was severe and bacterial colony growth was inhibited at levels between 0.1 and 0.5 mg Ag kg^{-1} soil (Murata et al., 2005). Their findings also suggested that soil denitrifying bacteria are susceptible to inhibition by Ag. In a study by Throbäck et al. (2007), Ag was added to soils in amounts ranging from 0.003 to 100 mg kg^{-1} dry weight. The immediate effect of Ag was that only less than 20%

of the activity of denitrifying bacteria in the control remained in samples with the highest Ag concentration. After 14, 30 and 90 days of incubation, the inhibition patterns were similar, and no recovery was observed. This would imply that in the presence of Ag, the conversion of nitrates to N_2 in soil is reduced due to inhibition of denitrifiers. There was also a reduction in the copy number of the copper nitrate-reductase-encoding nirK and a clear population shift to novel, presumably Ag-resistant nirK expressing strains.

In addition to the concentration of Ag in soils, the size of the Ag NPs is crucial to their anti-microbial activity. By examining the correlation between NP size distribution, photocatalytic ROS generation, intracellular ROS accumulation, and nitrification inhibition, Choi et al. (2008) observed that inhibition to nitrifying organisms correlated with the fraction of Ag less than 5 nm in the suspension. It appeared that these size ENPs could be more toxic to bacteria than any other fractions of ENPs or their counterpart bulk species. The study by Hänsch and Emmerling (2010) also suggested that Ag NP significantly inhibited microbial biomass and activity in soils. They performed a medium-term experiment (four months) by amending soils with Ag dose analogous to 3.2, 32 and 320 μg Ag kg^{-1} soil and found that Ag NP application had no influence on microbial biomass N, fluorimetric enzymes (Leucine-aminopeptidase, β-cellobiohydrolase, acid phosphatase, β-glucosidase, chitinase and xylosidase), soil pH and organic C. However, they found clear evidence that microbial biomass was significantly decreased while basal respiration was increased with increasing Ag NP application rate. In addition, metabolic quotient (basal respiration per unit of microbial biomass) was greater in the Ag NP treatments compared to the control. This indicated that the efficiency of substrate use was lowered in Ag NP treated soils and they speculated a changed soil microbial community composition due to Ag NP application after four months. Community level physiological profiles (CLPP), fatty acid methyl ester (FAME) assays and DGGE also revealed that metal NPs

(Ag, Cu or Si) caused a shift in bacterial community of a high latitude (>78°N) arctic soil and of three NPs examined, Ag NPs were highly toxic to these arctic consortia. Subsequent culture-based studies confirmed that one of the community-identified plant-associating bacteria, *Bradyrhizobium canariense*, appeared to have a marked sensitivity to Ag NPs (Kumar et al., 2011).

Other metal NPs have been found to be less toxic to soil microorganisms. When two types of nano-Al particles (with aluminum oxide, or carboxylate ligand coating, Alex and L-Alex, respectively) were amended to the soil, Alex Al resulted in a 50% reduction of light output at concentrations below 5000 mg L^{-1} soil suspension concentration while L-Alex showed a similar effect at around 17,500 mg L^{-1} and the control soil at 37,500 mg L^{-1}. Soil respiration studies showed that there were no statistical differences between the time and sizes of peaks in CO$_2$ production and the total mineralization of glucose (Doshi et al., 2008). Shah and Belozerova (2009) studied the influence of Si, Pd, Au and Cu NPs on microbial communities in a soil amended with potting mix (6.2% w/w). The NPs were added to the soil to give a final concentration of 0.013% (w/w) or 0.066% (w/w) and the treated soils were incubated for a period of 15 days. Their results on substrate utilization pattern studied using BIOLOG, number of colony forming units and FAME profile indicated that the influence of ENPs was statistically non significant. Further, no significant influence on the microbial communities of the soil over a short-term was noted even though the higher concentration was five times that of the lower concentration. Contrarily, Ge et al. (2011) have reported that metal oxide NPs may measurably and negatively impact soil bacterial communities. They exposed a grassland soil to different doses of NPs of TiO$_2$ (0, 0.5, 1.0, and 2.0 mg g^{-1} soil) and ZnO (0.05, 0.1, and 0.5 mg g^{-1} soil) in microcosms over 60 days. They found that both these NPs reduced microbial biomass (as indicated by declines in both substrate induced respiration, SIR and total extractable DNA) and bacterial diversity and composition (as indicated by T-RFLP analysis). The effect of ZnO NP was found to be stronger than that of TiO$_2$ NP. This is well supported by the findings of Du et al. (2011). They studied the effects of TiO$_2$ and ZnO NPs (10 g and 5 g in 110 kg soil respectively) on wheat growth and soil enzyme activities under field conditions and found that these NPs did not affect urease activity but significantly inhibited soil protease, catalase, and peroxidase activities which suggested that these NPs themselves or their dissolved ions were clearly toxic for the soil ecosystem. Likewise, Zn- and ZnO-NPs were found to inhibit the activities of dehydrogenase, β-glucosidase and acid phosphatase in soils under *Cucumus sativus* (Kim et al., 2011).

Similarly, the effects of nano-scale and micro-scale zerovalent iron (nZVI and mZVI) particles on general (dehydrogenase and hydrolase) and specific (ammonia oxidation potential, AOP) activities mediated by the microbial community in an uncontaminated soil were examined (Cullen et al., 2011). nZVI (diameter 12.5 nm; 10 mg g^{-1} soil) apparently inhibited AOP. They attributed the significant reduction in nitrite concentration recorded in the presence of nZVI to the confounding action of the particles on the nitrite product and not due to nZVI inhibition of microbial ammonia oxidation. Similarly, they found that nZVI and mZVI stimulated dehydrogenase activity but had minimal influence on hydrolase activity indicating the genuine response of a stimulated microbial population or an artifact of ZVI reactivity. Overall, they found no evidence for negative effects of nZVI or mZVI on the processes studied.

Though no changes or inhibition in soil microbial activity and related indices due to ENPs have been observed in the above studies, it is critical to note that most of these findings are based on incubation or microcosm studies or have been conducted in aqueous systems. Apparently, the results would vary if the experiments are repeated under field conditions. Besides, some of these studies focus on a single or very few variables while keeping all the experimental conditions constant. Hence, the results appear unsuitable for ecotoxicological evaluations of ENPs in the actual soil environment. This emphasizes the need for experiments that generate exclusive data on the effects on ENPs on microbial community/processes in field conditions or experimental conditions that exactly simulate the natural soil environment. Understandably, the white paper on nanotechnology drafted by EPA in February of 2007 finds no successful study assessing the potential toxicity of ENPs to soil microorganisms (US Environmental Protection Agency, 2007).

4. Influence of soil organic matter

Apparently, the studies we have reported indicate that metal NPs especially Ag is toxic to soil microorganisms, while the influence of C$_{60}$ on soil microbial activity is insignificant in the short-term. This could be due to the strong binding of nC$_{60}$ to soil organic matter (SOM; Tong et al., 2007). Due to adsorption by SOM the mobility of ENPs in the soil matrix is curtailed and hence their influence on the microbial populations is drastically reduced. ENPs can be strongly sorbed to soil surfaces and SOM making them less mobile or are small enough to be trapped in the inter-spaces of soil particles and might therefore travel farther than larger particles before becoming trapped in the soil matrix. The strength of sorption would, however, depend on the size, chemistry, aggregation behavior, conditions under which it is applied etc. (US Environmental Protection Agency, 2007). In fact, whether an ENP can be hazardous in soil depends not only on its concentration, but also on the likelihood of it ever coming into contact with microbial cells. It may also be noted that natural colloids and ENPs in the environment can interact with one another and also with other larger particles (Simonet and Valcárcel, 2009). Studies also demonstrate lack of toxicity from bulk materials (300 nm) of Ag-, CuO- and ZnO-NP (Gajjar et al., 2009), suggesting that aggregation of the ENPs into larger particles, possibly by factors present in the soil (natural colloids, SOM etc.) may reduce their antimicrobial activity. Therefore, assessing the stability of ENPs in the soil entails evaluating their ability to aggregate or interact with other particles (Zhu et al., 2006) because the adsorption of natural OM to the surfaces of natural colloids and ENPs is known to strongly influence, and in some cases control their surface properties and aggregation behavior (Stankus et al., 2011).

A major redeeming factor could be the ability of the soil to modify ENPs, like for instance ENP surfaces can be altered by chemical or biological modification or degradation. Cullen et al. (2011) found no negative effects of nano-scale zerovalent Fe on ammonia oxidizing potential after 14 days suggesting that nZVI had become passivated through reaction with soil components and is no longer capable of interference with the assay via nitrite reduction or ammonium production. In addition, ENPs can be coated by SOM; in this way, humic and fulvic acids (HA and FA) can inhibit the aggregation of CNTs in aqueous suspensions (Hyung et al., 2007; Neal, 2008) and soil (Shah and Belozerova, 2009). Short term bacterial toxicity of fullerene NPs was also reduced by natural OM (Li et al., 2008) and this coincides with the results on Ag NP (Fabrega et al., 2009a). The SOM can also affect the aggregation and deposition behavior of ENPs by adsorption. It has been demonstrated that the presence of HA in the back ground solution reduces the aggregation kinetics of fullerene NPs considerably due to steric repulsion induced by the HA macromolecules adsorbed on to the NP surfaces (Chen and Elimelech, 2007). This behavior is also evident from other studies which reported steric stabilization of fullerene NPs through OM adsorption (Chen and Elimelech, 2008; Duncan et al., 2008). Li et al. (2008) observed that sorption of nC$_{60}$ to soil reduced its bioavailability and antibacterial activity, and the sorption capacity strongly depended on the OM content of the soil. Adsorption of aquatic dissolved humic substances onto nC$_{60}$ and possible subsequent reactions were also found to eliminate nC$_{60}$ toxicity at HA concentrations as low as 0.05 mg L^{-1}. Also, the inhibition of bacterial viability counts by Ag NPs exposure was found to

be less in the presence of terrestrial HA due to adsorption/binding of Ag NPs (Dasari and Hwang, 2010). These findings indicate that SOM could significantly mitigate the potential impacts of ENPs on soil microorganisms.

Similarly, in the absence of FA, there was extensive sloughing of the biofilm bacteria into suspension implying Ag NP–*P. putida* interactions, while in the presence of FA, sloughing was reduced (Fabrega et al., 2009b). This suggested that soils rich in HA and FA could also negate the toxic effects of Ag NP. Conversely, coating by OM would also mean that these ENPs could have long residence times resulting in increased bioavailability (Cumberland and Lead, 2009). Studies by the several workers (Baalousha et al., 2008; Diegoli et al., 2008; Giasuddin et al., 2007; Hyung et al., 2007) have shown that humic substances and solution conditions such as pH affect surface properties and aggregation of fullerenes, CNTs, Au- and Fe-oxide NPs. Short term bacterial toxicity of fullerene NPs is reduced by natural OM (NOM; Li et al., 2008) and this coincides with the results on Ag NPs (Fabrega et al., 2009a). Also, the microbial communities in soil have an inherent ability to resist disturbances from heavy metals such as Ag NP and a capacity to recover from these (Throbäck et al., 2007). The ENPs could also react with ions in the soil and form complexed salts that are not toxic to the microorganisms (Shah and Belozerova, 2009). It is also possible that metal oxide NPs aggregate and tend to form relatively large agglomerates which reduce their nano-associated toxicity by reducing the potential direct interactions between ENPs and bacteria (Wu et al., 2010). Apparently, the information available indicates a complex interaction between natural (organic humic substances) colloids and ENPs of different types. The observed effects include aggregation, disaggregation and surface film formation and are all dependent on environmental conditions (Ju-Nam and Lead, 2008). In fact, a lot depends on the nature of the OM in question because NOM with relatively high hydrophobicity had a greater capability of inducing toxicity mitigation (Lee et al., 2011). However, it is obvious that interactions of NPs with SOM have not been thoroughly investigated, but are likely to be a function of particle size, shape and surface properties (specific surface area and surface charge). Besides, like in aquatic systems, NOM in soil may influence the surface speciation and charge of NPs, and thus affect their aggregation/deposition properties. These OM compounds may be sorbed to the surfaces of ENPs by various types of interactions, including electrostatic, hydrogen bonding, and hydrophobic interactions (Navarro et al., 2008; Ojamae et al., 2006). Therefore, it can be presumed that the interactions between ENPs and NOM may finally determine the ENPs' fate in soil. The formation of larger agglomerates of ENPs by high molecular-weight NOM compounds will reduce their toxicity and is likely to decrease their bioavailability. In contrast, solubilization by natural surfactants such as lower-molecular-weight NOM compounds will increase their mobility and further the bioavailability of ENPs (Navarro et al., 2008).

5. Response of soil microorganisms to ENPs

The idea that microorganisms are resistant, resilient, and functionally redundant is pervasive in ecology (Allison and Martiny, 2008). High degree of metabolic flexibility, physiological tolerance to changing environmental conditions (Meyer et al., 2004), high abundances, widespread dispersal, and the potential for rapid growth rates have also led to the suggestion that microbial communities will be resilient to change (Fenchel and Finlay, 2004). Besides, rapid evolutionary adaptation through horizontal gene transfer could allow sensitive microorganisms to adapt to new environmental conditions and quickly return the community to its original composition (Allison and Martiny, 2008).

Apparently, these studies demonstrate that soil microbial communities often are quite resilient to perturbations. Though a comprehensive understanding of the interactions between metal oxide NPs and

microorganisms especially bacteria is still at a very early age (Han and Gu, 2010), findings suggest that bacteria with a tolerance for a toxic agent may appear with time (Bååth, 1992) and the antimicrobial activity of ENPs could possibly be reduced by bacterial self protection-mechanism. For instance, *B. subtilis* (Gram-positive) responded to nC_{60} by altering membrane lipid composition, phase transition temperature, and membrane fluidity (Fang et al., 2007). Earlier, Thill et al. (2006) studied the impact of a water dispersion of CeO_2-NP (7 nm) on gram negative bacterium *E. coli* and found that positively charged CeO_2 at neutral pH displayed a strong electrostatic attraction toward bacterial outer membranes and Ce (IV) was reduced to Ce (III) at the surface of the bacteria. However, the specific enzymes involved in the process have not been identified. It was also observed that in the presence of toxic CuO- and NiO-NPs, *E. coli* cells appeared to accumulate as flocs. Apparently, stress levels did influence this aggregation capability because the cells with more toxic ENPs of NiO formed bigger flocs than those with CuO (Wang et al., 2010). This phenomenon is consistent with the fact that environmental stresses trigger expression of proteins to induce cell aggregation (Kwiatkowska et al., 2008). Bacteria also have other protective responses to environmental stresses (Wu et al., 2010). They showed that bacterial cells have extracellular protein to neutralize small amounts of toxic ions. However, ENPs can continuously release toxic ions to bacteria and such protective mechanisms could possibly become less effective. Sudheer Khan et al. (2011) found that bacteria secreted exopolysaccharides (EPS) that capped Ag NPs thereby reducing its toxicity. Such EPS capped Ag NPs showed less toxicity to *E. coli*, *S. aureus* and *Micrococcus luteus* compared to the uncapped ones. This suggested that capping of ENPs by bacterially produced EPS would serve as a probable physiological defense mechanism.

6. Conclusions and future research needs

The published literature suggests that among the ENPs, the antimicrobial activity of metal NPs to soil microbial communities holds great significance. Though such negative effects of pollutants, especially heavy metal contamination on microbial activity, biomass and diversity in soil have been amply demonstrated (Gremion et al., 2004), little information is available on how metal ENPs act in the soil matrices especially their adsorption to clay minerals, organic fractions, toxic substances, organic pollutants etc. Such interactions between organic pollutants and ENPs may result in a pollutant with increased toxicity or reduced toxicity or anything in-between. However, if the microbial cells adsorb the ENPs containing the adsorbed pollutant a toxic effect may result from the pollutant, the ENPs or from both (Nowack and Bucheli, 2007). Also, one of the main drawbacks of current investigations is the lack of information on transformations of ENPs in soil and detection in the presence of natural NPs like nano-clays, minerals, oxides and hydroxides of Al, Fe, and Mn, enzymes, organic fractions like humic substances, viruses and mobile colloids.

This highlights the need for more information on interaction of ENPs with soil components and more quantitative assessments of aggregation/dispersion, adsorption/desorption, precipitation/dissolution, decomposition, and mobility of ENPs in the soil environment (Klaine et al., 2008). Mobility in soil is dependent on the size of the ENPs, although it is the agglomerate size, not the primary size that is correlated with transportability. Many factors influence the mobility of ENPs in the soil, but size, charge, and agglomeration rate in the transport medium are predictive of ENP mobility in soil (Darlington et al., 2009). The existence and speciation of metal NPs in soil solution and knowledge on the interaction between their active sites and soil solution or other ions are essential for a better understanding of the interactions between metal NPs and microorganisms in the soil. However, the solution chemistry of metal NPs is quite limited and thermodynamic data such as solubility and reaction constants of NPs are unavailable.

Another major hurdle is the physico-chemical interactions between ENPs and bacterial cell surfaces. In general, ENPs are much larger than cations and anions in the soil and they do not carry but expose lot of active sites on the surface resulting in more complicated reactions. Also SOM coatings on the ENPs may alter the surface characteristics and reactivity of the NPs and this will make physico-chemical interactions even more complicated (Han and Gu, 2010).

Data is required on the long-term effects of ENPs on soil microbial populations in a range of soils with varying physico-chemical characteristics and soils from different ecosystems. The behavior of ENPs and their interactions with microorganisms in soils poor in organic matter, for example tropical soils under intensive cultivation or severely degraded soils would throw light on whether SOM and its components are critical factors involved in detoxifying ENP contaminated soils. Also, the effects of HA which are known to photosensitize transformations of several types of synthetic chemicals and xenobiotics including detoxification through oxidative coupling reactions mediated by enzymes in soil have to be thoroughly investigated. Besides, SOM and HA, presence or absence of excess salts like those encountered in saline and saline-sodic soils could affect the toxicity of ENPs. For instance, Ag NPs presented little or no impact on bacterial assemblages in estuarine sediments possibly due to environmental factors, in particular the chloride ions in estuary water affecting the chemistry and behavior of Ag NPs (Bradford et al., 2009). Likewise, Li et al. (2011) found that the addition of salts enhanced the aggregation of ZnO NPs and consequently affected the dissolution behavior and biological availability of the particles. Their study also showed the potential of soil extracts to mitigate the toxic effects of ZnO NPs on filter paper, which may also be attributed to the presence of salts and organic carbon.

It has also been suggested that though SOM coatings may make the ENPs less toxic to microbial communities, coated ENPs may be able to access the cell surface more readily than uncoated ones, due to the similar solubility of the surfactant with the cell membrane (Lubick, 2008). The morphological characteristics and size of the ENPs, their chemical and catalytic properties, speciation in intracellular sites, pathways of transport into bacterial cells, and the specific enzymes that assimilate or detoxify the metals or expel them from cells also need to be considered while studying their effects on soil microbial communities (Han and Gu, 2010). However, this appears extremely difficult due to the complexities involved in the behavior of ENPs and due to difficulties in measuring their toxicity in soils. This is further exacerbated by the presence of naturally occurring NPs of similar molecular structures and size ranges (e.g. Fe and Al oxides).

Overall, it is apparent from the studies done *in vitro* that ENPs pose a potential hazard to microorganisms. Studies done by incubating soils with ENPs, microcosm studies and pot experiments suggests that in most cases ENPs inhibit soil microbial activity (Table 1). This underlines the fact that the effect of ENPs on microbial community in soils under field conditions is still in its infancy, the smothering effects of SOM and HA on ENPs are still being speculated and the bacterial self protection mechanism on encountering ENPs in the soil matrix is yet to be extensively studied. Considering that attempts are being made to employ some of these ENPs as carrier materials for smart delivery of chemical fertilizers to crops (DeRosa et al., 2010), it is imperative that we set specific standards for the manufacture, use, and disposal of ENPs (Handy and Shaw, 2007). Therefore, conclusive evidences need to be obtained to draw strong conclusions about the potential toxicity of ENPs to microbial activity under field conditions and herein lies one of the main challenges in risk assessment of spreading ENPs.

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