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## Ecotoxicity of Nanomaterials in Soil

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### ABSTRACT

Rapid development in nanotechnology and wide spread use of nano products increased probability of their release in aquatic and terrestrial ecosystems. Unique properties of nanomaterials and ambiguity in their transformation, reactivity and toxicity put a new challenge in front of scientists, government regulators and public stakeholders. In addition vital importance of soil microorganisms in soil as well as their diversity and lack of clear investigations about nanomaterial toxicity or other negative impacts on soil microorganisms show the necessity for more attention in this issue. Therefore the research community needs to focus on understanding the reactivity, mobility, fate, persistence and effects of nanomaterials in terrestrial ecosystems. This paper is trying to discuss some negative impacts of synthetic nanomaterials on soil microorganisms.

**Keywords:** Nanomaterials, Ecotoxicity, Nanotoxicity, Soil Microorganisms, nanoecotoxicity

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### INTRODUCTION

In recent decades, the interest in environmental issues has increased very quickly. Not only scientists, but also other active members of the society (i.e., politicians, industries, general public), have paid much attention to all aspects related to environment, in general, and environment protection, in particular. Engineered nanomaterials (ENMs) like other types of industrial products may enter the environment through intentional and unintentional releases such as atmospheric emissions and solid or liquid waste streams from production facilities [53]. They have potentially negative impacts on human health and the environment [44]. This potential results from several properties that may permit mobility in the environment, coupled with the reactivity and potential toxicity of some ENPs [31]. The rapid onset of damage in organisms in different compartments of environments and ongoing funding and development of nanomaterials has worried some scientists, policy-makers, members of the public and industry and investors about their potential impacts on the health and safety of both humans and the environment, and has led to the development of the new field of nanotoxicology [25].

#### **Nanotechnology and Nanomaterials:**

Nanotechnology is a collective term that implies the capacity to design, characterization, production, and application of structures, devices, and systems at a nanometer scale. Nanotechnology thus has potential applications in a wide range of sectors, from energy (production, catalysis, storage), materials (lubricants, abrasives, paints, tires, sports ware), electronics (chips, screens), optics, remediation (pollution absorption, water filtering, disinfection), to food (additives, packaging), cosmetics (skin lotions and sun screens) and medicine (diagnostics, drug delivery). This width reflects a diversity of materials that are or will be used in the different applications [17]. It is estimated to far

exceed the impact of the industrial revolution and is projected to become a \$1 trillion market by 2015 and employ about 2 million workers [48] and currently, according to conservative estimates more than 800 consumer products containing nanoparticles or nanofibers are already in the market and a number of others are still to come [33].

#### **Ecotoxicity of nanomaterials:**

As a consequence of the increasing production of NMs of all types and the potential for their release in the environment, their toxicity needs to be addressed. In doing so, it is necessary first to determine the fate and behavior of manufactured NMs in the environment.

Three aspects seem important when assessing the impact of ENPs as pollutants ending up in the environment:

1) **Mobility:** for environmental exposure it is important to have empirical data or procedures to predict the persistence and mobility in air, soil and water and ability to move or from one recipient to another. Examples of parameters that may be needed to make predictions on environmental fate is chemical factors like adsorption capacity, degree of aggregation, photolytic degradation, dispersability, interactions with soil particles, tendency of nanoparticles to aggregate etc. it is still an open question whether we can expect to find individual free nanoparticles in the environment [35].

2) **Modification;** how and to which extent ENPs are modified by contact with the environment (and the consequences of such modifications on ecotoxicity and mobility). In the environment, one should of course consider exposure and toxicity to variety of relevant organisms, including the modifying effect of their living environment (soil, sediment, water), which is why environmental risk assessment is a far more complex task which may depend on e.g. scientific consensus on which test systems and environmental parameters that need to be included [17].

3. **Ecotoxicity:** The final issue to be considered in the context of environmental exposure is to what degree various nanoparticles are taken up by biota, whether they are metabolized or degraded, and at which rates they are excreted and the possible harm that ENPs can cause to organisms living in water, sediments and soils that they enter [17]. As a consequence the term “nano(eco-)toxicology” has been developed as a separate scientific discipline with the purpose of generating data and knowledge about NMs effects on humans and the environment [31].

Ecotoxicity measurements are conducted on different trophic levels including microorganisms, plants, invertebrates and vertebrates, and test systems have been standardized for some organisms and for some exposure conditions. But there are of course a far wider range of environmentally relevant organisms living in nature that are or may be used in non-standardized methods to test whether a substance has harmful effects on organisms or processes in the environment. For example, antibacterial effects of cerium oxide nanoparticles on *staphylococcus aureus* and zinc oxide nanoparticles on *Listeria monocytogenes* has been identified by researchers [1] [28].

Soil microorganisms are the largest unexplored reservoir of microorganisms on the earth. Considering their importance functional role for biosphere, a great deal of research is necessary before observed effects can be extrapolated to higher organisms such as mammals [17]. Although there are some indications that nanoparticles in the environment may have undesirable antimicrobial effects, it is impossible to say at this time what risks posed by nanoparticles are relevant and possibly of concern for organisms in the ecosystem [46] because there are many unknown aspects about them in relation to environment and living organisms.

In spite of importance of ecotoxicity studies of ENPs, few have taken into account the modifying effects of NMs on soil, sediment and water constituent. It can be due to the reason that the methods and tools for such task have not been well developed yet [4] and also they have such diverse properties and behaviors that it is impossible to provide a generic assessment of their health and environmental risks [24]. The shape, charge and size of different particles can influence their kinetic (absorption, distribution, metabolism and excretion) and toxic properties [10]. Furthermore many transformations, e.g. reaction with biomacromolecules, redox reactions, aggregation, and dissolution, may occur in both environmental and biological systems. These transformations and other will alter the fate, transport and toxicity of NMs [8]. As a consequence, even NMs of the same chemical composition which have different sizes or shapes can have vastly different toxicity [38].

#### **Nanomaterials in relation to soil constituents:**

The modification of ENPs after entering in contact with environmental matrix constituents, like ions, natural colloids and other charged surfaces are likely to affect not only mobility, aggregation etc., but also to modify toxicity characteristics [20]. There are many parameters that effect ecotoxicity of ENPs which should be considered

in researches on ecotoxicity of NMs. These parameters vary from NM physico-chemical characteristics to expected environmental concentrations to fate and transport mechanisms. The environmental implications of toxicity will also depend upon the ecological composition and structure of complex microbial communities. Interaction of an ENP with e.g. a charged surface of a larger particle cause them not be available for absorption in the same way or to the same extent as a freely suspended ENP, rendering it less bioavailable. Consequently, a far lower exposure to ENPs may be observed in an environmental matrix compared to what is experienced *in vitro* [17].

Practically nothing is known about how ENPs interact with soils and sediments [30]. Of particular relevance to manufactured NMs, soil colloids and other porous media may facilitate the movement of contaminants in soils and other porous media. Consequently, they may affect on degree of toxicity of manufactured NMs. Soil is the environmental matrix that is richest in natural nanoparticles, both as primary particles and agglomerates/aggregates. This is due to constant physical/chemical weathering and re-arrangement of its geogenic constituents coupled with a high biological activity that transform both dead organic matter and minerals. Sediment and soil constituents, like clay and organic matter have large specific surface areas (typically around 300-500 m<sup>2</sup>/g), and a high electrochemical surface charge that is likely to make them interact with charged particles, like many ENPs. Natural organic matter in water, sediments and soils also contain hydrophobic domains that are likely to interact with hydrophobic ENPs, like fullerenes and carbon nano tubes (CNTs). For example , the intraction between NMs and humic substances (HS) including natural organic matter result in a nano scale coating of NMs, analogous to protein coronas in mammalian systems ,that drastically changes their aggregation ,deposition and toxic properties [8] .While some constituents retain hydrophilic, polar substances, others strongly bind hydrophobic, non-polar compounds. Some ENPs, such as fullerenes and carbon nanotubes (CNTs), are non-polar and do not easily disperse or dissolve in water. In this manner they may resemble common hydrophobic organic contaminants, like polycyclic aromatic hydrocarbons (PAH) [17].

As described above, soils and sediments are complex porous media that are likely to constitute natural barriers against transport and remobilization of ENPs. The fate and bioavailability of ENPs dispersed in these systems thus strongly depend on the filtering properties resulting from these conditions [36]. The organic and mineral phsio-chemical compositions and structural heterogeneity of natural media is complex and must be taken into account to understand the transport and fate of nanoparticles under natural conditions [17].

Environmental factors like pH and ionic strength [2] together with the physical-chemical properties, structure and concentration of ENPs [39] may determine whether they are bound within or transported out of soils and sediments. However, interactions with dissolved constituents may also affect their mobility. As described previously, dissolved organic matter is a constituent of both surface waters and soil and sediment, and has recently been shown to interact with CNTs in a way that may enhance their dispersion and transport [13].

Zeta potential, i.e. the diffuse surface charge, as an important characteristic of colloid stability may link to the chemical nature of the dispersed nanomaterials and to the properties of the continuous phase (pH, dilution, temperature and inter atomic distance between others). Its measure gives good information on nanomaterials mobility, aggregation rates and interactions with surfaces: when its value approaches 0 mV massive aggregation occurs. Moreover, for magnetically charged particles, the dipole moment is a key feature in their characterization and appears to be related to the toxicity potential [22].

Furthermore, an interesting issue is how redox transformations may influence the transformation and fate of engineered nanoparticles [51]. Redox reactions occur in a wide range of environments, and are important for the degradation of organic matter, for generation of energy by chemolithotrophic organisms, and for the precipitation and dissolution reactions that influence sequestration and mobility of metals. To what extent nanomaterials will be transformed by redox processes in the environment and how these processes may influence toxicity or other hazards of various nanomaterials is still an open question [17]

#### **Using soil microorganisms as case study in nanoecotoxicology:**

Apart from microorganisms, there are many very useful organisms from different environments that may be used in ecotoxicity testing. In water it may be pertinent to use free living (pelagic) organisms or organism living on or in sediments (benthic organisms) depending on where the suspected harmful agent is found. Further it may be interesting to use organisms of different trophic level (from different steps in a food chain), from primary producers

to grazers and predators, as some environmental pollutants may accumulate in the food chain (biomagnification) [17].

Microorganisms are of great environmental importance because they are the foundation of aquatic and terrestrial ecosystems and provide key environmental services ranging from primary productivity to nutrient cycling and waste decomposition [41]. Microorganisms (mainly bacteria, but also fungi, protozoa and algae) play a very important role in maintaining soil health, ecosystem functions (eg. nutrient cycling) plant nutrition and plant growth promotion [17].

Therefore, selection of soil organisms for ecotoxicity studies can be done based on specific modes of exposure (contact, ingestion, and prey preferences), specific habitats (surface, shallow or deep sub-surface, aerated or anoxic environments, etc.) or specific functions (denitrification, bioperturbation, etc.) [17].

Bacteria form symbiotic relationships with legumes which provide a major source of fixed nitrogen for both these and other plants. Denitrifying bacteria play an important role in keeping waterways clean by removing nitrate from water contaminated by excessive fertilizer use. Bacteria form symbiotic relationships with all animals from insects to humans. Many of these bacteria aid their animal hosts to digest food, others perform more unusual functions. Consequently, understanding toxicity of NMs to microbes is important to evaluate the potential impacts of NMs in the environment.

Furthermore, microorganisms are convenient (model) test organisms because they grow rapidly and are inexpensive to culture; have a high surface-to-volume ratio, making them sensitive to low concentrations of toxic substances; and facilitate studies at many levels ranging from a single biochemical reaction in bacteria to complex ecosystems containing a diversity of microorganisms [41]. Also, it is highly likely that bacteria will influence NMs fate and behavior. Microorganisms (mainly bacteria, but also fungi, protozoa and algae) have the advantage that they are ubiquitous and highly diverse (filling a range of habitats and functions), small (permitting miniaturized tests) and with short generation times (permitting rapid tests). Also, they are in immediate contact with liquids and surfaces and absorb nutrients and other molecules from their environment directly through their cell walls [17].

Many microorganisms are also easy to culture, and easy to extract DNA from. The latter permits identification based on sequencing of DNA and more importantly to describe whether or not (or to which extent) certain genes related to toxicity protection or stress have been activated. Identification of multiple microorganisms in a single sample through molecular methods (DNA or other) permits us to describe the composition (or diversity) of complex microbial communities and changes in composition due to a suspected harmful agent [48]. Measurements of such changes are often far more sensitive than tests based on isolation, pure culture and testing of individual microorganisms [41].

Microbial ecotoxicity tests can investigate survival, reproductive capacity, and mutation as well as non lethal toxicity endpoints [17]. Calculation of a minimal inhibitory concentration or minimal bactericidal concentration offers a standardized method to compare the lowest level of toxicant that prevents bacterial growth for the minimal inhibitory concentration or that actually reduces the number of viable cells for the minimal bactericidal concentration. In bioluminescent tests, diminished bioluminescence of certain bacteria, such as *Vibrio fischeri*, suggests that the test substance has antimicrobial activity [16].

#### **Mechanisms of nanomaterials Toxicity:**

The characteristics of NPs that influence toxicity include size, surface area, morphology, and dissolution. To date, screening studies using in vitro approaches have observed toxicity from metal NPs at lower concentrations than toxicity from carbon-based NPs [7].

While toxicity mechanisms have not yet been completely elucidated for most NMs, possible mechanisms include disruption of membranes or membrane potential, oxidation of proteins, genotoxicity, interruption of energy transduction, formation of reactive oxygen species, and release of toxic constituents[55]. These toxicity mechanisms may result from various factors such as: high surface area to volume ratio, surface charge, hydrophobic and lipophilic groups may allow them to interact with proteins and membranes, complementary effects of nanostructures which cause inhibition of enzyme activity, bioaccumulation and chemical composition which increase their reactivity [33].

However, unintentional toxicity mechanisms can be difficult to isolate and vary widely even within the same class of NM, such as fullerenes or nanosilver. For example, fullerol (C60) [OH]<sub>x</sub>, (the hydroxylated form of C60) generates singlet oxygen and can behave as a potent oxidizing agent in biological systems but it is not noticeably cytotoxic [34]. Coating C60 with polyvinyl pyrrolidone produces a NP that generates singlet oxygen that can cause lipid peroxidation and other cell damage [18]. Still other studies with fullerene water suspensions (nC60) have shown antibacterial activity in the absence of light or oxygen, negating the exclusive influence of singlet oxygen [21]

Certain NMs such as quantum dots cause toxicity to bacterial cells by releasing harmful components, such as heavy metals or ions which they have in their core (ie: CdSe, CdTe, CdSeTe, ZnSe, InAs, or PbSe) and in their shells (ie: CdS or ZnS). Less is known of the stability of quantum dots in the environment, other than that half lives are likely to be quite long (months, years) and vary with photolytic conditions [43]. The soil-dwelling amoeba *Dictyostelium discoideum* has been shown to incorporate avidin and conjugated CdSe-containing quantum dots by endocytotic pathways [15]. Evidence that quantum dots may enter a wide variety of cell types by endocytosis raises a potential concern for their long term effects, especially as they may be retained within different tissues and organs for some time [11].

The toxicological interactions between NMs and proteins are related to either the NM physically interacting with proteins or the NMs producing ROS (Reactive Oxygen Species) or other damaging radicals. The generation of reactive oxygen species (ROS) is an important toxicity mechanism of ENPs. ROS include oxygen radicals have one or more unpaired electrons: such as superoxide anion (O<sub>2</sub><sup>-</sup>), peroxide (O<sub>2</sub><sup>-·</sup>), hydroxyl radical (·OH), and singlet oxygen (1O<sub>2</sub>). They are e.g. formed in mitochondria as oxygen is reduced along the electron transport chain. But despite their beneficial activities, radicals possess an unpaired electron, which makes them highly reactive can clearly be toxic to cells and there be able to damage [17], cell membranes, cellular organelles, all macromolecules including lipids, proteins, and nucleic acids contained in DNA and RNA [12]. Several *in vitro* studies on the toxicity of ENPs have shown generation of ROS, e.g. by TiO<sub>2</sub> [19] and fullerenes [37]. On the other hand, some authors have found that ENPs, including e.g. fullerenes, may also *protect* against oxidative stress [3]. This apparent dichotomy underlines the need for research on nanoparticle-cell interactions and mechanistic aspects of ENP metabolism in organisms and specific cells [17].

NMs that generate ROS can damage iron-sulfur clusters that behave as cofactors in many enzymes, leading to Fenton chemistry that catalyzes the production of more ROS generation [14]. Reactive oxygen species can also lead to the formation of disulfide bonds between sulfur-containing amino acids, thus disturbing the structure and function of the protein [41].

Silver NPs and titanium dioxide are among the best studied NMs with respect to microbial toxicity. Such materials are established as antimicrobial agents, and their nanocrystalline forms may act similarly [26]. Silver NPs may cause toxicity via multiple mechanisms but actual mechanism by which silver nanoparticles interfere with bacteria is as yet unclear. Morones *et al.* (2005) reported several possible causes: Silver NPs adhered to the surface alter the membrane properties, therefore affecting the permeability and the respiration of the cell; they can penetrate inside bacteria and cause DNA damage, and they can release toxic Ag<sup>+</sup> ions.

Some researchers suggest that silver nanoparticles damage bacterial cells by destroying the enzymes that transport the cell nutrient and weakening the cell membrane or cell wall [40]. In their study of *E. coli* bacteria, they found that nanosilver damaged and pitted the bacteria's cell walls and accumulated in the cell wall, leading to increased cell permeability and ultimately cell death. *E. coli* is often used as a model for gram negative bacteria, suggesting that these results could be more broadly relevant. However, other researchers believe nanosilver destroys the ability of the bacteria's DNA to replicate. It is believed that silver ions interact with thiol groups of proteins, resulting in inactivation of vital enzymes [6]. Degradation of lipopolysaccharide molecules, forming pits in the membrane, and changes in membrane permeability due to nanosilver have also been reported [40].

Interactions of NMs with nucleic acids have applications in DNA labeling or DNA cleavage. Nucleotides can be tagged with NPs, such as quantum dots, which act as labeling agents for bioimaging applications [5]. As with NMs that are made to traverse the cell membrane, iron oxide NPs can be modified into non viral NP gene transfection vectors to carry genetic information into the cell [52]. Quantum dots can also nick supercoiled DNA [9]. Titanium dioxide NPs, such as those used in sunscreen, indirectly damage DNA because of ROS production, which can lead

to DNA strand breaks, cross-linking, and adducts of the bases or sugars [49]. Cerium dioxide NPs may themselves be transformed after contact with living cells, oxidize membrane components involved in the electron transport chain, and cause cytotoxicity [45].

Photosensitive metallic and metal oxides that generate ROS as well as fullerenes are used for photodynamic therapy, targeting cells and DNA [50]. In contrast to the beneficial applications of NM–DNA conjugation, fullerenes have been reported to bind DNA and cause deformation of the strand, adversely impacting the stability and function of the molecule [54]. Photosensitive fullerenes can cleave double stranded DNA on exposure to light, although this is highly dependent on the type of the fullerene derivative [42].

Electron transport phosphorylation and energy transduction processes may be disrupted if membrane integrity is compromised or if a redox-sensitive NM contacts membrane-bound electron carriers and withdraws electrons from the transport chain. Fullerene derivatives have been reported to inhibit *E. coli* respiration of glucose [23].

These data and similar literatures highlight the need for more information on the interaction of NPs with soil components and more quantitative assessments of aggregation/dispersion, adsorption/desorption, precipitation/dissolution, decomposition, and mobility of manufactured NPs in the soil environment. This information will aid the interpretation of terrestrial ecotoxicity test data and will inform the correct protocols for the assessment of the ecotoxicity of NPs in soils.

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