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# Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi

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**Abstract** Developments in nanotechnology are leading to a rapid proliferation of new materials that are likely to become a source of engineered nanoparticles (ENPs) to the environment, where their possible ecotoxicological impacts remain unknown. The surface properties of ENPs are of essential importance for their aggregation behavior, and thus for their mobility in aquatic and terrestrial systems and for their interactions with algae, plants and, fungi. Interactions of ENPs with natural organic matter have to be considered as well, as those will alter the ENPs aggregation behavior in surface waters or in soils. Cells of plants, algae, and fungi possess cell walls that constitute a primary site for interaction and a barrier for the entrance of ENPs. Mechanisms allowing ENPs to pass through cell walls and membranes are as yet poorly understood. Inside cells, ENPs might directly provoke alterations of membranes and

other cell structures and molecules, as well as protective mechanisms. Indirect effects of ENPs depend on their chemical and physical properties and may include physical restraints (clogging effects), solubilization of toxic ENP compounds, or production of reactive oxygen species. Many questions regarding the bioavailability of ENPs, their uptake by algae, plants, and fungi and the toxicity mechanisms remain to be elucidated.

**Keywords** Toxicity · Nanoparticles · Fullerenes · Carbon nanotubes · Carbon black · Silver nanoparticles · TiO<sub>2</sub> · Organic matter

## Introduction

Engineered nanoparticles (ENPs), with sizes smaller than 100 nm in at least one dimension, have received a lot of attention and concern recently due to their rapidly increasing applications in various areas of the economy, such as textiles, electronics, pharmaceuticals, cosmetics, and environmental remediation (Dunphy Guzman et al. 2006b; The Royal Society & The Royal Academy of Engineering 2004). They are some of the most important products of nanotechnology, whose benefits and drawbacks are believed to well exceed those of the industrial revolution. On one hand, some optimists claim that nanotechnology can reverse the harm done by industrialization (Borm 2002). On the other hand, in vitro studies, so far, have put a damper on these claims. Investments in nanotechnology are thus increasing rapidly worldwide. The total global investment in nanotechnologies was around \$10 billion in 2005 (Harrison 2007) and it is estimated that the annual value for all nanotechnology-related products will be \$1 trillion by 2011–2015 (Roco 2005). Accordingly, the

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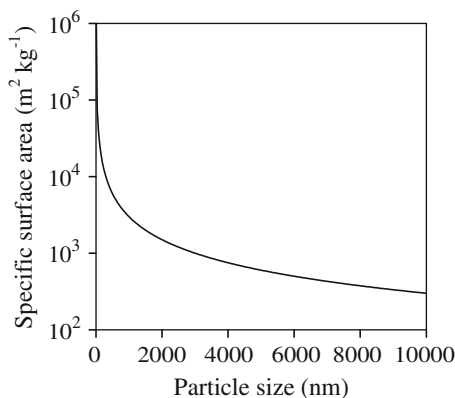
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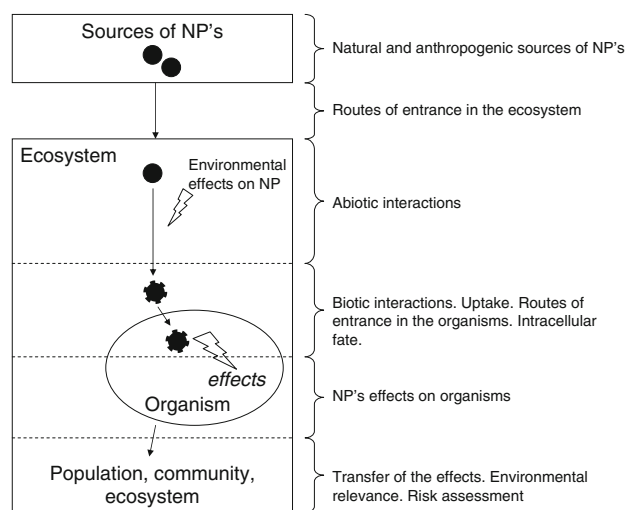
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**Fig. 1** Relationship between specific surface area ( $\text{m}^2 \text{kg}^{-1}$ ) of a spherical particle and its size (diameter in nm) with a density of  $1000 \text{ kg m}^{-3}$  as an example. Specific surface area increases as particles become smaller

current annual global production of ENPs was on the order of  $10^3$  tons in 2004, which is expected to increase further to  $10^4$ – $10^5$  tons per year after 2010 (The Royal Society & The Royal Academy of Engineering 2004). This production includes ENPs made for use in agricultural applications (Kuzma and VerHage 2006) and environmental remediation, such as zero-valent iron (Zhang 2003). However, because of their widespread use in consumer products, it is expected that ENPs will find their way into aquatic, terrestrial, and atmospheric environments (Nowack and Bucheli 2007), where their fate and behavior are largely unknown. The unique properties of ENPs, such as high specific surface area (Fig. 1), abundant reactive sites on the surface as a consequence of a large fraction of atoms located on the exterior rather than in the interior of ENPs, as well as their mobility, could potentially lead to unexpected health or environmental hazards (Maynard et al. 2006; Wiesner et al. 2006). Therefore, organisms, and especially those that interact strongly with their immediate environment such as algae, plants, and fungi, are expected to be affected as a result of their exposure to ENPs. There is a consensus amongst both proponents and skeptics that the potentially adverse effects ENPs could have on humans as well as whole ecosystems need to be widely examined in this early phase of nanotechnology (Colvin 2003; Dunphy Guzman et al. 2006b; Nel et al. 2006; Oberdörster et al. 2005).

In this review, we examine the environmental behavior and ecotoxicity of nanoparticles (NPs) to algae, plants, and fungi. The term NP is used to refer to natural and unintentionally produced nanoparticles, which often have a wider distribution of sizes, while the term ENPs is used to refer to engineered nanoparticles that are designed and intentionally produced, with generally more narrowly defined sizes. Sources and behavior of NPs in atmosphere, soils, and aquatic systems, as the main habitats for algae,



**Fig. 2** The logical chain of events accounting for the toxicity of NP starts with the sources of NPs and their entrance routes into the ecosystem. Then, the NPs will experience abiotic interactions because the conditions prevailing in the different atmospheric, aquatic and terrestrial environments, leading to physical and chemical alterations (graphically represented as changes at the NPs' surface). These alterations will greatly determine the fate of the NPs in the environment and thus their bioavailability to organisms. Once in the proximity of organisms, interactions might occur at biological interfaces, resulting in the entrance of NPs into these organisms. Once inside the organisms, NPs may cause various toxic effects and might be transferred through food webs, thus affecting communities and ecosystems

plants, and fungi, are discussed first, then the toxicity of ENPs. Three key topics are emphasized: (1) sources, transformations, and fate of nanoparticles (NPs) in the environment, (2) biotransformations that ENPs can experience in contact with algae, plants, and fungi, and then the entrance and fate to these organisms, and (3) the underlying mechanisms of ENPs' toxicity and their effects on algae, plants, and fungi, and how these toxic effects might be transferred through food webs, thus affecting communities and whole ecosystems. The interaction between these three areas has been summarized in Fig. 2.

### Sources and environmental behavior of NPs

#### Natural and anthropogenic sources of NPs

NPs are actually not new and have a history as long as that of the Earth itself. For example, NPs could be produced during a volcanic eruption through processes like Aiken-mode nucleation, as a result of simultaneous emission of substantive nuclei (e.g., sulfuric and nitric acids) (Aiken 1884), and in hydrothermal vent systems (Luther and Rickard 2005). However, it was not until the appearance of

internal combustion engines, power plants, and extensive burning of fossil fuels with the advent of the industrial revolution that their amounts increased significantly and became a potential risk to the environment (Biswas and Wu 2005). With a foreseeable further development of nanotechnology, there will be a substantial increase in the production and release of ENPs.

Therefore, NPs can be produced either intentionally, as ENPs, or unintentionally, with the latter including natural sources such as aerosols from volcanic eruptions, forest fires, pollen fragments, and viruses as well as anthropogenic sources such as power plants, vehicles, coal combustion, frying, and welding, and include nanoparticles such as soot, black or elemental carbon. In aquatic systems, NPs include metal sulfide nanoclusters emanating from hydrothermal systems (Luther and Rickard 2005). At the same time, the physicochemical properties may be different for the NPs from different sources, which may further affect NPs' interactions with organisms, although their toxicity is found to be mainly size or surface area dependent (Nel et al. 2006). NPs from unintentional sources are mostly polydisperse/heterogeneous, containing sulfide, sulfate, nitrate, ammonium, organic carbon, elemental carbon, and trace metals, and have irregular shapes, whereas ENPs are monodisperse/homogeneous and regularly shaped (Sioutas et al. 2005).

Although a budget for NPs in the atmosphere, soils, and aquatic systems is currently lacking, it is conceivable that a significant amount of NPs from an increasing range of applications will find their way into these environments, where plants, algae, and fungi live. Besides direct emission into the atmosphere or photochemical formation therein, ENPs, as they are used in sunscreens, detergents, paints, printer inks, or tires, can also enter the environment through accidental spills during their production and transportation, wear and tear, and the final disposal of the ENP-containing products. In particular, there are certain types of ENPs that are made for the purpose of environmental remediation, such as dechlorination of groundwater pollutants and reclamation of land lost to forest fires (Masciangioli and Zhang 2003; Waychunas et al. 2005; Yue and Economy 2005; Zhang 2003). A lifecycle assessment of the release of ENPs into the environment is thus imperative for the establishment and implementation of effective and protective regulatory policy.

#### NP behavior and fate in the atmosphere

NPs in the atmospheric environment may come from either point/stationary or nonpoint/mobile sources. A portion of these NPs are directly emitted from combustion sources. Others are formed through nucleation and condensation processes of the hot supersaturated vapors when being

cooled to ambient temperature. Furthermore, chemical reactions in the atmosphere may lead to chemical species with very low saturation vapor pressures, which will finally produce particles by nucleation (Biswas and Wu 2005; Friedlander and Pui 2004; Sioutas et al. 2005). During the course of gas to particle conversion (nucleation) in the latter two processes, the gaseous molecules are first combined into ultrafine nuclei (3–20 nm) through binary water-sulfuric acid, ternary water-sulfuric acid-ammonia, or ion-induced nucleation (Kulmala 2003; Kulmala et al. 2004). Then the size of these nuclei will increase due to the condensation of organic or inorganic vapors on the nuclei, with a growth rate of 1–20 nm h<sup>-1</sup>; the nucleation rate of particles with a diameter of 3 nm lies between 0.01 and 10<sup>5</sup> particles cm<sup>-3</sup> s<sup>-1</sup>. The size distribution of the newly formed particles is mainly determined by processes such as condensation/evaporation and dilution, while coagulation and deposition play minor roles (Zhang and Wexler 2004). Therefore, any environmental or meteorological factors (temperature, relative humidity, atmospheric turbulence, etc.) that are involved in these processes may affect the NPs' concentration in the atmosphere. For example, Chang et al. (2004) found that samples taken at different combustion and exhaust temperatures showed higher particle number concentrations at 645°K than at 450°K. Thus it is expected that the exposure of organisms to ENPs will be strongly determined by the environmental conditions prevailing in each ecosystem.

Although there is a lack of knowledge about the fraction of NPs from each of the formation processes mentioned above, emission inventories suggest that motor vehicles are the primary sources of fine and ultrafine (nano)particles in the atmosphere (Schauer et al. 1996; Shi et al. 2001). As the importance of traffic emission sources and the potentially high transportability of NPs in the air, a lot of work has been carried out to determine the concentration and size distribution of ultrafine particles near highways (Hinds 1999). Both spatial and temporal variations of these parameters have been observed. The particle concentration was mostly found to decrease exponentially with downwind distance from the freeway due to atmospheric dispersion and coagulation (Zhu et al. 2002a, b). In these studies, the measured particle size ranged from 7 to 300 nm with particle number concentration mostly accounted for by 10–20 nm particles, and the particle concentration decreased from  $2 \times 10^5$  on the freeway to  $5 \times 10^4$  particles cm<sup>-3</sup> 300 m downwind. One exception is from the work of Morawska et al. (1999), who did not observe statistically significant differences in fine particle number concentrations for distances up to 200 m away from the road. At the same time there is also a diurnal variation of the concentration of NPs in the atmosphere, which is mainly determined by two counteracting processes:

reduced temperature may increase particle emissions, while lower traffic may reduce it. Zhu et al. (2006) found that particle number concentration measured 30 m downwind from the freeway was 80% of the previous daytime measurements (e.g.,  $1.5 \times 10^5$  versus  $1.1 \times 10^5$  particles  $\text{cm}^{-3}$ ). Similarly, higher particle concentrations were observed in winter, as a result of lower temperatures and smaller extent of dilution (Jeong et al. 2004; Stanier et al. 2004). Jeong et al. (2004) measured the ultrafine particles (10–500 nm) in Rochester, New York, USA from December 2001 to December 2002. More than 70% of the total measured number concentration was associated with 11–50 nm ultrafine particles with a concentration of about 7000 particles  $\text{cm}^{-3}$  in winter and 4000 particles  $\text{cm}^{-3}$  in summer. Although a recent study evaluating the aerosol generation during the handling of ENPs such as carbon nanotubes showed an insignificant release (Maynard et al. 2004), it remains unknown to what extent ENPs may contribute to the ultrafine (nano)particles in the atmosphere, while their behavior might be similar to natural ones of similar size.

#### Aggregation, deposition, and mobility of NPs and ENPs in aquatic systems

The surface properties of NPs are one of the most important factors that govern their stability and mobility as colloidal suspensions or their aggregation into larger particles and deposition in aquatic systems. The stability of NPs as colloidal suspensions governs their mobility in aquatic systems. Stable colloidal suspensions of NPs are a prerequisite for efficient interactions of NPs with algae, which may lead to uptake or toxic effects. In soils, the mobility of NPs in pore water is an essential condition for interactions with plant roots or fungi hyphae. Therefore, the surface properties of various NPs have to be briefly discussed. Due to the complexity of the surfaces of natural NPs, we focus in this review mainly on the surface properties of ENPs.

Metallic ENPs are usually coated with inorganic or organic compounds, such as citrate, cysteine, carbonate or surfactants such as sodium dodecyl sulfate to maintain the stability of the colloidal suspension (Mafune et al. 2000). Therefore, surface properties of ENPs in aqueous suspensions are strongly dependent on the composition of these coatings, which typically results, at neutral pH, in a negative charge of the ENPs, which then results in their stabilization with respect to aggregation (Mafune et al. 2000; Mandal et al. 2001; Munro et al. 1995). Surface properties of metal oxide NPs are also determined by their acidity constants and zero-point of charge (Giammar et al. 2007; Hristovski et al. 2007; Kormann et al. 1991; Schindler and Stumm 1987). For example,  $\text{TiO}_2$  ENPs are expected to be positively charged at  $\text{pH} < 6$  and negatively

charged at  $\text{pH} > 7$  (Dunphy Guzman et al. 2006b; Ridley et al. 2006), whereas  $\text{SiO}_2$  particles are generally negatively charged, as their zero-point of charge is located at around pH 2 (Hiemstra et al. 1996). Furthermore, some elemental metal NPs may have similar surface properties to their oxidized counterparts, as their surfaces are mostly coated by a layer of passivating oxide (Nurmi et al. 2005). Nonmetallic ENPs such as carbon nanotubes and fullerenes have hydrophobic surfaces and are not readily dissolved in water. These ENPs may be solubilized by functionalization with polar groups at their surfaces. Otherwise, the surfaces of hydrophobic carbon nanotubes are likely to interact preferentially with hydrophobic or amphiphilic compounds.

Particle aggregation and deposition are closely related phenomena (Wiesner et al. 2006). Aggregation describes the interaction between two mobile objects, whereas deposition refers to the attachment of a mobile particle to an immobile phase (e.g., collector) (Elimelech and Omelia 1990). The limited research that has been carried out on ENP aggregation and deposition suggests that prior principles on colloidal transport in aqueous media (i.e., Smoluchovsky's equations and DLVO theory) may still apply to ENPs, except that the Brønsted concept needs to be applied for supersmall NPs ( $< 10$  nm) (Brant et al. 2005; Derjaguin and Landau 1941; Kallay and Zalac 2002; Lecoanet et al. 2004; Lecoanet and Wiesner 2004; Smoluchowski 1917; Verwey and Overbeek 1948). According to these theories, particle deposition/aggregation kinetics can be defined as a two-step process of particle transport followed by attachment (Elimelech and Omelia 1990). The transport of colloidal particles (1 nm to 1  $\mu\text{m}$ ) is determined by convection, diffusion (Brownian motion), as well as external forces, whereas attachment onto other particles/surfaces is controlled by the colloidal interaction forces at short distances of operation.

The deposition/aggregation process is determined by the NPs' surface properties, which are mainly dependent on parameters such as temperature, ionic strength, pH, particle concentration and size, etc. (Dunphy Guzman et al. 2006a; Elimelech and Omelia 1990; Filella and Buffle 1993; Kretzschmar and Sticher 1997; Lecoanet et al. 2004). For example, an increase in the ionic strength compresses the electric double layer, thus decreasing the electrostatic repulsion between two objects with the same charge. The energy barrier will then decrease and the attachment probability becomes closer to unity. However, other forces may be involved in the deposition/aggregation process, such as steric repulsion/attraction, hydration effects, hydrophobic interactions, magnetic attraction, or the charge may be heterogeneously distributed on the particle surface (Metcalf et al. 2006), all of which can further complicate the interactions and remain to be examined. In addition, NPs' association with either natural organic matter (NOM) (Buffle et al. 1998)

or artificial organic compounds designed to enhance the NPs' properties or solubility will further increase the complexity of the interactions. In fact, most NOM has a biological origin including the organic matter released from plants, algae, and fungi (e.g., proteins, polysaccharides, nucleic acids, lipids, etc.). For example, fibrillar exopolymers, which are stiff, long-chain acid-rich polysaccharides exuded by phytoplankton and bacteria, have dramatically different effects on NPs than more flexible humic-type substances (see "Interactions of NPs with organic matter"). They are thought to play a key role in the formation of transparent exopolymer particles (TEP), which in turn form the matrix for aggregation of larger particles in freshwater and marine environments (Santschi 2005). Therefore, a better understanding of the NPs' aggregation, deposition, and mobilization will help us to better predict the NPs' fate in the environment as well as their biological effects.

### Interactions of NPs with organic matter

As a ubiquitous component of aquatic systems, NOM may influence the surface speciation and charge of NPs, and thus affect their aggregation/deposition properties. These organic matter compounds may be sorbed to the surfaces of ENPs by various types of interactions, including electrostatic, hydrogen bonding, and hydrophobic interactions (Hug 1994; Ojamae et al. 2006). According to Buffle et al. (1998), three groups of compounds with different biophysical properties may be distinguished in NOM: (1) rigid biopolymers, including the polysaccharides and peptidoglycans produced by phytoplankton or bacteria (Myklestad 1995), (2) fulvic compounds mostly from terrestrial sources, originating from the decomposition products of plants, and (3) flexible biopolymers composed of aquagenic refractory organic matter from a recombination of microbial degradation products.

For the latter two groups the major influence is the modification of the particle or collector's surface charge due to their high surface charge density (Santschi 2005). Dispersed particles will only be destabilized when their surface charge is nearly neutralized, otherwise the stability will be increased as a result of the electrostatic or steric repulsion (Chen and Elimelech 2007; Hyung et al. 2007). Kretzschmar and Sticher (1997) found that the hematite surface charge was reversed from positive to negative and its attachment efficiency to a sandy soil decreased from 1 to 0.01 when coated with  $4 \text{ mg l}^{-1}$  humic acid. On the other hand, rigid biopolymers can induce aggregation/deposition through gel formation (Santschi et al. 1998; Verdugo et al. 2004). Ferretti et al. (2003) examined the flocculation of hematite particles by a comparatively large rigid polysaccharidic compound: schizophyllan. A two-stage flocculation mechanism was proposed, with particles adsorbed onto the schizophyllan associations, which were

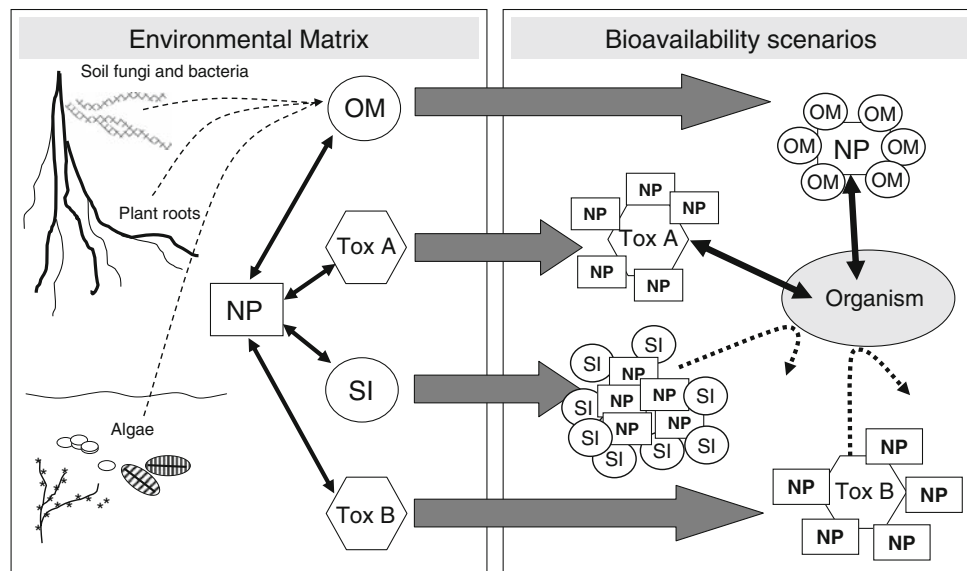
later combined together to form large flocs during the second stage. An enhanced aggregation of alginate-coated hematite NPs in the presence of calcium, strontium and barium cations was also observed as a result of the 'egg-box structure' of alginate and gel formation (Chen et al. 2007a). Stable dispersions of cerium oxide NPs have been obtained using polyacrylic acid, which has similar properties to humic and fulvic acids (Sehgal et al. 2005). Furthermore, carbon nanotubes are known to be solubilized by biopolymers (Karajanagi et al. 2004; Liu et al. 2006; Star et al. 2002). Therefore, the interactions between ENPs and NOM may finally determine the ENPs' fate in aquatic systems. The formation of larger aggregates by high-molecular-weight NOM compounds will favor the removal of ENPs into sediments 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 (Fig. 3).

Besides NOM, several artificially synthesized organic compounds are now being used to stabilize ENP suspensions in aquatic systems (Dubois et al. 2007; Pellegrino et al. 2004; Wang et al. 2003; Yu et al. 2007; Zhang et al. 2007a). These compounds can be generally categorized into two groups based on their effects on ENP stabilization. For the first group hydrophobic surfactants on the ENP surface are replaced by small molecules containing polar groups on both ends, whereas the amphiphilic compounds of the second group can encapsulate NPs and form stable micelles with their hydrophilic end outside. The formation of a stable finely dispersed aqueous colloidal solution of fullerene  $C_{60}$  and  $C_{70}$  was reported in the presence of  $\gamma$ -cyclodextrin (Andrievsky et al. 2002; Boulas et al. 1994). Similarly poly-vinylpyrrolidone can also stabilize  $C_{60}$  and carbon nanotube suspensions by wrapping the aggregates inside (Lyon et al. 2006; O'Connell et al. 2001; Yamakoshi et al. 1994). Carbon nanotubes can form stable suspensions with the addition of sodium dodecylbenzene sulfonate and sodium dodecyl sulfate (Islam et al. 2003; Jiang et al. 2003; Moore et al. 2003). Therefore, in the presence of appropriate organic compounds, NPs will have a longer residence time in aquatic systems, or enhanced mobility in soils, and may thus interact more efficiently with algae or with plant roots (Fig. 3).

### Interactions with organisms and ecotoxicity of ENPs

#### Entry of ENPs into algae, plants, and fungi

As opposed to animals, most cells of plants, algae, and fungi possess cell walls that constitute a primary site for interaction and a barrier for the entrance of ENPs into their



**Fig. 3** Scenario of nanoparticles' (NP) interactions with toxicants (Tox A and B), salt ions (SI), and organic matter (OM) such as humic acids or compounds released by plants, fungi, bacteria, and algae. Some compounds present in environmental matrices might increase the NPs' stability (OM) and thus bioavailability (represented as solid

arrows entering organisms), whereas others (salt ions) might foster the aggregation of NPs, thus reducing their bioavailability (represented as dotted arrows not entering organisms), or physically restraining NP-organism interactions. In other cases, NPs' bioavailability might be either increased or decreased

cells. Major cell wall components are carbohydrates, linked to form a rigid complex network, and proteins (Heredia et al. 1993; Knox 1995). The functional groups, such as carboxylate, phosphate, hydroxyl, amine, sulfhydryl, and imidazole contained in these biomolecules (Vinopal et al. 2007) offer a range of distinct active sites. Cell walls in plants and algae mainly consist of cellulose, whereas those for fungi consist of chitin. Algae will typically have glycoproteins and polysaccharides in their cell walls. However, diatom algae represent a special case, with cell walls composed of hydrated silicon dioxide. Cell walls are semipermeable, allowing the passage of small molecules while limiting the passage of larger molecules. The diameter of pores across the cell wall, which has a thickness ranging from 5 to 20 nm, determines its sieving properties (Fleischer et al. 1999; Fujino and Itoh 1998; Madigan et al. 2003; Zemke-White et al. 2000). Thus, only ENPs and ENP aggregates with a size smaller than that of the largest pore are expected to pass through the cell wall and reach the plasma membrane. Permeability of the cell wall might change during reproduction, with the newly synthesized cell wall more permeable to ENPs (Ovecka et al. 2005; Wessels 1993). Moreover, the interactions of the cells with ENPs might induce the formation of new pores, which might be bigger than usual and thus increase the internalization of the ENPs through the cell wall.

After passing through the cell wall, ENPs meet the plasma membrane. The potential entry routes of ENPs through this bilayer lipid membrane have been discussed

recently (Moore 2006). During the endocytic processes, the plasma membrane forms a cavity-like structure that surrounds the ENP and pulls it into the cell. The ENPs also might cross the cell membranes using embedded transport carrier proteins or ion channels. As soon as the ENPs enter the cell, they may bind with different types of organelles (e.g., endoplasmic reticulum, Golgi, and endo-lysosomal system) and interfere with the metabolic processes there, possibly as a result of the production of reactive oxygen species (ROS). For example, the swelling of the endoplasmic reticulum, vacuolar changes, and phagosomes were explicitly seen in macrophage cells exposed to high doses of single-wall nanotubes (Jia et al. 2005). Furthermore, the nucleus of macrophage cells may experience degeneration, enlargement, and rarefaction at high concentrations of multiwall nanotubes (Jia et al. 2005).

As compared with algae and fungi, plants might also be exposed to NPs in atmospheric and terrestrial environments. Airborne NPs will be attached to leaves and other aerial parts of plants whereas roots will interact with waterborne or soil-material-associated NPs. Therefore, one can expect that plant communities with higher leaf area indexes (LAI) will also have a higher interception potential for airborne ENPs, thus increasing their entrance into trophic webs. For example, typical LAIs of spruce (*Picea abies*) forests in Southern Germany ranged between 5.3 and 7.9, and the total leaf area of single trees reached up to 750 m<sup>2</sup> (Tenhunen et al. 2001). Thus, forests can function as very efficient traps for airborne ENPs, but also

undercanopy vegetation showing LAI ranging from 0.27 to 3.3 (Kostner 2001) may also act as an efficient ENPs trap. Apart from the mere leaf area surface, evapotranspiration (ET) should be a good estimator for assessing to what extent ENPs could be taken up into the plant via the stomata. Köstner et al. (2001) give a very detailed description and discussion of ET in forest stands, pointing out the significance of undercanopy for gas exchange and sink for dry deposition.

Accumulation of airborne iron particles on plants leaf surfaces has been shown to be reduced on waxy and smooth leaf surfaces, while the accumulation increased on the nonwaxy and wrinkle surfaces (Da Silva et al. 2006). Once on the leaf surface, ENPs might penetrate the plants via the bases of trichomes or through stomata and be translocated to different tissues. The accumulation of NPs in photosynthetic surfaces might provoke foliar heating due to stomata obstruction and to alteration of the gas exchange (Da Silva et al. 2006). This heating might affect the plant physiology. In soils, a potential ENP entrance mechanism in plants is via endocytosis, which was observed during the growth of root hair cells (Ovecka et al. 2005).

ENP studies on fungi have focused on the potential of fungi to synthesize ENPs (Mandal et al. 2006). Extracellular biosynthesis of silver ENPs from ionic silver has been shown in *Fusarium oxysporum* (Ahmad et al. 2003), and is believed to be catalyzed by certain enzymes. At the same time gold microwires have also been synthesized using two species of *Aspergillus* and *Neurospora* (Sugunan et al. 2007). In this study, fungi were exposed during a week to gold NPs (15 nm diameter) that were surface functionalized with glutamate, aspartate, and polyethylene glycol. The consumption of these organic compounds by the growing fungi resulted in the assembly of the gold microwires. Finally, ENPs might also enter the fungi cells through endocytosis (Fischer-Parton et al. 2000).

#### Adverse effects of ENPs on algae, plants, and fungi

Of the limited studies about the effects of ENPs on algae, plants, and fungi, both direct and indirect effects have been found. Direct toxic effects of ENPs on organisms are mainly determined by their chemical composition and surface reactivity. Their greater surface area per mass (Fig. 1 and Table 1), compared with larger-sized particles of the same chemistry, renders ENPs more reactive biologically (Oberdörster et al. 2005). This greater reactivity might cause catalysis of redox reactions upon contact with organic molecules (Zhang 2003), and also impact on photosynthetic or respiratory processes. The photo-induced electron transfer capacity of some ENPs, such as the fullerenes (Imahori et al. 2003), suggests that photosynthetic or respiratory processes will be impacted as a consequence

**Table 1** The most widely studied ENPs and their properties

Type	Diameter <sup>a,b</sup> (nm)	Specific surface area (m <sup>2</sup> g <sup>-1</sup> )
Fullerene (C <sub>60</sub> )	0.72	~10000
Silver (Ag)	10	9–11
Titanium oxide (TiO <sub>2</sub> )	5	200–220
Zinc oxide (ZnO)	20	~50
Iron oxide (Fe <sub>2</sub> O <sub>3</sub> )	20–50	~50
Copper oxide (CuO)	30–50	~13.1
Quantum dots	1–10	100–1000
Single-walled carbon nanotubes	1–2 (diameter) 5–30 μm (length)	~400
Multiwalled carbon nanotubes <sup>c</sup>	<8 (OD) 2–5 (ID) 10–30 μm (length)	~500

<sup>a</sup> The diameter given is that of a single nanoparticle and often the smallest size commercially available

<sup>b</sup> Nanoparticles in solution may form aggregates resulting in larger particles

<sup>c</sup> OD represents outside diameter and ID means inside diameter

of ENPs penetrating the cell wall and the membrane, and reaching cytosol.

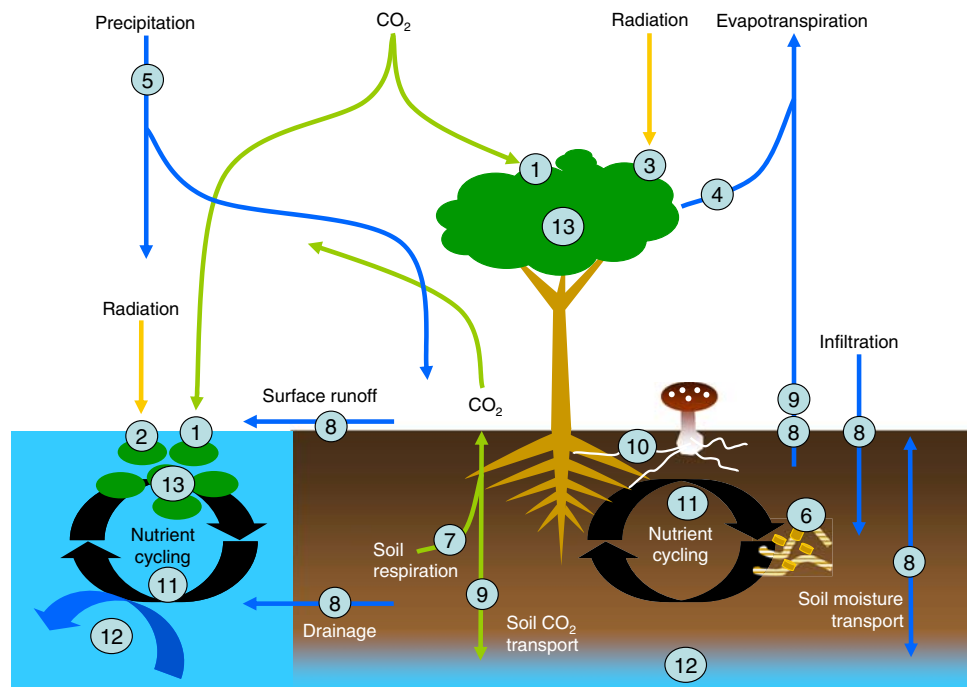
Toxicity of TiO<sub>2</sub> ENPs to the green algae *Desmodesmus subspicatus* has been found to be dependent on the ENPs' specific surface area. The smallest particles showed a clear concentration-effect relationship, whereas the larger ones caused less toxicity (Hund-Rinke and Simon 2006). However no measurements of aggregation size, which could possibly influence inhibitory effects, were reported. Effects of alumina (Al<sub>2</sub>O<sub>3</sub>) NPs on root growth have also been shown to be related to their size rather than their chemical composition (Yang and Watts 2005). At the same time Zn and ZnO ENPs were shown to affect growth in radish, rape, and ryegrass (Lin and Xing 2007). Since neither supernatant from centrifugation nor filtrated Zn and ZnO solutions showed significant phytotoxic effects, toxicity has been related to the ENPs rather than to the release of Zn<sup>2+</sup>. However, the authors mention that dissolution on the root surface cannot be ruled out at present. Furthermore, silver ENPs (AgNP) may provoke membrane alterations, increase its porosity, and disrupt the capacity of bacteria cells to regulate transport through the membrane (Pal et al. 2007; Sondi and Salopek-Sondi 2004). This increased membrane porosity also may further facilitate the entrance of ENPs into cells (Morones et al. 2005).

As for the indirect effects of ENPs, they are caused mainly by the physical restraints or the release of toxic ions (e.g., metal ENPs) or the production of ROS. Moreover, ENPs themselves may serve as pollutant carriers (see "Interactions of ENPs with other pollutants"), thus

enhancing or reducing the bioavailability of other toxic substances (Fig. 3). As an example of physical restraint, the accumulation of ENPs on the surface of photosynthetic organisms may inhibit photosynthetic activity because of shading effects, i.e., reduced light availability, rather than because of toxicity of the ENPs themselves (Fig. 4). As a function of the tendency to aggregate and combine with other organic materials (Chen and Elimelech 2007), sorption of nanoparticles to algal cells is to be expected. TiO<sub>2</sub> particles were shown to adsorb onto the algal cell surface, resulting in a 2.3-fold increase of cellular weight (Huang et al. 2005). Similarly, aggregates of carbon black bound to sperm cells reduced fertilization success of *Fucus serratus*, a marine seaweed (Fernandes et al. 2007). Adsorption of larger ENP aggregates to cell walls might also alter the cellular acquisition of essential nutrients, either through clogging of the walls or nutrient adsorption.

ENPs might produce reactive oxidant species (ROS) upon their interaction with organisms or with agents present in the environment (e.g., ultraviolet radiation). ROS production is especially relevant in the case of ENPs with photocatalytic properties such as TiO<sub>2</sub> (Kus et al. 2006) upon ultraviolet (UV) exposure (Rodriguez-Moya 2007; Zhao et al. 2007). Accordingly, damaging effects of

TiO<sub>2</sub> ENPs on bacteria have been shown to be enhanced by sunlight or UV illumination (Adams et al. 2006). Other ENPs, such as silver NPs and fullerenes, also showed the capacity to produce ROS upon UV exposure (Badireddy et al. 2007; Rodriguez-Moya 2007). Thus, it may be expected that ROS defense mechanisms based on ascorbate, glutathione, alpha-tocopherol (vitamin E), or enzymatic scavengers of activated oxygen such as superoxide dismutase (SODs), peroxidases, and catalases (Asada 1992; Noctor and Foyer 1998), might give protection against ROS-mediated ENP effects. At the same time, toxicity of ENPs may be partly due to their release of toxicants (Brunner et al. 2006; Franklin et al. 2007; Navarro et al. 2007). Ionic silver (Ag<sup>+</sup>) released from AgNP inhibits respiratory enzymes and induces oxidative stress upon generation of ROS (Kim et al. 2007; Pal et al. 2007). Ag<sup>+</sup> may also bind to sulfur- and phosphorus-containing molecules (S-adenosylmethionine, cysteine, taurine, glutathione, etc.) involved in cell antioxidant defense (McDonnell and Russell 1999; Pappa et al. 2007), and may result in the depletion of the intracellular concentration of these molecules (Hussain et al. 2005). Thus, AgNP was found to reduce cell-specific growth rate, photosystem II quantum yield, and chlorophyll *a* content of a marine



**Fig. 4** Simplified scheme of some terrestrial and aquatic ecosystem processes. Among other impacts, ENPs' effects on photosynthetic organisms may reduce the fixation of CO<sub>2</sub> (1); ENPs adsorbed (2) or deposited (3) on photosynthetically active surfaces might reduce light availability or gas exchange (4) and thus photosynthesis; ENPs present in the atmosphere might increase the nuclei available for raindrop formation (5), thus altering precipitation; ENPs' impacts on

bacteria, fungi, and other edaphic fauna (6) might affect soil respiration (7), and other soil-texture-related processes such as transport of liquids (8) or gases (9), also modifying symbiotic relationships (10). Together, this might lead to impairments in three key services provided by ecosystems, i.e., nutrient cycling (11), water deperation (12), and biomass production (13)



phytoplankton (*Thalassiosira weissflogii*), and these toxic effects were accounted for by the release of  $\text{Ag}^+$  (Miao et al. 2007). Similarly, results from Navarro et al. (2007) indicate that toxicity of AgNP to the photosystem II quantum yield of a freshwater alga (*Chlamydomonas reinhardtii*), also depended on the release of  $\text{Ag}^+$ . Both studies showed that about 1% of the AgNP was released as  $\text{Ag}^+$ . In the case of Navarro et al. (2007),  $\text{Ag}^+$  concentrations were too low to explain the toxicity exhibited by AgNP, thus suggesting the importance of the AgNP-cell interaction. Franklin et al. (2007) also compared the toxicity of ZnO ENPs with that of  $\text{Zn}^{2+}$ . It was found that toxicity of ZnO ENPs may be partly related to the solubility of the ENPs.

On the other hand, organisms may excrete compounds as a feedback response, to alter the ENPs' toxicity. Exudation of metal chelates from root systems, such as siderophores, may either reduce the bioavailability of toxic metal ions (Dong et al. 2007) released from metallic nanomaterials by complexation (Fig. 3), or can increase metal uptake by plants under conditions where uptake is diffusion limited (Degryse et al. 2006). Such compounds may also influence the dissolution rate of metals from metallic NPs or from metal oxide NPs. At the same time, algae can produce substances which can induce ENP flocculation or metal ion chelation and thus reduce the bioavailability of both ENP and metal ions they released (Soldo et al. 2005). Exopolymeric substances production has been shown to increase in algae upon exposure to ENPs, and may thus contribute to detoxification mechanisms (Miao et al. 2007). Figure 3 illustrates that ENP bioavailability can be both enhanced (Terashima and Nagao 2007) and reduced (Dunphy Guzman et al. 2006a) by abiotic properties of the surrounding matrix. No studies are currently available about ENP toxicity to fungi. Only studies about the biosynthesis of ENP and nanotubes on fungal hyphae have been performed, as described in the section "Entry of ENPs into algae, plants, and fungi".

#### Positive effects of ENPs on algae, plants, and fungi

Besides the toxicity that ENPs may cause in algae, plants, and fungi, they may also have some positive effects. It was found that fullerenes may act as antioxidants, preventing lipid peroxidation induced by superoxide and hydroxyl radicals (Wang et al. 1999).  $\text{TiO}_2$  NPs have also been found to induce spinach seed germination and plant growth by regulating the germination of aged seeds and its vigor (Zheng et al. 2005). Furthermore, the presence of  $\text{TiO}_2$  NPs was observed to increase the dry weight, chlorophyll synthesis, and metabolisms in photosynthetic organisms. These positive effects are possibly due to the antimicrobial properties of ENPs, which can increase strength and

resistance of plants to stress. At the same time, ENPs could also sequester nutrients on their surfaces and thus serve as a nutrient stock to the organisms, especially those ENPs having high specific surface area (Table 1). Algae living in extreme habitats (the liquid water between snow crystals) show the capacity to accumulate mineral particles on their cell walls (Luetz-Meindl and Luetz 2006). These mineral particles have been hypothesized to be important for the survival of these algae living in low-nutrient habitats. Therefore, it is expected that ENPs containing essential elements might also be attached to the algae and supply the nutrients for their growth.

#### Interactions of ENPs with other pollutants

As a result of their remarkably high surface area to volume ratio and complexing capability, ENPs may adsorb pollutants, which might change the transport and bioavailability of both the ENPs and the pollutants in natural systems (Fig. 3), and alter their toxic effects. Trace-metal ion speciation might be altered by NPs (especially oxide and oxide-coated NPs), therefore altering their bioavailability and potential toxicity (Fig. 3). At the same time, organic pollutants may interact with hydrophobic ENPs such as carbon nanotubes and fullerenes (Chen et al. 2007b; Gotovac et al. 2006, 2007; Hu et al. 2007; Wang et al. 2007). The toxicity of diuron to green algae was reduced in the presence of carbon-black ENPs (Knauer et al. 2007). And fullerenes were found to decrease the toxicity of various chemicals to daphnids and algae as a result of their decreased bioavailability (Baun et al. 2008). On the contrary, the toxicity of phenanthrene to the green alga *P. subcapitata* and the zooplankton *D. magna* increased despite 85% sorption of phenanthrene to  $\text{C}_{60}$  aggregates, indicating that sorbed phenanthrene was still available for the organisms (Baun et al. 2008). The bioavailability of phenanthrene to plant roots also increased upon its adsorption to alumina ENPs (Yang and Watts 2005). Furthermore, the presence of  $\text{TiO}_2$  ENPs has been shown to increase the accumulation of cadmium and of arsenate in carps because of their strong sorption capacity for these elements (Sun et al. 2007; Zhang et al. 2007b). ENPs' potential to enhance or reduce agrochemicals bioavailability and toxicity (Fig. 3) might entail changes in the dosage of these compounds, and alter their impacts on nontarget organisms.

#### Considerations for toxicity testing

The present standard toxicity tests on photosynthetic organisms involve the assessment of the impacts of toxicants on growth or photosynthesis of green algae (*Scenedesmus vacuolatus*) and duckweed (*Lemna minor*)

for aquatic systems, and various terrestrial plant species such as wheat or cress, which are grown in soil, but no specific tests exist for fungi. Terrestrial tests cover either bacteria or more general endpoints of microbial activity (e.g., soil respiration, activity of various enzymes). Concerning exposure, this raises some problems, in particular when trying to test hydrophobic ENPs in water: they will usually aggregate and thus be barely available to the organisms. In soils, hydrophobic ENPs can interact with humic substances and thus be easily available to organisms exploiting them, mainly bacteria and fungi. However, toxicity testing in soils in most cases requires high test concentrations, due to the manifold interaction potentials within this complex matrix (Fig. 3). For deriving principles of toxic mechanisms, cell cultures or plants grown in liquid culture might be useful alternatives. Still, we can only speculate here since data on ENP toxicity for terrestrial and aquatic plants simply do not exist, except for the studies mentioned above.

Some key issues need particular consideration in testing the toxicity of ENPs. Interactions of ENPs with organisms are expected to be dependent on their size, shape, chemical composition, charge, surface structure and area, solubility, and aggregation state. The physical and chemical characterization of ENPs and the media is thus a prerequisite for defining exposure conditions. Actually, the preparation of exposure media containing ENPs that are poorly soluble in aqueous solutions has been discussed as a major focus of future research aimed for the development of standardized toxicity testing protocols (Oberdörster 2007). The presence of impurities or release of materials which could influence the toxic effects of ENPs should also be considered. In the case of metallic ENPs, toxicity can be altered through release of metal ions, which necessitates the quantification of soluble metal ions in the exposure medium. The interpretation of toxicity results must therefore be based on particle characteristics that better explain the toxicity observed.

Considering that ENP toxicity mechanisms might be different in acute and chronic tests, ecotoxicity experiments should include examinations of both short- and long-term effects. The very limited number of studies testing the ecotoxicity of NP has been carried out with concentrations that could be claimed to be so high that they are not realistic for environmental exposure concentrations. Ecotoxicity tests should also consider testing different ENP concentrations with special concern for concentration-induced aggregation effects. It may be speculated that in the lower concentration range ENPs will tend to show less aggregation and lead to a disproportionately higher uptake and effects than expected from high concentrations. On the other hand, higher ENP concentrations may result in increased cell density, shading, and clogging effects,

leading to responses that are not due to toxicity of the ENPs. Therefore, a clear dose-response relationship may not be observed. However, these problems may be overcome if different endpoints are adopted. Furthermore, as there may be substantial interactions between ENPs and other pollutants (Fig. 3), examination of ENP toxicity alone is not sufficient. Standardized mixture and toxicity scenarios should be considered to assess the environmental risks of ENPs.

#### Ecological relevance of ENPs' effects in aquatic and terrestrial ecosystems

Evaluation of the risks that ENPs pose to the environment involves a comparison of environmental concentrations with those that are toxic to organisms. Both types of data are lacking. No estimates of environmental concentrations of ENPs exist at the time of writing this review. Moreover, identification of the pathways of ENPs into the environment (Nowack and Bucheli 2007) and quantification of the fluxes through the ecosystems are needed. Other basic information required includes the behavior, fate, and bio-availability of ENPs once in the ecosystems, which are dependent on the chemical characteristics of the receiving medium (see above). Figure 4 gives a generalized scheme of ENPs' potential effects on ecosystems.

In aquatic ecosystems, productivity of algae might be compromised not only because of the direct toxic effects of ENPs, but also because of indirect effects derived from physical restraints. ENPs adsorbed to algae might result in shadowing or in increased cellular weight (Huang et al. 2005) and cell sinking. Both effects will reduce the light availability for photosynthesis, thus reducing the biomass available for animals. Because the key role of microbial loops is nutrient recycling in aquatic ecosystems (Stone and Weisburd 1992), the direct effects of ENPs on bacterioplankton might also reduce the productivity of surface waters.

To date, virtually no study exists on the effects of NPs on soils. However, given the evidence from studies performed with bacteria, negative effects are definitely expected. The antifungal and antimicrobial activity of ENPs may seriously threaten free-living nitrogen-fixing bacteria and symbiotic relationships involving fungi, bacteria, and plants (mycorrhiza, rhizobia in legumes, lichens, etc.). At the ecosystem level, this would result in reduced plant nutrient availability and aggravated succession of seriously disturbed ecosystems, which often depends on microbial primary colonizers such as algae, cyanobacteria or lichens. Furthermore, ENPs may affect the role played by the fungi to protect host plants against phytopathogens or oxidative stress (Hildebrandt et al. 2007), thus having effects at the ecosystem level

(Schutzendubel and Polle 2002). Fungi and bacteria are the prevailing diet of soil animals, which not only foster and buffer their activity but also affect the soil's physical condition through digging and mixing. Therefore, essential ecosystem services provided by plants and soil communities, such as biomass production, organic matter breakdown, nutrient recycling, groundwater purification or soil creation, stability, and infiltration capacity might be affected (Fig. 4).

Some ENPs, such as quantum dots, may be taken up by bacteria (Kloepfer et al. 2003), and due to their long residence time in cells (Hardman 2006), be transferred through food webs. Significant bioaccumulation has been predicted for carbon-based NPs because of their strong partition into membranes (Dunphy Guzman et al. 2006b). Once in the organisms, the fate of ENPs will be strongly dependent on the physicochemical characteristics of the internal medium of these organisms. The acidic conditions and higher surfactant concentrations prevailing in animal digestive systems may lead to changes in particle aggregation, and thereby also affect particle uptake in the organism and the transfer to the next trophic levels. Moreover, trophic transfer studies should consider the multiple toxic mechanisms exhibited by ENP: chemical surface reactivity, their particle nature, and the solubilization of nanomaterial components. As an example, it is possible to imagine ENP as miniaturized toxic delivery systems through food webs, by releasing compounds or reacting against biological molecules at each trophic level without a remarkable loss of toxicity. Therefore, these unique ENP characteristics might lead to more persistent impacts on ecosystems than other toxicants.

#### Final remarks

Because of the environmental use of ENPs (Hong and Otaki 2006; Kim and Lee 2005; Peller et al. 2007) and their unintentional release (Zhang 2003) the exposure of algae, plants, and fungi to these new materials is a reality. However, there is a remarkable lack of information on some key aspects, which prevents a better understanding and assessment of the toxicity and ecotoxicity of ENPs to these key ecosystem organisms. Many challenging questions remain unanswered, among them: (a) the relevant ENP concentrations in the different aquatic, terrestrial, and aerial environments, (b) the physical and chemical characterization of the ENPs and exposure routes, (c) the mechanisms allowing ENPs to pass through cellular membranes and cell walls, (d) the specific properties that are related to ENPs' toxic effects, and (e) the mechanism underlying ENPs' trophic transfers. The pluridisciplinary approaches needed to address these questions stress the paramount importance of collaborative efforts between

ecotoxicologists, toxicologists, biologists, chemists, biophysicists, and analytical researchers.

Moreover, frameworks allowing the extrapolation of *in vitro* results to natural systems, or to assess the risks of forthcoming ENPs based on previous knowledge, are needed. In this regard, the use of ENP standards for toxicity studies, claimed from several forums, seems a priori unrealistic, because of the enormous and continuously growing number of different ENPs. On the other hand, the standardization of new ENP toxicity testing methods sounds more feasible. Databases, based on these well-established toxicity tests, may contribute to elucidate some of the knowledge gaps identified above.

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