Chapter 3
Ecotoxicity of Metallic Nanoparticles and Possible Strategies for Risk Assessment

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3.1 Introduction

Nanoparticles are defined as particulate matter, usually with nanoscale dimensions (1–100 nm), whose properties are diverse from their bulk form (Auffan et al. 2009). Various distinctive properties of nanoparticles such as electronic (Kim et al. 2007), optical/photonic (Chan et al. 2013), magnetic (Mornet et al. 2006), and catalytic (Nasrollahzadeh et al. 2015) have significant roles in daily human life. Fundamentally, nanoparticles are categorized in two groups: (i) carbon-containing nanoparticles and (ii) metal-containing nanoparticles. Carbon-containing nanoparticles are made of carbon nanotubes and fullerenes. However, most of the metal-containing nanoparticles are made from metals such as gold (Au), iron (Fe), silver (Ag), copper (Cu), and metal oxides such as titanium dioxide (TiO_2), antimony oxide (Sb_2O_3), cerium dioxide (CeO_2), copper oxide (CuO), nickel oxide (NiO), iron oxide (FeO), and zinc oxide (ZnO).

Metallic nanoparticles are important because of their physical, chemical, and optoelectronic properties. Metallic nanoparticles have been used in various products with different purposes, such as sensors (Li et al. 2007), as catalysts in various processes (Carnes and Klabunde 2003), drug delivery (Hola et al. 2015), sunscreens (Gulson et al. 2015), solar-driven energy production (Sau et al. 2010), and in pollutant remediation (Kamat and Meisel 2003; Choopun et al. 2009; Raman and Kanmani

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2016). Additionally, nanoparticles have been shown to inhibit microorganism growth by exhibiting antibacterial, antiviral, and antifungal properties (Padmavathy and Vijayaraghavan 2008; Khatami et al. 2015; Rai et al. 2016). As metallic nanoparticles are widely used, their exposure is likely to increase substantially, and they interact negatively with microorganisms, green plants, animals, human beings, and their surrounding environment (Navarro et al. 2008; Wise et al. 2010; Singh et al. 2016, 2019; Shweta et al. 2017, 2018; Arif et al. 2018; Vishwakarma et al. 2018). Therefore, detailed understanding of their synthesis, interaction, and possible risk valuation would offer a foundation for harmless use of nanoparticles.

3.2 Synthesis of Metallic Nanoparticles

Synthesis of metallic nanoparticles is a timely area of research because their use is widespread. Various physical and chemical methods are used for the production of metallic nanoparticles (Tripathi et al. 2017; Koul et al. 2018). For the synthesis of metal oxide nanoparticles, stabilized precipitation and flame pyrolysis are commonly used methods (Christian et al. 2008). Monodisperse nickel phosphide (Ni_2P) nanorods and nanoparticles are made by a one-step solution-phase route (Li et al. 2015). Moreover, there is growing interest in the controlled synthesis of metallic nanoparticles (Wiley et al. 2005; Xia et al. 2009). However, these methods have certain drawbacks because of the use of poisonous chemicals and radiation and are an expensive process. Therefore, academic research is shifting toward biological synthesis of metallic nanoparticles, as it is rapid, feasible, and more productive relative to its cost. In this context, microorganisms have an increasingly critical role as they can provide inorganic materials either intra- or extracellularly for the synthesis of nanoparticles (Simkiss and Wilbur 1989; Mann 1996). Numerous microorganisms such as bacteria (Shahverdi et al. 2007; Saifuddin et al. 2009; Pantidos and Horsfall 2014), actinomycetes (Abdeen et al. 2014; Golinska et al. 2014), algae (Singaravelu et al. 2007; Aruoja et al. 2009; Abboud et al. 2014), and fungi (Mukherjee et al. 2001; Ahmad et al. 2003; Yadav et al. 2015) are used for nanoparticle synthesis. Also, peptides (Tomczak et al. 2007), starches (Kumar et al. 2014), and almost all parts of plants have been used for the synthesis of metallic nanoparticles.

3.3 Application of Nanoparticles

Nowadays, metal-based nanoparticles have become one of the main and increasing aims of nanotechnology, as these particles are usually used in cosmetics, antibacterial agents, tires, stain-resistant clothing, optic devices, toothpaste, sensors, food additives, and data storage (Fig. 3.1).

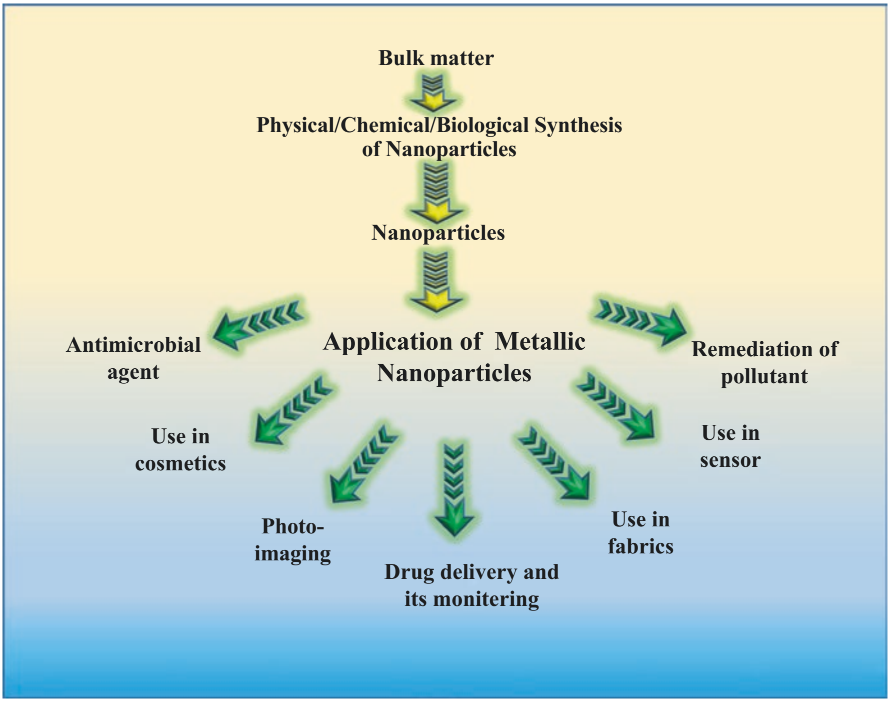

Fig. 3.1 Application of metallic nanoparticles

As antibacterial agents, these particles are widely used in medical applications, food storage, and water treatment (Bosetti et al. 2002; Cho et al. 2005; Singh et al. 2008; Espitia et al. 2012). In the textile industry, silver nanoparticles are used to prepare cotton fibers that exhibit antibacterial activity (Durán et al. 2007). It has been reported that Ag or Au nanoparticles extracellularly produced from *Fusarium oxysporum* can be used to prevent or to reduce the infection of *Staphylococcus aureus* (Durán et al. 2007). Moreover, metallic nanoparticles are of great scientific importance regarding their catalytic activity in various metal-based reactions (Hvolbæk et al. 2007). Metallic nanoparticles also show superior catalytic activity in the reduction and removal of dye. For instance, gold nanoparticles are reported to catalyze the reduction of dye in the presence of stannous chloride (Gupta et al. 2010). Silver and gold nanoclusters were reported to catalyze the reduction of methylene blue dye ($C_{16}H_{18}ClN_3S$) by arsine in micellar medium (Kundu et al. 2002). Köhler et al. (2008) reported that the catalytic activities of nanoparticles enhanced the bleaching of the organic dyes. However, catalytic activity of metallic nanoparticles also varies from metal to metal. For instance, the Ag nanoparticle was found to be superior to Au and Pt colloid in catalyzing chemiluminescence from the luminol–hydrogen peroxide system (Guo et al. 2008). Despite the aforementioned antibacterial and catalytic activity of metallic nanoparticles, the optical properties of a metallic nanoparticle also offer a manageable tool for particle sorting and sensing, for instance, in optoelectronic devices (Djurišić et al. 2010; Choi et al. 2013) and in sensing devices (Ankamwar et al. 2005).

In the medical field, metallic nanoparticles are used to develop an aggregation-based immunoassay for anti-protein A (Thanh and Rosenzweig 2002), and for treatment of B-chronic lymphocytic leukemia (Mukherjee et al. 2007) and oral cancer (El-Sayed et al. 2005).

Nanoparticles have also found application in remediation of contaminated environments (Li et al. 2006). There are several studies on the application of nanoparticles for remediation of various pollutants such as metals, organic pollutants, and dyes (Mak and Chen 2004; Hoch et al. 2008; Cheng et al. 2013; Zhao et al. 2016). However, as the benefit obtained from the intended use of nanoparticles for remediation is balanced by potential risk, it is therefore obligatory to assess the probable environmental risk.

3.4 Toxicity of Metallic Nanoparticles

The toxicity of nanoparticles is principally the result of their small size, their large surface area compared to volume, and reactive facets. Metallic nanoparticles show toxic effects on various organisms (Table 3.1). Ge et al. (2011) reported that TiO_2 and ZnO nanoparticles reduced the biomass of a microbial community. Among microbial communities, nitrogen-fixing bacteria are an important component of the soil ecosystem as they maintain soil health and fertility. Cherchi et al. (2011) reported bactericidal effects of TiO_2 nanoparticles in *Anabaena variabilis*. Toxicity of nanometal oxides in aquatic ecosystems has also been studied by various research groups (Blaise et al. 2008; Lee et al. 2009; Pradhan et al. 2012; Miller et al. 2012). Federici et al. (2007) reported that the gills of *Oncorhynchus mykiss* showed sensitivity toward TiO_2 nanoparticles. Furthermore, TiO_2 nanoparticles were reported to inhibit the growth of *Desmodesmus subspicatus* at higher concentrations (Hund-Rinke and Simon 2006). However, the toxicity of metallic nano-sized particles in an aquatic ecosystem is debatable (Sharma 2009) as their physicochemical properties are dissimilar from their ionic and bulk form (Christian et al. 2008). Moreover, soluble ions released from metallic nanoparticles appear be the main cause of ecotoxicity (Aruoja et al. 2009). Green plants are also affected by metallic nanoparticles as these particles enter into the plant by various means such as stomata, cuts or wounds, and through the roots. Zn and ZnO nanoparticles negatively affect the growth of *Raphanus sativus* (radish), *Brassica napus* (rape), and *Lolium perenne* (ryegrass) (Lin and Xing 2007). Similarly, Yang and Watts (2005) reported the toxicity of alumina nanoparticles in *Brassica oleracea* (cabbage), *Daucus carota* (carrot), *Zea mays* (corn), *Cucumis sativus* (cucumber), and *Glycine max* (soybean). Metallic nanoparticles are known to induce effects on human health, as they cause gastroduodenal corrosive injury (Liu et al. 2004), a cytotoxic effect on glomerular and

Table 3.1 Toxic effect of metallic nanoparticles on organisms

Nanoparticles	Organisms	Effects	References
TiO_2 nanoparticles	*Daphnia magna*	Bioaccumulation may interfere with food intake and ultimately affect growth and reproduction	Zhu et al. (2010)
TiO_2 nanoparticles	*Daphnia magna*	Caused mortality	Lovern and Klaper (2006)
ZnO and TiO_2 nanoparticles	*Escherichia coli*	Induced oxidative stress and DNA damage leading to reduced viability of *E. coli*	Kumar et al. (2011a)
ZnO and TiO_2 nanoparticles	*Salmonella typhimurium*	Both ZnO and TiO_2 nanoparticles were significantly internalized in the *S. typhimurium* cells in a concentration-dependent manner and these nanoparticles exhibited weak mutagenic potential	Kumar et al. (2011b)
Ag nanoparticles	Bacteria	Ag nanoparticles inhibited soil-denitrifying bacteria	Throbäck et al. (2007)
Ag nanoparticles	Bacteria	Inhibited the nitrifying organisms	Choi et al. (2008)
TiO_2 and ZnO nanoparticles	Bacteria	TiO_2 and ZnO nanoparticles reduced both microbial biomass, bacterial diversity, and composition	Ge et al. (2011)
CuO, NiO, ZnO, and Sb_2O_3 nanoparticles	*Escherichia coli*, *Bacillus subtilis*, and *Streptococcus aureus*	Toxic to microorganisms: toxicity order was CuO > ZnO > NiO > Sb_2O_3 nanoparticles	Baek and An (2011)
CeO_2 nanoparticles	*Synechocystis* PCC6803 and *Escherichia coli*	*E. coli* was sensitive to the 'direct' effects of nanoparticles, whereas *Synechocystis* was protected by extracellular polymeric substances, preventing direct cellular contacts	Thill et al. (2006)
Ag nanoparticles	Zebrafish	Induced oxidative stress and apoptosis	Choi et al. (2010)
Au nanoparticles	Phytoplanktonic alga (*Scenedesmus subspicatus*) and a benthic bivalve (*Corbicula fluminea*)	Mortality was 20% at lowest contamination condition; the highest reached 50% in algae. Au nanoparticles were adsorbed by the algal cell wall, leading to progressive intracellular and wall disturbances. In bivalves these nanoparticles bioaccumulated and penetrated into the gills and digestive epithelia to cause oxidative stress	Renault et al. (2008)
Ag nanoparticles	*Mytilus edulis*	Au nanoparticles accumulated in digestive gland causing oxidative stress	Tedesco et al. (2010)

tubular renal cells (Pujalté et al. 2011), and toxic effects on the pulmonary system (Moos et al. 2010). Karlsson et al. (2009) assessed the effect of metallic nanoparticles on human cell lines and reported that nanoparticles are perhaps more toxic than their bulk forms.

3.4.1 Uptake of Metallic Nanoparticles

The detailed mechanisms of biological uptake of metallic nanoparticles are not well known. However, it has been hypothesized that uptake of nanoparticles in animal bodies takes place through the gut (Baun et al. 2008) by various mechanisms such as diffusion through cell membranes, via endocytosis and adhesion (Geiser et al. 2005; Kim et al. 2007). In other biotic components (plants, algae, fungi) the cell wall restricted the entry of nanoparticles as it acts as a barrier. However, small-sized nanoparticles enter the cell via the pores in the cell wall (Zemke-White et al. 2000), and further interaction of these small-sized nanoparticles with the cell wall might increase the pore size, resulting in the internalization of large-sized nanoparticles (Navarro et al. 2008). After passing through the cell wall, endocytosis takes place (Navarro et al. 2008), and inside the cell nanoparticles bind with various cellular structures, thereby inhibiting cellular activity or damaging the cell organelles. Plants interact more frequently with nanoparticles by the presence of stomata, cuts or wounds, and the large surface area of leaf and roots (Navarro et al. 2008).

3.4.2 Mode of Action of Nanoparticles

Internalized metallic nanoparticles inside the cell interfere with several biological mechanisms, such as causing disruption of the membrane potential, and destabilization and oxidation of protein, and affect the stability of nucleic acid, stimulate the production of free radical species called reactive oxygen species (ROS), disrupting energy flow and releasing toxic compounds (Klaine et al. 2008). Gold nanoparticles have been reported to puncture the cell membrane (Tsao et al. 1999) and alter the cell shape and enzymatic activity (Liu et al. 2004).

Metallic nanoparticles also generate oxidative stress in biological systems by the production of ROS. ROS disrupt the influx and efflux of electrons and ions, disrupt membrane permeability, and reduce glutathione content inside the cell (Limbach et al. 2007). ROS increase the permeability of cell membrane by oxidization of double bonds of fatty acids. It has also been reported that TiO_2 nanoparticles have photocatalyst properties (Khus et al. 2006) and, with exposure to ultraviolet radiation (Zhao et al. 2007) produce ROS thereby causing DNA damage. Photosensitive silver nanoparticles have been shown to break the double-stranded DNA upon exposure of to UV light (Badireddy et al. 2007). It has also been reported that CeO_2 nanoparticles cause oxidation of membrane-bound complexes of respiratory electron transport chain (Thill et al. 2006). Moreover, quantum dots cause oxidative destruction (Hardman 2006), and heavy metals or metal ions released from quantum dots are toxic to the living cells (Klaine et al. 2008). Silver ions discharged from the metallic nanoparticles interact with functional thiol groups (derived from the cysteine residues) of enzymes (Matsumura et al. 2003) and inhibit the respiratory enzymes (Kim et al. 2007).

3.5 Ecotoxicology Assessment and Possible Strategies

According to the U. S. Environmental Protection Agency (EPA), “risk is a measure of the probability that cause damage to life, health, property, and/or the environment.” Before assessing the biotic hazard, it is desirable to assess the physical and chemical properties of nanoparticles. The various techniques for analysis and characterization of metallic nanoparticles include membrane filtration (Akthakul et al. 2005; Howell et al. 2006), size-exclusion chromatography (Wang et al. 2006), and photon correlation spectroscopy, used to determine the size and sometimes the shape of metallic nanoparticles (Chrastina and Schnitzer 2010). Additionally, transmission electron microscopy (TEM) (Jose-Yacaman et al. 2001; Chrastina and Schnitzer 2010), the scanning electron microscope (SEM) (Rai et al. 2009), and atomic force microscopy (AFM) (Viguie et al. 2007) are used to gather evidence about the configuration, arrangement, charges, and force of nanoparticles. The complex arrangement of crystal metallic nanoparticles can be resolved by X-ray diffraction (XRD) and energy-dispersive X-ray (EDX) techniques (Rai et al. 2009). However, it is difficult to analyze the properties of nanoparticles because their concentration in the environment is below the detection limit and test samples also carry unwanted nanoparticles (Lead and Wilkinson 2006). After the analysis and characterization of metallic nanoparticles, standard toxicity tests on organisms are used to assess the impacts of nanoparticles. For aquatic threat evaluation, an algal growth inhibition assay is commonly used, and *Pseudokirchneriella subcapitata* is an ideal organism for envisaging lethal threats to primary producers (Aruoja et al. 2009). Toxicological effects of metallic nanoparticles on diverse algal species, such as *Chlamydomonas reinhardtii* (Navarro et al. 2008; Chen et al. 2012; Melegari et al. 2013), *Desmodesmus subspicatus* (Hartmann et al. 2010), *Dunaliella tertiolecta*, and *Chlorella vulgaris* (Oukarroum et al. 2012), have also been studied. Also, *Vibrio fischeri*, a naturally luminescent bacterium, is widely used for ecotoxicological studies, and the bacterial luminescence inhibition assay is economical and easy to perform (Mortimer et al. 2008). There are various aspects for understanding the risk assessment of nanoparticles, such as dose of nanoparticles, exposure time, and endpoint measurement. Furthermore, short- and long-term laboratory experiments and development of a coordinated approach are still needed for assessing the toxicity.

3.6 Conclusions

Because of the wide application of metallic nanoparticles, their unintentional release and exposure pose a serious hazard to organisms and their environments. Only a few areas have been covered for the assessment and testing of the hazardous effects of metallic nanoparticles. Therefore, there is a requirement for data on the long-term effects of metallic nanoparticles, in vivo interactions of metallic nanoparticles, applied methods, databases of well-established toxicity tests, and the establishment of testing guidelines to enhance the transparency and comparability of obtained data.

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