Review Article

Nanotoxicity of Materials on Marine and Aquatic Organisms

Jiss James*, Prachi Saxena, Rajendran. N.
School of Bio Sciences and Technology, VIT University, Vellore, 632014, Tamil Nadu, India.
***Corresponding author's E-mail:** jissclarajames@yahoo.com

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ABSTRACT

With the increase in industrialization and the use in technology there is an increase in the use of nanomaterials recently. This has led to the increase in their release into the environment. There are various sources of entry of nanoparticles into the environment. Nanoparticles enter the environment through volcanic eruptions, waste water effluents, accidental spillages, industrial run-offs etc. The process and rate of degradation of these nanoparticles is unknown and thus they tend to build up in the environment. Such nanoparticles have major impacts on the ecosystem and the organisms. Nanoparticles affect the microorganisms to the macro organisms and some of the nanoparticles are known for antimicrobial activities. The microorganisms are more adversely affected while the organisms larger in size are less affected since the effect of the nanoparticles on these organisms varies according to their size. Some of the nanoparticles like the silver nanoparticles have known antimicrobial effect and their presence may lead to the killing of the pathogenic as well as the beneficial microorganisms in the environment which may affect to various environmental processes like disturbances in the biogeochemical cycles and other processes involving the microorganisms. Nanoparticles also tend to accumulate in the marine and aquatic environments and thus affecting the aquatic microorganisms to the macro organisms. However, the effects of many nanoparticles on the ecosystem are also not fully known. In this review, uses of nanoparticles, their entry into the aquatic and marine environment, and their impact on the organisms present in those environments have been discussed.

Keywords: Nanoparticles, Nanotoxicity, Fullerenes, Plankton, Microorganisms, Environment.

INTRODUCTION

The term 'nano' is derived from the Greek word 'nanos' which means 'dwarf'. Nano is also used as a prefix in scientific terms and it means one billionth of a metre. Nanotechnology is the design, characterization, production and application of structures and devices by controlling their shape and size at the nanometre level.[1] Nanotechnology is a growing scientific field and has many applications in many various fields, like electronics, medicine, food, energy, and aerospace. Nanomaterials are materials having atleast one dimension below 100 nm. This group consists of the nanoparticles which are defined as materials with at least two dimensions between 1 and 100 nm.[2, 3] Nanomaterials possess unique catalytic, mechanical, and optical properties, and electrical conductivity mainly due to their size in nanometres.[4]

TYPES OF NANOMATERIALS

Nanomaterials are of four major types and they are described below.

Carbonaceous nanomaterials

Carbonaceous nanomaterials include fullerenes, carbon nanotubes (CNTs) and amorphous carbon nanoparticles. They originated with the discovery of 60 carbon atom containing compounds known as fullerenes, produced by evaporating graphite. Carbon nanotubes are cylindrical derivatives of fullerenes. Carbon nanotubes are of two types i) The multi-walled carbon nanotubes are concentric cylinders with a length up to 10 nm diameter up to 5–40 nm in diameter. ii) Single-walled CNTs have a single cylinder. These carbon nanomaterials have a variety of applications in electronics catalysis chemical sensing and cell biology.[5-8]

Metal based nanomaterials

This category of nanomaterials include nanogold, nanosilver and metal oxides, such as titanium dioxide, zinc oxide, cerium dioxide, molybdenum trioxide, bismuth trioxide, lithium cobalt dioxide, indium tin oxide.[4] These are commonly used and synthesized in most of the laboratories. Metal oxides like zinc oxide and titanium dioxide are used extensively in sunscreens, cosmetics, and bottle coatings because of their ability to block ultraviolet radiations. Production of metal oxides for use in skin care products is estimated to be 1,000 tonnes/ year in 2005–2010.[9]

Silver and gold nanoparticles are other metal based nanoparticles and of these two, silver nanoparticles are more widely studied. To induce antimicrobial effect, silver is generally used in the nitrate form. Silver nanoparticles are used in molecular diagnostics, in therapies, and in devices that are used in several medical procedures. They are well studied because silver ions and silver-based compounds are toxic to microorganisms including 16 major species of bacteria.[10, 11] Silver ions damage the plasma membrane and cause the release of K+ ions from bacteria.[12] They cause harm to both the beneficial and the pathogenic microorganisms. Thus these nanoparticles can disturb the ecosystem and cause ecological problems when released into the environment as microorganisms play a key role in the biogeochemical cycles and various other processes.

Semiconductor nanocrystals

Semiconductor nanocrystals are also known as quantum dots. They are closely packed semiconductor crystals comprised of hundreds or thousands of atoms, and whose size is in the order of a few nanometers to a few hundred nanometers. The optical properties of quantum dots depend on their size. They are made of metals or semiconductors such as cadmium selenide (CdSe), cadmium telluride (CdTe), CdSeTe, indium phosphide (InP), or zinc selenide (ZnSe).

Dendrimers

These nanomaterials are nanosized polymers made from branched units. The surface of a dendrimer has numerous chain ends, which perform specific chemical functions. Their size, topology, flexibility, and molecular weight can be controlled. They are used in different fields like biology, material sciences, and surface modification.

SYNTHESIS OF NANOMATERIALS

The synthesis of nanoparticles or materials has been carried out either by natural or synthetic methods and these main methods have been described below.

Synthetic nanomaterials

There are various methods to synthesize nanoparticles. Three commonly used methods for the synthesis are physical vapour deposition (PVD), chemical vapour deposition (CVD) and solution-based chemistry. Other methods of synthesis include electrochemical synthesis and physical methods.[13] The three major methods of synthesis have been described below in brief.

Physical vapour deposition

In this method, no chemical reaction takes place instead the vapour is created in a physical manner. There are various sub techniques involved in this out of which sputtering, pulsed laser deposition, and thermal evaporation are the commonly used techniques.

Chemical vapour deposition

In this process, one or more volatile precursors chemically react and/or decompose on the substrate surface to produce the desired material deposit. The difference in CVD and PVD is that chemical reaction is must in CVD.

Solution based chemistry

Materials which have a complex stoichiometry are often difficult to synthesize via vapour deposition techniques. In this case solution based chemistry is used. This method has been used to synthesize metal oxide films. Zinc oxide is one of the metal oxides synthesized by using solution based chemistry.

Natural nanomaterials

Nanoparticles have existed naturally from the beginning of the Earth's history. Some sources of natural nanoparticles are given in table below.

Table 1: Examples of naturally occurring nanoparticles and their sources.

Location of nanoparticles	Particle types	References
Volcanic dust	Bismuth oxide nanoparticles	14
Volcanic ash	Cristobalite (crystalline silica)	15
Ocean surface micro layer (SML)	Colloids, sub-micron components of phytoplankton, and carbon particles	16,17
Soil	Mineral particles, colloids	18
Ice cores	Carbon nanotubes, carbon fullerenes and silicon dioxide nanocrystals	19

These are the nanomaterials produced in nature. The synthesis of nanomaterials can also be done in a natural way using biological methods. This method of synthesis is called green synthesis and is given below.

Green synthesis

Most of the chemical methods of synthesis of nanoparticles are expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks. Thus there is a need of environment friendly methods of nanoparticle synthesis which could be attained by green synthesis. Nanoparticle synthesis by biological methods like using microorganisms, enzymes, fungus, and plants or plant extracts are possible eco-friendly alternatives to chemical and physical methods.[20-25]

USES OF NANOMATERIALS

There are a wide range of applications of nanomaterials. The unique structure and properties of nanomaterials have made them popular among material developers. These materials as fillers in plastics, as coatings on surfaces, and as UV-protectants in cosmetics are some of the already available applications.[26] New applications for nanomaterials are being worked on in hundreds of laboratories in various parts of the world.

Carbon nanotubes are used in shielding and absorbing electromagnetic radiation, field emission, thermal conductivity, hydrogen storage, adsorption, catalyzing, etc. Due to their size in nanometres, nanomaterials are also used in antifouling agents. The particles of nanomaterials are held in the coating lattice and are not readily leached out of the coating by the physico-chemical forces of the environment, while slowly releasing ions thus help provide longer-term anti-fouling character.[26] Nanoemulsions which include latex and other formulations are used in paints and surface coatings. They are also used in cosmetics, sunscreens and other similar cream formulations, and in medicine for drug delivery. Iron and other bimetallic nanoparticles are used for water and soil remediation. Dendrimers are used in biology as DNA transecting agents, therapeutic agents for prion diseases, formation of hydro gels, drug delivery, DNA chips, and ex vivo amplification of human blood cells.[4] Nanoparticles are incorporated into fabrics such as nylon, polypropylene and other polymers to provide long-term antimicrobial character even in harsh environments.[26]

ENTRY OF NANOMATERIALS INTO THE ENVIRONMENT

There is an increase in the production and also use of nanomaterials of all types. This has led to their increased release into the environment which may increase the harmful effects on ecosystem. Nanomaterials cause harmful effects to both terrestrial and aquatic environments. Nanoparticles on land contaminate soil, migrate into surface and ground waters, and interact with the microorganisms, flora and fauna. Nanoparticles present in the solid wastes, wastewater effluents, direct discharges, and accidental spillages enter the marine and aquatic systems by wind or rainwater runoff.[4] Industries use nanoparticles for the synthesis of various products. The waste water effluents of these industries are let out into various water resources. This is one of the major entry points of nanoparticles into the aquatic and marine environments. The industrial waste dumped into landfills may also contain nanoparticles. These nanoparticles are often washed off into the water resources with rainwater and thus are another entry point into the aquatic and marine environments. Nanomaterials also exist naturally in the environment. For example nanoparticles are found in volcanic dust, glacial ice cores. In soils, natural nanoparticles include clays, organic matter, iron oxides, and other minerals. There are several mechanisms that lead to the production nanoparticles in the environment and these are geological or biological. Geological mechanisms include physicochemical weathering, volcanic activity etc. The geological processes produce inorganic nanoparticles, but biological mechanisms typically produce organic nanomolecules.[27]

FATE OF NANOMATERIALS IN THE ENVIRONMENT

The behaviour of nanomaterials in the various ecosystems such as natural waters, sediments or soils is complex and involves several processes.[28,29] Nanomaterials tend to undergo aggregation and form colloidal dispersions in water. They may also be adsorbed onto various surfaces like the exterior surfaces of organisms. Environmental pH, salinity, hardness of water and the presence of natural organic matter are some of the factors which influence the fate of nanomaterials in the ecosystems.

Fate of nanomaterials in the terrestrial environment

Nanomaterials move through the soil matrix, at different rates, some nanoparicles like Cu are retained in the soil matrix at a higher rate compared to others like ZnO nanoparticles. Leaching of ions from the parent NPs are also observed over a period of time.[30]

Fate of nanomaterials in the fresh water environment

The fate and behaviour of nanomaterials are different in freshwater and marine environments.[27] Chemical composition, mass, particle number and concentration, surface area concentration, size distribution (including polydispersity of the primary particle and the nature of any aggregates), specific surface area, surface charge/zeta potential, surface contamination, and the nature of NP shell and any capping agents, stability, and solubility (using a 1-nm cut off between dissolved and nanoparticles fractions) are some of the factors which come into role when it comes to the physicochemical properties of nanoparticles in relation to behaviour in natural waters.

Fate of nanomaterials in the marine environment

The marine environment is more alkaline, has higher ionic strength, than the fresh water environment. The marine environment exhibits changes in physicochemical characteristics with depth and this may influence aggregation and colloid chemistry. The high ionic strength of seawater compared to freshwater will lead to cause aggregation. Studies suggest that even small increases in salinity above that of freshwater can decrease colloid concentrations by aggregation and precipitation processes.[31]

ORGANISMS IN THE MARINE ENVIRONMENT

The oceans cover about 71% of the Earth's surface. Due to the depth of the ocean it contains much more volume which is habitable compared to that on land. The habitats in the marine environment include everything from the tiny layers of surface water in which organisms are trapped due to surface tension between the ocean and atmosphere, to the depths of the oceanic trenches. Examples of habitats in the marine environment include coral reefs, kelp forests, seagrass meadows, the surrounds of seamounts and thermal vents, tide pools, muddy, sandy and rocky bottoms, and the open ocean (pelagic) zone. A large proportion of all life forms on Earth exist in the ocean. A wide diversity of organisms is found in the marine environment. Organisms in the sea are described below.

Microorganisms

They are organisms that are too small to be observed in sufficient detail by the unaided human eye and are single cellular organisms. This includes any organism smaller than 0.1 mm. They are the basis of the ocean food web.

Plankton

They are the organisms that are suspended in the mass of water and are carried by the sea currents. Plankton are composed of phytoplankton and zooplankton. Phytoplankton are the primary producers, having size in the range of 40–80 mm in size. Zooplankton are primary consumer having size in the range of 1–2 mm.[32]

Benthos

Benthos are the organisms that live on the bottom of the ocean, and can be stationary such as corals and sponges or move such as worms, some types of fish, many molluscs, etc.

Nektons

This group includes all the animals that can move smoothly enough to overcome currents and actively swim in water. The most common animals that belong to it are vertebrates, many types of fish, reptiles like tortoises and water snakes, sea mammals such as whales, dolphins, sperm whales, etc. They are generally secondary and tertiary consumers placed at the end of the food chain.

EFFECT OF NANOMATERIALS ON AQUATIC ORGANISMS

Effect of nanomaterials on microorganisms

Microorganisms are of great importance as they form the foundation of aquatic ecosystems and they play various roles ranging from nutrient cycling to waste decomposition. Nanomaterials cause adverse effects on many bacterial species. Titanium dioxide and silver nanoparticles are well studied for their adverse effects on microorganisms.[4] In *E. coli* cells the direct interaction of silver nanoparticles with cell membranes affects the regulation and transport though the membrane and accumulation of intracellular reactive oxygen species takes place.[33,34,35] Titanium oxide leads to solar disinfection of *E. coli* through photo catalytic activity and reactive oxygen species.[36] Nanomaterials like fullerenes also inhibit the growth of *E. coli* by interfering with the energy metabolism.[37] MgO has antibacterial activity against *B. sutbilis* and *S. Aureus*.[38] Other nanomaterials like carbon nanotubes, quantum dots, ZnO also have antimicrobial properties.[4]

Effect of Nanomaterials on phytoplankton

The toxicity of nanomaterials on phytoplankton and accumulation of nanoparticles in phytoplankton can directly or indirectly affect the entire aquatic ecosystem as they are the primary producers in the aquatic systems. There is a formation of stable nanocrystals on marine phytoplankton when exposed to cadmium.[39] Thus metal nanoparticle exposure and subsequent uptake can arise from the presence of appropriate conditions for crystal formation on the surface of the organism, even in the absence of nanoparticles in the original polluting material.[39] Iron nanoparticles are toxic to a few species of marine phytoplankton like *Thalassiosira pseudonana*, *Dunaliella tertiolecta*.[32] These nanocrystals on the surfaces of these phytoplankton enter the food chain as they are the primary producers. Iron nanoparticles also cause inhibition of growth of marine phytoplankton species *Isochrysis galban*.[32]

Chloroplasts in the algae take up the solar energy to synthesize glucose from carbon dioxide and water while generating oxygen. Chlorophyll is an important component in the photosynthetic reaction. The main types of chlorophyll present in phytoplankton photosynthesis are chlorophyll *a, b* and *c*.[40] The chlorophyll *a* content was observed to decrease as the Fe_3O_4 nanoparticle concentration increased in *Chlorella vulgaris*.[40] Fe_3O_4 nanoparticles have a significant toxic effect on CO_2 absorption and the net photosynthetic rate. Malondialdehyde (MDA) which is an important marker of lipid peroxidation and cellular oxidative damage, in *Chlorella vulgaris* gradually increases as the Fe_3O_4 nanoparticle concentration increases.[40] This indicated that stress caused by Fe_3O_4 nanoparticles increased the MDA content in *Chlorella vulgaris*, exacerbates lipid peroxidation of *Chlorella vulgaris*, and leads to membrane damage in the algal cells.[40] Thus Fe_3O_4 nanoparticles have significant effects on the contents of chlorophyll a, MDA and GSH, amount of CO_2 absorbed, net photosynthetic rate, SOD activity and inhibition of ·OH generation in *Chlorella vulgaris*.[40]

The green algae *Chlamydomonas reinhardtii* and *Pseudokirchneriella subcapitata* when exposed to cadmium quantum dots show physical changes in the cellular structure of algal cells, to a spherical shape from the normal crescent or sickle shape of unexposed cells.[41] Carbon black nanoparticles have adverse effects on reproduction and embryo development in the marine macroalgae *Fucus serratus* resulting in a reduced fertilization success and higher incidence of incorrect polar body axis alignment.[42]

Effect of nanomaterials on crustaceans

Crustaceans have the ability to sequester toxic metals in granules in the hepatopancreas and other tissues. *Daphnia magna* is a cladoceran which is a common crustacean found in freshwater lakes and ponds.[43] Daphnids are standard US EPA test organisms.[44] Daphnids take up nanomaterial like fullerenes, which leads to altered moulting and decreased reproductive output. If exposed to higher concentrations of fullerenes, increased rates of mortality was observed in Daphnids.[43] *D. Magna* is able to ingest the nanoparticles. This ingestion and/or accumulation of nanoparticles are dependent on the size of the particles, since *D. Magna* is a filter-feeder.[45] The smaller particles, especially those less than 50 microns in diameter, are more easily ingested by daphnids while large particles are too difficult to process and thus daphnids restrict them from entering the filter chamber or reject them through movements of their postabdominal claw.[46] Nanoparticles can also adhere to the body surfaces of *D. magna*. This was observed due to hydrophobic properties of the nanoparticles. Hydrophobic substances adhere easily to the negatively charged biological surfaces.[47] In the *Acartia tonsa* which is a marine copepod, adhesion of C60 fullerene nanoparticles to the exoskeleton and antennae was observed.[48] *Artemia salina* (brine shrimp) is a zooplankton used as a feed for larval fish in aquacultures.[49] They have an important role to play in the energy flow of the food chain in marine environment. Artemia are filter-feeders like daphnids that can readily ingest fine particles smaller than 50 µm.[49] TiO_2 nanoparticles accumulate in Artemia.[49] Extended exposure of Atremia to TiO_2 induces oxidative stress manifested with marginal mortality.[49]

Effect of nanomaterials on molluscs

Bivalves have the ability to filter large volumes of water, processing microalgae, bacteria, sediments, particulates, and natural nanoparticles, and tend to accumulate different chemicals in their tissues.[50] Uptake of nanoparticle agglomerates, leads to stimulation of lysozyme release, oxidative burst and nitric oxide production.[50] Nanoparticles enter the digestive gland cells of blue mussels and cockles by endocytosis. Marine bivalves such as *Mytilus edulis* take up nanoparticles by endocytosis.[51] C60 fullerene in *Mytilus edulis* hemocytes, induces cytotoxicity.[50] Mussels and oysters more efficiently capture and ingest nanoparticless that are incorporated into agglomerates compared to those freely suspended (100 nm) nanoparticles.[52]

In the freshwater bivalve *Elliptio complanata* accumulation CdeTe quantum dots agglomerates takes place. As a result lipid peroxidation and DNA strand breaks in gills and digestive gland were observed.[53] Accumulation of C60 fullerene agglomerates in oysters (*Crassostrea virginica*) induced digestive gland lysosomal destabilization and abnormal development in larvae.[50] Exposure of the endobenthic marine bivalve *Scrobicularia plana* to CuO Nanoparticles induced copper accumulation and increase in whole tissue activities of the antioxidant enzymes SOD, catalase and GST, of metallothionein-like protein content, and affected burrowing behaviour.[54]

Table 2: Nanomaterials and aquatic organisms being affected*

Nanomaterials	Organisms affected	Effect
Carbon-containing fullerenes Fullerene C60	Largemouth bass (*Micropterus salmoides*), *Daphnia magna*, *Hyalella azteca*, Fathead minnow *(Pimephales promelas)*, Japanese medaka *(Oryzias latipes)*, Zebrafish *(Danio rerio)*,*Pseudokirchneriella subcapitata*, Oysters *(Crassostrea virginica)*	Increase in lipid peroxidation, delayed molting and reduced offspring production, delayed embryo and larval development.
Carbon nanotubes		
Multiwalled	*Stylonychia mytilus*	Damage to micronucleus, macronucleus, and membrane, growth inhibition.
Single-walled	*Daphnia magna*, Rainbow trout *(Oncorhynchus mykiss)*, Zebrafish embryos	
Double-walled	Zebrafish embryos	
Carbon black	*Daphnia. magna*, *Lymnaea stagnalis*, Zebrafish embryos	
Metallic		
Copper	Zebrafish (*Danio rerio*)	Deformities and abnormalities observed in the embryos, growth inhibition.
Silver	Zebrafish (*Danio rerio*)	
Iron	*Thalassiosira pseudonana*, *Dunaliella tertiolecta* and *Isochrysis galbana*	
Silver	Rainbow trout (*Oncorhynchus mykiss*), *E. coli*	
Metal oxides		
TiO2	*Daphnia magna*, Rainbow trout (*O. mykiss*), green algae (*Desmodesmus subspicatus*), *Artemia salina* (brine shrimp)	Decreases of NaK -ATPase activity observed in the gills and intestine. Oxidative stress–linked effects observed.
ZnO	Microalgae (*P. subcapitata*)	
CuO	*Scrobicularia plana*	
MgO	*B. subtilis, S. aureus*	Antimicrobial.
Quantum dots		
Cadmium telluride (CdTe)	Freshwater mussels (*Elliptio complanata,*)	Increase in lipid peroxidation, DNA Damage.
CdSe/ZnS	*Chlamydomonas reinhardtii Pseudokirchneriella subcapitata*	
Others		
Nonionized fluorescent polystyrene nanoparticles	Japanese medaka (*O. latipes*)	Accumulation in the brain, testis, liver, and blood
Negatively charged polystyrene fluorescent particles	*Daphnia magna*	Rapid uptake into the bodies.

*[4, 50, 53, 54, 59]

Effect of nanomaterials on fish

In the liquid phase nanoparticles exist as emulsions or suspensions. Due to their size in nanometres nanomaterials have easy access to the water flowing between the secondary lamellae of the gills and to the gill surface layers. In addition, any water drunk by the fish will present nanoparticles to the gut mucosa. Nanoparticles cause inflammation of the gill and injury to the gut at low concentrations.

Fish gills are sensitive to nanoparticles.[55] Fish when exposed to fullerens exhibit signs of lipid peroxidation in the brain.[4] C60 induces mortality within 6 to 18 h of exposure in adult fathead minnow (*P. promelas*).[43] Nano-sized copper particles causes harm to fish gills of Zebra fish *(Danio rerio)* through a different but as-yet unknown mechanism. $Fe2O_3$ nanoparticle aggregates cause a delay in embryo hatching, malformation in some zebra fish embryos and larvae, and eventually mortality.[40] The impact of Fe2O3 on zebra fish is associated with the aggregation and sedimentation of Fe2O3 nanoparticles.[40] The aggregation and sedimentation of Fe2O3 on the surface of embryos causes toxicity.[40] The hatching delay of zebra fish embryos may be due to direct adherence/adsorption of Fe2O3 aggregates on the surface which causes a change in the surface mechanical properties or by interferes with the digestive function of the chorionic hatching enzyme.[56]

Exposure of TiO_2 nanoparticles to rainbow trout (*Oncorhynchus mykiss*) causes respiratory problems and other sub lethal effects in the fish.[57] Nanoparticles have a significant effect on medaka (*Oryzias latipes*) fish and their embryos. Nanoparticles were also detected in the brain, testis, liver, and blood of medaka.[58] Largemouth bass (*Micropterus salmoides*) when exposed to C60 fullerenes show significant lipid peroxidation in brains. Total glutathione levels were also marginally depleted in gills of this fish.[59]

CONCLUSION

With the rapid advances in science and technology and the potential uses and applications of nanomaterials there will be increase in their production and thus their corresponding release into the environment. Hence, it is essential that research to solve the problems and overcome the challenges associated with nanomaterials be accomplished. The nanomaterials in the terrestrial ecosystems tend to be washed off into the aquatic systems. Thus more research is needed to understand the interactions of nanoparticles with the aquatic organisms and their mechanism of toxic action. Adsorption of nanomaterials to organics such as humic acids or phytoplankton in the water, or onto sediments and soils, greatly increase the concentration of nanomaterials locally. Since other organisms will ingest sediment and phytoplankton, thus leading to the entry of nanoparticles into the food chain. Thus adverse effects are not only on phytoplankton which are primary producers but also on the primary, secondary and tertiary consumers. Till date only a few of the adverse effect of nanoparticles on aquatic organisms is studied, much more research is needed in this area.

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