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Source, Transformation and Ecotoxicity of Silver Nanoparticles in Aquatic Ecosystem: A Review

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ABSTRACT

Nanotechnology is one of the fastest growing technologies nowadays. Today, various companies are using engineered nanoparticles to create innovative products ranging from agriculture to pharmaceuticals. Nanotechnology is expected to become a trillion-dollar industry in the next decade. As the use of nanotechnology in industry increases, so will the presence of nano-waste in the environment. Aquatic ecosystems will be the most affected one as most nano-wastes will enter the aquatic environment through sewage and industrial effluents. This is why it is important to better understand the impact of engineered nanoparticles on aquatic ecosystems. Among the types of nanoparticles used in industry, silver nanoparticles are the most common and the most widely used. Therefore, it is very important to find out where from the silver nanoparticles come in aquatic ecosystem, what kind of interaction it has in the aquatic environment, how it is transformed there and what kind of toxic effect it has on aquatic organisms.

Keywords Silver nanoparticle, Aquatic ecosystem, Aquatic organisms, Bioaccumulation, Transformation.

INTRODUCTION

The definition of nanoparticle is that all particles with a diameter of approximately 1 to 100 nanometers in any dimension are called nanoparticle. Compared with normal materials, nanoparticles have several noble characters such as increased surface area, pore volume which are not found in bulk materials (Breznan *et al*. 2018). Due to these properties, nanoparticles can show high rate of reaction, ion exchange and adsorption (Breznan *et al*. 2018). Due to the unique chemical, biological and optical properties of nanoparticles compared to ordinary materials, their use is quite advantageous in various industries. Products that use engineered nanoparticles are called nano-enabled products (NEPs). The demand for these nano enabled products is increasing day by day. The global market for NEPs in 2016 was 32.9 billion US dollars and is predicted to reach 125 billion US dollars by 2024 (Research and Markets 2018). The use of engineered nanoparticles can be seen in a variety of industries such as fertilizers, textiles, detergents, water purification, pharmaceuticals, biomedical, print, paint, cosmetic. It may sound surprising but it is true that in just one decade nanotechnology

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Fig.1. Sources of silver nanoparticles in aquatic ecosystem.

has gone from research and development stage directly into our daily use products (Hansen *et al.* 2020). Knowingly or unknowingly, we use all those products in our daily life. As the use of these NEPs has increased exponentially, so has the amount of nano-waste. After their use, NEPs eventually end up in terrestrial or aquatic ecosystems as nano-wastes. Among these, wastes generated from NEPs used in print, textile, detergent, water purification, pharmaceutical or cosmetic industries mainly end up in the aquatic environment as industrial effluents or sewage. Among the types of nanoparticles used in industry, silver nanoparticles are the most common. Due to its inbuilt anti-microbial properties, it is used in various consumer products such as cosmetics, personal care products, pharmaceuticals, packaging, detergent, handwash, saving cream. (Pulit-Prociak and Banach 2016). Silver nanoparticles are used directly in more than 250 consumer products (Anjum *et al.* 2013). Therefore, the presence of silver nanoparticles as nano waste in aquatic ecosystems is quite high. In this review paper, I have tried to find out what is the source of silver nanoparticles in aquatic environment, what kind of interaction it has in aquatic environment, how it is transformed and what kind of toxic effect it has on aquatic organisms. So that before any new nano product made of silver nanoparticles is marketed, we can be aware of the potential harmful effects and take appropriate measures.

Entry of silver nanoparticles into the aquatic environment

Due to the wide spread of nanotechnology in modern times, the amount of disposal of various nanoparticles in the aquatic environment has also increased considerably. Since the aquatic environment acts as a sink for various industrial run-offs and sewage, the amount of disposal of various nanoparticles is highest in the aquatic environment. Silver nanoparticles can enter the aquatic environment in different ways such as during production, utilization or disposal (Tolaymat *et al*. 2017, Bundschuh *et al*. 2018). Industrial waste from production facilities where products containing silver nanoparticles are made can enter the aquatic environment through run-off processes. One study found that 15% of the total nano silver found in the Rhine River came from the textile and plastics industry (Pulit-Prociak and Banach 2016). Silver nanoparticles from wastewater treatment plants can also leach into the aquatic environment. Products containing silver nanoparticles can leach into the aquatic environment through sewage after use. Even products containing silver nanoparticles can be exposed to the aquatic environment through leakage and accidents during transport (Walters *et al*. 2016). Some fertilizers and pesticides containing silver nanoparticles currently used in agriculture are also exposed to the aquatic environment through surface run-off processes (Baalousha and Lead 2015). Fig. 1 shows the different sources of silver nanoparticles in aquatic ecosystem. As the concentration of silver nanoparticles in the aquatic environment is increasing day by day, they are easily entering the food chain and accumulating. This results in damage to the organisms living in the aquatic ecosystem. Not only this, the silver nanoparticles are being aggregated and precipitated causing harm to the benthic organisms. Since the damage potential of nano silver depends on its fate and transformation, we first need to know how these silver nanoparticles change or transform in the aquatic environment.

Transformation of silver nanoparticles in the aquatic environment

How silver nanoparticles behave in the aquatic environment or how harmful they are to aquatic organisms depends on their interaction with the surrounding environment (Banu *et al.* 2021). After entering the aquatic environment, silver nanoparticles can remain in the form of suspensions or aggregate to form aggregates (de Souza *et al*. 2019). Silver nanoparticle's size, shape, ionic strength, surface coating, pH of water, water temperature, presence of other organic matter in water has important influence on its stability (Banu *et al*. 2021). The above factors may also alter the toxicity of silver nanoparticles in the aquatic environment. Fig. 2 lists the environmental and physicochemical factors that regulate stability and toxicity of silver nanoparticles in aquatic ecosystem. The release of silver ions from a silver-containing compound is the cause of the toxicity of that silver-containing compound and since silver ions are released faster from silver nanoparticles than the bulk material, the toxicity of silver nanoparticles is also higher than the bulk material (Yu *et al*. 2013). Experiments have shown that small-sized silver nanoparticles easily release silver ions, so the toxicity of small-sized silver nanoparticles is also higher than that of large-sized silver nanoparticles (Dobias and Bernier-Latmani 2013). The stability and toxicity of silver nanoparticles also depend on the ionic environment they experience in water. As experiments have shown that low concentrations of chloride ions in water easily release silver ions from silver nanoparticles, which impose greater toxicity on zebrafish embryos (Lee *et al*. 2018). The pH of water also controls the toxicity and stability of silver nanoparticles. It was observed that at high pH silver nanoparticles undergo aggregate formation and at low pH they release silver ions very rapidly into water. That is why the toxicity of silver nanoparticles is more in acidic environment (Peretyazhko *et al*. 2014). Water temperature can also alter the toxicity and stability of silver nanoparticles. Aggregate formation is reduced at higher temperatures but aggregate formation is faster at lower temperatures, so silver ions are released faster at higher temperatures and exhibit greater toxicity (Steinmetz *et al*. 2020). Various organic substances present in water such as humic acid, fluvic acid reduce the toxicity of silver nanoparticles as they form a coating on the nanoparticles which inhibits the release of silver ions (Adegboyega *et al*. 2013). The type of surface coating on silver nanoparticles also greatly controls their stability and toxicity. As shown silver nanoparticles with phytochemical coating are more stable and less toxic (Zhang *et al*. 2019).

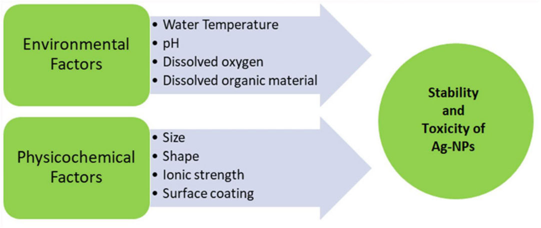

Fig. 2. Factors that interfere stability and toxicity of silver nanoparticles in aquatic ecosystem.

Entry of silver nanoparticles into aquatic organisms

Silver nanoparticles can enter into the body of aquatic organisms by two ways. Either directly into the body from water or through food. Due to the small size of silver nanoparticles, they can easily penetrate various biological barriers (such as gills or other surface epithelium) and enter the body. Microscopic silver nanoparticles can penetrate the semipermeable membranes of bacteria and fungi, the cell wall of phytoplankton, the cuticle present outside the body of zooplankton or the gills and the gut epithelium of large aquatic animals. Sometimes silver nanoparticles accumulate on the surface of the body. Later it enters the body by processes such as endocytosis, phagocytosis. Thus, silver nanoparticles enter organisms at different trophic levels. After entering the organism, silver nanoparticles will either accumulate in the body or it will be excreted in the excretion process (Banu *et al*. 2021). Whenever silver nanoparticles accumulate in the organism, their concentration in the food chain will increase in the process of biomagnification and show more toxicity.

Ecotoxicity of silver nanoparticles on aquatic organisms

Silver nanoparticles behave differently after entering different organisms and their toxic effects are not the same on all organisms. Also, the toxicity of silver nanoparticles varies between freshwater and seawater organisms. That is why the toxicity of silver nanoparticles on each organism needs to be discussed separately to get a holistic understanding of its toxicity.

Effects on bacteria

In aquatic ecosystems, bacteria play an important role in nutrient cycling, mainly as decomposers. Silver nanoparticles interact with thiol groups of various bacterial proteins to inactivate them (Morones *et al*. 2005). Silver nanoparticles alter membrane permeability and disrupt electron transport chains of bacteria (Morones *et al*. 2005). Also, silver nanoparticles disrupt bacterial membrane permeability and bacterial DNA replication (Feng *et al*. 2000). Toxic effects of silver nanoparticles have been observed on both gram-positive and gram-negative bacteria. Experiments have shown that gram negative bacteria are more susceptible to silver ions and less susceptible than gram positive bacteria. As a result, the toxicity of silver nanoparticles on gram positive bacteria is less effective (Völker *et al*. 2013). One experiment showed that silver nanoparticles smaller than 5 nanometers can reduce the growth of nitrifying bacteria (Reidy *et al*. 2013).

Effects on phytoplankton

The food chain of aquatic ecosystems begins with phytoplankton. Therefore, the harmful effect of silver nanoparticles on phytoplankton will have a significant impact on the entire aquatic ecosystem. Experiments have shown that within one to two hours of exposure, silver nanoparticles can alter phytoplankton cell morphology, increase oxidative stress, increase membrane damage, decrease thylakoid number, decrease chlorophyll content, decrease photosynthetic rate, increases lipid peroxidation, inhibits the electron transport chain (Miao *et al*. 2010, Li *et al*. 2015, Leonardo *et al*. 2015). As growth is disrupted, mortality also increases (Li *et al*. 2015). An experiment on *Chlamydomonas reinhardtii* showed that silver nanoparticles reduced their rate of photosynthesis (Navarro *et al*. 2008). An experiment on *Ochromonas danika* showed that silver nanoparticles reduced the growth rate of the algae (Miao *et al*. 2010). An experiment on *Chara vulgaris* and *Pithophora oedogonia* showed that silver nanoparticles disrupted their cell walls and caused various cell abnormalities (Dash *et al*. 2012). However, the exact mechanism by which silver nanoparticles enter the algal body is not yet known (Zhang *et al*. 2020). A very recent experiment showed that silver nanoparticles caused growth inhibition, decreased cell diameter, reduced chlorophyll-a content and increased lipid accumulation in the freshwater alga *Scenedesmus* sp. and the marine diatom *Thalassiosira* sp. (Pham 2019).

Effects on macrophyte

Aquatic plants, also known as macrophytes, are also an important and integral part of aquatic ecosystems. Macrophytes provide habitat for various organisms, regulate various biogeochemical cycles and act as primary producers. Silver nanoparticles are taken up from water by these macrophytes and accumulate in roots, leaves and other parts. Silver nanoparticles can destroy the cell membrane of aquatic plants, increase ROS generation, damage DNA, reduce ATP production (Tripathi *et al*. 2017). In *Lemna minor* and *Eichhornia crassipes*, silver nanoparticles were observed to accumulate in root, stem, leaf tissue and cause necrosis. As a result, plant growth rate and metabolic rate is reduced (Rani *et al*. 2016). In the aquatic plant *Spirodela polyrhiza* and in the sea grass *Cymodocea nodosa*, silver nanoparticles have been shown to increase oxidative stress, reduce the number of thylakoids, destroy cytoskeleton, photo system II and leaf rhizomes are extensively damaged (Jiang *et al*. 2014, Mylona *et al*. 2020).

Effects on zooplankton

Zooplanktons play a very important role in aquatic ecosystems. Basically, they act as a bridge between producer and higher organism. It is through these zooplankton that any toxic material reaches the body of higher organisms. *Daphnia* is the zooplankton on which various scientists have tested the toxicity of silver nanoparticles. Tests have shown that silver nanoparticles at very low concentrations can cause growth inhibition, reprotoxicity in *Daphnia*. At higher concentrations it can even cause death of *Daphnia* (Yan and Wang 2021). Scientists have also observed that the toxicity of silver nanoparticles to *Daphnia* is reduced if the amount of natural organic material in the water is high (Cupi *et al*. 2016). Silver nanoparticle toxicity on *Daphnia* also decreases as the amount of sunlight increases (Zhang *et al*. 2015). On the other hand, if the amount of dissolved organic carbon in the water increases, then the toxicity of silver nanoparticles on *Daphnia* increases (Pokhrel *et al*. 2013). Silver nanoparticles not only cause harm to zooplanktons, but are easily biomagnified from zooplankton to subsequent trophic levels. One study showed that zooplankton *Artemia salina* biomagnified silver nanoparticles several times to the next trophic level (Babaei *et al*. 2022). Similar effects were found from water flea (*Moina macrocopa*) and blood worm larvae (*Chironomus* sp.) (Yoo-Iam *et al*. 2014). An experiment on *Artemia nauplii* showed that silver nanoparticles caused DNA damage, altered hatching and even caused death (Arulvasu *et al*. 2014). An experiment on *Artemia salina* showed that silver nanoparticles slightly increased the oxidative stress of the organism, changed antioxidant defense mechanisms, and accumulated AgNP in the gut and other body compartments, but did not cause mortality (Demarchi *et al*. 2020).

Effects on higher aquatic animals

In aquatic ecosystems, Annelida, Arthropoda, Mollusca and fish occupy the primary, secondary and tertiary consumer trophic levels. They feed on phytoplankton, macrophytes and zooplankton. It is through them that any toxic substance reaches the body of birds and mammals. Therefore, their role in the aquatic ecosystem is very important and studying the toxic effect of silver nanoparticles on them is also very important. A test on the earthworm *Eudrilus eugeniae* showed that silver nanoparticles of 180 to 200 nanometers caused fibrosis, lipofuscin like deposits, gut tissue disruption in this earthworm (Samrot *et al*. 2018). In mussel *Dreissena bugensis* silver nanoparticles change kinetics of acetylcholinesterase and cause protein, lipid aggregation (Auclair *et al*. 2022). Zebrafish (*Danio rerio*) is a very common model for toxicity studies in aquatic systems. Experiments have shown that the chorionic pores in zebrafish embryos are large, allowing silver nanoparticles to penetrate easily (Chen *et al*. 2020). Silver nanoparticles can damage the yolk sac membrane in zebrafish embryos at low concentrations and cause dose dependent lethality. At higher concentrations these nanoparticles can induce reduced yolk sac and bent tail in zebrafish embryos (Osborne *et al*. 2013). In adult zebrafish, silver nanoparticles also cause various types of damage such as disrupts sodium-potassium ion channels of gill, alter the microbiota of the male gastrointestinal tract (Ma *et al*. 2018). A recent study showed that silver nanoparticles cause toxicity in brain, oocyte, liver and muscle tissue of adult zebrafish, resulting in easy degradation of unsaturated fatty acid chains and even affecting reproduction rate (Seyedi *et al*. 2021). Silver nanoparticles in *Labeo rohita* degenerate hepatocytes, form apoptotic bodies in liver, cause necrosis and hyperplasia of gill lamellar cells, cause DNA damage (Sharma *et al*. 2016, Khan *et al*. 2018). In *Cyprinus carpio* maximum damage from Ag-NP was observed on intestine and gill. Long chain omega-3 unsaturated fatty acids are oxidized resulting in reduced membrane fluidity that prevents normal physiological function of the gills and subsequently inflammation and lamellar damage are observed (Xiang *et al*. 2020). It has also been shown that increased levels of antioxidant enzymes are associated with increased susceptibility to tumor formation (Khosravi-Katuli *et al*. 2018). Silver nanoparticles present in municipal sewage can cause inflammation and immunosuppression in rainbow trout *Oncorhynchus mykiss* (Banu *et al*. 2021). A concentration of 0.15 mg/L Ag-NP can cause cytotoxicity, necrosis of nucleus and mitochondrial damage in hepatocytes (Ostaszewska *et al*. 2018). 8.9 mg/L AgNP alters blood cell morphology in *O. mykiss*, increases the amount of reactive oxygen species and alters cell signaling pathways (Shabrangharehdasht *et al*. 2020). Silver nanoparticles in Tilapia (*Oreochromis mossambicus*) cause damage to gill tissue, increase oxidative stress, increase protein carbonyl activity, cause epithelial hyperplasia and telangiectasia (Sibiya *et al*. 2022).

Effects of green synthesized silver nanoparticles on aquatic ecosystem

Since chemical production of nanoparticles requires a lot of energy and produces many harmful by-products, there has been an increase in green nanoparticles production since last decade (Nath *et al*. 2022). If a phytochemical or a microbe-derived compound is used as a reducing agent during the preparation of silver nanoparticles, a solid layer of that organic compound is formed on the nanoparticles, which greatly reduces the toxicity of the silver nanoparticles (Rafique *et al*. 2017, Khoshnamvand *et al*. 2020). Various tests on *Daphnia*, Zebra fish, *Labeo rohita* showed that green synthesized silver nanoparticles exhibited less toxicity than chemically synthesized silver nanoparticles (TulabyDezfuly *et al*. 2017, Renuka *et al*. 2020, Khan *et al*. 2017). However, some other studies have shown that green synthesized silver nanoparticles are also quite harmful to aquatic organisms (Krishnaraj *et al*. 2016, Ramachandran *et al*. 2018). Moreover, there is a need for sufficient research on how long these green synthesized silver nanoparticles are stable in water, how long the organic coating on them is maintained, whether they do aggregate formation. Only when we have enough research papers with the above-mentioned data, we shall be able to accurately say how much less harmful the green synthesized silver nanoparticles really are to aquatic organisms.

CONCLUSION

Just as every discovery of science brings some blessing to human civilization, it also brings some unintended curse. The same is the case with nanotechnology. Just as nanotechnology has shown the potential for incredible innovative power in various industrial fields, it has also raised concerns about its potential for toxicity and since silver nanoparticles are so common among all types of nanoparticles, there is reason for scientists to think about their toxicity. Effluents from factories using silver nanoparticles, like most industrial effluents, easily target aquatic ecosystems. Similarly, household products containing silver nanoparticles can easily mix with sewage water after use, which ultimately fall in aquatic ecosystems. Fertilizers and pesticides containing silver nanoparticles in agricultural fields can easily enter aquatic ecosystems even by being washed away by rainwater. Therefore, it is very important to know what kind of damage silver nanoparticles cause in aquatic ecosystems. The above discussion shows that silver nanoparticles have harmful effects on organisms living at all trophic levels. By the biomagnification method, their quantity has also increased in the subsequent trophic levels. Since fish living in aquatic ecosystems are widely used to provide protein in human diet, silver nanoparticles will enter our body through fish protein and cause toxicity to our vital organs. Although various tests have shown that green synthesized silver nanoparticles are less harmful, more research is needed on them. More research is also needed on how different abiotic factors and biotic factors in water are changing stability and toxicity of silver nanoparticles. Much more research is needed on the molecular mechanism by which silver nanoparticles enter different organisms and the pathway by which they accumulate. In most of the studies that have been done, scientists have observed harmful effects of silver nanoparticles under laboratory conditions on one or two species. Studies on the entire food chain or food web are difficult. Therefore the number of such research papers are also very few. Again, the effect of silver nanoparticles on an organism in laboratory conditions may not show the same effect in the real aquatic environment. That is why I think it is necessary to study the effects of silver nanoparticles on aquatic ecosystems through a holistic approach so that the overall picture become clear to us. Only then we can take any sustainable measures regarding the application of silver nanoparticles in various industries and consumer products which will be good for both the environment and people.

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