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SILVER NANOPARTICLES IN THE AQUATIC ENVIRONMENT: A SYNTHESIS OF TOXICITY IN FISH

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Abstract: Silver nanoparticles are one of the most exploited metal nanoparticles due to their unique properties. They are synthesized via chemogenic and biogenic routes. Their widespread applications have resulted in their unintentional release into various environmental matrices, ultimately making way to the aquatic environment. Silver nanoparticles have a broad-spectrum antimicrobial efficacy, which coupled with their increasing concentration in the environment has raised concerns about their safety and ecotoxicity. Fishes are most likely to be affected in aquatic ecosystems. Studies have reported toxic effects of silver nanoparticles in different fishes at various levels of organisation i.e. haematological, histological and biochemical level. This review aims to summarize all the available information on biological effects and establish an understanding of the toxicity mechanism of silver nanoparticles to illuminate associated potential risks and safety.

Keywords: Aquatic environment, Ecological risk, Fishes, Medicine, Silver nanoparticles, Toxicity.

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INTRODUCTION

Nanoparticles are wide class of materials having at least one dimension between 1 and 100 nm. Based on different shapes, nanoparticles can have up to three dimensions. Nanoparticles can be uniform or may comprise the following layers: (1) the surface layer, the outer most layer of nanoparticles, which generally consists of very small size molecules, metal ions or polymers, (2) shell layer, which is of different chemical composition than the core, (3) core layer, centre of the nanoparticles (Joudeh and Linke, 2022). The large surface area to volume ratio amplifies their reactivity and efficiency in catalytic reactions and shows significant anti-microbial potential even at low concentrations, making them suitable for several biomedical applications like drug delivery, medical imaging etc. (Yin *et al.*, 2020).

Among all the nanoparticles synthesised and characterised so far, silver nanoparticles (AgNPs) are the most significant ones due to their potential applications in commercial uses. They are typically composed of 20-15,000 silver atoms. AgNPs are widely known for their anti-microbial activities, which makes them very useful in nanomedicine for wound healings, drug delivery as anti-microbial coatings in textiles, biomedical devices and treatment (Pachaiappan *et al.*, 2023; Ramanayaka *et al.*, 2023).

Due to their broad-spectrum antimicrobial and remediation properties, AgNPs have diverse applications in medicine, textiles, agriculture, food storage, and environmental remediation (Biswas *et al.,* 2024). Extensive use of AgNPs results in their release into the aquatic environment leading to nanopollution, which raises ecotoxicological concerns owing to its harmful effects on aquatic organisms (Rai and Biswas, 2018; Auclair *et al.,* 2019; Biswas and Sarkar, 2019). The predicted environmental concentrations of AgNPs in water might reach from µg/L to mg/L range (Wang *et al.,* 2022), which are considered to be toxic in any environmental setting (Kühr *et al.,* 2018).

Silver can occur in four oxidation states (Ag, Ag^{+}, Ag^{2+}, and Ag^{3+}) in aquatic environment (Ibrahim, 2020). Although, the exact mechanism of AgNPs toxicity is still not well understood due to lack of information, it is believed that the toxicity of AgNPs is caused due to release of silver ions from the nanoparticles upon entering into the environment and its absorption by living organisms (Rai *et al.,* 2018; Cáceres-Vélez *et al.,* 2019). AgNPs can react with ions, ligands and colloids present in aqueous solution depending on the environmental conditions. Hence, AgNPs can end up in the environment and pose serious threat to the exposed organisms (Biswas *et al.,* 2018). Because of the broad-spectrum efficacy of AgNPs as antibacterial agent, they can cause toxicity to non-target aquatic and soil organisms (Wei *et al.,* 2023).

Fishes occupy an important position in aquatic food chain. The amniotes i.e. reptiles, birds, mammals including human beings consume fishes as a source of protein (Verma, 2017; Verma and Prakash, 2020). If the nanoparticle in question exhibits any toxicity to fishes, there is a high chance of transmitting that toxicity along the food chain (Roy and Nath, 2022). Many studies report the toxic effects of chemically synthesized nanoparticles (Younas *et al.,* 2022). Exposure to higher levels of AgNPs results in different degrees of toxic manifestation in fishes in the form of inflammation, metabolic stress, immune suppression, biochemical disturbance, and growth depression which depends on the concentration, duration of exposure and size of the nanoparticles (Hedayati *et al.,* 2019; Mabrouk *et al.,* 2021). Chemically synthesized AgNPs can inhibit acetylcholine esterase (AChE), impair T-lymphocytes recruitment, cause imbalance in blood plasma potassium and chloride level and increase cortisol level in fish exposed to nanoparticles (Bandeira *et al.,* 2020). Other studies also report histopathological changes in gill epidermis, liver and kidney of fish (Younas *et al.,* 2022).

Numerous studies have reported the toxic effect of AgNPs on fish but still there is a lack of proper and systematic information about the nature and fate of AgNPs in the environment and its implication on toxicity. This review article will outline the toxic effects of AgNPs in fishes at different organisational levels, such as haematological, histological and biochemical level.

RELEASE OF SILVER NANOPARTICLES INTO THE ENVIRONMENT

Although AgNPs may occur naturally, but their over exploitation has played a significant role behind its growing concentration in the environment. AgNPs are one of the most exploited nanoparticles due to its unique anti-microbial potential. Their increasing production with the current rate of 500 ton per year, and widespread industrial uses have raised the chances of environmental contamination in general, and the aquatic environment, the inevitable destination, in particular (Ihtisham *et al.,* 2021).

AgNPs are used in nano-coating of textiles, personal care products, medical devices, food containers, cosmetic products, paints and nano-functionalised plastics etc. (McGillicuddy *et al.,* 2017; Rai and Biswas, 2018; Biswas *et al.,* 2024). Their broad-spectrum antimicrobial nature can make it harmful for non-target soil and aquatic living organisms. Release of AgNPs into the environment can take place through direct or indirect mode. Direct release includes aerial deposition, sewage discharges from industrial facilities and household areas into water bodies, accidental spills during manufacturing and transport etc. and indirect release are discharges from organic, inorganic fertilizers and engineered plant growth substances used as fertilizer substitutes. In both the cases AgNPs can be accumulated in soil and underground water. Despite these facts, AgNPs are still used in more than 250 consumer products around the world. Globally, the AgNPs market size has expanded from $2.20 million to $2.42 million within 2021-2022 time periods, with growth rate of approximately 16% anticipated for the period of 2023-2028 (Noori *et al.,* 2024). They ultimately enter water bodies through sewage discharge, atmospheric dry and wet deposition, and waste leachate (Forstner *et al.,* 2020).

The generation of AgNPs in United States is reported to be up to 2,500 ton per year from which approximately 150 ton is released in sewage sludge and 80 ton in surface waters (Tripathi *et al.*, 2017). In terms of their concentrations in different environmental sectors, studies have reported presence of AgNPs (in European Union) in sediments ranging from 0.19 to 470.6 μg/kg and in surface water 0.03-2.79 ng/L (Giese *et al.*, 2018). Dumont *et al*. (2015) reported the AgNPs concentrations of 0.3 ng/L in rivers in large southern European cities and Xiao *et al*. (2019) reported that silver nanoparticles concentrations in Taihu Lake reached 0.77 ng/L.

The concentrations of AgNPs throughout Waste Water Treatment Plants were estimated to be in the range of μg/L to ng/L, and most of their sizes belonged to below 100 nm category (Cervantes-Avilés *et al.,* 2019). The present day predicted environmental concentrations of AgNPs in water might reach levels in the μg/L range and, in some cases, even mg/L range (Wang *et al.,* 2022). These estimates underscore the potential presence and implications of AgNPs in aquatic ecosystems.

FATE OF SILVER NANOPARTICLES IN THE ENVIRONMENT

As the aquatic ecosystem is inevitably the final destination for AgNPs, their fate is influenced by factors like water salinity, dissolved organic matter, and the presence of other ions. AgNPs can stay in suspension or undergo dissolution, oxidation, and adsorption upon entering in aquatic environment. In freshwater, AgNPs tend to remain stable due to dissolved organic matter, while in highly saline waters, they tend to aggregate and dissolve more readily (Li *et al.,* 2020).

In natural water AgNPs may be transformed through several ways which include oxidative dissolution, chlorination, and sulfidation. The silver ions (Ag^+) released from AgNPs can also be reduced under certain conditions to produce new silver nanoparticles (Zhang *et al.,* 2018). Zhang *et al.* (2016) in a study outlined that the physico-chemical behaviour of AgNPs in the aquatic environment is of complex nature and there remains a knowledge gap regarding the kinetics of nanoparticles dissolution. They also mentioned that the assumptions regarding Gibbs free energy of formation of AgNPs in aquatic ecosystems and release of silver ions (Ag^+) through dissolution of AgNPs doesn't fully describe their actual fate. The transformation of AgNPs in wastewater is comparatively homogenous, because most of the silver nanoparticles enter the wastewater streams after being transformed into sulfides (Zhao *et al.,* 2021).

TOXICITY OF SILVER NANOPARTICLES IN FISH

Silver nanoparticles act as a double-edged sword with both beneficial and harmful effects. They are known for their broad-spectrum efficacy as anti-microbial agent, but concern about their safety is raised when non-target organisms are subjected to its increasing concentrations (Liao *et al.,* 2019; Ghosh *et al.,* 2025). Once AgNPs enter into aquatic environment, they usually oxidize into silver ions (Ag^+), which are significantly toxic to aquatic organisms such as fish. (Galazzi *et al.,* 2019). Determining the toxic effect of AgNPs on fish is crucial for assessing their ecological impact. Numerous studies have documented the toxicity of AgNPs in fish at various levels, including haematological, biochemical, and histological. Exposure to silver nanoparticles can cause a range of adverse effects in fish, affecting their blood composition, metabolic processes, and tissue structure (Rajkumar *et al.*, 2016).

Fishes can be exposed to AgNPs through various routes, including, waterborne exposure, and dietary exposure (Fig. 1). Uptake of AgNPs by fish can take place through dietary exposure to algae, weeds, insects, and worms, which are also capable of accumulating silver nanoparticles (Dube and Okuthe, 2023). Uptake can also take place through drinking water. Waterborne exposure occurs when fish are directly exposed to AgNPs in the water column (Mona *et al.*, 2023). Kleiven *et al.* (2018) reported that *Salmo salar* gills accumulated high concentration of silver than the gastrointestinal tract after waterborne exposure to AgNPs, however, dietary exposure resulted into the opposite scenario.

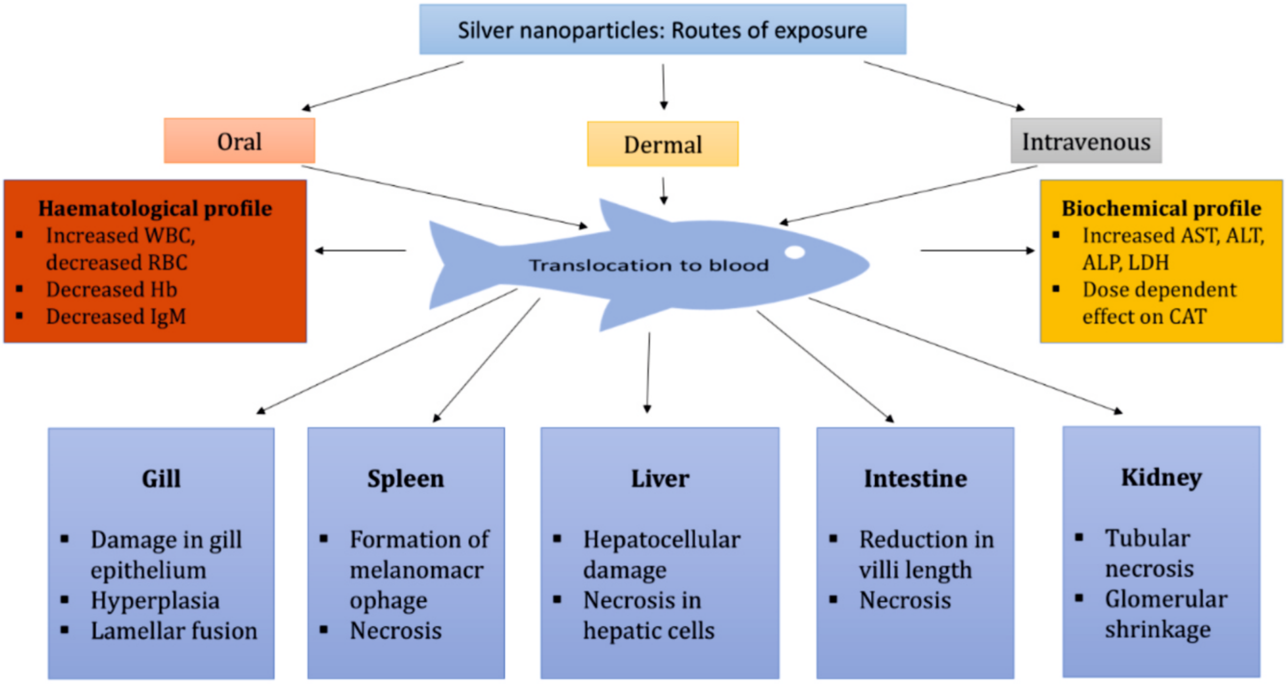

Fig. 1: Routes of exposure and toxic effects of silver nanoparticles on fish.

The toxicity of silver nanoparticles in fishes at different levels is elaborated below:

1. Toxicity of silver nanoparticles in fish at haematological level

Once inside a cell, AgNPs interact with various intracellular components, impacting cellular processes and potentially leading to toxicity (Djanaguiraman *et al.*, 2024). A study reported that AgNPs exhibited a number of toxic effects on common carp *Cyprinus carpio* upon exposure. The red blood cells (RBCs) and white blood cells (WBCs) count differed significantly in respect to the control (Vali *et al.*, 2020). Rajkumar *et al.* (2016) reported significant alteration in red blood cells, white blood cells, haemoglobin (Hb) and total protein level in *Labeo rohita* upon exposure to AgNPs. In another study exposure to AgNPs caused decrease in blood red blood cells, white blood cells, haemoglobin level and serum total protein level in *Oreochromis niloticus* (Ibrahim, 2020). In the adult common molly (*Poecilia sphenops*), exposure to AgNPs resulted in decreased red blood cell and white blood cell level while, serum total protein concentration increased (Vali *et al.*, 2022).

Another study on AgNPs toxicity in rainbow trout (*Oncorhynchus mykiss*) showed similar alteration in red blood cells (RBCs), white blood cells (WBCs) and haemoglobin levels in the treated group (Shabrangharehdasht *et al.*, 2020). Joo *et al.* (2018) conducted a detailed study on toxicity of AgNPs on *Oncorhynchus mykiss*. The results indicated a concentration-dependent increase in thrombocyte, monocyte, and large lymphocyte and decrease in white blood cell (neutrophils and lymphocytes) count in the AgNPs treatments.

Studies have reported that AgNPs can cause morphological changes in red blood cells and induce damage to hematopoietic tissue leading to negative effect to red blood cell count. Upon entering the bloodstream, AgNPs can generate reactive oxygen species within the red blood cell leading to lipid peroxidation, protein oxidation, and nucleic acid damage, which ultimately compromises the cell's structure and function (Vali *et al.,* 2022). Studies have reported that these nanoparticles can trigger the immune system in fishes, which is represented as increased white blood cell count (Bantu *et al.,* 2017). They can also attach to haemoglobin molecule, specifically to the heme, tryptophan and amide residues, potentially disrupting its structure and function. This interaction can lead to a conformational change in haemoglobin, potentially causing it to unfold. Furthermore, AgNPs can facilitate electron transfer, forming a charge-transfer complex with haemoglobin-heme, which can contribute to oxidation. The interaction is dose and time-dependent (Qian *et al.,* 2015).

2. Toxicity of silver nanoparticles in fish at histological level

Silver nanoparticles cause histological damage in fish tissues, including the gills, liver, and muscles, leading to various alterations like hyperplasia, necrosis, and vacuolization (Ostaszewska *et al.,* 2016). A study reported histological changes in gill tissues of African catfish (*Clarias garepinus*) treated with silver nanoparticles. The histopathological changes observed were aneurism, epithelium lifting, subepithelial edema and hyperplasia of interlamellar epithelium, curling of secondary lamellae, necrosis of epithelial cell, hypertrophy and proliferation of erythrocytes, bifurcation and fusion of filament, increase in number and size of mucous cells in gill tissues (Sayed *et al.,* 2020).

Rajkumar *et al.* (2016) in his study indicated that the AgNPs-treated *Labeo rohita* fish groups exhibited significant alterations in gill tissues, which include proliferation of bronchial chloride cells ultimately leading to lamellae fusion and formation of localized aneurism. The aneurism increases risk of rupture, which can result in severe haemorrhage and other complications or death. Another study reported alterations of gill tissues in AgNPs treated *Cyprinus carpio*. Alteration in the sense of damage, atrophy, shortening of secondary lamella, degeneration, and necrosis at different concentrations of AgNPs were reported (Kakakhel *et al.,* 2021). This study also clearly observed histological alterations in intestinal tissues, which include some, shaded intestinal villi mucosal epithelial cells and missing of small number of epithelial cells.

Another study demonstrated the formation of hyperplasia, edema and lifting of the gill epithelium, and lamellar fusion of the gills, and hemosiderosis, hemorrhage, hydropic swelling, and pyknotic nuclei in the liver of *Carassius auratus* as a result of silver nanoparticles toxicity (Abarghoei *et al.,* 2016). Naguib *et al.* (2020) reported histopathological changes in liver of *Clarias gariepinus* treated with AgNPs, which include proliferation of hepatocytes, pyknotic nuclei, infiltrations of inflammatory cells, melanomacrophages aggregation, cytoplasmic vacuolation, dilation in the blood vessel, hepatic necrosis, rupture of the wall of the central vein, and formation of apoptotic cells in the liver. Another study reported damage, necrosis and cell lysis in intestinal villi of *Cyprinus carpio* treated with AgNPs (Kakakhel *et al.,* 2021). AgNPs also caused nephrotoxic effect in zebrafish (*Danio rerio*). The study recorded tubular necrosis, cell damage, loss of epithelial lining, glomerular shrinkage in the kidney of the treated fishes, which is evidence of direct cytotoxic effect of AgNPs (Okuthe and Siguba, 2025).

3. Toxicity of silver nanoparticles in fish at biochemical level

Silver nanoparticles can significantly impact the biochemical processes in fish, leading to various adverse effects like oxidative stress, enzyme alterations, and changes in immune function. They can interfere with metabolic pathways, impair enzymatic activities, and disrupt the balance of antioxidants, potentially leading to tissue damage and reduced fish health. Rajkumar *et al.* (2016) reported that tissue-damaging enzymes like acid phosphatase (ACP) and alkaline phosphatase (ALP) levels were significantly higher in the silver nanoparticles-treated fish tissues of gill, liver and muscle when compared with control tissues of *Labeo rohita*.

Another AgNPs toxicity study on *Oreochromis mossambicus* reported increased activity of serum enzymes aspartate transaminase (AST), alanine transaminase (ALT), acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in treated group compared to control (Sivan *et al.,* 2024). Vali *et al.* (2020) observed decreased albumin and globulin level and escalated serum glucose, cortisol, alanine transaminase, and alkaline phosphatase level in AgNPs treated *Cyprinus carpio*. The study also reported enhanced catalase activity in the treated fish serum.

Hypophthalmichthys molitrix when treated with AgNPs, showed significantly higher activities of serum alanine transaminase (ALT), aspartate transaminase, lactate dehydrogenase, white blood cells, acetylcholinesterase and catalase (Younas *et al.,* 2022). Sivan *et al.* (2024) reported increased serum aspartate transaminase, alanine transaminase, acid phosphatase, alkaline phosphatase and lactate dehydrogenase activity in *Oreochromis mossambicus* treated with AgNPs, while antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase activity decreased.

FACTORS AFFECTING THE TOXICITY OF SILVER NANOPARTICLES

The cytotoxicity of AgNPs is influenced by several factors, including nanoparticle size, concentration, surface chemistry, and cell type. The size of AgNPs has a significant impact on their toxicity, influencing cell viability, lactate dehydrogenase activity, and reactive oxygen species generation. Smaller silver nanoparticles often exhibit higher toxicity compared to larger ones, likely due to factors like increased surface area and reactivity, which affects their cellular uptake and interaction (Menichetti *et al.,* 2023).

It is well-known that surface area, volume ratio, and surface reactivity all are significantly influenced by particle size. As particles become smaller, the surface area to volume ratio increases drastically, resulting in larger proportion of surface atoms and higher potential for surface interactions and reactivity (Joudeh and Linke, 2022). Properties like sedimentation velocity, mass diffusivity, attachment efficiency, and deposition velocity are significantly influenced by particle size. Smaller particles generally have higher diffusivity and lower sedimentation rates, while larger particles exhibit the opposite. The size dependency influences nanoparticles interactions with biological and solid surfaces, affecting their deposition, attachment, and overall behaviour (Yusuf *et al.,* 2024). Zhang *et al.* (2020) reported the differences between neurotoxic effects of 20nm and 70nm AgNPs. The results revealed that 20 nm and 70 nm AgNPs significantly reduce neuronal cell viability, with the smaller AgNPs exerting stronger toxic effects than the larger ones. Another study by Zhang *et al.* (2018) reported that the smaller size (10 nm) AgNPs caused higher toxicity in *Azotobacter vinelandii* than the larger (50 nm) ones.

The surface chemistry of AgNPs significantly impacts their interaction with cells and can be modified through surface coatings to enhance their properties. AgNPs in conjugation with specific molecules can enable novel functions, improve colloidal stability, and influence the way they interact with cells (Umapathi *et al.,* 2022). Surface coatings on AgNPs influence their shape, aggregation and dissolution rate, which in turn affect their toxicity (Fahmy *et al.,* 2019). AgNPs induced cytotoxicity is influenced by several factors like coating materials, size, aggregation, and dissolution ratio, leading to variations in the mechanisms and extent of toxicity. The core mechanisms of AgNPs influenced toxicity involves reactive oxygen species generation, depletion of antioxidant defence systems, and mitochondrial dysfunction, all of which can be altered by surface coating (Noga *et al.,* 2023).

Polyvinylpyrrolidone-coated AgNPs are more stable than citrate-coated counterparts in OECD recommended media, especially in presence of chloride. Citrate coatings initially help stabilize silver nanoparticles and reduce their toxicity, but they are destroyed upon drying the nanoparticles into a powder, leaving it in a pristine state that still causes significant cytotoxicity upon re-dissolution. In contrast, polyvinylpyrrolidone -coated AgNPs retain good stability and have negligible toxic effects on HaCaT keratinocytes when the dried powder is re-dissolved (Tejamaya *et al.,* 2012). However, previous studies reported that uncoated AgNPs significantly decrease cell viability in a time-and dose-dependent manner, and coating is used to provide protection against cytotoxicity (Akter *et al.,* 2018).

CONCLUSION

Widespread applications of AgNPs have resulted in its unintentional release into the environment. Silver nanoparticles in different environmental sectors inevitably find its way into aquatic environment. Entering into aquatic environment they undergo various trans-formations, which alter its toxicity. Toxicity of AgNPs also depends on its particle size or surface chemistry. It is very important to understand the toxic effects of AgNPs in fishes, because it outlines the potential harmful effect of AgNPs in vertebrates. Also, fish is an important source of food for humans. So, harmful effect of silver nanoparticles in fishes can pose direct serious threat to human beings. Proper characterization of AgNPs is needed to define its size, surface morphology etc., which in turn will help in determining its toxic effect. Furthermore, extensive research is needed to earn mechanistic insights into the toxicity of AgNPs.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest relating to this article or its content.

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