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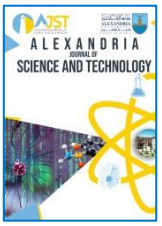

Review

Potential health risk effects of silver nanoparticles on aquatic ecosystem: Regulations and guidelines

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ABSTRACT: The increased production and application of silver nanoparticles (AgNPs) raise concerns about environmental exposure and potential health effects. This systematic review article provides a comprehensive overview of the current knowledge regarding impacts of AgNPs, applications, as well as their environmental impacts to aquatic ecosystem. They also highlight the various regulations or guidelines for testing and assessing the risks of AgNPs that showed long-term adverse toxic effects on aquatic organisms and ecosystems. Long-term health effects of exposure to AgNPs are unclear and required further studies for controlling these risks. More studies are needed to investigate bioaccumulation and trophic transfer of AgNPs in natural aquatic ecosystems as well as induced cytotoxicity. The collected data in this review article will help developing regulations and guidelines to limit release of AgNPs into environment to protect marine ecosystems from potential risks and to develop strategies limited related health hazardous.

1. INTRODCTION

AgNPs are a subject of extensive research and scrutiny within the scientific and regulatory communities. However, increasing NPs use has raised concerns about potential toxicity to living organisms and environment. The field of nanotechnology is rapidly evolving, and new information about the risks and benefits of NPs is constantly emerging. One of the key areas of concern surrounding NPs is the potential impact on human health. Among all NPs, AgNPs are the most widely used NPs [1]; well commercialized worldwide [2, 3]. AgNPs are nanoscale zero charge (Ag^0) particles exhibited unique physicochemical properties and biological activity and are highly desirable in various fields, including biomedicine and industrial applications.

Surprisingly, in a list of 1015 NPs containing products present on the markets, 259 products contain AgNPs [4]. In 2023, AgNPs were used in 331 different product types in 42 countries and 15 industries, with a global production of 700 tons and a continuing growth trend [5]. The Organization for Economic Co-operation and Development (OECD) has highlighted AgNPs as high interest due to widespread applications in several daily

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products and inherent properties [6]. Over the years, AgNPs have emerged as one of the best metal NPs due to their large surface-to-volume ratio and success and efficiency in combating various pathogens [7]. These NPs are considered ecofriendly alternatives to conventional Ag compounds such as $AgNO_3$, as they offer numerous advantages such as high stability, enhanced antimicrobial activity and low toxicity.

Applications of AgNPs

In recent years AgNPs have gained significant attention due to their unique physicochemical properties such as their high surface area, strong antimicrobial activity, high electrical conductivity, excellent thermal stability and potential applications in various fields [8-11]. AgNPs had numerous industrial applications, some of which will be illustrated.

Antimicrobial agent due to impeccable ability as an antibacterial, antifungal, and antiviral agent, are effective against a broad spectrum of bacteria, viruses, and fungi [12, 13]. AgNPs kills all tested microorganisms. I*n vitro* antimicrobial tests performed against different bacterial strains such as *S. progenies*, *K. pneumonia*, *P. mirabilis*, *E. fecalis*, *S. pneumoniae*, *Aeruginosa*, *E. coli* and *S. aureusand* their efficiency against bacteria exceeded standard antibiotic in terms of inhibition zones and minimum inhibitory concentration (MIC). High antimicrobial activity due to participation on demolition bacterial cell process, permeation lipid layers and penetrate cell membrane, form H.B. with active centers of cell constituents and perturbing respiration. Uptake rate or entrance into cell and kill microorganism. Protective agent increases lipophilicity and penetration rate to cell membrane. AgNPs activity against different microorganisms depends either on impermeability of cells membranes and differences in cells ribosome of microbes. By lipophilicity, AgNPs penetrate lipid layer of cell membrane, binding and convert super coiled DNA strands into open circular DNA [1].

Recently, AgNPs have been employed in the livestock, poultry industries and aquaculture to combat pathogens [7]. Interestingly, one of the nanomaterials most used in water treatment are AgNPs disinfection [8].

Biomedicine and biomarkers: AgNPs have shown great potential in the field of biomedicine [14]. They exhibit excellent antimicrobial activity against a broad spectrum of microorganisms, including bacteria, fungi, and viruses. This property makes enabled application for wound dressings, surgical instruments, and drug delivery systems. AgNPs are used in a variety of medical devices, such as catheters, implants, and surgical instruments and in hand sanitizers, disinfectants, and wound dressings. Additionally, AgNPs have been investigated for their potential in cancer therapy, as they can selectively target cancer cells while sparing healthy cells [15, 16].

AgNPs is the effective antimicrobial agent treating various wound infections. Although there are many studies reporting Ag(I) ion as antimicrobial agent and low antimicrobial resistance, high concentrations causes' severe cytotoxicity was mitigated by addition of secondary ion or chemical with keeping antimicrobial activity. Zn(II) ions coupled to AgNPs effectively promotes osteogenic functions by enhancing cell proliferation, differentiation and osteoblast marker gene expression due to structural similarity to many proteins. AgNPs are commonly combined with OH-apatite in biocompatible osteoconductive scaffold materials. TiO_2NPs and Ag-co-substituted nano-OH-apatite (HA)/polyamide-66 composite scaffolds materials *in vivo* in a rabbit model of experimental osteomyelitis. Scaffold: were potent antibacterial activities against both *E. coli* and *S. aureus* bacterial cells and supported pre-osteoblastic cell proliferation; biocompatible less toxic. Mg co-substituted with Ag and HA to enhance bone re-sorption and new bone tissue generation process. Mg reduced AgNPs toxicity and increasing cell viability. The antibacterial biocompatibility strontium (Sr) co-substituted in Ag-HA coated bone implants. Sr enhanced (pre-osteoblastic cell proliferation and bone formation, number of bone-forming sites and bone mineral density and inhibited osteoclast activity. Cu and B improved biological activity [17]. Toxicity: depends on: (inversely proportional to particle size, surface chemistry, coating agent and bulk chemical composition; limited clinical applications. AgNPs cytotoxic to osteoblasts (depends on the dose by increasing oxidative stress) and osteoclasts, damaging DNA and negatively impact biocompatibility of orthopedic implants. Beyond the musculoskeletal system, safety of AgNPs decreased by its ability to bypass normal host phagocytic defense and reach blood and brain [17].

Osteomyelitis clinical therapies trials recommended AgNPs filling bone voids after resections and facial reconstruction, re-infection prophylaxis, and treatment of chronic syndromes such as osteoarthritis. Quantum dot particle size AgNPs (Å scale) treated osteomyelitis). Obstacles are: No clinical trials listed on National Institute of Health (NIH) Clinical Trials website about nanotechnology treating osteomyelitis and difficult differentiation between chronic and acute syndromes. In trial examining pain reduction, AgNPs were used in conjunction with standard of care, $Ca(OH)_2$ in patients that have necrotic pulp after a root canal. AgNPs-traditional $Ca(OH)_2$ paste combination decreased pain than native $Ca(OH)_2$ paste. AgNPs decreased pain and infection of necrotic root, by antimicrobial molecular mechanism [19]. Trial of synthetic bone graft was compared to an autologous bone graft for patients with degenerative disk disease or grade-3 spondylolisthesis, all participants received posterolateral spinal fusions, with an autologous graft on left side and Nano Bone being used on right. Patients are being evaluated with spinal CT scans at one year postfusion surgery. Osteomyelitis treatment was not aimed. This product was nanocrystalline structure osteoconductive scaffold could potentially be appealing in future as a conduit for enhancement postsurgery bone [19].

TEM micrographs showed particle size of AgNPs and AgNPs@activated carbon (AC) composite showed AgNPs adhered to AC matrix. AgNPs (control sample was prepared by photoreduction of $AgNO_3$ by acetic acid. Well-dispersed spherically shaped non aggregated or agglomerated NPs. Quantum dots particles size below 21 nm homogeneously distributed at regular inter-particle distance and loaded and incorporated homogenously and well dispersed onto AC matrix, (**Figure 1**) [1].

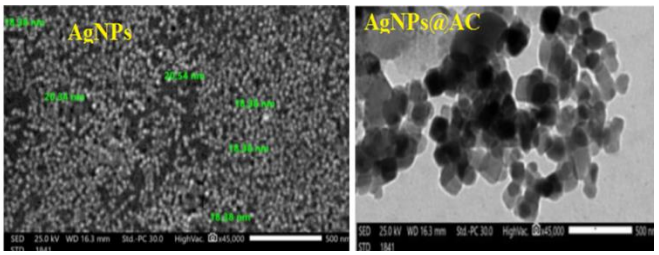

Figure 1. TEM micrographs (Adapted with CCBY 4.0 permission, Ref. [1], Copyright 2021 Taylor&Francis).

Particle size distributions of AgNPs and Ag@AC composite by were determined by dynamic light scattering, (**Figure 2**). Two peaks around 10, and 80 nm signified particle size distribution of AgNPs and AgNPs@AC respectively. The increment from particle size distribution of AgNPs to that of composite reflected the successful loading of well AgNPs dispersed on AC matrix.

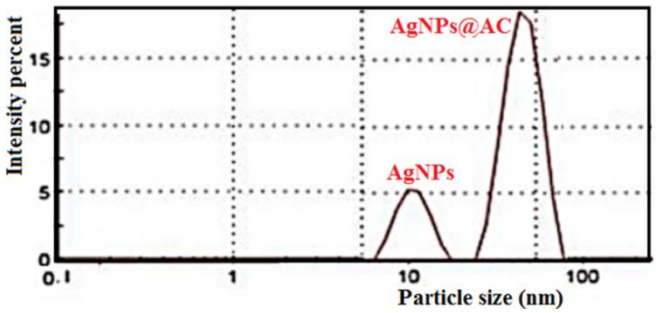

Figure 2. Particle size distribution of AgNPs and AgNPs@AC composit (Adapted with CCBY 4.0 permission, Ref. [1], Copyright 2021 Taylor&Francis).

AgNPs were effective electro catalyst for oxygen reduction reaction in microbial fuel cells [1], solar cells, batteries and chemical sensors for heavy metals environmental pollutants. Composites AgNPs and AgNPs@AC have particle sizes of 7 nm and 1000 nm, respectively. The composite was good antibacterial agent to VERO *(ATCC ccl-81)* cells. Zeta potential -25 mV confirmed thermodynamic stability against coagulation. For many Gram-positive and Gram-negative bacteria. The composite was strong antibacterial, 75.72% anti-inflammatory effect at 500 μgmL^{-1}. The maximum non-toxic concentration 78.125 gmL^{-1} corresponds to antiviral activity 96.7% against hepatitis A. virus. It is a candidate for pharmaceutical formulations such as anti-COVID N95 masks. At 160 μgmL^{-1}, antioxidant activity was 42.74% [1].

Localized surface plasmon resonance give AgNPs unique optical properties such as many applications in optical devices such as surface-enhanced Raman spectrophotometer, AgNPs was utilized for curing infections, wound mending& rapid healing due to antimicrobial effect and efficacy against antibiotics resistant bacteria. AgNPs killing above six hundreds pathogenic infections bacteria. AgNPs has high antibacterial activity to *Staphylococcus aureus*, Corticium *salmonicolor* and *Escherichia coli*. Inhibition efficiency for *S.aureus* 99.9% at 5 ppm. The effective dose (ED_{50}) for *C. salmon color* was 27.2 ppm. AgNPs@AC composite was potent antibacterial against many pathogenic Gram positive and Gram-negative bacteria. Concentration 60 μgmL^{-1} inhibited all tested microbial pathogens except *Bacillus subtilus* (ATCC 6633) and *Candidia albicans* (ATCC 10231). The highest sensitivity to 100 μgmL^{-1} was shown by *Enterococcus Fecalis* (ATCC 29212) and *Pseudomomas aeroginosa* (ATCC 9027). Inhibition zones in (**Figure 3**) (relative to standard antibiotic) showed potent performance of AgNPs@AC composite than AgNPs. This composite was recommended for combating bacterial plant diseases in preference than many synthetic bactericides [1].

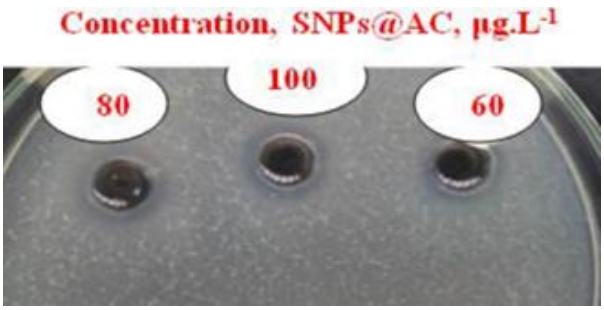

Figure 3. Antagonistic effects of concentrations AgNPs against Enterococcus Fecalis (ATCC 29212) [1].

AgNPs inhibited biosynthesis of bacterial cell, binding protein and lipid macromolecules of cell wall and stimulating expression cytokines by intestinal epithelial cells. AgNPs@AC: anti-inflammatory *in vivo via* inhibition denaturation of tissue proteins (a documented causes of inflammatory and arthritic diseases *via* production auto antigens). The composite was recommended for developing anti-inflammatory therapy. Concentrations: 100, 300, and 500 μgmL^{-1} inhibited denaturation bovine serum albumin protein with 75.72% protection efficiency at 500 μgmL^{-1}. Composite reduced inflammation in eosinophilic chronic rhinosinusitis mouse model, (**Figure 4**) [1].

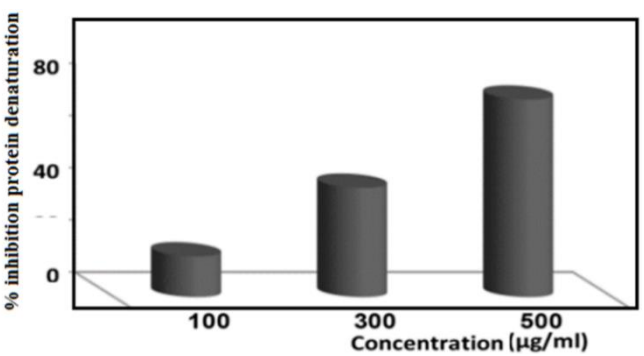

Figure 4. Inhibition of AgNPs@AC to protein denaturation (Adapted with CCBY 4.0 permission, Ref. [1], Copyright 2021 Taylor&Francis).

Composite cytotoxicity on VERO cell line initiated in kidney tissue derived from normal, adult African green monkey. Maximum non-toxic concentration (MNTC) 78.125 μgmL^{-1} used for antiviral assay against HAV gave 96.666% antiviral

activity (Table 1) and (**Figure 5**), Adapted with CCBY 4.0 permission [1], Copyright 2021 Taylor&Francis). Hence, composite have good preventative and therapeutic characteristics that effectively reduce viral infection, probably by blocking cell-virus interaction depends on particle size and zeta potential.

A low 78.125 µgmL^{-1} composite concentration inhibited HAV (10^5 cells/200µL) by 96.67%. HAV activity decreased to 3.33%. Antiviral activity was evaluated, excluding all side effects on host VERO cells to only clarify effect of safe concentration and subsequently non-toxic concentration to ensure virus viability because any toxic concentration was undetected. Composite showed Antiviral cytotoxicity on VERO cell line and MNTC evaluated antiviral activity. The highest concentration was avoided to prevent toxicity to host cells. (**Figure 5**) explained composite cytotoxicity and safe concentration for antiviral activity [1].

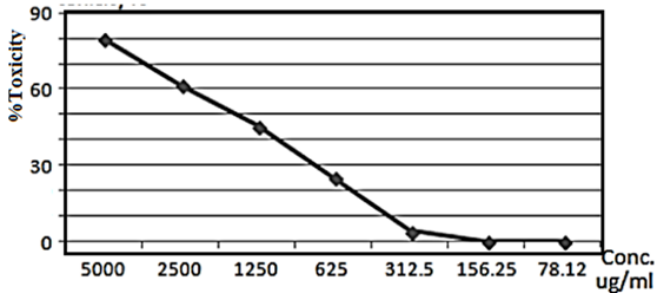

Figure 5. Effect of composite on toxicity of VERO cells (Adapted with CCBY 4.0 permission, Ref. [1], Copyright 2021 Taylor&Francis)..

Electronics: AgNPs are used in a variety of electronic devices, such as solar cells, batteries, heat dissipation, photography, sensors. High electrical conductivity and thermal stability enabled ideal applications Inkjet inks contain dispersed AgNPs in optical electronic circuits [11].

Heterogeneous catalysis AgNPs are used as effective catalyst in a variety of chemical reactions, especially in reduction of organic compounds [10, 11]. Hydrogenations and oxidations reactions of unsaturated hydrocarbons, bond formation of C-C, C-S, C-N, photochemical reactions, organo synthesis of chiral compounds, and cycloaddition reactions. Quantum dot size decreasing treatment dose in environmental and medicinal applications [5]. Various hazard free, eco-friendly synthesis methods of AgNPs include chemical, gamma-irradiation, thermal decomposition, photochemical and biosynthesis techniques. Two approaches stabilized AgNPs against coagulation. Capping by chemical stabilizers such as cyclodextrin or deposition on large surface area substrates such as TiO_2 powders [10].

Textiles: AgNPs are used in textiles (clothing, bedding, and other textiles to impart antimicrobial and odor-resistant properties [20].

Food packaging: AgNPs are used in food packaging as active packaging materials in the food industry to extend the shelf life of food products by inhibiting growth of spoilage-causing microorganisms [21]. AgNP-containing food packaging has been shown to be effective against a wide range of foodborne pathogens such as *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*. AgNPs prevented biofilms formation on food contact surfaces, reducing the risk of foodborne illnesses [22. 23].

1.2 Environmental behavior of AgNPs

Excessive discharge of AgNPs, whether from feeds or pollutants, poses hazards to edible crop plants in soils. Concentrations 100, 300 and 500 ppm AgNPs on *Lupinustermis* L. stress (growth, lipid peroxidation, total antioxidants, proline, total protein, carbohydrate, polyphenols and seedling leaf ultrastructure. High concentration inhibited, while low concentration (100ppm) stimulated seed germination which directly proportional to the growth parameters. Transmission electron micrographs showed that high concentration (500 ppm) accumulated in leaves, impact chlorophyll (decreased content and altered chloroplast structure and consequently, protein and carbohydrate metabolism. High concentration (in contrary to low concentration) caused generation of reactive oxygen species which increase total phenolic compound, proline, lipid peroxidation and H_2O_2. AgNPs toxicity was concentration dependent, and it was beneficial below 100 ppm [24].

The widespread use of AgNPs in various consumer products raises concerns about their potential accumulation in the environment, especially considering the estimated annual global AgNPs production [25] damaging ecosystems balance and representing a threat to human health [26]. AgNPs are the most producing NPs and released extensively to both aquatic and terrestrial environments [27]. It is important to carefully assess the potential AgNPs risks and benefits before releasing them into aquatic environments because in addition to their several beneficial effects, negative impacts on aquatic organisms and ecosystem function have been reported [26, 27].

However, AgNPs have emerged as a potential threat to aquatic ecosystems and the fauna within them [28-30]. Aquatic ecosystems are considered as one of the most vulnerable to contamination due to their ability to receive and accumulate large amounts of pollutants, including nanomaterials, from different sources [31]. It has been hypothesized and suggested that AgNPs concentration of in an aquatic ecosystem in µg or mg L^{-1} are yet toxic to any environment [32]. It has been reported that mutable properties and the small particle size AgNPs are toxic and dangerous to the ecosystems [33]. AgNPs environmental behavior is influenced by their physical properties, environmental transformations, and environmental conditions [26]. Key factors include particle size, capping agents, dissolution, adsorption, and aggregation. Dissolution is a critical process that affects bioavailability and ecotoxicity, and is influenced by factors such as pH, ionic strength, and natural organic matter. The mode of action of AgNPs is still not fully understood, and eco-toxicity data showed low repeatability and reproducibility, particularly for microorganisms, algae, and cell lines. However, it is essential to carefully evaluate their potential environmental impacts to ensure sustainable and responsible use [34-36].

Table 1: Effect of composite against HAV on VERO cell line

Test	Conc (µg.mL^{-1})	Mean O.D.	Viability	Toxicity	% Viral activity	%Antiviral
Control	0.00	0.223	100.0	0.00	0.00	0.00
HAV	0.00	0.103	46.2	53.811	100	0.00
Composite	78.13	0.219	98.2	1.793	3.333	96.67

Toxicity Mechanism of AgNPs

Although AgNPs toxicity mechanism is still uncertain, numerous studies have confirmed the Trojan-horse mechanism as responsible for AgNPs toxicity [37-40] which potentially leads to cellular autophagy, apoptosis or necrosis.AgNPs toxicity may partly was proposed to follow Trojan-horse type mechanisms. This means that AgNPs uptaked up by the cell as particles and dissolve inside the cell causing a high local concentration of Ag(I) ion, however there may also be an external dissolution of AgNPs with subsequent Ag(I) ion-related toxicity. Hence, it seems that both particulate and ion-based mechanism of action may be at play, as further discussed by other authors [41-43].

Ag is found in four different oxidation states (0, +1Ag, +2 and +3) in the aquatic environment [33, 34]. Though the exact estimates of AgNPs toxicity are still hindered due to lack of acquaintance [28]. It has been proved that the Ag NPs are not much stable and able to be dissolved in aqueous solutions, so it releases Ag+ progressively [44, 45]. It has been accepted that the toxicity caused by AgNPs is due to the release of Ag (I) ion into the environment that was absorbed by living organisms [46]. AgNPs exhibited toxicity to aquatic organisms, with effects mediated by dissolved Ag(I) ions or direct interaction with cells [26, 47].

The potential long-term effects of AgNPs exposure on aquatic ecosystems

The potential long-term effects of AgNPs exposure on aquatic ecosystems are a growing concern. However, the release of AgNPs into the environment can have long-term effects on aquatic organisms [48]. 2013). NPs can be taken up by organisms through ingestion, dermal contact, or gill uptake. AgNPs have been shown to be toxic to a wide range of aquatic organisms, including phytoplankton, zooplankton, invertebrates, and fish [49].

Toxicity: Once inside an organism, NPs can cause a variety of toxic effects, including oxidative stress, inflammation, genotoxicity, and developmental abnormalities [49].

Oxidative stress: Oxidative stress is a major mechanism of AgNPs toxicity that has been mostly attributed to the generation of reactive oxygen species (ROS) in cells [50, 51] through various mechanisms, including the release of Ag ions, the interaction with biomolecules, and the activation of cellular signaling pathways. ROS can cause oxidative damage to lipids, proteins, and DNA, leading to cellular dysfunction and death [52]. AgNPs induce lipid peroxidation, which is the oxidative damage of lipids. Lipid peroxidation damage cell membranes and lead to cell death. In addition, AgNPs caused protein oxidation, which can alter protein structure and function. Protein oxidation can lead to the loss of enzyme activity and other protein functions. AgNPs generated reactive ROS through several mechanisms: Direct interaction with molecular oxygen to form ROS, such as superoxide anions and hydroxyl radicals. Indirect generation *via* induce production of ROS by activating various cellular signaling pathways, such as NADPH oxidase pathway and mitochondrial electron transport chain. AgNPs soluble in biological fluids to release Ag(I) ion reacted with biomolecules to generate ROS [52].

Inflammation: AgNPs can activate the inflammatory response in aquatic organisms by stimulating production of pro-inflammatory cytokines and chemokines [10, 11, 53]. Inflammation is a complex process that involves the recruitment of immune cells and the release of inflammatory mediators. Oxidative stress can activate inflammatory signaling pathways and lead to production of inflammatory mediators [54]. Inflammation is a normal response to injury or infection, but chronic inflammation can contribute to a variety of diseases, such as cancer, cardiovascular disease, and arthritis. Ag NPs can impair the immune function of aquatic organisms, making them more susceptible to infections and diseases. This can lead to increased mortality and reduced productivity of marine invertebrate populations [54, 55].

Genotoxicity: induced DNA damage such as DNA strand breaks, DNA base modifications, DNA adducts, and chromosomal aberrations [57]. DNA damage can lead to mutations which can increase the risk of cancer and other genetic diseases [58]. AgNPs interfere with the normal development of aquatic organisms, Developmental causing a variety of abnormalities, such as impaired larval growth, developmental delays, and morphological defects [57]. These effects can have significant implications for the survival and reproductive success of exposed organisms [59]. AgNPs toxicity to aquatic organisms is a complex issue that depends on a variety of factors:

- **Physicochemical properties**: AgNPs: particle size, shape, surface charge, and composition all influence their toxicity. For example, smaller AgNPs are generally more toxic than larger ones. Quantum dot AgNPs are more likely to induce oxidative stress than larger particles. Surface charge: Positively charged AgNPs are more likely to induce oxidative stress than negatively charged particles [2, 3, and 37]. Presence of a surface coating on AgNPs reduce induced oxidative stress.
- Exposure concentration: is also a factor inducing of oxidative stress [28, 50, 51, 54, 58].
- Exposure route: AgNPs can be taken up by aquatic organisms through various routes, including ingestion, inhalation, and dermal contact. Exposure route affect distribution and toxicity of AgNPs in the organism [3, 26].
- Specific characteristics of the exposed organism: The sensitivity of marine organism invertebrates to AgPs can vary depending on their species, life stage, and

physiological condition. For example, larvae and juveniles are often more sensitive to Ag NPs than adults, and organisms that live in polluted environments may be more tolerant to Ag NPs than those that live in clean environments [4, 9].

Reported the long-term effects of AgNPs exposure on marine ecosystems

On assessing chronic toxicity of AgNPs to aquatic organisms:

Bactericidal AgNPs have been reported to cause in vitro and in vivo effects in organisms other than bacteria, e.g. in vertebrates [60, 61], Mus musculus [63] and invertebrates [Drosophila melanogaster, Caenorhabditis elegans and Eisenia fetida [64]. Studies have shown that when AgNPs are released into aquatic ecosystems, they can undergo transformations and release toxic Ag(I) accumulated in aquatic organisms, leading to harmful effects on their health and survival [65]. Furthermore, the high concentration of AgNPs and their potential to be oxidized in the environment can result in toxicity for living organisms [66]. Several studies have investigated the long-term AgNPs effects on aquatic organisms and ecosystems [67].

Secondary organisms like crustaceans (*Daphnia magna*) can be affected by AgNPs in water or by ingestion of primary producers and among these effects can be cited the abnormal swimming and decrease of reproduction [68]. In several studies, *Daphnia* exposure to higher concentrations AgNPs resulted in decreased cumulative offspring with reducing growth and reproduction in a dose-response manner [69, 70] Acute and chronic toxicity of AgNPs to crustaceans, has not been adequately investigated. *In vitro* chronic toxicity of different concentrations (0.2, 0.5 µg/L) AgNPs to freshwater micro - crustacean *Daphnia lumholtzi* for 21 days [71]. Results showed concentration higher than 0.2 µg/L caused toxicity on the reproduction rate and with a concentration lower than 0.5 µg/L did not have an adverse effect on maturation of *D. lumholtzi* during 21 days of the exposure period compared with control. In several studies, *Daphnia* was exposed to a higher AgNPs concentration resulted in reducing growth and reproduction in a dose response manner. Decreased cumulative offspring was also reported in previous studies [69, 70] at AgNPs exposures of 50 and 100 µg/L, respectively.

Malformations in embryos of zebrafish due to exposure to AgNPs have also been reported [72]. 2011). Several studies have demonstrated that AgNPs can be toxic to algae [73], fish [74], snails [75] and plants [76]. The long-term effects of AgNPs in zebrafish [60] exposed to environmentally relevant concentrations of Ag NPs (0-50 µg/L) for 120 days. The study found that chronic exposure to environmentally relevant concentrations of Ag NPs had significant adverse effects on zebrafish. These effects included oxidative stress, neurotoxicity, reduced growth, impaired reproduction, and increased mortality. AgNPs exposure significantly increased oxidative stress in zebrafish, as measured by increased levels of reactive oxygen species (ROS) and lipid peroxidation. The increase in oxidative stress and mortality was dose-dependent, with the highest concentration (50 µg/L) causing a 2-fold increase in ROS levels and 20% increase in mortality compared to the control respectively. The highest concentration (50 µg/L) causing a 20%, 50% and 50% decrease in growth, in the number of eggs produced and in locomotor activity respectively compared to the control. Ag NPs exposure significantly impaired neurotoxicity in zebra fish, and increased anxiety-like behavior [77].

AgNPs acute toxicity on the goodeid fish *Chapalichthys pardalis*. Fish were exposed to different concentrations of AgNPs (2-32 mg L^{-1}) for 96 h to determine the LC_{50} and performed subchronic tests (21 days) using sublethal AgNPs concentrations (equivalent to CL1 and CL10). Acute toxicity tests evaluated AgNPs toxicity by oxidative stress, macromolecular and metabolic biomarkers in three organs (live, gills and muscle). Subchronic exposure (21 days) to sublethal AgNPs concentrations (LC1 and LC10) caused oxidative stress in *C. pardalis* adults, evidenced by decreased antioxidant enzyme activity and increased lipid and protein oxidation. AgNPs also reduced macromolecule levels and increased energy consumption, as indicated by decreased glucose levels. Integrated biomarkers response analysis revealed the greatest effect in organisms exposed to LC10, with the liver and gills being the most severely affected organs. The study found that exposure to AgNPs caused oxidative stress and affected macromolecular and metabolic biomarkers in the goodeid fish *Chapalichthys pardalis*. These effects were dose-dependent, with higher concentrations of AgNPs causing more severe effects. These findings highlight the acute and chronic toxic effects of AgNPs on endemic fish species, raising concerns about their potential impact on aquatic ecosystems [74].

AgNPs is very toxic to fish fauna, and therefore it is very important to establish AgNPs toxicity [77]. Small AgNPs easily penetrated inside fish tissues such as the liver, gills, brain, and muscles [28], hence, AgNPs migrated from fish to human, causing contamination. Among all aquatic organisms, fish is one of the most used aquatic organisms for assessing environmental toxicity and toxic effects.

The potential toxicity of the sublethal AgNPs concentrations on the neotropical fish *Prochilodus lineatus*. After 5 and 15 days, total Ag accumulation, oxidative stress markers (antioxidant enzymes, lipid peroxidation and antioxidant capacity against peroxyl radicals), aspartate and alanine aminotransferases activities (ALT, AST) were analyzed in gills and histopathological changes were recorded(morphometric analysis, proportion of the secondary lamellae available for gas exchange, reaction indexes, and organ index -Igills-) that included mucus cell count (MCc). After 15 days the Ag accumulation was five times higher than after 5 days in the case of 25.0 µg L^{-1} exposure. After 5 days, regarding oxidative stress, all enzyme activities were inhibited. The decreases in ALT activity and antioxidant capacity were evident after 2.5 µgL^{-1} AgNP for 15 days. LPO levels and AST activity increased after the highest time of exposure and AgNPs concentration and the same occurred with Igills. MCc increased after 15 days at both AgNPs concentrations. Gill alterations included hemorrhage, aneurisms, hypertrophy and the presence of fusions. The presence of low AgNPs concentrations, in short and subchronic exposures, generates alterations in stress biomarkers and in the structure of this vital organ that are the gills. Overall, reported the study [28] provides evidence for the AgNPs potential risks to human health through the food chain [28].

The chronic effects of AgNPs on the freshwater amphipod *Hyalella azteca*. Aamphipods were exposed to different concentrations of AgNPs (0, 1, 10, and 100 µg/L) for 28 days.

Ag NPs exposure did not significantly affect the survival of *H. azteca* at any concentration. Ag NPs significantly reduced the growth and number of offspring of *H. azteca* at all concentrations. The reduction in growth and number of offspring was dose-dependent, with the highest concentration 100 µg/L causing 40% and 70% decrease in growth and number of offspring respectively compared to the control. AgNPs exposure upregulated expression of genes involved in oxidative stress and inflammation in *H. azteca*. The upregulation of these genes was dose-dependent, indicating that AgNPs induced oxidative stress and inflammation in the amphipods [78].

Toxicity of AgNPs and $AgNO_3$ (25, 50, 75 and 100 µg/L) to the Mozambique tilapia (*Oreochromis mossambicus*) over a 7-day subacute exposure period. Ag accumulation was detected in the gills of tilapia exposed to both AgNPs and $AgNO_3$.Histological examination revealed gill tissue damage in fish exposed to both AgNPs and $AgNO_3$.AgNPs and AgNO3 induced oxidative stress in the gills, as evidenced by increased lipid peroxidation and protein carbonyl activity. The findings suggest that Ag accumulation in the gills is a major factor contributing to the toxicity of AgNPs and $AgNO_3$ in aquatic organisms. Enzymatic antioxidants (GST, GPx, SOD, and CAT) and non-enzymatic antioxidants (MT, GSH) were affected by AgNPs and AgNO3 exposure. Non-specific immunological parameters (LYZ, MPO, and RBA) were altered in the blood serum of fish exposed to AgNPs and $AgNO_3$. Neurotoxic effects were observed in the brain tissues of fish exposed to AgNPs and $AgNO_3$, as indicated by decreased acetylcholine esterase activity (AChE). The study demonstrated that both AgNPs and $AgNO_3$ can cause toxic effects in tilapia, including oxidative stress, tissue damage, and alterations in antioxidant and immunological parameters [79].

Toxicity of a series of sub-lethal concentrations of AgNPs on gill membranes of freshwater carp (*Cyprinus carpio*). Changes in membrane fatty acid (FA) profile, lipid peroxidation, and membrane fluidity were recorded at a high concentration (1.25 mg/L) AgNPs exposure. Ag NPs exposure alters the fatty acid profile of gill membranes, increasing the proportion of saturated fatty acids and decreasing that of polyunsaturated fatty acids. AgNPs exposure causes significant lipid peroxidation in gill membranes, as evidenced by increased levels of malondialdehyde (MDA), reduce the membrane fluidity of gill membranes, as measured by the fluorescence anisotropy of 1, 6-diphenyl-1,3,5-hexatriene and inhibited antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR), indicating impaired antioxidant defense mechanisms. On the other hand, AgNPs exposure upregulates expression of genes involved in lipid metabolism and oxidative stress response, including fatty acid synthase, acetyl-CoA carboxylase and glutathione peroxidase. Histopathological analysis shows abnormal gill morphology after AgNPs exposure, including epithelial hyperplasia, lamellar fusion, and increased mucus production. AgNPs exposure decreased the activities of antioxidant enzymes such as SOD, CAT, and GR, indicating impaired antioxidant defense mechanisms [80].

On the other hand, other analyzes have shown that the prolonged exposure to AgNPs cannot be very harmful to the aquatic ecosystem [81]. For example, demonstrated that the chronic exposure to AgNPs or $AgNO_3$ during 90 days does not significantly affect the phytoplankton biomass and the diversity of aquatic plants and animals. The bioaccumulation and toxicity of blood-mediated AgNPs in freshwater fish were reported [60]. Fish were exposed to AgNPs for 21 days at concentrations range from 0.1 to 100 µg/L. Bioaccumulation was observed in different organs of the fish, with the highest concentrations found in the liver and gills followed by the intestines. The study found that AgNPs accumulated in the liver and gills of fish and caused oxidative stress and inflammation in these organs. The oxidative stress was associated with increased ROS levels and lipid peroxidation. Oxidative stress and inflammation damage cells and tissues leading to organ dysfunction. In the liver, oxidative stress and inflammation can lead to liver damage and impaired liver function. In the gills, oxidative stress and inflammation can damage the delicate gill tissues and impair gas exchange. At the highest concentration, AgNPs had little influence on fish behavior. The results suggest that blood mediated AgNPs can bioaccumulate in fish and cause adverse effects at environmentally relevant concentrations [49].

- Investigating bioaccumulation and trophic transfer of AgNPs in marine food webs: Researchers are using Lab. and field studies to track the uptake, accumulation, and transfer of AgNPs in marine food webs. These studies are examining the potential for AgNPs to accumulate in higher trophic level organisms, such as fish and marine mammals, and the potential risks to their health [49].
- **Bioaccumulation and trophic transfer**:

AgNPs bioaccumulate in aquatic organisms and be transferred up food chain through predation. This can lead to the accumulation of Ag NPs in higher trophic level organisms and may pose a risk to their health. This has been demonstrated in several studies.

AgNPs bioaccumulated and trophic transfer in a marine food chain consisting of phytoplankton (*Skeletonema* costatum), copepods (*Acartia tonsa*), and fish (*Oryzias melastigma*) [82]. The results showed bioaccumulation in all three organisms. The BAF for AgNPs in fish was 1.4, indicating that AgNPs were not significantly biomagnified in food chain [83]. AgNPs could be transferred from phytoplankton (*Isochrysis galbana*), to copepods (*Oithona davisae*) to fish (*Oryzias melastigma*) in a simplified marine food chain. The BAF for AgNPs in fish was 0.5, indicating that AgNPs were not significantly biomagnified in food chain. The bioaccumulation and trophic transfer of Ag NPs of different sizes (20 nm and 100 nm) in a marine food chain consisting of phytoplankton (*Isochrysis galbana*), copepods (*Oithona davisae*), and fish *(Oryzias melastigma).* The results showed that AgNPs could bioaccumulate in all three organisms. The BAF for AgNPs in fish was higher for the 20 nm AgNPs (2.1) than for the 100 nm AgNPs (1.1), indicating that the smaller AgNPs were more biomagnified in the food chain [84].

The small AgNPs can easily penetrate inside the fish tissues such as the liver, gills, brain, and muscles and migrate from fish to human, which might lead to Ag contamination. Particle size influenced their migration ability. Smaller AgNPs were more easily transferred and accumulated in the tissues of both fish and mice. This suggests that smaller AgNPs may pose a greater risk to human health through the food chain [28].

The long-term exposure of common carp, *Cyprinus carpio* to blood-mediated AgNPs (B-AgNPs) (0.03, 0.06 and 0.09 mg/L)

and recorded bioaccumulation and histological alterations in various organs. The B-AgNPs were primarily accumulated in the liver, followed by the intestine, gills, and muscles. Bioaccumulation led to histopathological changes, including gill tissue damage and necrosis. The highest AgNPs concentration 0.09 mg/L caused histopathological alterations in both gills and intestine, including degeneration, shedding, and cell lysis. The study suggests that AgNPs can induce toxicity in fish, warranting further investigation into their environmental impact. Antioxidant enzyme activities (GST, GR, and CAT) were altered in liver and gills, with varying responses depending on the concentration and organ [67].

Several recent studies on bioaccumulation and trophic transfer of AgNPs indicates that Ag NPs can bio magnify in marine food chains.

Stable isotope tracers to investigate the trophic transfer and bio magnification of AgNPs in a simplified marine food chain consisting of phytoplankton (*Thalassiosira pseudonana*), copepods (*Acartia tonsa*), and fish (*Oryzias melastigma*). Ag NPs efficiently transferred from phytoplankton to copepods to fish. The biomagnification factor (BMF) of A NPs was 1.6 in copepods and 2.1 in fish. Biomagnification factor BMF for AgNPs in fish was 1.6, indicating that AgNPs were biomagnified in the food chain. AgNPs could impair the reproductive success of fish [67, 85].

A mathematical model was used to investigate bioaccumulation and trophic transfer of AgNPs in a marine planktonic food web. The model predicted that Ag NPs could bioaccumulate in all trophic levels of the food web, with the highest concentrations occurring in top predators. The model also predicted that Ag NPs could be transferred from phytoplankton to zooplankton to fish through both dietary exposure and waterborne exposure. AgNPs could cause oxidative stress and DNA damage in fish [9].

The trophic transfer and biomagnification of AgNPs in a marine benthic food web consisting of sediment, polychaetes (*Neanthes japonica*), and fish (*Oryzias melastigma*). The results showed that AgNPs could be transferred from sediment to polychaetes and fish. The BAF for Ag NPs in fish was 2.1, indicating that Ag NPs were biomagnified in the food web [86].

AgNPs affected behavior of fish in a marine benthic food web. They exposed juvenile turbot (*Scophthalmus maximus*) to different concentrations of AgNPs and observed changes in their swimming behavior and predator avoidance response. Fish exposed to AgNPs exhibited decreased swimming speed, increased erratic swimming, and reduced time spent in the open area of the tank. On the other hand, AgNPs exposure also impaired the predator avoidance response of fish. Fish exposed to AgNPs were less likely to respond to a simulated predator attack and showed reduced escape responses. The changes in behavior suggested that AgNPs can have sublethal effects on fish, even at relatively low concentrations. Altered swimming behavior and impaired predator avoidance response could make fish more vulnerable to predation and other environmental stressors. The study highlights the potential for AgNPs to disrupt the fish behavior in marine ecosystems. Further research is needed to investigate the long-term effects of AgNPs on fish behavior and to assess the ecological risks associated with AgNP pollution in the marine environment [86].

Overall, these studies on bioaccumulation and trophic transfer of AgNPs in aquatic food webs have provided important new insights into the potential AgNPs risks to aquatic ecosystems. It is important to note that the studies cited above were conducted in laboratory settings on studies in animals and cell cultures. More research is needed to investigate the bioaccumulation and trophic transfer of AgNPs in natural aquatic ecosystems and health human's risks in, particularly the risks associated with the consumption of seafood contaminated with AgNPs. More effort is needed to further investigate these risks and to develop strategies to mitigate the potential impacts of AgNPs pollution on marine ecosystems and to develop regulations and guidelines to limit the release of Ag NPs into the environment and to protect marine ecosystems and human health from the potential risks of AgNPs pollution. Extent of bioaccumulation and trophic transfer of AgNPs depends on several factors including:

1. Concentration, size, and shape of AgNPs in the environment, the duration of exposure. The effects of different AgNP sizes and shapes on bioaccumulation and trophic transfer: Studies have shown that smaller AgNPs are more likely to be bioaccumulated and transferred up the food chain than larger AgNPs. Additionally, Ag NPs with irregular shapes is more likely to be bioaccumulated than Ag NPs with spherical shapes.
2. The species of marine organism. Studies have shown that different marine organisms have different abilities to bioaccumulate and transfer Ag NPs. For example, filter-feeding organisms, such as mussels and oysters, are more likely to bioaccumulate AgNPs than carnivorous fish.
3. The specific ecosystem in which the AgNPs are released. Studies have shown that the presence of dissolved organic matter (DOM) in seawater can reduce the bioavailability of Ag NPs and therefore reduce their bioaccumulation and trophic transfer. Additionally, higher temperatures can increase the bioavailability of AgNPs and therefore increase their bioaccumulation and trophic transfer.

Human Health Implications of AgNPs

The increasing use of AgNPs in consumer products, such as textiles, cosmetics, and food packaging, raises concerns about their potential human health effects [87]. The release of AgNPs into the environment could pose a risk to both aquatic ecosystems and human health [10, 11]. AgNPs impregnated in coal of water filters used in home treatment devices can represent risks to human health due to Ag^{+} ion release in purified water [8].

A complete removal of toxic AgNPs was carried out using modified electrocoagulation (EC) at low energy power (3V, 200 mA) at optimum operational conditions: Aluminum (Al) anode, electrolyte conductivity, pH and electrodes spacing. Percent removal efficiency (%Re) was 97% for Al at 30 mA cm^{-2} at 3.1 kW h m^{-3}, current efficiency 95% at optimized operational conditions including current density, electrolysis time, and pH and electrodes materials. Lab. scale reactor can be scaled up for pilot plant. Redox reactions at electrodes surfaces controlled %Re. The yellowish orange couloir of AgNPs completely disappeared from the treated effluent. Up to 200 ppm were removed effectively using Al anode, pencil cathode, pH 4, low current density 30 mA cm^{-2} at agitation speed 50 rotation per min [88].

Removal methods: sequenialbatch reactor, sludge activation, filtration, RO, slow adsorption (pH adjusted) and ion-exchange were hindered by complexity, high costs and formed sludge. Expensive adsorbents activated carbon, clays, zeolites, cellulosic materials, graphene and biochar experience pores closure. Adsorbed AgNPs on AC give toxic Ag(I) ions. Micro porous adsorbent rarely recycled, required energy and toxic chemicals for activation and modification and interference by other pollutants. Slow coagulation by alum ($Al_2(SO_4)_3$, $FeCl_3$, poly $AlCl_3$ give sludge. Chemical neutralization of these concentrated chemicals form secondary pollutants. AgNPs not completely removed by EC using conc. sodium citrate stabilizer in 90 min. at 30 mA cm^{-2}. The obstacles in scaling up electrocoagulation for removal AgNPs are the literature survey was inconsistent and inadequate and needs further studies to optimize operational conditions (electrodes materials for saving electricity and time space pH; initial concentration, and reactor design [88].

Furthermore, the sewage sludge resulting from wastewater treatment is often used as fertilizer for agricultural soils, thus, toxic AgNPs (disinfectant) can be leached to aquatic systems and enter in food web by the primary producers. AgNPs could accumulate in fish and other seafood that are consumed by humans, posing a potential health risk. Humans can be exposed to AgNPs through ingestion of contaminated seafood or through dermal contact with contaminated water or sediment [89].

The amount of AgNPs that is consumed in seafood is likely to be very small. However, even small amounts of AgNPs could pose a health risk, particularly for people who consume large amounts of seafood or who are exposed to AgNPs from other sources. The potential health risks associated with the consumption of seafood contaminated with AgNPs are not fully understood. Relationship between AgNPs and its possible toxicity to human health is relatively new. Some studies have shown that Ag NPs can be toxic to mammalian cells [90].

AgNPs can induce cytotoxicity, genotoxicity, and oxidative stress in various cell types, including lung cells, skin cells, and immune cells [91]. Furthermore, AgNPs can cross the blood-brain barrier and accumulate in vital organs, such as the liver, kidneys, and brain [38]. Some studies have also shown that the interaction between Ag NPs and mammalian cells can cause lesions in the genetic material [36, 92].

AgNPs can induce chromosomal breaks and genotoxic damage. AgNPs have been shown to be toxic to human cells in vitro, and there is some evidence that they may also be toxic to humans in vivo [10, 11]. However, there are a number of potential risks that have been identified, based on studies in animals and cell cultures. These potential risks include [93]:

Toxicity to digestive system: AgNPs toxic to the digestive system, causing inflammation and damage to the lining of the gut. This can lead to a number of symptoms, including abdominal pain, nausea, vomiting, and diarrhea.

Impaired nutrient absorption: AgNPs interfere with absorption of nutrients from food, such as Fe and Zn leading to nutritional deficiencies and associated health problems.

Systemic toxicity: AgNPs can be absorbed into the bloodstream and distributed to other organs and tissues in the body. This can lead to systemic toxicity, affecting the brain, liver, kidneys, and other organs.

Genotoxicity: AgNPs damage DNA, leading to mutations and an increased risk of cancer.

Reproductive toxicity: AgNPs can impair reproductive function, both in males and females. This can lead to reduced fertility and an increased risk of birth defects.

Further research are needed to better understand the health risks associated with the consumption of seafood contaminated with AgNPs.

Evaluating AgNPs effects on ecosystem processes (Environmental Fate and Ecotoxicity)

Understanding AgNPs environmental fate and ecotoxicity is crucial for assessing their potential risks and developing appropriate risk management strategies [94]. AgNPs can enter the environment through various routes, including wastewater discharge, agricultural practices, and consumer products [32]. AgNPs persist in the environment for long periods and significantly impact the environment and the fauna within it ([8]. Once released, AgNPs can undergo transformations, such as aggregation, dissolution, and surface modifications, which can influence their behavior and toxicity. Release mechanisms of AgNPs in aquatic ecosystems can occur through various processes. These mechanisms include the long-term release of retained AgNPs, the stability of AgNPs in various chemical environments, and the factors influencing the mobility of AgNPs in different substrates. AgNPs behavior and toxicity in aquatic environments are strongly influenced by their specific chemical species rather than just their total concentration. Studies show that the concentrations of Ag NPs in sediments are predicted to be 1–5 µg kg–1 in most model studies [95].

AgNPs released into environment, can undergo chemical and biochemical conversions, leading to the release of Ag ions which are highly toxic and can accumulate in aquatic organisms, causing irreversible damage and disorders. AgNPs can have toxic effects on aquatic organisms and can also have indirect effects on ecosystems by altering the behavior and interactions of organisms [42]. However, the ecotoxicological AgNPs effects are highly dependent on their size, shape, surface coating, and concentration, highlighting the need for further research to understand their environmental impacts [96, 97]. Research has shown that the presence of dissolved organic carbon and other hydrogeochemical properties of water can affect the distribution and physical properties of AgNPs in aquatic environments [98]. Moreover, various factors such as solution pH, ionic strength, and ionic constituents control the aggregation of Ag NPs, influencing their behavior in aquatic systems [99]. Therefore, it is important to study the behavior of AgNPs in aquatic ecosystems and understand their potential impacts on fauna [100]. AgNPs can affect important ecosystem processes, such as nutrient cycling, energy flow and community structure. For example, AgNPs inhibited growth of phytoplankton, which are primary producers in marine ecosystems. AgNPs can also be toxic to algae, which are important primary producers in aquatic ecosystems [101]. High concentrations of Ag NPs can inhibit algal growth and photosynthesis, leading to disruptions in the food chain and lead to changes in the abundance and diversity of other aquatic organisms. AgNPs can also affect the behavior of marine invertebrates, which can have indirect effects on ecosystem processes. AgNPs presence in aquatic ecosystems can disrupt

the balance of the ecosystem and potentially harm the fauna within it.

Resistance development

Aquatic organisms may develop resistance to AgNPs over time, as has been observed with other types of pollutants. This could make it more difficult to control the effects of AgNPs in marine ecosystems in the long term.

Synergistic effects: AgNPs may interact with other pollutants in the environment and produce synergistic effects, which can be more toxic than the effects of either pollutant alone. For example, AgNPs have been shown to increase toxicity of other metals, such as Cu, and Zn to marine invertebrates.

Developed prediction models for long-term AgNPs effects on marine ecosystems: Researchers are developing mathematical models to predict the fate and transport of AgNPs in marine ecosystems and to assess potential risks to marine organisms and ecosystem processes. These models are being used to identify areas that are most at risk from AgNPs pollution and to develop strategies to mitigate the risks [75].

Regulatory framework and risk assessment

The increasing concerns about the environmental and health impacts of AgNPs have prompted regulatory agencies worldwide to develop guidelines and frameworks for their safe use. As more information becomes available on the potential AgNPs risks to aquatic ecosystems, it is likely that more specific regulations and guidelines will be developed to limit their use and release into aquatic environments. Several countries, including the United States, European Union, and Canada, have implemented regulations and guidelines to control the production, use, and disposal of nanomaterials, including AgNPs. These regulations and guidelines help to ensure that nanomaterials are used safely. However, the regulatory landscape for AgNPs is still evolving, and there is a need for harmonized approaches to ensure the safe and sustainable use of these materials. Risk assessment methodologies, including hazard identification, exposure assessment, and risk characterization, are essential for evaluating the potential risks associated with AgNPs and informing risk management decisions. The guidelines for testing and assessing AgNPs risks of vary from country to country and organization to organization. However, there are some general principles that are common to most guidelines.

1. Physicochemical Characterization:

The first step in testing AgNPs is to characterize their physicochemical properties *via* measuring particle size, shape, surface area, and surface charge [1]. It is also important to identify the chemical composition and any coatings or stabilizers that may be present.

2. Environmental Fate and Transport:

The next step is to assess the environmental fate and transport of AgNPs. This includes studying how AgNPs move through different environmental compartments (e.g., water, soil, air) and how they interact with other chemicals and organisms.

3. Toxicity Testing: assess potential risks of AgNPs to human health and the environment. This testing can be conducted on a variety of organisms, including fish, invertebrates, plants, and bacteria. Toxicity tests can measure a variety of endpoints, such as survival, growth, reproduction, and genotoxicity.

4. Risk Assessment: Final step process is integration results of the physicochemical characterization, environmental fate and transport studies, and toxicity testing to determine the potential risks of AgNPs. Risk assessment informed decision-making about the use and regulation of AgNPs. Examples of specific regulations or guidelines that control AgNPs release into environment, (Table 2).

AgNPs widely discharged in aquatic systems from the broad uses as corrosion inhibitors and anticorrosive antimicrobial protective coating. Example: antibacterial composite silica and AgNPs coating for titanium [103]. AgNPs improved protection efficiency of polyurethane coatings for mild steel in NaCl [104]. AgNPs-polymethyl methacrylate composite superhydrophobic anticorrosive adherent coating [105]. Controlled release active antimicrobial corrosion coatings AgNPs/SiO_2 core-shell. AgNPs was used as an efficient corrosion inhibitor for aluminum in HCl [106] and AgNPs was recommended for corrosion control copper alloy during acid pickling [107].

By developing and implementing regulations or guidelines to control release AgNPs into environment, governments and organizations can help to reduce AgNPs risks of to human health and environment. However, there is a growing recognition that more specific regulations are needed to address AgNPs risks.

Approaches overcome AgNPs toxicity (Potential strategies mitigation or prevention oxidative stress:

- **Reduce exposure to AgNPs:** The most effective way to reduce oxidative stress caused by AgNPs is to reduce exposure to AgNPs-containing products. Avoiding or limiting the use of these products, such as antimicrobial sprays, clothing, and food packaging.
- **Use antioxidants:** Antioxidants can help to neutralize ROS and protect cells from oxidative damage. Some antioxidants that have been shown to be effective in mitigating AgNPs-induced oxidative stress include vitamin C, vitamin E, and N-acetylcysteine.
- **Enhance body's own antioxidant defenses:** Immunity antioxidant defenses can be enhanced by eating a healthy diet rich in fruits, vegetables, and whole grains. Exercise and regular physical activity can also help to boost the body's antioxidant defenses.
- **Use Ligands: Develop new methods to remove AgNPs:** Researchers are developing new methods to remove AgNPs. For example, some studies have shown that AgNPs can be removed from body using chelation therapy. Metal chelators are substances that can bind to metal ions and prevent them from interacting with biomolecules. Some metal chelators that have been shown to be effective in mitigating AgNPs-induced oxidative stress include EDTA and dimercaptosuccinic acid (DMSA).

Develop AgNPs particles with reduced oxidative potential: Researchers are developing new types of AgNPs with reduced oxidative potential, using AgNPs in combination with other materials. For example, AgNPs coated with a protective layer of biocompatible material have been shown to have reduced oxidative potential.

Table 2: Projects for mitigation AgNPs toxicity

Project Assessing AgNPs	AgNPs focus	Citation
US Environmental Protection Agency (EPA) Toxic Substances Control Act (TSCA).	EPA's research program toxicity to aquatic organisms, fate in water bodies, and potential bioaccumulation in food chain. Developing water quality criteria (numeric values establish maximum AgNPs permissible pollutant amount in water without impact human health or aquatic life).	US Environmental Protection Agency (1976). Toxic Substances Control Act (TSCA). Public Law 94-469, 90 Stat. 2003.
International Maritime Organization (IMO)	Guidelines for control and management of ships' ballast water and sediments (BWM Convention). Prohibited or restricted discharge into marine environments through ballast water.	International Maritime Organization. (2004). International Convention for Control and Management of Ships' Ballast Water and Sediments, 2004.
London convention, Protocol (Prevention of Marine Pollution by Dumping of Wastes, other Matter)	Preventing marine pollution by regulating dumping of wastes and other matter into sea. Dumping AgNPs hazardous into ocean may be prohibited or restricted.	International Maritime Organization (2006). London Convention. Protocol on prevention Marine Pollution by Dumping Wastes and Other Matter, 1972 as amended in 1996.
European Union's Registration, Evaluation, Authorization; Restriction of Chemicals Regulation	Manufacturers and importers must be registered at the European Chemicals Agency (ECHA). Registration dossier must include information on physicochemical properties, environmental fate and potential risks to human health and environment. In 2019, AgNPs added to REACH Candidate list of substances of very high concern (SVHCs).	European Commission (2006). Regulation 1907/2006 of European Parliament, Council: 18, 2006 (registration, evaluation, authorization and chemicals restriction, established ECHA, amending directive 1999/45/EC, repealing Council Regulation (EEC) 793/93, Commission Regulation 1488/94, Council Iirective 76/769/EEC, Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.
European Union Horizon 2020: nano-solutions	Long-term effects on marine organisms and ecosystems. Chronic toxicity tests, bioaccumulation and field studies.	[26, 47]
National Institute of Environmental Health Sciences (NIEHS)	Health and ecological effects on Aquatic Environments (organisms including marine invertebrates). Chronic toxicity tests, bioaccumulation and mechanism.	[4]
California University, Santa Barbara project	Fate and effects on marine ecosystems and mitigation strategies to risks with a focus on Southern California. Field studies: experimental and modeling.	[82]
Plymouth Marine Lab. (Assessing Environmental Risks to Marine Ecosystems)	Effects on marine organisms and ecosystems in United Kingdom.	[83]
Woodrow Wilson International Center's Project on Emerging Nanotechnologies	Promote responsible nanomaterials use, including AgNPs.	Emerging Nanotechnologies. (2015). Nanotechnology and Environment. Woodrow Wilson International Center for Scholars.
National Science Foundation (NSF)	Environmental risks to Marine Ecosystems. A range of studies, including Lab. and field studies, to assess AgNPs risks to marine organisms and ecosystems.	[102]

Conclusion

These studies provide evidence that Ag NPs can have long-term toxic effects on aquatic organisms and ecosystems. Long-term health effects of exposure to AgNPs are not yet fully understood, but there is concern that they could potentially cause adverse effects. More research is needed to better understand the potential risks of AgNPs and to develop strategies to mitigate these risks. More research is needed to investigate bioaccumulation and trophic transfer of AgNPs in natural aquatic ecosystems. This research will help to inform the development of regulations and guidelines to limit the release of Ag NPs into the environment, to protect marine ecosystems from the potential risks of Ag NP pollution and to develop strategies to minimize human exposure to these NPs.

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