

Ecotoxicology of Engineered Silver Nanoparticles, with a Special Focus on their Interactions with Phytoplankton

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Abstract: The cost-effectiveness and characteristic properties of nanoparticles have enabled the field of nanotechnology to gain traction in the past few decades. Silver nanoparticles (AgNPs), in particular, have found application across fields, in medicine, cosmetics, and other consumer products. AgNPs are antimicrobials, and while this property has enabled them to replace traditional bactericides on the market, it is also a point of concern when thinking about the disposal and accumulation of these nanoparticles in the environment. In past studies, AgNPs have proven to be biotoxic to phytoplankton- the marine algae that form the base of the aquatic trophic food chain and drive the global carbon and nutrient cycles. The mechanisms of AgNP ecotoxicology are up for debate: some studies show that silver ions leach from the nanoparticle, and have an affinity towards the thiol groups of the cell membranes, which enables them to increase its permeability and hamper the cell's functionality; other studies suggest that the electrostatic nanoparticles themselves damage the cell membranes. It is also worth noting that the interactions between AgNPs and the components (both biotic and abiotic) influence the physicochemistry of nanoparticles, making them more or less toxic. Therefore, this paper suggests better, mesocosm-based experiments to assess the ecotoxicology of AgNPs, so as to better define the safety limits and assess the bioavailability of nanoparticles during the coming era of nanotechnology.

Keywords: ecotoxicology, engineered nanoparticles, silver nanoparticles, phytoplankton, cytotoxicity

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I. INTRODUCTION

Nanotechnology is at the heart of technological innovation of the 21st Century. Nanoparticles, due to their nanoscale dimensions, have characteristic properties different from those of their bulk materials. These properties are attributed to quantum effects, larger surface area, and self-assembly, which affect the optical, electrical, thermal, and magnetic behaviours of the materials. As a consequence, nanoparticles have found an application in fields ranging from agriculture to medicine to energy¹⁻⁷.

As with other nanomaterials, the production and use of silver nanoparticles, or AgNPs, have also seen a meteoric rise. Distinctive physio-chemical properties such as surface-enhanced Raman scattering⁸, high electrical and thermal conductivity⁹, catalytic activity¹⁰, and non-linear optical properties¹¹ have led to AgNPs being at the centre of much of the innovation using nanomaterials. AgNPs are used in a growing number of applications across a range of commercial consumer products, such as coating material, cosmetics, and storage containers¹².

Most importantly, AgNPs are antimicrobials¹³, and their ability to eliminate pathogenic microbes have made them central to the progress of nanomedicine. Multilayer films containing silver nanomaterial show antibacterial and anticoagulant properties and are used for surface modification in medical devices and cardiovascular implants¹⁴. Silver nanomaterials have also been used as surgical mesh, fabrication of artificial joint replacements, and wound dressing¹⁵.

As the production and usage of nanoparticles are further integrated into the commercial supply chains, the incidental release of these nanoparticles will become more likely. Large amounts of nanosilver end up in rivers, lakes, estuaries, and coasts through sewage and industrial discharges¹⁶. While small quantities of AgNPs and other nanoparticles have proven to be harmless to human cells, researchers around the world are now assessing the potential toxicity of AgNPs to aquatic life. Natural nanoparticles have always been present in the environment, but the introduction of engineered nanoparticles (ENPs) threatens to destabilise their present harmless quantities. The physicochemical properties strongly influence their behaviour, fate, and environmental functions of the ENPs. These properties typically include size, morphology and shape, surface coating, surface charge.

This paper will focus on one particular component of the vast aquatic life: phytoplankton. The well-being of phytoplankton is tied directly with the health of their respective water bodies. Phytoplankton form the basis of aquatic food webs, using sunlight, nutrients, carbon dioxide, and water to produce oxygen and nutrients for other organisms. Aside from being responsible for ocean's productivity, phytoplankton also drive the global

carbon cycle and regulate the chemistry of water bodies. Phytoplankton are extremely sensitive to changes in aquatic chemistry. Planktonic organisms interact rapidly and strongly with their ambient environment and are directly affected by exposure to engineered nanoparticles¹⁷.

The following review aims to elucidate the potential ecotoxicity of AgNPs when released in aquatic waterbodies, with a focus on how their interactions with phytoplankton affects the algae. In order to do so, we will investigate how properties characteristic to AgNPs make them a destabilising factor in phytoplankton community. To begin with, we will establish how natural and engineered nanoparticles are different from one another. We then explore the different and debatable mechanisms using which AgNPs can be toxic to phytoplankton, and how the interaction between AgNPs and biotic & abiotic components of the water body stands to influence the bioavailability of these nanoparticles. Finally, we suggest some prospects for future research into the ecotoxicology of AgNPs.

II. RESULTS AND DISCUSSION

While the field of nanotechnology gained traction only in the late 20th and the early 21st Century, nanoparticles are a phenomenon of nature, and form and occur in all spheres of Earth²⁰. Natural nanoparticles (NNPs) can be generated by various chemical, physical, or biological processes in nature, such as chemical interactions of volcanic ash or chemical weathering of minerals, and can even be found in bacteria and yeasts as sulfur or selenium NPs²¹. Since their Nobel-Prize winning discovery by Robert F. Curl Jr., Sir Harold Kroto, and Richard E. Smalley in 1985²², fullerenes have been identified everywhere in nature: on carbon-rich rocks from 600 million years ago²³ to planetary nebulae²⁴. As has been extensively discussed by Michael Hocechella in his work on natural nanoparticles, it is essential to consider whether an engineered nanomaterial is a new type of exposure, or whether, due to the presence of a similar material in nature, organisms in distinct spheres have been in contact with it for an extended period of time²⁵. Not only do NNPs inform the nano-engineering process, but also provide the foundation of research into the adverse effects nanoparticles can have on ecosystems or human and animal health.

Incidentally released nanoparticles (INPs) and engineered nanoparticles (ENPs) make up the pool of anthropogenically released nanoparticles (ANPs), and the release of both incidental and engineered nanoparticles into the environment alters the nature and amount of NNPs²⁶. INPs arise as a by-product of anthropogenic activities, and such particles have become unintentionally abundant since the inception of the Industrial Revolution²⁷. For example, the release of plastic materials into the environment is one of the major pollution-related issues, and recent studies confirms that microplastics further degrade into nano-sized particles²⁸.

While INPs are more abundant globally due to their unchecked release into the environment, ENPs have been produced for less than a century. For example, engineered Silica nanoparticles, which are today used for DNA detection and medical imaging²⁹, were first produced in the 1960s³⁰. From 2010 onwards, the presence of ENPs in consumer products has become commonplace³¹, and the increasing commercial application calls for a deeper dive into the possible harmful effects of the ENPs. In order to assess the health risks of nanoparticles, it is important to understand their toxicity mechanisms. A recent study confirmed the neurotoxicity of ENPs through different mechanisms such as membrane damage, cell cycle interference, reactive oxidation-species formation, and accumulation of autophagosomes³². Besides direct emission into the environment, ENPs are also prone to accidental spills during their production and transportation, wear and tear, and the final disposal of ENP-containing products. This is a matter of urgent concern, especially in the case of nanoparticles being used for environmental remediation. AgNPs are one such category of metallic nanoparticles³³.

As mentioned before, silver (Ag) has historically been used as an antimicrobial agent. Ag ions are one of the most phytotoxic metal ions³⁴. AgNPs, which contain 20 to 15,000 silver atoms and usually has a diameter of 100nm, exhibit remarkable antimicrobial activity, even at low concentration³⁵. This can be owed to the large ratio of surface area to volume. As such, AgNPs have found attractive applications in textiles, cosmetics, healthcare products, wound dressings, food packaging films, and corneal replacements to name a few. AgNPs have replaced traditional antibiotics such as tetracycline and streptomycin, owing to their ability to eliminate multi-drug resistant (MDR) bacteria³⁶. AgNPs have been reported to be effective at killing both gram-positive and gram-negative bacteria³⁷.

Different studies report different mechanisms of silver nanoparticles' antimicrobial effect. The most widely-accepted and reported of these mechanisms is that AgNPs continually release silver ions. These ions show electrostatic attraction to sulphur-based, or thiol proteins, and can increase the permeability of the cytoplasmic membranes by adhering to them and disrupting the bacterial envelope. An increase in membrane permeability affects the cell's ability to properly regulate transport activity through the plasma membrane.

Free silver ions can also deactivate respiratory enzymes and generate harmful reactive oxygen species (ROS). Increase in oxidative stress in microbes is a clear indication of toxicity caused by heavy metal ions such

as the Ag ion. AgNPs can also produce ROS and free radical species such as hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻), and singlet oxygen³⁸. This extracellular and intracellular increase in ROS can disrupt cell membrane functionality and modify bacterial DNA. Ag ions can also disrupt DNA-related metabolism by interacting with the sulphur and phosphorus present in the nucleic acid.

A study from 2008 used fluorometry to examine the short-term toxicity of Ag⁺ and AgNPs to *Chlamydomonas reinhardtii*³⁹, an important alga often used as a model for studies of fundamental processes like photosynthesis. AgNPs contributed to the toxicity as a source of Ag ions, decreasing the photosynthetic yield of *C. reinhardtii*. In a majority of studies, the photosynthetic yield of the algae decreased as a function of increasing total Ag concentrations, i.e., AgNP toxicity acted as a function of total silver dissolved.

For commercial use, nanoparticles are often provided with surface-coating to enhance the stability of the ENPs. Another study from the same authors examines the role of differently coated silver nanoparticles in the toxicity to algal photosynthesis in *C. reinhardtii*⁴⁰. This study attested that the role of nanoparticle coatings in toxicity to the alga was minimal. This surface-coating served as intermediate reservoirs from which Ag⁺ could be released, and in modulating interactions of AgNP with algae, lead to further release of Ag⁺.

The other mechanism given for cytotoxicity of AgNPs includes direct contact between the nanoparticles and the bacterial cell wall. Just as in the case of Ag ions, the positively charged nanoparticles confer an electrostatic attraction between the AgNPs and the negatively charged cell membrane. Using transmission electron microscopy, researchers revealed that after coming into contact with AgNPs, the cell membrane of *E. Coli* was disrupted, becoming circumferential, and numerous electron-dense pits could be seen at the damaged sites on the membrane⁴¹. In other cases, AgNPs penetrated inside the cell and hampered vital cell functionality⁴². AgNPs often interact with ribosomes and denature them, causing inhibition of protein synthesis⁴³.

A study giving proof for this alternate mechanism of cytotoxicity used AgNPs with β-D-glucose capping⁴⁴, which ensures very low dissolution of Ag⁺ from the nanoparticles. In their experiment, AgNPs-G were very stable in culture medium over time, since the dissolution degree was between 1% and 5% at 1 and 10 days respectively. No loss of glucose was observed and the toxicity later recorded could be attributed to AgNPs only.

It is worth noting that the ecotoxicology of AgNPs greatly depends upon their interactions with abiotic and biotic components. In the above examples, the exposure of AgNPs to phytoplankton further modulates the release of Ag ions into the water. The physicochemistry of AgNPs is also rapidly altered by abiotic variables, like pH, salinity, dissolved organic matter, and dissolved oxygen content. ENMs entering the aquatic ecosystem undergo dynamic transformations as they interact with the organic and inorganic components present in the environment, and attain a unique “environmental identity”⁴⁵. An eco-corona forms around the nanoparticle and gives it new surface properties⁴⁶. The three major ways in which AgNPs can transform in aqueous environments is by dissolution, aggregation, and reactive oxygen species (ROS) generation⁴⁷, and these transformations are crucial to consider when predicting the cell-NP interaction.

A study made use of two common phytoplankton: *Pithophora oedogonia* and *Chara vulgaris*⁴⁸. Alterations in pigment content were used as a parameter to measure the effect of AgNPs on both, and over the course of the experiment, the total chlorophyll in both algal species exhibited significant reduction. However, the decrease was more pronounced in *C. vulgaris* than in *P. oedogonia*. This difference in reduction was attributed to the harder cottony assemblages of filaments in the latter taxon, which could resist any interaction with AgNPs⁴⁹. Another study by He et Al. assessed the short-term toxicity of citrate-stabilised AgNPs to *Chattonella marina*, a genus of marine raphidophytes commonly associated with red tides. The study explored the dynamic interaction between the biological produce of *C. marina* and the physicochemical properties of AgNPs. *C. marina* generates superoxide (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radicals (OH⁻)⁵⁰, which mediate the transformation of AgNPs into Ag(I) via electron-charging and discharging. In turn, Ag(I) toxicity can enhance ROS generation which leads to further alteration in algal metabolism.

Apart from undergoing changes themselves, AgNPs also run the risk of negatively modifying abiotic components necessary for the survival of phytoplankton. A study from 2011 reported the results of ENP interactions with exopolymeric substances (EPS) from three marine phytoplankton species: *Amphora sp.*, *Ankistrodesmus angustus* and *Phaeodactylum tricornutum*⁵¹. EPS are high-density, colloidal polymers released by microorganisms that assemble into microgels, and they are an important part of the aquatic organic carbon cycle. However, interactions with ENPs can alter their hydrophobic or ionic mechanisms of assembly. Over time, these changes can culminate in deleterious environmental impact due to a change in organic carbon flux, the microbial ecosystem, and the marine trophic cycle.

III. CONCLUSION

Nanotechnology progresses unabated, at a pace ecotoxicology research must aim to keep up with. NNPs have always existed in nature, but ENPs are different physiochemically and their introduction and accumulation in water bodies can be detrimental to the environment. While the mechanism of their biotoxicity is still up for debate, AgNPs are inherently toxic, given their ability to release silver ions, as has been seen in the multiple experiments conducted in the field. The primary reason why we need to better understand the ecotoxicology of ENPs is that their physicochemistry is rapidly altered by their surroundings. The interactions of ENPs with these biotic and abiotic variables can alter their bioavailability to aquatic life. This physicochemical variability makes it difficult to correctly assess safe limits of exposure and can lead to ecological catastrophes in the future. As the nanoparticle market continues to gain traction and AgNPs become a part of more everyday products, we must be prepared with more responsible ways to dispose of them. In case their accumulation, much like the accumulation of other such pollutants, is inevitable, we need to scale up the research making use of mesocosms and microcosms to predict the biotoxicity and bioavailability of AgNPs. Given what we already know about the interaction between AgNPs and phytoplankton, experiments using marine algae can be a good place to start.

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