



The impact of silver nanoparticles on microbial communities and antibiotic resistance determinants in the environment[☆]

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ABSTRACT

Nanosilver (NAg) is currently one of the major alternative antimicrobials to control microorganisms. With its broad-spectrum efficacy and lucrative commercial values, NAg has been used in medical devices and increasingly, in consumer products and appliances. This widespread use has inevitably led to the release and accumulation of the nanoparticle in water and sediment, in soil and even, wastewater treatment plants (WWTPs). This Article describes the physical and chemical transformations of NAg as well as the impact of the nanoparticle on microbial communities in different environmental settings; how the nanoparticle shifts not only the diversity and abundance of microbes, including those that are important in nitrogen cycles and decomposition of organic matters, but also their associated genes and in turn, the key metabolic processes. Current findings on the microbiological activity of the leached soluble silver, solid silver particulates and their respective transformed products, which underpin the mechanism of the nanoparticle toxicity in environmental microbes, is critically discussed. The Article also addresses the emerging evidence of silver-driven co-selection of antibiotic resistance determinants. The mechanism has been linked to the increasing pools of many antibiotic resistance genes already detected in samples from different environmental settings, which could ultimately find their ways to animals and human. The realized ecological impact of NAg calls for more judicious use of the nanoparticle. The generated knowledge can inform strategies for a better ‘risks versus benefits’ assessment of NAg applications, including the disposal stage.

1. Introduction

The use of engineered nanoparticles is growing rapidly, with broad applications ranging from electronics and renewable energy, to healthcare, food and agriculture (Tsuzuki, 2009). Nanoparticles (NPs) are particles of 1–100 nm size, with unique physicochemical and biological properties when compared to their larger micron-sized counterparts (Fabrega et al., 2011a). In general, NPs can be classified into metal, metal and metalloid oxides (inorganic) NPs (e.g. gold, silver, iron oxide and silicon dioxide), carbon-based NPs (e.g. carbon nanotubes and nanofibers) and organic NPs (e.g. dendrimers, liposomes and micelles) (Ealias and Saravanakumar, 2017). Surveys on companies have revealed that nanosilver (NAg) is among the most manufactured and used nanoparticles (Keller et al., 2013; Piccinno et al., 2012) with enhanced

characteristics, including high electrical and thermal conductivity, as well as catalytic activity (Fabrega et al., 2011a; Frattini et al., 2005). NAg are also well-known for their potent antimicrobial activity against bacteria, fungi and even viruses (Fabrega et al., 2011a; Faiz et al., 2018; Gunawan et al., 2013). With the relatively low manufacturing cost (Capek, 2004), antimicrobial NAg has been incorporated in many applications; in medical devices, such as in wound dressings and catheters to fight infections, as well as in increasing arrays of consumer products and appliances, from cosmetics and textiles, to food packaging and refrigerators (Ballottin et al., 2017; Carbone et al., 2016; Ge et al., 2014; Lin et al., 2013).

This Review intends to comprehensively discuss current findings on the environmental studies of NAg, to identify impacts of the nanoparticle on microbial communities and pools of antibiotic resistance

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determinants. The widespread application of NAg, with an estimated 55 to 360 tones annual global production of the nanoparticle (Lazareva and Keller, 2014; Piccinno et al., 2012), has led to its release into the environment. Studies have indicated presence of the nanoparticle in different environmental settings, from direct release into natural waters and soil, to accumulation in wastewater treatment plants (WWTPs). In soil, the concentrations of total silver range from 0.01 to 126 mg/kg (Huang et al., 2019). In water and sediment, the total silver concentration may range from 0.01 to 300 µg/L and 0.2–150 mg/kg, respectively (WHO, 2002). Increasing number of research inquiries have deduced that environmental presence of NAg has the potential to alter the microbial communities and in turn, the ecologically important processes. Silver-induced changes in the balance and physiology of microbial communities have been implicated on nutrient cycle processes, the most studied one being the nitrogen cycle, with inhibition of nitrification, for example, has been reported in soil studies (Beddow et al., 2017; Huhe et al., 2016; Shin et al., 2012). Shifts in microbial communities have also been linked to the development of silver-resistant population due to genetic mutations under the silver selection pressure, as well as acquisition of resistance genes in mobile genetic elements by the microbes (Das et al., 2012b; Gunawan et al., 2013; Ma et al., 2016). Quite recent studies have also indicated that silver has the potential to enrich antibiotic resistance genes (ARGs) in microbial communities. The so-called co-selection mechanism has been seen in environmental settings, including in rivers and WWTPs (Ma et al., 2016; Siddiqui et al., 2018). The Review also takes into account the often overlooked natural transformations of the nanoparticle (Fig. 1). NAg will undergo aggregation and chemical transformations into many different silver species, each with unique microbiological activity (Fabrega et al., 2011a; Zhang et al., 2016, 2018c). The physicochemical characteristics of the nanoparticle, i.e. size, shape and surface functional group, along with the characteristics of the surrounding medium, will collectively affect the transformation. Studies have reported that NAg co-exists with its transformed silver products in the environment, and that these silver species are relatively stable, capable of exhibiting toxicity on microbial communities (Settimio et al., 2014; Zhang et al., 2016, 2018b). Following a critical analysis of the most important findings on NAg microbiological activity in environmental settings, we then place our perspectives to identify future work to fill in the knowledge gaps.

2. Transformations of nanosilver in the environment

NAg is released into the environment through a number of pathways (Fig. 2). The nanoparticle is accumulating in oceans and rivers as a result of wastewater treatment effluent discharge as well as flow of stormwater into natural waters. Presence of silver has also been detected in soils, being associated with the use of NAg-containing products, such as pesticides and plant growth-promoting sprays (Hänsch and Emmerling, 2010), as well as disposal of NAg-containing products in landfills and the

use of biosolids (sludge from wastewater treatment plants) in agriculture (Fabrega et al., 2011a; Whiteley et al., 2013). The latter can also lead to further release of silver into natural waters via runoffs (Whiteley et al., 2013). NAg can also enter the atmosphere via emissions from waste incineration plants (Gottschalk et al., 2009), which ultimately accumulate in soil and natural waters through the wet or dry deposition process (Anjum et al., 2013).

NAg undergoes aggregation and chemical transformation in the environment (Levard et al., 2012; Zhang et al., 2018b), with the corresponding transformation products having been indicated to exhibit unique toxicity on microbes (Zhang et al., 2018b). Herein, we discuss how the nanoparticle interact with the natural compounds in the environment, but first, we will describe the origins of the nanoparticle microbiological activity.

2.1. Microbiological activity of the leached soluble silver and solid silver particulates

The toxic effects of NAg on environmental microbes can manifest in the form of growth inhibition and lethal cell damages (Hachicho et al., 2014). Studies have reported such effects on soil bacteria *Pseudomonas putida* (Fabrega et al., 2009; Hachicho et al., 2014; Matzke et al., 2014; Sudheer Khan et al., 2015) and *Bacillus subtilis* for example, (Gambino et al., 2015), as well as on many nitrogen cycle bacteria, including the nitrogen-fixing and nitrifying bacteria (Beddow et al., 2014; Yuan et al., 2013; Zhang et al., 2018a). In regard to the mechanisms, research inquiries have indicated multiple origins of the nanoparticle toxicity, whereby, apart from the more established findings on the microbiological activity of the leached silver ions (Ag^+) (De Matteis et al., 2015; Faiz et al., 2018; Jorge de Souza et al., 2019), increasing number of studies have also reported the activity of the solid silver particles that remain after leaching (Faiz et al., 2018; Sotiriou and Pratsinis, 2010). When in contact with aqueous system, NAg undergoes oxidative dissolution to leach silver ions (Fig. 3). The partial dissolution process occurs in two stages. In the fast dissolution stage, Ag_2O layer dissolves and the leached Ag^+ that adhered to the surface of the particle is desorbed. The slow rate determining step is the oxidation of NAg surfaces (Liu and Hurt, 2010; Zhang et al., 2018b). The overall NAg dissolution reaction is shown in Equation (1):



The released Ag^+ will react with a cocktail of inorganic and organic moieties in the environment, transforming into an array of silver species with different toxicity effects. The silver ion itself is active, capable of inflicting cell membrane disruptions as well as targeting cytoplasmic biomolecules in bacteria (Levard et al., 2012; Liu and Hurt, 2010; Zhang et al., 2016, 2018b). Studies have indicated the Ag^+ -targeting of iron-sulfur clusters that are present in cellular proteins, including in many important enzymes (Lemire et al., 2013; Ranquet et al., 2007). For

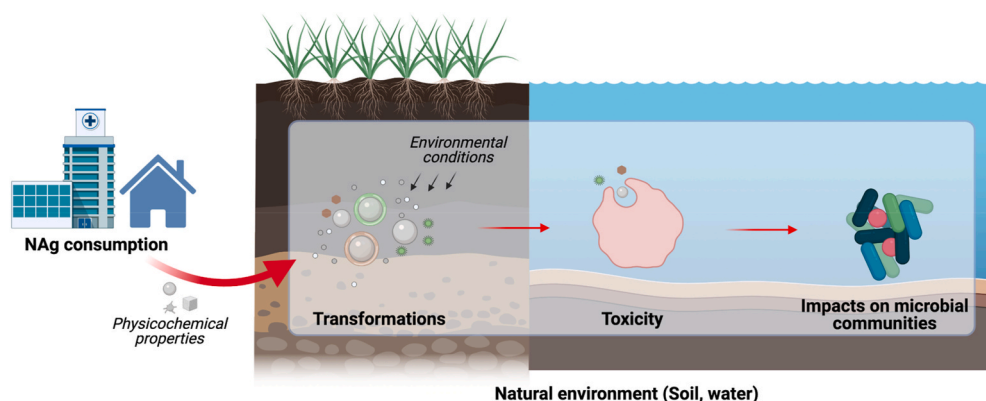


Fig. 1. Environmental release of NAg and impacts on microbial communities. Physicochemical properties of NAg (e.g. size, shape, surface functional groups) along with environmental parameters (e.g. presence of dissolved organic matters, pH, ionic strength, sunlight exposure) affect the natural transformations of the nanoparticle into different silver species, imparting unique toxicity characteristics on microbes, in turn, shifting the microbial composition, metabolic activities as well as pools of ecologically important genes.

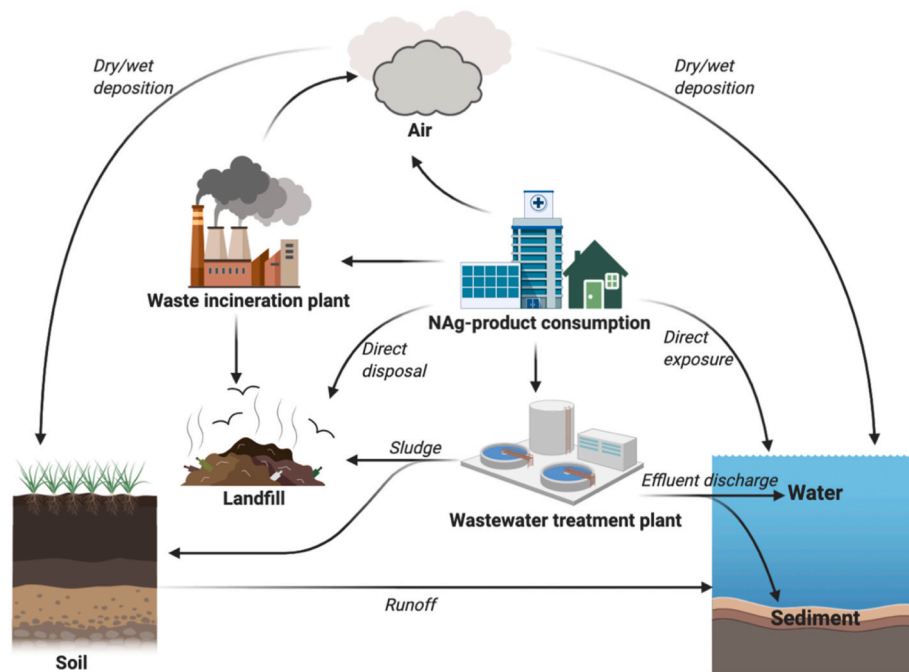
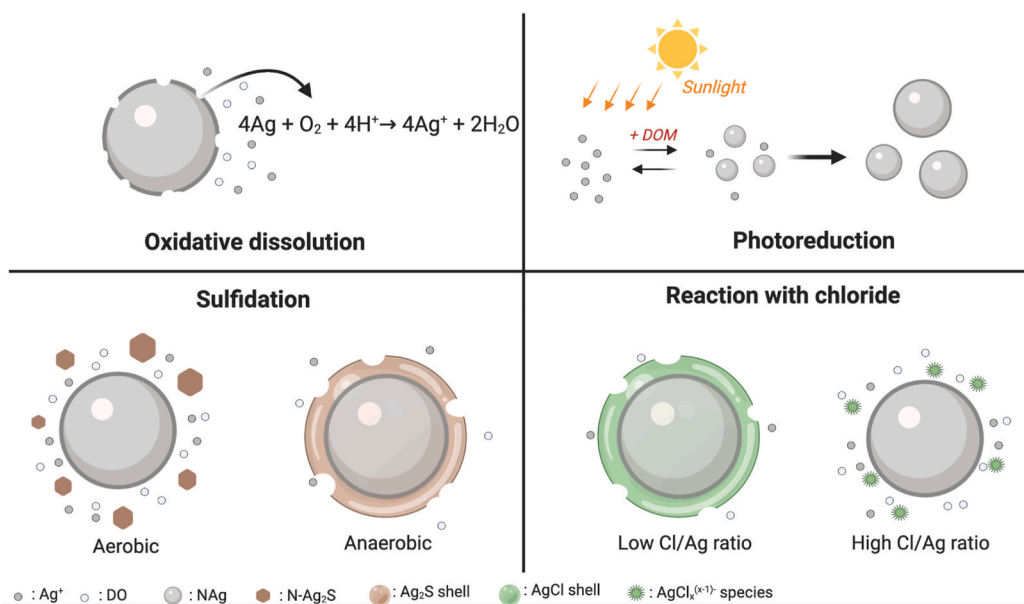


Fig. 2. NAg release pathways into natural environments and wastewater treatment plants. In general, soil and aquatic (water and sediment) environments are the major sinks for the nanoparticle. Airborne NAg-containing emissions will ultimately deposit in soil and aquatic environments through dry/wet deposition.



levels of reactive oxygen radical generation in environmental bacteria (Faiz et al., 2018). Studies have observed cellular uptake of the particles, even in the biofilm forms of growth (Behra et al., 2013; Eymard-Vernain et al., 2018; Faiz et al., 2018; Kim et al., 2011; Von Moos et al., 2014). An electron microscopy analysis (TEM-EDS) for example, detected the presence of silver particles in *B. subtilis* cells following exposure to NAg (d = 10 nm, citrate-coated) (Kim et al., 2011). Adsorption has been indicated by many studies as the initial interaction of nanoparticles with environmental microbes (Von Moos et al., 2014). NAg can physically adsorb on bacteria, like those seen with the soil bacteria *B. subtilis* and *P. stutzeri* (Pasricha et al., 2012). Microbes typically exhibit a net negative surface charge due to proton dissociation from functional groups that are present in their most outer envelope layer at the typical water pH range (Gregory, 2006; Morones et al., 2005; Neal, 2008). This could facilitate adsorption of NAg on microbes via electrostatic attraction, in particular particles with a net positive surface charge (positive zeta potential) (Von Moos et al., 2014). Apart from electrostatic attraction, other forces, such as the van der Waals forces, hydrophobic interactions and hydrogen bonding, and receptor-ligand interactions, can also involve in the adsorption phenomena (Neal, 2008; Sondi and Salopek-Sondi, 2004). How exactly the cellular presence of silver particles exerts their toxicity, however, still remains unclear. Already seen in mammalian cells, studies have hypothesized the Trojan horse-type effects in bacteria, whereby intracellular leaching of silver ion occurs following uptake of the particles (Hsiao et al., 2015; Long et al., 2017), and therefore, the subsequent targeting of cellular biomolecules, such as iron-sulfur clusters in proteins (Lemire et al., 2013; Xu and Imlay, 2012) and respiratory chain enzymes (Gordon et al., 2010; McQuillan et al., 2012), that could lead to the formation of the reactive oxygen radicals.

Research inquiries, interestingly, have indicated that silver ion is more toxic when compared to the solid silver particles, at least on some environmental microbes. Exposure of *P. putida* to ionic silver (from AgNO₃) were associated with lower effect silver concentrations (EC) than NAg with various surface coatings (uncoated, citrate- and tannic acid-coated) and sizes (d = 8–60 nm) (Hachicho et al., 2014; Matzke et al., 2014). For example, the EC₅₀ (silver concentration causing 50% growth inhibition relative to the cell-only control) of AgNO₃ was 0.1 mg Ag/L compared to the significantly higher 250 mg Ag/L for NAg (d < 10 nm) (Hachicho et al., 2014). Beddow et al. (2014) also noticed such differences on nitrogen cycle microbes. At 0.5–50 mg Ag/L, uncoated NAg (d = 118–188 nm) exhibited up to 2-fold lower inhibition effects on the nitrification potential rates of *Nitrosomonas europaea*, *Nitrosospira multiformis*, and *Nitrosococcus oceanii* than the ionic silver exposure at comparable silver concentrations (as AgNO₃). Regardless, despite the potential differences in the toxicity characteristics, taken together, these studies highlight the major contributions of both the leached silver ions and the solid silver particles on the overall NAg toxicity on environmental microbes. Current studies have also indicated that NAg exposure can alter the gene expression profiles of environmental microbes. Lu et al. (2020) studied the effects of the nanoparticle (d = 10 nm, citrate-coated) on freshwater bacteria and reported changes in the expression of genes that could result in inhibition of energy metabolisms as well as DNA replication and repair. Other study indicated that microbes develop strategies to cope with NAg toxicity. Meier et al. (2020) observed higher expression of oxidative stress response genes (e.g. superoxide dismutase that neutralizes superoxide radicals) as well as efflux pump genes (e.g. CusA, a cation pump capable of Ag⁺ ion efflux) in soil microbes following exposure to NAg (PVP-coated).

In the next sections, we describe the potential further transformation of the leached silver ions and the solid silver particles in the environment, and the effects on their microbiological activities. The studies are being focused on single species environmentally-relevant organisms to first clarify the changes in the respective toxic effects, prior to discussing the nanoparticle impacts on microbial communities.

2.2. Aggregation and hetero-aggregation and effects on toxicity

Aggregation is one of the most important ‘physical’ transformations that affects the interactions of NAg with microbes. It is a process whereby nanoparticles interact to form larger clusters as a result of the inter-particulate attractive van der Waals forces (Hartmann et al., 2014; Shevlin et al., 2018). In general, aggregation renders the nanoparticle less mobile, in many cases depositing on sediments and in turn, less available to organisms that are present in the corresponding aqueous environment (Behra et al., 2013). In the following, we will discuss how the presence of soluble and particulate moieties in the environment, as well as environmental parameters such as pH and ionic strength, influence the aggregation process of NAg.

Studies have shown that the natural presence of dissolved organic matters (DOMs) in the environment can affect the extent of NAg aggregation (Pokhrel et al., 2013; Zhang et al., 2017). A recent work by Fernando and Zhou (2019) reported the concentration-dependent NAg aggregation (d = 22 nm, uncoated) in the presence of humic acid (HA), one of the major DOMs presents in aquatic and soil environments. The study observed less particle aggregation when in the presence of environmentally-relevant HA concentration (1–20 mg/L in aquatic environment), in contrast to the relatively high degree of aggregation seen when in the absence of the DOM. At higher concentration of HA (20–250 mg/L, referring to its natural presence in soil), however, the nanoparticles were found to be engulfed by the HA molecules, increasing the aggregates’ hydrodynamic diameter. NAg aggregation is also ionic-strength- and pH-dependent. Zhang et al. (2016) reported a lower critical coagulation concentration (CCC, the minimum concentration of counterions to induce aggregation) at pH 8 when compared to pH 4, which means that, at comparable ionic strength, the nanoparticle would aggregate at higher extent at the higher pH. The nature of the organic matters seems to also have important roles on NAg aggregation. Presence of lower MW (molecular weight) DOMs (<30 kDa) was recently reported to decrease the extent of aggregation of PVP-coated NAg (d = 27 nm) in both mono and divalent cation systems, while higher MW DOMs (>30 kDa) were associated with enhanced aggregation (Yin et al., 2015). Studies have linked the latter observations to presence of functional groups in DOMs, such as the aromatic and reduced sulfur and nitrogen contents (Louie et al., 2015; Yu et al., 2018).

Further, it is important to note that, in the environment, the aggregation phenomena can occur not only between particles of NAg, but also between NAg and particles that are naturally present, referred to as hetero-aggregation. Among the major ‘native’ particles that can interact with NAg are iron oxides, illite (Liu et al., 2015), montmorillonite (Liu et al., 2015; Zhou et al., 2012) and kaolin (Wang et al., 2015), which are present, for example, in aquatic environment as clay minerals. A study has observed NAg-kaolin aggregation (d = 40 nm, citrate-stabilized), and found that the extent of the hetero-aggregation was higher at alkaline pH than acidic pH, being associated with the lower critical coagulation concentration at alkaline pH (Wang et al., 2015). Further, in relation to the NAg-clay mineral interactions, seasonal changes can affect the fate of the nanoparticle in the environment. One example is the transformation of the clay mineral goethite to hematite due to dehydration in arid seasons (Cornell and Schwertmann, 2003), which may release the previously retained NAg by goethite into aquatic environment (hematite is thought to have less NAg sequestering capacity) (Wang et al., 2018).

In summary, it is evident that aggregation of NAg, including the nanoparticle hetero-aggregation with other particles in the environment, are affected by the complex interactions of various factors, the major ones being the presence of DOMs, pH and ionic strength. Aggregation of NAg has been indicated to lower the toxicity of the nanoparticle on environmental microbes. A current study reported a benign effect of highly aggregated NAg on *Bacillus megaterium* and *Escherichia coli*, as well as the fungus *Cryptococcus neoformans*, under high ionic strength conditions (Bélteky et al., 2019). Exposure of the microbes to

the nanoparticle aggregates (citrate-capped NAg, $d = 28$ nm) in high ionic strength systems (with presence of NaCl) saw little effects on cell viability, while the same nanoparticles with minimal aggregation (no NaCl) killed more than 75% of the cell population. The study also observed similar effects with other surface-functionalized of NAg (green tea extract-capped, $d = 88$ nm). Specific environmental conditions however, such as presence of low molecular weight DOMs, acidic pHs as well as low ionic strength, have been indicated to lessen and slowed the aggregation of NAg, which could render the nanoparticle more bioavailable and potentially toxic to microbes.

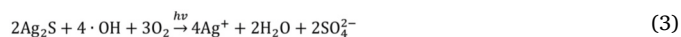
2.3. Chemical transformation of nanosilver in the environment

While the solid silver particles undergo the aggregation process, the leached silver ions, once released from the nanoparticle, will undergo chemical transformation to form various silver species due to reactions with the ubiquitous presence of silver complexing and precipitating agents in the environment. These new silver species as herein described, can exist as 'free' species, while some also structurally exist as part of the nanoparticle.

2.3.1. Sulfidation and effects on toxicity

Sulfidation of NAg can occur in the environment under both aerobic and anaerobic conditions (Fig. 3). In aerobic conditions and in the presence of sulfide (in the forms of HS^- or H_2S), 'indirect' sulfidation mechanism has been proposed, whereby silver ions that leached from the nanoparticles react with sulfide to form nano-sized Ag_2S particulates (Liu et al., 2011; Zhang et al., 2018b). 'Direct' sulfidation, on the other hand, occurs under anaerobic conditions and typically higher concentrations of sulfide, with Ag_2S shells forming on the surface of NAg (therefore, the Ag– Ag_2S core-shell structure) due to the lack of silver ion leaching (Liu et al., 2011). The presence of dissolved organic matters (DOMs), in particular humic acid, have been reported to increase the rate of sulfidation (Liu et al., 2011; Thalmann et al., 2016). Sulfides, e.g. HS^- can interact with non-polar groups of DOM and studies have indicated that the organic matters, due to their stronger interactions with NAg, can replace presence of functional groups on NAg surface, for example the citrate coating (used as stabilizer) (Gunsolus et al., 2015). The nanoparticle-DOM interaction is hypothesized to carrying sulfide closer to the nanoparticle surface (Thalmann et al., 2016).

Sulfidation of NAg to Ag_2S has been shown to reduce the nanoparticle toxicity on environmental organisms, seen with bacteria that are commonly present in soil and water, e.g., *E. coli* (Reinsch et al., 2012), nitrifying bacteria (Choi and Hu, 2009), the fungi *Phanerochaete chrysosporium* (Guo et al., 2016), and even with zebrafish embryos (Levard et al., 2013a). Not only that Ag_2S has been deduced by many studies to be biologically less active, but also, its formation is thought to lead to lesser silver ion leaching from the nanoparticle, in particular in the case of Ag– Ag_2S core-shell formation (Levard et al., 2013a; Zhang et al., 2018b). Stable presence of Ag_2S in the environment has also been indicated, even at low sulfide concentrations (Fletcher et al., 2019). Interestingly, studies have shown that sulfidized NAg can revert to being bioavailable and therefore toxic to organisms, under specific conditions, for example, when in the presence of Fe(III) and light under aerobic condition. This is a two-step process (Equations (2)–(4)), wherein Ag_2S particles transform back to silver particles via the photo-induced Fe (II)/Fe(III) redox cycling reactions (Li et al., 2016; Zhang et al., 2018b):



2.3.2. Reaction with chloride and effects on toxicity

NAg can also undergo chemical transformation through reactions

with the ubiquitous chloride (Cl^-) in environment. A variety of silver species can form depending on the Cl/Ag ratio (Fig. 3). Studies have shown that at relatively low Cl/Ag ratios (less than 5×10^3), AgCl shells will form on the surface of the nanoparticle, while at relatively high ratios ($>5 \times 10^4$), soluble Ag–Cl complexes ($\text{AgCl}_x^{(x-1)-}$) will form (Li et al., 2018; Zhang et al., 2018b). The formation of the Ag– AgCl (core-shell) structures is thought to decrease the bioavailability, and in turn, toxicity of the nanoparticle. AgCl is biologically inert, with the core-shell formation also reducing the extent of Ag^+ leaching from the nanoparticle (Levard et al., 2013a). In contrast, the soluble Ag–Cl complexes have been indicated to exhibit toxicity effects on environmental microbes (Levard et al., 2013b; Xiu et al., 2011). Using *E. coli* as the model bacterium, Li et al. (2018) supported this notion. Firstly, under dark condition, the work showed that NAg ($d = 20$ nm, citrate-coated, 5 mg Ag/L) toxicity increased upon increasing the Cl^- concentration from 0.01 M to 0.1 and 0.5 M, which is most likely associated with changes in silver speciation, that is, the formation of the bioavailable soluble Ag–Cl complexes at the latter systems with relatively high Cl/Ag ratios, as opposed to the inert AgCl formation at lower Cl/Ag ratios. Interestingly, the toxicity effects increased following further UV irradiation. The study found that apart from the enhanced rate of Ag^+ leaching, the irradiation facilitates generation of oxygen radicals. An increased level of superoxide radical ($\text{O}_2^{\cdot-}$) was observed in NAg systems at relatively low Cl^- concentration (0.01 M). At higher Cl^- concentration, there was less $\text{O}_2^{\cdot-}$ -generated, instead, an increased presence of other reactive oxygen radical, the hydroxyl radical ($\text{OH}\cdot$), was detected (Li et al., 2018). The findings highlight the genuine potential of NAg toxicity in chloride-containing environmental settings, in particular those with high concentrations such as oceans and riverbeds, with the effects being further enhanced by the potential sunlight-induced formation of oxygen radicals.

2.4. Nanosilver speciation in soil and effects on toxicity

As shown by scholarly studies, the physicochemical speciation of NAg in soil is a complex process, perhaps even more so than in the aquatic systems. Soil is an intricate matrix of solid, solution and gas phase systems, with which the nanoparticle can interact (Anjum et al., 2013; Chen, 2018). A number of studies have addressed the physical transformation of NAg, in particular the hetero-aggregation process, considered as one of the major transformation of the nanoparticle in soil due to the abundant presence of 'native' particles (Hotze et al., 2010). Hoppe et al. (2016) studied the hetero-aggregation of NAg ($d = 30$ nm, citrate-stabilized and $d < 20$ nm, stabilized with polyoxyethylene and polyoxyethylene glycerol trioleate) in the presence of soil minerals (e.g. silica, goethite, and kaolinite) and concluded that the process is more likely than the NAg–NAg aggregation in soil. The studies further found that the presence of these 'native' particles can potentially retain the nanoparticle in soil through the aggregation process, which is more favorable at acidic conditions ($\text{pH} \leq 5$) (Hoppe et al., 2014, 2016). Notably, the hetero interactions involve a complex interplay between the nanoparticle, types of mineral as well as the solution chemistry (Hoppe et al., 2016).

NAg can also undergo chemical transformation in soil, with current studies reporting similar processes as those observed in the aquatic environments. The nanoparticle has been indicated to primarily sulfidize in soil, while also reacting with chloride to certain extent. Li et al. (2017) added NAg ($d = 11$ nm, PVP-coated) and in independent experiments, AgNO_3 , into paddy soils and after 28 days, found that most of the silver in both the nanoparticle and ionic systems, were present as the biologically inert Ag_2S (>94% of added silver), with relatively minor presence of metallic Ag^0 , AgCl , Ag_3PO_4 and Ag_2O . Although note that, Ag_2S can re-transform to the toxic metallic Ag^0 and even re-dissolve into Ag^+ ions under specific conditions. This could be the underlying reason, at least in part, for the reported toxic effects of Ag_2S on soil microbes, such as the ammonia-oxidizing bacteria (Schlich et al., 2018). Further studies by

VandeVoort et al. (2014) observed the effect of the nanoparticle surface functional group on the sulfidation. The work detected formation of Ag_2S , as well as Ag(I) – humic acid complexes following 30 days incubation of PVP-coated NAg ($d = 15$ nm) in test soils with presence of humic substances, while essentially only the Ag(I) – humic acid complexes were detected in the case of uncoated NAg ($d = 50$ nm). Such interactions of the nanoparticle with dissolved organic matters (DOMs) in soil matrix, as reviewed by Chen (2018), have been associated with electrostatic interactions, van der Waals forces and hydrophobic interactions.

There is evidence that microbes can also affect NAg transformation in the environment. The nanoparticle, for example, has been indicated to interact with microbial secreted proteins, the so-called secretomes. Eymard-Vernain et al. (2018) studied the sulfidation of NAg ($d < 100$ nm, polyvinylpyrrolidone (PVP)-coated) in the presence of whole cells of the soil bacterium *B. subtilis* and observed different speciation of the nanoparticle when compared to those exposed to the isolated secretomes originating from the bacterium. The work detected formation of Ag_2S nano-sized particles, both crystalline and amorphous forms, in the live-cell *B. subtilis* system, while Ag – Ag_2S core-shell particle structures were found in the isolated secretome system (no bacteria). Although the study did not measure the level of sulfide in both systems, it is hypothesized that the different Ag_2S speciation was linked to sulfur metabolism by *B. subtilis* which may affect sulfide concentration in the system. Interestingly, only ~20% of silver was sulfidized in the secretome system, in comparison to the significantly higher ~80% sulfidation seen in the cell system. The observations highlight the potential effects of microbes and their associated physiological activities on silver speciation in the environment.

3. Nanosilver-induced shifts in microbial communities: effects on nitrogen cycle and organic matter decomposition

Microbes are important for ecological processes. Microbes are the engines for nutrient cycling, decompose organic matters, as well as forming the base of the food chain (Ahmad et al., 2011). The accumulating presence of the toxic NAg in the environment has led to escalating concerns on potential shifts in microbial community profiles (Das et al., 2012a). In previous sections, we have seen how NAg exerts toxicity on single species environmental microbes. Microbes, however, predominantly grow as multi-species communities in the environment with complex metabolic interactions (Konopka, 2009). This has prompted scholarly studies, summarized in Table A.1 (Supplementary material), to investigate how presence of NAg can change the community abundances and diversities, and equally important, their metabolic functions. A number of approaches have been used to elucidate the ecological implications of the nanoparticle, from DNA- and RNA-based studies that enable partial community analyses (e.g. real-time polymerase chain reaction (PCR) of specific genes) and even whole community microbial profiling (e.g. metagenomic (DNA), and metatranscriptomic (gene expression) analysis), to studies of microbial metabolisms, which include detecting changes in nitrification and carbon consumption rates.

3.1. Community and metabolic shifts in aquatic environments (water and sediment)

In response to the increasing reports of NAg release into rivers, estuaries and oceans (Beddow et al., 2017; Das et al., 2012b; Doiron et al., 2012; Fabrega et al., 2011a), studies have indicated NAg-induced changes in the metabolic activities of microbes sampled from these natural waters. Inhibition in respiration was observed in stream water microbial communities in the presence of the nanoparticle ($d = 21$ nm, PVP-coated) (Colman et al., 2012), although the effect seemed to be significant only at relatively high NAg concentration, in this case at 75 mg Ag/L, relative to the control systems (representative microcosms isolated from the environment, with no added silver). At

environmentally-relevant concentration of NAg, Das et al. (2012b) reported lower affinity of extracellular enzymes to their substrates in natural water bacterial communities. The effect, for example seen with the hydrolysis enzyme alkaline phosphatase, was concentration-dependent. A 10- to 100-fold reduction in the enzyme-substrate affinity and therefore the enzyme activity, were detected at NAg concentrations ($d = 1$ –10 nm, polyacrylate-coated) greater than 0.1 mg Ag/L, while only minimal affinity changes were found at lower nanoparticle concentrations.

Studies have also shown that presence of NAg could shift the composition of aquatic microbial communities. Using denaturing gradient gel electrophoresis (DGGE), Das et al. (2012a) reported changes in the water bacterial communities sampled from a stormwater management pond following exposure to environmentally-relevant concentrations of 0.01–1 mg Ag/L NAg ($d = 1$ –10 nm, polyacrylate-coated). After five days exposure, the community composition shifted by as much as 50–60% relative to the control microcosms, and the effects were indeed milder at the lower NAg concentrations (0.01 and 0.02 mg Ag/L). Such silver-induced changes have also been observed in marine microbial communities. Presence of NAg ($d = 20$ nm, polyallylamine-coated) decreased the richness of a marine microbial community, from 44 OTUs (operational taxonomic units, classifying microorganisms based on their sequence similarity) detected in the control microcosms, to 41 OTUs at the relatively low 0.005 mg Ag/L concentration and to 28 OTUs at 0.05 mg Ag/L. Ionic silver at equivalent concentrations (supplied as AgNO_3) were associated with more severe effects, with lower numbers of 27 and 20 OTUs detected in the 0.005 mg Ag/L and 0.05 mg Ag/L systems, respectively (Doiron et al., 2012). The latter is in agreement with the earlier described differences in the toxicity characteristics of the different forms of silver, with the ion seemingly more toxic than solid silver particles.

NAg effects on microbial community also extend to the surface-attached dense growth of biofilms, which, unlike their free-living planktonic growth counterparts, are enclosed by protective polymer matrix, and are therefore more resilient to external stress. Fabrega et al. (2011b) exposed marine biofilm to 2 mg Ag/L NAg ($d = 65$ nm, citrate-coated). After four days, although the major bacterial groups were still present in the biofilms, the study observed changes in their relative abundances, based on terminal restriction fragment length polymorphism (T-RFLP) and phylogenetics analyses. For instance, the relative abundance of γ -Proteobacteria was lower in the silver-treated samples when compared to the control microcosms, while, the exposure seemed to increase the relative abundance of Cyanobacteria. Another important observation, a recent study has reported the impact of NAg on microbial decomposers. At the relatively low ~0.005 mg Ag/L exposure, Tili et al. (2017) reported ~50% less bacterial biomass and ~70% less fungal biomass in stream water microcosms, following 25 days exposure to NAg ($d = 25$ nm, citrate-coated). The bacterial composition shifted more rapidly upon exposure to ~0.05 mg Ag/L concentration, whereby significant shift in microbial composition represented by clear separation in non-metric dimensional scaling (NMDS) ordination were seen after just 5 days, while the fungal biomass remained unaffected until 25 days.

The release of NAg into aquatic environment also affects microbial population in the sediments. Studies have shown that presence of the nanoparticle shifts the bacterial composition and even the abundances of genes that have important roles in nitrogen cycle. Jiang et al. (2017) reported a ~50% decrease in the relative abundance of the nitrification bacteria *Nitrospira* spp in the lake sediment following exposure of microcosms to the environmentally-relevant 0.5 mg Ag/L NAg concentration ($d = 20$ nm, PVP-coated), when compared to the control microcosms (note that nitrification is a process in nitrogen cycle that involves oxidation of ammonia (NH_3) to nitrite (NO_2^-), then nitrite to nitrate (NO_3^-)). In another study, the presence of NAg ($d = 17$ –40 nm, methoxypolyethylene-glycol-coated) was found to lower the abundance of bacterial *amoA* gene, that encodes the alpha-subunit of ammonia

monoxygenase (the enzyme that catalyzes the conversion of ammonia to hydroxylamine, the first step in ammonia oxidation) by ~75% and ~65% in low-salinity and mesohaline sediments, respectively, after 14 days. The effects were observed at the 'high-end' of environmentally relevant NAg concentration, of 50 mg Ag/L. Interestingly, the study also detected inhibition of the nitrification potential rates of the microbial communities, by 20-fold in low-salinity sediments relative to the control microcosms, and by 2-fold in mesohaline sediments, after just one day (Beddow et al., 2017).

The nanoparticle has been reported to also affect denitrification process (a nitrogen cycle process that convert nitrate and nitrite to gaseous forms of nitrogen) in sediments. Added presence of NAg (d < 100 nm, uncoated) at 10 mg Ag/L in sediment isolated from Taihu Lake, China as well as ionic silver at equivalent silver concentration, were found to inhibit the rate of N₂ gases release, by 32% (nanoparticle effect) and 44% (ion effect), respectively, relative to the control microcosms, again indicating the more significant effect of silver ion (Liu et al., 2018). The findings are in agreement with the observed decrease in the relative abundance of *nirK* and *nirS* genes (encode nitrite reductase enzymes that catalyze nitrite reduction to ammonium), and indeed, in the abundance of *Paracoccus* spp, a group of bacteria known to carry the genes. The study further noticed that the extent of inhibition of the denitrification process is less in the presence of functionalized NAg, whereby PVP-coated nanoparticle was associated with higher N₂O and N₂ release when compared to the uncoated nanoparticle. The chemical transformation of NAg, in this case the formation of nano-sized Ag₂S particulates (sulfidation phenomenon), also reduced the extent of denitrification inhibition. Indeed, in comparison to the PVP-coated NAg, the sulfidized PVP-coated particles exhibited even less inhibition effect on denitrification (Liu et al., 2018). The latter is in agreement with the earlier described notion that Ag₂S is biologically less active.

Up to this stage, we have seen how the release of NAg into aquatic environments has the potential to shift the microbial community composition both in sediments and waters. The shifts, which were also seen in biofilm forms of growth, can disturb the physiological functioning of the microbial communities. The presence of NAg has been indicated to inhibit the nitrogen cycle, lowering the abundance of important nitrification and denitrification microbes and genes. Interestingly, these adverse effects seem to be more severe with the ionic form of silver, indicating the significant impact of silver ion leaching from the nanoparticle on microbial communities. Furthermore, studies have found that NAg can affect the flow of nutrients across the water-sediment surface, already seen in the agricultural canal water-sediment interface. The water-sediment interface is where NAg could settle following the likely aggregation of the nanoparticle once released into aquatic environments (Buchkowski et al., 2016). Presence of NAg has been shown to reduce the N₂ flux rates across the interface, while increasing oxygen consumption of microbial communities, relative to the no-added silver control, which is thought to in turn affect the microbial community composition in both water and sediments. Notably, transformations of NAg, such as the sulfidation process, can affect the toxicity of the nanoparticle on microbial communities.

3.2. Community and metabolic shifts in wastewater treatment systems

Research efforts have investigated the potential effects of the nanoparticle on the microbial communities associated with wastewater treatment plants (WWTPs), in particular those that are present in the activated sludge process, a biological treatment to remove nutrients from wastewater prior to discharge into natural waters. *Bacteroidetes* and *Proteobacteria*, which are typically present in the activated sludge communities (Larsen et al., 2008), have been shown to be sensitive to the presence of NAg. Using DGGE analysis, Sun et al. (2013) studied activated sludge microcosms isolated from a WWTP and observed significant reduction in the relative abundance of the two Gram-negative bacterial phyla, relative to the control microcosms following exposure

to the environmentally-relevant 1 mg Ag/L NAg (d < 15 nm, PVP-coated). *Bacteroidetes* have major roles in sugar degradation in wastewater treatment, while *Proteobacteria* are essential for floc formation, which will determine important sludge parameters, including flocculation, settling and dewaterability (Nielsen et al., 2002). Therefore, changes in the abundance of these bacterial group would disrupt the organic matter decomposition process as well as the characteristics of activated sludge, lowering the overall wastewater treatment efficiency (Kragelund et al., 2008; Sun et al., 2013). This has been corroborated by other studies, reporting decreasing rates of organic matter removal upon exposure of activated sludge in sequencing batch reactors (SBRs) to increasing 0.1–50 mg Ag/L NAg (d = 13 nm, PVP-coated) concentrations. The organic matter removal efficiency decreased from 95% in the untreated (no added silver) SBR, to 20% when in the presence of 50 mg Ag/L NAg after 24 h. The finding is further supported by a 16S rRNA gene sequencing analysis, also indicating a decreasing relative abundance of *Bacteroidetes* in the silver system (Jeong et al., 2014).

Research inquiries have also reported effects of the nanoparticle on nitrifying bacteria communities in WWTPs. Nitrification bacteria (both the ammonia- and nitrite-oxidizing) are essential for biological nitrogen removal in WWTPs, whereby nitrification is often the rate-limiting step (Wagner et al., 2002; Yang et al., 2014). A significant (~50%) decrease in the relative abundance of *Nitrosomonas* spp (ammonia oxidation) and an almost complete disappearance of *Nitrosococcus* spp (ammonia oxidation) were observed by Yang et al. (2014) when comparing the activated sludge microbial profile in the presence and absence of NAg (d = 35 nm) at 40 mg Ag/L. The findings are consistent with the detected 4-fold decrease in the nitrification gene *amoA* copy number. Moreover, the abundance of *Chloroflexi* spp, a group of bacteria that involve in carbon oxidation (as well as nitrification) also decreased by 2-fold following the nanoparticle exposure. Further, it is worth noting that the study also observed more pronounced microbial community shifts in the NAg-treated activated sludge when compared to those treated with ionic silver (supplied as AgNO₃). The apparent passivated effects of ionic silver could associate with the likely predominant transformation of Ag⁺ into AgCl and AgCl₂⁻, as evident from the equilibrium speciation modeling, suggesting that only ~10% of the total ionic silver existed as free Ag⁺ ions (Yang et al., 2014). The NAg effect on the WWTP nitrifying bacteria communities is apparently less at relatively low silver concentrations. Zhang et al. (2014) only observed little changes in the relative abundance of *Nitrosomonas* spp and *Nitrospira* spp (nitrite oxidation), before and after NAg exposure (d = 6 nm) at 0.1 mg Ag/L in a membrane bioreactor activated sludge system. Doolette et al. (2013) did not detect any major changes on the nitrification and methanogenesis activities (the latter is an anaerobic digestion process to produce methane from organic matters) in a simulated activated sludge system in the presence of the nanoparticle (d = 40 nm, PVP-coated) at 2.5 mg Ag/L. The latter study also found presence of Ag₂S in the sludge system and deduced that the likely sulfidation of the nanoparticle had lessen its effects on the nitrification and methanogenesis activities (Doolette et al., 2013).

The evidence of decreasing relative abundance of bacterial communities that have important roles in organic matter decomposition and nitrogen cycle, supports the notion that the presence of NAg could lower the efficiency of the corresponding biological processes in WWTP, which could result in the treated wastewater containing higher than acceptable levels of nutrients upon discharge into rivers and oceans. The latter would have multitude effects on the natural environments, including the infamous algal bloom that could disturb the food chain balance.

3.3. Community and metabolic shifts in soil

The frequent use of WWTP biosolids in agriculture (with studies already reporting presence of silver in biosolids, as mentioned earlier) as well as NAg-containing products, such as pesticides, including their disposal, warrant investigation on the effects of the nanoparticle on soil

microbial communities. Studies have indeed observed NAg-induced shifts in soil bacterial communities, including those that are in close proximity with plant roots. After just one day, Colman et al. (2013) reported a decrease in community richness from 150 ± 9 OTUs in control soil added with biosolids (no added NAg) to 101 ± 22 OTUs when in the presence of NAg-spiked biosolids at relatively low silver concentration (0.14 mg Ag/kg soil, d NAg = 21 nm, PVP-coated). Another study with suburban vegetable soils, found decreasing relative abundance of specific bacterial phyla (including *Acidobacteria*, *Actinobacteria*, *Cyanobacteria* and *Nitrospirae*), as well as the viable counts of (cultivable) ammonia- and nitrite-oxidizing bacteria with increasing NAg concentration (d = 50 nm), from 0 to 100 mg Ag/kg soil, relative to control soil microcosms (Wang et al., 2017). The potential effects on nitrogen cycle bacteria were also seen in NAg studies on soil collected from low arctic sites. Kumar et al. (2014) observed decreasing relative abundance of the nitrogen-fixing *Rhizobiales* from ~10% in control soil microcosms to $\leq 0.7\%$ when in the presence of the nanoparticle (d = 20 nm) at the relatively high 660 mg Ag/kg soil concentration (at genus level, a significant 370-fold decrease in the relative abundance of *Rhizobia* was detected), although the study also saw 5-fold increase in the relative abundance of the ammonia-oxidizing *Nitrosomonadales*.

Research inquiries have reported bacterial community changes in rhizosphere, small regions of soil near plant roots that have been known to harbor microbiomes. Sillen et al. (2015) observed shifts in the bacterial community structure in the rhizosphere soil following NAg exposure (d = 20 nm, uncoated) at the environmentally relevant 100 mg Ag/kg soil concentration, although the extent of the shifts were less than those in the corresponding bulk regions of the soil. The shifts in microbial composition were most likely associated with the detected changes in physiological activities in the rhizosphere. Following the nanoparticle exposures, the study saw decreasing enzymatic hydrolysis activity (as indicated by the fluorescein diacetate assay that measures cellular esterase activity, in this case, in soil bacteria and fungi) and less carbon consumption by the microbial communities in the rhizosphere (the latter indicated by the consumption of different types of carbon sources, e.g. xylose, malic acid, and hydroxybenzoic acid). Other studies have also reported disruptions in the soil microbial activity, again on enzyme activities, as observed by Colman et al. (2013) on leucine amino peptidase (~50% decrease relative to control non-treated soil microcosms) and phosphatase (~30% decrease relative to the control microcosms) due to an exposure to PVP-coated NAg (d = 21 nm) at 0.14 mg Ag/kg soil. The enzymes cleave the amine and phosphoester bonds, respectively, and are therefore essential for degradation of organic matters. Further study by Kumar et al. (2014) showed a halved microbial respiration activity in arctic tundra soil when in the presence of NAg (d = 20 nm, 660 mg Ag/kg soil).

Studies have indicated that metal-based nanoparticles and their transformation products exhibit different impacts on soil microbial communities (Sun et al., 2021). This seems to also apply to NAg, as observed by a number of research inquiries studying the effects of the sulfidized form of the nanoparticle on soil microbial communities. When compared to NAg and Ag^+ (as AgNO_3), Doolette et al. (2016) reported weaker effects of nano-sized Ag_2S particulates, not only on the soil community composition in general (based on changes in the composition of OTUs), but also on the *amoA* gene abundances and in turn, the nitrification rates, following 28 days exposure to the respective silver species at 0.1–5590 mg Ag per kg soil. In a more quantitative study, Schlich et al. (2018) observed 80% inhibition of ammonia-oxidizing bacterial activities (relative to untreated control) upon 14 days exposure of soil samples to NAg (d = 15 nm, NM-300K-stabilized) and Ag^+ (as AgNO_3), with comparable effect seen only after 140 days in the case of nano-sized Ag_2S exposure. Indeed, studies have also indicated the potentially weaker effects of Ag_2S on organic matter decomposition. Peixoto et al. (2020) reported inhibition of β -glucosidase activity, enzyme that breaks down complex carbohydrates into simple sugars, evident only after 28 days exposure of soil samples to nano-sized Ag_2S ,

while treatment with Ag^+ (as AgNO_3) already exhibited substantial effects immediately upon exposure. Note that the trends were in line with the observed shifts in microbial community composition, again, seen after 28 days for the Ag_2S , while immediately for Ag^+ exposure (Peixoto et al., 2020).

In summary, studies have shown the potential impacts of NAg, as well as the transformed products, although weaker for the latter (sulfidized NAg), on soil microbial communities. Presence of the nanoparticles not only inhibited nitrogen cycle processes and organic matter decompositions through shifts in microbial composition, changes in the abundances of key genes and enzyme activities, but also can adversely impact plant growth, due to decreasing plant nutrient intake as a result of the altered microbiomes in the rhizosphere.

4. Nanosilver-driven changes in environmental pools of antibiotic resistance determinants

Antibiotic resistance is now a global health crisis. The issue has been associated with the indiscriminate use of antibiotics in clinical settings, as well as in livestock and agriculture (Kardas et al., 2005), with clinicians and scientists calling on their judicial use to slow the development and spread of resistance. There has been known release of antibiotics into soil and aquatic environments, imposing selective pressure on natural microbial communities (Allen et al., 2010). Studies have shown that other toxic agents, including metals (Mahbub et al., 2020; Pal et al., 2017) could act as driving force for the increasing antibiotic resistance gene (ARG) pools in the environment, through the mechanism of co-selection.

In the context of the ecological impact of NAg, understanding how metals can co-select ARGs is important, with the environmental presence of the nanoparticle potentially shifting the ARG profile in microbial population, even in the absence of antibiotics (Pal et al., 2017). Studies have shown that the co-selection is frequently associated with cross-resistance, co-resistance, or co-regulation/co-expression phenomena (Fig. 4). Cross-resistance occurs when the same genes are conferring resistance to multiple compounds (Chapman, 2003; Imran et al., 2019). In the case of multi-drug resistant *Acinetobacter baumannii* for instance, an efflux pump mechanism is responsible for resistance towards heavy metals, metalloids and antibiotics (Fournier et al., 2006). Similarly, silver-resistant *E. coli* mutants showed cross-resistance to cephalosporin antibiotics due to the shared active efflux mechanism (Li et al., 1997). Co-resistance, on the other hand, occurs when different genes that confer resistance to different compounds, are present in the same genetic element (Baker-Austin et al., 2006; Pal et al., 2017). As an example, a recent study reported the presence of *pcoA-D-E* (copper resistance genes), *silE-P* (silver resistance genes) as well as antibiotic resistance genes, including the beta-lactams (*bla_{CTX-M}*), quinolones (*oqxAB*) and aminoglycosides (*aac-Ib-cr*) resistance genes, in plasmids (a mobile, extrachromosomal DNA element that can replicate independently) collected from *E. coli* isolated from livestock animals (Fang et al., 2016). Another mechanism that can promote the co-selection of ARG by metals is the co-regulatory/co-expression mechanisms, i.e. when a regulatory system controls the expression of multiple resistance genes that confer resistance to different compounds (Baker-Austin et al., 2006; Pal et al., 2017). For the latter, the regulatory protein CzcRS, for example, has been found to increase the expression of the CzcCBA efflux system conferring resistance to cadmium, zinc and cobalt, while also, at the same time, decreasing the expression of the OprD porin conferring resistance to carbapenem antibiotics in the pathogenic environmental bacterium *Pseudomonas aeruginosa* (Perron et al., 2004).

Scholarly works have indicated the potential of silver-driven co-selection of ARGs, with studies performed on laboratory strains and environmental bacteria. Laboratory strain of the pathogenic environmental bacterium *Mycobacterium smegmatis* that was pre-exposed to NAg exhibited increased resistance to isoniazid, a common mycobacterial antibiotic, with the minimum inhibitory concentration (MIC) of the

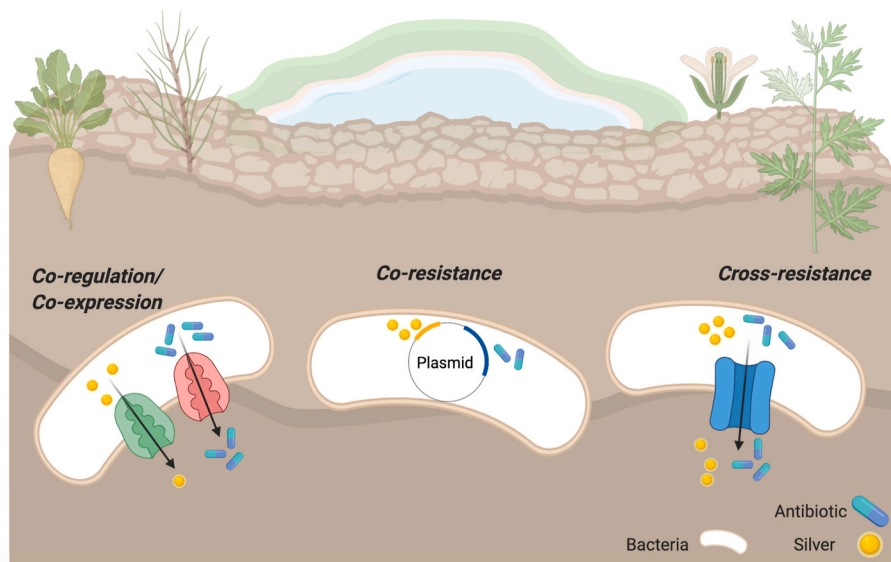


Fig. 4. Silver/antibiotic defense mechanisms in bacteria that associate with the silver-driven co-selection of antibiotic resistance.

antibiotic elevated by 4-fold when compared to the wild-type strain. The bacterium was also found to develop resistance to silver, while no resistance to other toxic metals, in this case Cu, Co and Zn ions, were observed, suggesting that the antibiotic resistance is distinctively linked to the silver resistance (Larimer et al., 2014). Although it remains unclear as to how the silver resistance determinant is associated with that of the antibiotic, the study indicated a silver-driven co-selection of ARGs. In other studies, *E. coli* and *Staphylococcus aureus* that were pre-exposed to NAg exhibited increased resistance to antibiotics of various modes of action (penicillin, chloramphenicol, and kanamycin) with their MICs and minimal biocidal concentrations (MBCs) raised by 2- to 8-fold. In fact, a lower extent of membrane damage and cellular oxidative stress were observed upon exposures of the bacteria to the antibiotics, relative to the non-NAg pre-exposed cells. The latter study further hypothesized that NAg elevate antibiotic resistance through stimulation of cellular ROS generation, in turn, promoting higher resilience to stress (Kaweeterawat et al., 2017).

4.1. Co-selection of antibiotic resistance genes in aquatic environments (water and sediments) and soil

Current studies on environmental samples, in particular those from natural waters, have found not only an elevated presence of ARGs, but also, silver resistance genes, with the observations being associated with the metal co-selection potential. Analysis of surface water samples from a highly contaminated river (stretches of Yamuna River, India) revealed an abundant presence of the extended-spectrum β -lactamases (ESBLs) genes (conferring resistance to β -lactam antibiotics) and silver resistance genes (*sil* genes). Among the collected 399 bacterial isolates, 30% (121 isolates) were ESBL producers with *bla*_{CTX-M} being the most prevalent ESBL genes and of those *bla*_{CTX-M} isolates, more than 70% have at least one *sil* genes (*silE*, *silP* or *silS*). Further, a conjugative gene transfer study between a plasmid-free *E. coli* strain and the ESBLs- and *sil* gene(s)-harboring bacterial isolates as donors, showed transfers of the antibiotic and silver resistance genes into the *E. coli* recipient, indicating that both resistance genes are located in the same mobile genetic elements, therefore, a co-resistance phenomena. The observations, along with the evidence of high prevalence of ESBL and *sil* genes in the polluted river, most likely refer to silver-driven co-selection of ARGs (Siddiqui et al., 2018). Another study reported presence of silver in surface water samples, which was associated (quantitative correlation with Spearman's Rank Correlation Coefficient) with the detected presence of *sul1* gene

(conferring resistance to sulfonamides), again, indicating a silver-driven co-selection of ARG, although the genetic link between the antibiotic resistance gene and any presence of silver resistance gene (i.e. the possibility for the cross-/co-resistance or co-regulatory/co-expression) was not investigated in the study (Garner et al., 2016). Similarly, Reddy and Dubey (2019) observed a statistically significant correlation ($p < 0.05$) between the abundance of silver resistance genes and various ARGs, including the tetracycline, sulfonamide and streptomycin resistance genes in the surface water of a polluted river (Ganges, India).

In contrast to those found in surface waters, research inquiries have observed only minimal changes in the pool of antibiotic resistance determinants in sediments with presence of silver, although these studies are still limited in numbers. Mühling et al. (2009) created microcosms from estuarine sediments and spiked the microcosms with NAg ($d = 59$ nm) of up to 2 mg Ag/L. The study observed comparable number of colonies growing on antibiotic-containing plates of the spiked microcosms when compared to the control (no added silver, being validated by multivariate statistical analyses). Differences in NAg speciation in the sediments and surface water could be a factor for the different nanoparticle effects on the pools of resistance determinants. The generally higher extent of NAg aggregation is thought to, at least in part, reduce the bioavailability of the nanoparticle in sediments and hence the impact on microbial communities. Another study reported a statistically significant correlation between the abundance of *sul1* genes and silver levels in riverine sediments, although to a lesser extent when compared to that in the corresponding bulk water (Garner et al., 2016). The latter observation could imply a more favorable silver-driven co-selection of specific ARGs, in this case the *sul1* genes, in sediments. The impact of NAg on the antibiotic resistance determinant pools in soil environment is still largely unexplored. Chen et al. (2019) found no statistically significant change ($p > 0.05$) in the abundance of ARGs in general, following exposure of soil microcosms, as well as plant phyllosphere to NAg ($d = \sim 20$ nm and ~ 50 nm, uncoated) at 100 mg Ag/kg concentration, the latter are the above-ground regions of plant that harbor microbiomes (Hirsch et al., 2008). A notable increase in the relative abundance of efflux pumps genes however, was detected, which indicates a silver-driven co-selection (Chen et al., 2019).

Up to this stage, it is evident from scholarly studies that the silver-driven ARG co-selection phenomena is more apparent in water microbial communities than in sediments. The distinct effects most likely associate with the different NAg speciation in those environments, which in turn affecting toxicity on microbes. While the impact of NAg on

soil ARG pools is still largely unexplored, given the complexity of soil composition and soil chemistry, it is anticipated that the co-selection phenomena, if any, would be more challenging to elucidate.

4.2. Co-selection phenomena in wastewater treatment systems

Co-selection of ARGs has been indicated to also occur in wastewater treatment plants, that is, in the biological process systems. Ma et al. (2016) dosed sequencing batch reactors (SBRs) with NAg (d = 52 nm) and in parallel experiments, with ionic silver, initially at 0.1 mg Ag/L and sequentially increased every 14 days to 1, 10, and 20 mg Ag/L concentrations. The metagenomics analyses revealed that the different forms of silver co-select different ARGs. In the NAg-dosed SBRs, specific ARGs, i.e. aminoglycoside resistance genes, mainly the genes responsible for streptomycin resistance protein A (*strA*) and multidrug efflux pump subunit AcrB (*acrB*) were found in greater abundance relative to the undosed SBRs system. NAg-dosed SBRs were also associated with higher abundance of metal resistance genes. At comparable silver concentrations in the Ag⁺-dosed reactors, bacitracin resistance genes, mainly the undecaprenol kinase (*dgkA*) and undecaprenyl diphosphatase (*uppP*) genes, as well as macrolide-lincosamide-streptogramin (MLS) resistance genes, mainly the erythromycin resistance gene (*ermF*), were the most abundant (Ma et al., 2016). The different co-selections are thought to result from the different microbiological activity of NAg and Ag⁺. As earlier described in Section 2 of this Review, both forms of silver have been shown to exert complex toxicity mechanisms on microbes, targeting the cell envelope components, cellular proteins and DNA (Gunawan et al., 2020; Valentin et al., 2020). The nanoparticle when compared to the ionic form of silver, however, is associated with more toxicity origins, with studies increasingly reporting the antimicrobial contribution of the solid silver particulates that remain after leaching (Faiz et al., 2018; Sotiriou and Pratsinis, 2010). Further analysis of the metagenome profiles revealed greater abundance of mobile genetic elements in the silver-dosed reactors relative to the undosed ones. For the NAg-dosed reactors, higher abundance of integrons (mobile genetic elements that encode an integrase enzyme, often associated with antibiotic resistance) were observed, while higher abundance of plasmids were seen in the Ag⁺-dosed reactors. Further correlation analysis revealed significant link between the abundance of integron and *strA* (streptomycin ARG) in the NAg systems, while for the Ag⁺ systems, the abundance of plasmids were correlated to *dgkA* and *uppP* (bacitracin ARGs) as well as *ermF* (macrolide ARG). Both integrons and plasmids are, therefore, likely contributors to the silver-driven shifts in the ARG profiles (Ma et al., 2016). In other study, Kim et al. (2016) investigated the effects of NAg sulfidation on the ARG profile in thermophilic anaerobic digesters. Using quantitative PCR, the work found no significant effects of sulfidized NAg (as well as Ag⁺, supplied as AgNO₃) on *sul1*, *tet(W)* and *tet(O)* (both tetracycline ARGs), as well as *intI1* (integrase gene in class 1 integron) abundances in the systems. It is apparent that sulfide-rich environments, which promote silver sulfidation, would reduce the biological impacts of NAg (as well as Ag⁺), including the co-selection of ARGs (Kim et al., 2016). In other study, Miller et al. (2013) spiked thermophilic anaerobic digesters (53 °C) with 0.01–1 mg Ag/L NAg (d = 45 nm, citrate-stabilized) and also found minimal differences in ARG abundance when compared to non-silver-added control digester. The work suggested that the operating parameters of a system in WWTPs, such as the solid retention time (SRT) and temperature, could also affect the co-selection potential.

Taken together, research inquiries have indicated the ARG co-selection phenomena in wastewater treatment systems, in response to the presence of NAg. It is also apparent that different silver species co-select different ARGs, again, highlighting the effects of NAg transformation on the co-selection phenomena.

5. Conclusion and future outlook

In summary, the release of NAg into the environment can shift the balance and physiology of microbial community as well as, intriguingly, altering the antibiotic resistance determinant pools in environments. These effects are found to vary across different environmental settings, from surface water and sediments to soil and wastewater treatment plants (WWTPs), and are affected by the unique speciation of the nanoparticle. NAg can undergo aggregation and chemical transformations depending on the characteristics of the environmental surroundings, which include the presence of organic matters, complexing and precipitating agents, pH and ionic strength, even the presence of minerals. The nanoparticle has been indicated to not only lower the abundance of microbes that are important in nitrogen cycle and degradation of organic matters, but also the associated genes for these ecologically important processes. These adverse effects can disrupt the ecological balance. For instance, a deficiency of nitrogen-based nutrients could result from the decreased abundance of nitrogen-fixing, ammonia- and nitrite-oxidizing bacteria and genes seen in sediment and soil studies, which can affect plant growth and higher organisms in the food chain. Surface water and soil studies have also noted a lowered abundance of bacterial and fungal biomass, as well as, an inhibited activity of enzymes that are essential for breaking down organic matters. In WWTP studies, the observed decrease in the abundance of ammonia-oxidizing bacteria and genes as well as bacteria that are involved in sugar degradation are expected to lower the process efficiency for the biological removal of nitrogen compounds and organic matter. This could lead to the release of nutrient-rich effluents into natural waters, which is a major factor in the environmentally damaging algal bloom. NAg has also been indicated to co-select for antibiotic resistance genes (ARGs), as well as, interestingly, the transferable mobile genetic elements that harbor the ARGs. The phenomena could increase the resistance gene pools and facilitating their spread in both natural environments and WWTPs, which could ultimately find their way to animals and human through microbe-to-microbe gene transfers and the food chain. Scholarly studies, up to this stage, have also deduced generally stronger effects of ionic silver when compared to NAg, highlighting the significance of the silver ion leaching from the nanoparticle.

We propose three future research priorities to more thoroughly understand the ecological impact of NAg.

1. Firstly, to elucidate the mechanisms of the nanoparticle-induced shifts in microbial diversity and richness, including changes in the abundance of genes that are involved in nutrient cycle and organic matter decomposition. Research efforts have investigated the modes of NAg toxicity on single species microbes, which form the basis to decipher the nanoparticle effect at the community level. Studies should next enquire the potential effect of NAg on the cell-to-cell communication systems in microbial community. Presence of external stressor, like NAg, can affect the so-called quorum sensing (QS) mechanism, whereby microbes are producing and sensing signaling molecules, and in turn, orchestrating the expression of specific defense genes to protect the community. Deciphering how NAg affect the synthesis of cellular metabolites that are involved in the QS mechanism can give in-sights into the resultant community-associated response to the nanoparticle, and indeed advances in molecular biology has enabled fingerprintings of these metabolites.
2. Secondly, to investigate any potential effects of the already known presence of other heavy metals on NAg speciation and in turn, the nanoparticle toxicity. Research inquiries have reported, for example, the dissolution of the biologically inert Ag₂S to release the toxic Ag⁺, when in the presence of MnO₂, one of the naturally occurring oxidants (Shi et al., 2018). The study will give us a better picture on the true scale of NAg impact on microbial community in the different environmental settings.

3. Thirdly, in regard to the NAG-driven co-selection of ARGs, further work can focus on identifying the environmental microbes that harbor the resistance genes, at least at the genera level. More evidence should be obtained on the indicated correlation between the ARG enrichment and the phenomena of silver/antibiotic cross-/co-resistance as well as co-regulation/co-expression. The knowledge is key when assessing the potential for increasing abundance of antibiotic-resistant pathogens in the environment and their subsequent travel to animals and human through transfers of mobile genetic elements. Research inquiries should also cover more environmental settings, including soil, in which the ARG co-selection studies are still very limited.

Taken together, the realized ecological impacts of NAG call for more careful use of the nanoparticle, that is, to only use it when needed, and with clear antimicrobial targets. Apart from shifting the balance and dynamics of microbial communities, the environmental release of the nanoparticle has been indicated to further complicate the issue of antibiotic resistance. The knowledge can inform strategies for better assessment of the risks versus benefits of NAG applications, as well as, equally important, for a more effective regulations on the management and disposal of products.

Author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2021.118506>.

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