Speciation and lability of Ag-, AgCl- and Ag₂S-nanoparticles in soil determined by X-ray absorption spectroscopy and Diffusive Gradients in Thin Films

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Abstract

Long term speciation and lability of silver (Ag^-), silver chloride (AgCl^-) and silver sulfide nanoparticles (Ag_2S -NPs) in soil were studied by X-ray absorption spectroscopy (XAS), and newly developed 'nano' Diffusive Gradients in Thin Films (DGT) devices. These nano-DGT devices were designed specifically to avoid confounding effects when measuring element lability in the presence of nanoparticles. The ageing profile and stabilities of the three nanoparticles and AgNO_3 (ionic Ag) in soil were examined at three different soil pH values over a period of up to 7 months. Transformation of ionic Ag, Ag-NP and AgCl -NPs were dependent on pH. AgCl formation and persistence was observed under acidic conditions, whereas sulfur-bound forms of Ag dominated in neutral to alkaline soils. Ag_2S -NPs were found to be very stable under all conditions tested and remained sulfur bound after 7 months of incubation. Ag lability was characteristically low in soils containing Ag_2S -NPs. Other forms of Ag were linked to higher DGT-determined lability, and this varied as a function of ageing and related speciation changes as determined by XAS. These results clearly indicate that Ag_2S -NPs, which are the most environmentally relevant form of Ag that enter soils, are chemically stable and have profoundly low Ag lability over extended periods. This may minimise the long-term risks of Ag toxicity in the soil environment.

Introduction

Silver nanoparticles (Ag-NPs) constitute a major group of engineered nanomaterials (ENMs) increasingly found in consumer products. Indeed, recently revised databases of nano-products available in the USA¹ and European² markets confirm silver (Ag) as the most commonly identified element in consumer nanotechnology products currently in distribution. Most of these products exploit the antibacterial properties of Ag,³ but other nanoparticle-specific characteristics such as the high reactivity and enhanced optical properties of Ag-NP have also been utilised in a wide range of research and industry sectors.⁴⁻⁵ On the other hand, these special properties have raised significant concerns regarding the potential for Ag-NPs to pose unique risks upon their release into the environment. Assessing nanoparticle-related risks has proven challenging, however, due to their complex physical, chemical and biological pathways and transformations in the environment.⁶

A large proportion of the Ag used in consumer products is predicted to be released into the wastewater system⁷⁻⁸ and there is now a wealth of evidence indicating that the majority of Ag released via this pathway transforms into silver sulfide (α -Ag₂S, acanthite) or other sulfur-bound forms of Ag.⁹⁻¹³ Once formed, Ag₂S is highly insoluble ($K_{sp} = 6 \times 10^{-51}$) and is orders of magnitude less toxic than free Ag⁺ or Ag-NPs.¹⁴⁻¹⁵ This transformation can therefore reduce the ecological risks associated with the release of Ag⁺.¹⁶⁻¹⁸ For example, the toxicity of Ag₂S (not necessarily Ag₂S-NPs) to fathead minnow (*Pimephales promelas*) is 15,000 times lower than Ag⁺ and 50 times lower than silver chloride (AgCl).¹⁶ Similarly, Hirsch¹⁹ observed no statistically significant decrease in the survival rates of *Hyaella azteca* exposed to Ag₂S-amended freshwater sediments compared to control populations, even at doses as high as 753 mg Ag/kg. At the nanoscale, Ag₂S nanoparticles (Ag₂S-NPs) have reduced toxicity to bacteria (*Escherichia coli*),¹⁷ fish (*Danio rerio*, *Fundulus heteroclitus*), nematodes (*Caenorhabditis elegans*) and plants (*Lemna minuta*)¹⁸ relative to other forms of Ag. Furthermore, Ag-NPs transformed into “Ag-S phases” through the activated sludge treatment process did not affect nitrification or methanogenesis rates during

anaerobic digestion,¹³ indicating that this important microbial activity was not negatively affected.

Together, the available evidence suggests that, at least in the short term, sulfidation of Ag-NPs effectively acts as a “natural antidote to their toxicity”¹⁸ and may significantly reduce the risks posed by Ag-NPs.

Over extended time scales, however, little is known about the stability or bioavailability of Ag₂S (and particularly Ag₂S-NPs) in environmental compartments. One of the most significant endpoints of the Ag₂S formed from dissolved Ag or Ag-NPs in the wastewater transport and treatment system is soil. This is because biosolids (stabilised sewage sludge) are used in many countries as soil amendments to help provide organic matter and nutrients to improve crop production,²⁰ and since Ag₂S is insoluble, it is likely to accumulate over time in biosolid amended soils. Previous studies have examined the stability of Ag⁺ or Ag-NPs applied directly to soils or terrestrial environments,²¹⁻²³ but it is now recognised that these scenarios are of limited relevance as they are not the most probable forms of Ag released via the wastewater-biosolids-soil pathway.^{9-10, 24} In more recent studies, Ag-NPs have been mixed with sewage sludge or biosolid slurries just prior to application to soils.²⁵⁻²⁷ These treatments were found to adversely affect soil microbial activities and populations even at low mg/kg levels,²⁵ with a predicted no-effect concentration of 0.05 mg/kg of a standard Ag-NP (NM-300K) concluded in one study.²⁷ This is a more appropriate approach as the Ag is added within a relevant matrix (biosolids) applied to soil^{25, 27}; however, it is still not realistic since Ag₂S is usually the end product of Ag-NPs in biosolids from a wastewater treatment facility when applied to land. Furthermore, these studies did not directly determine the speciation of Ag in these soils after the extended ageing. In fact, the long term speciation, stability, and lability of Ag₂S-NPs (aspects that are critical in determining their environmental impact), have not been investigated in any environmental compartment to date.

Nanoparticles have a high specific surface area due to their extremely small size. This makes them more reactive and may render them more vulnerable to external conditions. For example, lead

sulfide (PbS) also has a low solubility ($K_{sp} = 9.0 \times 10^{-29}$), but its dissolution rate is reportedly dependent on particle size, with the area-normalised dissolution rate for PbS nano-crystals an order of magnitude higher than that of micro-crystals.²⁸ If Ag₂S-NPs behave similarly, then the reduction in toxicity postulated as a result of Ag-NP sulfidation may not be stable in the long-term, and the true level of the associated risks may be underestimated. Furthermore, some ligands (e.g. methionine) can enhance the dissolution of Ag₂S, whereas humic acids and thiol containing ligands effectively suppress this process,²⁹ and even if dissolution does occur in soils, reduction of Ag⁺ to metallic Ag may subsequently take place, particularly under alkaline conditions.³⁰ Recently, a small but potentially significant remobilisation of intact Ag₂S-NPs was observed in short term (24 h) batch retention and release experiments, particularly in the presence of environmentally relevant ligands such as thiosulfate and citrate.³¹ Thus, despite the low solubility of Ag₂S that seemingly infers its stability, both nanoparticle-specific dynamics and soil chemistry are likely to greatly affect its long term fate. Therefore, in order to assess the long term risks of Ag-NPs in the environment, there is a need to evaluate the stability and fate of Ag-derived nanomaterials and how they compare to those of pure Ag-NPs and other soluble Ag salts.

In this study, we investigated the long term transformation profiles of Ag₂S-NPs in soils together with that of a soluble Ag salt (AgNO₃) and two other Ag based nanomaterials: Ag-NPs with citrate coating and AgCl nanoparticles (AgCl-NPs). These three types of laboratory-synthesised NPs were added to the same soil at 3 different pH values and Ag speciation and lability were examined over an ageing period of up to 7 months. Silver speciation in soils was determined using Ag K-edge X-ray absorption spectroscopy (XAS) and labile Ag was measured using a DGT (Diffusive Gradients in Thin films) device newly designed for measuring element lability in the presence of nanoparticles.³²

Materials and methods

Synthesis of silver-, silver chloride and silver sulfide nanoparticles

Silver nanoparticles (Ag-NPs) were synthesised as previously described, by reduction of silver nitrate (AgNO_3) with sodium borohydride (NaBH_4) and using citrate as the capping agent.^{3, 33} Silver chloride nanoparticles (AgCl-NPs) were synthesised in ethylene glycol with polyvinylpyrrolidone (PVP, 40,000 M_w) capping agent.³⁴ They were resuspended in ultrapure water after centrifugation. Silver sulfide nanoparticles (Ag_2S -NPs) were prepared by reaction of AgNO_3 with elemental sulfur (S). Briefly, 7.1 mg of S was dissolved in 10 mL of warm ethanol with the aid of sonication, then added drop-wise to a 50 mL of 1 mM AgNO_3 solution containing 200 mg/L PVP, at 95 °C under vigorous stirring. The reaction was allowed to continue for 5 hours in the dark. The resulting solution had a turbid dark brown appearance. The NPs were either diluted (with MilliQ water) or concentrated (by centrifugation) before being applied to the soils. All nanomaterials were characterised by dynamic light scattering (DLS, Zetasizer ZS, Malvern Instruments Ltd), scanning electron microscopy (SEM, Quanta 450 FEG-ESEM, FEI Company) and by differential centrifugal sedimentation (DCS, DC24000 UHR, CPS Instruments). Powder X-ray diffraction was also used in the case of Ag_2S - and AgCl-NPs to check that the desired species had formed.

Preparation and ageing of Ag nanomaterials in soil

Soil from an agricultural area in South Australia was used in these experiments. In order to assess the effect of soil pH on Ag stability, the soil (with an original pH of 6.5 in 0.01 M CaCl_2) was subdivided and adjusted to three different pH values with either 0.1 M HNO_3 or 0.1 M KOH. This was followed by leaching for 20 h at 200 % water holding capacity (WHC) with chloride-free artificial rainwater to remove excess NO_3^- and K^+ , and to ensure consistency across tests. Between each

treatment, the soils were dried at 37 °C. The final pH values established were 5.7, 6.9 and 7.8 (when measured in a 1:5 soil: 0.01 M CaCl₂ suspension) and 5.7, 7.3, and 8.5 (when measured in a 1:5 soil:water suspension).

Silver was spiked at a nominal concentration of 42 mg/kg as AgNO₃, citrate coated Ag-NPs, Ag₂S-NPs or AgCl-NPs. The concentration was selected based on experimental considerations, as a sufficiently high concentration was required in order to ensure that Ag speciation could be reliably determined by XAS in the complex soil environment. Five grams of soil was used for each treatment and was brought to 60% WHC with water. The samples were aged in 70 cm³ polypropylene containers at 20°C in the dark, and were thoroughly mixed at each sampling time, i.e. at $t = 0$, 2 weeks, 4 weeks and 3 months. Ag₂S-NP treatments (most relevant form for soils) were kept incubating for longer than the other treatments in order to investigate their speciation over a longer ageing period, and were also sampled after 7 months of ageing. The samples (ca. 1 g each with moisture) were collected into 1.5 mL Eppendorf tubes and were stored in the dark at -20°C under argon and freeze-dried prior to XAS analysis. Due to transport and handling, the dried samples were exposed to ambient temperatures on several occasions (less than a week in total), but were otherwise kept in the dark in the freezer until 2-3 days prior to XAS analysis. Soils freshly spiked at the XAS facility ("fresh") were also measured to compare against the "stored" $t = 0$ samples.

X-ray absorption spectroscopy

X-ray absorption spectra were collected at the wiggler XAS Beamline at the Australian Synchrotron. The storage ring was operating at 3 GeV and 200 mA in top-up mode. Spectra were recorded at the Ag-K absorption edge (25.514 keV) using a liquid N₂ cooled Si (311) double crystal monochromator. The sample spectra were collected at room temperature using a 100-element HP-Ge

fluorescence detector (*Canberra*), and spectra of a reference Ag foil were recorded in parallel in transmission mode. The reference spectra served for energy calibration purposes via the first inflection point of the first derivative. Due to time constraints, spectra from the 2 and 4 week samples were only acquired from the pH 6.9 AgNO₃ and Ag-NP treatments. Inorganic Ag standards analysed included silver-nitrate (AgNO₃), -chloride (AgCl), -sulfide (Ag₂S), -sulfate (Ag₂SO₄), -oxide (Ag₂O), -carbonate (Ag₂CO₃), and -phosphate (Ag₃PO₄), as well as Ag bound to cysteine, histidine, zeolite, ferrihydrite, and Suwannee River humic and fulvic acids. Nanoparticle standard spectra (Ag-NPs, AgCl-NPs and Ag₂S-NPs) were acquired from a paste made by mixing the suspension with cellulose powder. Spectra of the Ag₂S-NP soil treatments aged for 7 months were collected at the Materials Research Collaborative Access Team (MRCAT) beamline 10-ID, Sector 10, at the Advanced Photon Source (APS) of the Argonne National Laboratory. Some standard spectra (AgCl-NPs, Ag bound to cystine and to ferrihydrite) previously collected at the APS were also used in the analysis.²⁴

The fluorescence XAS spectra were averaged using the pre-processing tool Sakura, and Athena³⁵ was used for background subtraction and normalisation over the range of -120 eV to +220 eV around the Ag K-edge. Linear Combination Fitting (LCF) was performed using Athena over the X-ray Absorption Near Edge Structure (XANES) region (-25 eV to +100 eV), using a combination of the standards identified with low SPOIL values by Target Transformation (TT), and from related experiments and literature.^{26, 33, 36}

Deployment of Diffusive Gradients in Thin Films

Diffusive gradients in thin films (DGT) (DGT Research Ltd., UK) devices were used to measure the labile concentration (C_{DGT}) of Ag in the aged soils. These DGT devices, termed here nano-DGT, were modified forms of the conventional DGT devices, as a 1 kDa dialysis membrane had been placed in front of the diffusive gel.³² This prevents nanoparticles coming into contact with the diffusive gel and

confounding the results by contributing directly to the accumulated Ag.^{32, 37} For the DGT measurement the ageing treatments were performed separately from those for XAS analysis, using the pH 5.7 and 6.9 (in CaCl₂) soil treatments. Replicate samples were aged in separate containers and analysed at 3 sampling times: $t = 0$, 2 weeks and 4 weeks, corresponding to the time scale over which the largest changes in Ag speciation were observed in the XAS studies.

At each sampling time, approximately 12 g of the wet soil was weighed out into suitably sized polypropylene containers for the deployment of the DGT devices. The contact zone between the rim of each sample container and the DGT device was sealed with parafilm in order to avoid sample loss or drying, and each assembly was pressed and tapped to ensure good contact between the soil and the DGT surface. After 48 h, the devices were retrieved and rinsed with ultrapure water, disassembled and the resin layer eluted in Eppendorf tubes with 1 M HNO₃ for analysis with ICP-MS. The soils were digested with microwave heating according to a modified US EPA Method 3051A, where reverse aqua regia (3:1 v/v concentrated HNO₃ : HCl) was used instead of aqua regia. Ag concentrations were determined using an Agilent 8800 Triple Quadrupole ICP-MS and these results were used to calculate the C_{DGT} . Additionally, manganese and iron concentrations were measured as C_{DGT} to interrogate the redox conditions of the soil over the first 4 weeks.

Results and Discussions

Characterisation of Nanomaterials

The results of NP characterisation are shown in Table 1 and Figure 1 (SEM images), and are discussed in the supporting information (SI). Powder XRD data (Figure S2) confirmed the formation of AgCl- and α -Ag₂S-NPs (the sewage sludge relevant phase)³⁸ and the XANES spectra of Ag-NPs, Ag₂S-NPs

and AgCl-NPs also indicated that the synthesis protocols had produced the desired forms of nanomaterials (Figure S1).

Formation and stability of Ag₂S in soils revealed by XANES

XANES spectra of soils spiked with AgNO₃, Ag- and AgCl-NPs all showed changes over the 3 month period, providing clear evidence of their reactivity within the soils (Figure 2). Ag speciation determined by LCF for the fresh, 3 month and 7 month (Ag₂S-NPs only) samples are shown in Table 2 (the complete set of fitted parameters can be found in the Tables S2-S4). In contrast, the spectra of Ag₂S-NP treated soils were almost invariant over time. It should be noted that the XANES profiles of Ag₂S and thiol-bound Ag (Ag-cysteine) were very similar to one another. Therefore in this article, the term “S-bound” in the samples will collectively refer to the formation of Ag₂S, Ag-cysteine and Ag-cystine unless otherwise stated; the latter included for completeness in S-bound Ag.

Analysis of the spectra clearly shows that the relative concentration of S-bound forms of Ag increases in almost all cases (Table 2). This is also demonstrated in Figure 3 when analysing the ageing behaviour of Ag-NPs and AgNO₃ at pH 6.9. This is expected given the high reactivity of Ag compounds and their known affinity to sulfur containing ligands. Furthermore, some pH dependence is also suggested by this data. Notably, AgNO₃ and Ag-NPs aged in a similar manner in neutral to alkaline soils (pH 6.9 and 7.8), whereas the acidic soil behaves differently with lower sulfidation/thiolation rates and higher occurrence of AgCl. Silver chloride can form at low pH under oxic conditions in aqueous systems³⁶ and it has been observed in an aged soil of pH 5.17 spiked with 200 mg/kg Ag (as AgNO₃).²⁶ On the other hand, Ag-cysteine can form at neutral to higher pH under similar redox conditions³⁶ and is often observed in systems with higher levels of organic carbon.^{26, 30} Ag₂S and Ag-cysteine can also form under reducing conditions.^{22, 24} In the soil used in this experiment organic carbon levels were low and

the initial redox potential was at least +109 mV. Furthermore, the Mn and Fe C_{DGT} data (Figure S4) indicated that the redox conditions did not change significantly over the first 4 weeks; the period over which the largest transformations were observed according to the XANES analyses. Thus, while the bulk soil did not become anaerobic, low redox conditions may have been created in small pockets or micropores within the soils where oxygen diffusion is limited, leading to the microbial production of bisulfide and the sulfidation of Ag. Inorganic sulfides (e.g. CuS, ZnS or FeS) can also sulfidise Ag even under oxic conditions and may have contributed to the process if they had pre-existed in the soil.³⁹⁻⁴⁰ We also note that Ag_2CO_3 formation was observed in neutral and alkaline soils freshly spiked with $AgNO_3$ (Table 2 and S2). However, the higher solubility of Ag_2CO_3 results in its disappearance over prolonged periods.²² There was also a slight difference in the “fresh” and “stored” $t = 0$ samples indicating some sulfidation had also occurred during transport but was negligible for the nanoparticles (Table S4).

In all cases, S-bound Ag constitutes the dominant fraction in the neutral and alkaline soils after 3 months (Table 2). In contrast, AgCl remains the most abundant species in acidic soils in all treatments with the exception of the Ag_2S -NP treatment. In the case of the AgCl-NP spiked systems, the prolonged stability of these NPs may be attributable to their larger initial particle size causing them to sulfidise more slowly. It may also be possible that the particle surface had been passivated as a result of Ag_2S surface layer formation; this could reduce dissolution from the remaining AgCl core.¹⁸ Formation of S-bound forms is clearer in the higher pH samples, which is consistent with the findings for the other starting materials. As mentioned above, it is difficult to separate the various forms of Ag bound to reduced S on the basis of XANES spectra. Resolving these species is possible in the extended X-ray absorption fine structure (EXAFS) regions²³ but the Ag concentrations in these samples were too low to obtain useful EXAFS data within reasonable acquisition times.

Importantly, it is clear that Ag_2S -NPs remained stable under the conditions tested in this experiment over the whole 7 month ageing period with $\geq 90\%$ of Ag remaining S-bound in all treatments.

This is also immediately evident from the XANES spectra (Figure 4) as the profiles from soil freshly spiked with Ag_2S -NPs and after 3 months of ageing are nearly identical. Furthermore, in the case of soils from these samples (in which a single chemical species dominates), the extended XAS articulate differences between the sulfide and the cysteine bound forms more clearly than the XANES data and suggests that Ag is indeed present as Ag_2S -NPs (Figure S3). This result, together with the apparent sulfidation of initially different Ag forms, presents a clear case about the mid to long term fate of Ag nanomaterials in soils: Ag nanomaterials in neutral or slightly alkaline soils transform into S-bound forms *and* remain in this form for at least 3 months, or at least 7 months in the case of Ag_2S -NPs. Thus, given the extremely low solubility of Ag_2S ⁴¹ and the low toxicity of thiol-bound forms (as in Ag-cysteine⁴²), the risk of dissolution of Ag nanomaterials and subsequent toxic effects is significantly reduced under these conditions. In the case of acidic soil: if Ag enters the soil as Ag_2S -NPs (as it would via the major wastewater–biosolids–soil pathway), it remains stable as Ag_2S , but other Ag species may also result in the formation of AgCl , also characterized by low solubility, which stays dominant over the 3 month period tested here.

Lability of Ag in the soils as measured by DGT

Diffusive Gradients in Thin films (DGT) are devices that are capable of determining the amount of labile components within an environmental matrix. They are often used to provide a surrogate measure of the bioavailable fraction of key nutrients and pollutants.⁴³ The major advantage of using DGT is that the calculated concentrations represent diffusion limited, time averaged concentrations of labile elements in the environment in which the device is deployed. This measurement includes metal resupplied from the solid phase in response to the non-equilibrium brought about by depletion of the labile pool of metals at the surface of the device. This feature effectively mimics the process that would

occur when metals are taken up by plants or organisms; thus the calculated concentrations, C_{DGT} , are correlated to potential bioavailability. DGT devices have previously been used to quantify a range of metals and nutrients in soils.⁴³⁻⁴⁵ For Ag, DGT have been applied to investigate the bioavailable Ag from Ag-NPs in aqueous media⁴² and from sediments spiked with ionic or nanoparticulate Ag,⁴⁶ but overall, the available literature on Ag analysis by DGT is limited. Indeed, this is the first application of the newly developed nano-DGT devices to Ag and its nanomaterials.

Figure 5 shows the labile Ag concentrations as measured by DGT (C_{DGT}) from soils spiked and aged with $AgNO_3$, Ag-NPs or Ag_2S -NPs in the same soil at two different pH values. It can be observed that C_{DGT} is considerably higher in the neutral pH soil than in the acidic soil particularly when the Ag is added as $AgNO_3$. This result suggests greater potential bioavailability of Ag in this system. This agrees with the XANES fitting data, where a significant formation of Ag_2CO_3 is observed at pH 6.9 and 7.8, but AgCl is shown to dominate the speciation at low pH (Table 2). When the dissolved concentrations are calculated using the K_{sp} values (8.46×10^{-12} for Ag_2CO_3 and 1.77×10^{-10} for AgCl) and considering the stoichiometry of the dissolution reaction for pure Ag_2CO_3 against AgCl minerals, the Ag concentration would be over 15 times higher for Ag_2CO_3 . In a mixed, dynamic system (as in these soils), C_{DGT} would be a function of more than just these variables, but given that i) at least two thirds of total Ag is attributable to AgCl and Ag_2CO_3 at $t = 0$, and ii) the other form is the stable Ag-cysteine (at pH 6.9), the relative contributions of chloride and carbonate species in the soil at pH 5.7 and 6.9 would explain the higher Ag C_{DGT} in the alkaline soil. The difference is not as evident in the case of the Ag-NPs (both have $C_{DGT} < 1 \mu g/L$), where AgCl and metallic Ag dominate at pH 5.7 and 6.9, respectively. Metallic Ag is also characterised by low Ag lability.³⁰

Over time, the labilities of Ag at pH 6.9 undergo distinct changes. In the case of $AgNO_3$, the eventual dissolution of Ag_2CO_3 and the increasing formation of S-bound Ag lead to a substantial decrease in C_{DGT} . Soil spiked with Ag-NPs exhibits an increasing trend in C_{DGT} , which may be due to a

slow release of Ag from Ag-NPs; however, this cannot fully explain the difference since it would be expected that the released Ag at such a slow rate is captured by strongly binding ligands (e.g. Cl, S, or thiols). Conversely, at pH 5.7, both AgNO₃ and Ag-NPs are almost immediately transformed into AgCl. This makes their chemical behaviours similar over time with consistently low C_{DGT} over the 4 week period. Indeed, the speciation of Ag after 3 months is near-identical to the speciation in freshly spiked soil, in both AgNO₃ and Ag-NPs treatments.

Most importantly, the lability of Ag₂S-NPs is very low in both soil systems. The contribution of Ag₂S-NPs to any labile pools of Ag in the soils is extremely low from the outset, and the little change in speciation observed between $t = 0$ and 3 months indicates that they will remain low in the long-term.

Further discussions and implications for environmental science

In this study, the combined analyses of XAS and DGT data provided comparative insights into the fate of Ag nanomaterials that may enter soil as AgNO₃, Ag-NPs, AgCl-NPs and most importantly, Ag₂S-NPs. These findings are of critical value in the risk assessment of Ag-NPs. From the perspective of chemical speciation, the stability and persistence of Ag₂S-NPs in soil is evidently high. While some small transformations are suggested by the XANES data, analysis of the data in k-space suggests that the core speciation remained dominantly as Ag₂S (Figure S3). Clearly, pH is only one of many variables that may contribute to Ag transformations (e.g. Eh, organic carbon content, seasonal variations, microbial contributions),³⁰ and biosolids were not applied together in this study which may also affect the sulfidation rates. However, it is evident that Ag₂S-NPs were significantly more stable than the other inorganic Ag forms tested with very low levels of labile Ag in the soil environment. Thus, given that the vast majority of Ag being released to soil environments is likely to be transformed into Ag₂S prior to application, the probability of releasing elevated levels of dissolved Ag under current conditions appears

to be quite low. Consequently, the risk assessment of Ag-NPs that enter the soil environment via the wastewater system can be considerably simplified: the chemical fate of Ag-NPs is dominantly Ag₂S and the potential toxicity of Ag₂S (NPs or otherwise) *through dissolution* is minimal.

However, it is important to note that Ag₂S-NPs may also potentially exert toxicity by other mechanisms. For example, it has previously been demonstrated that Ag₂S applied via a sludge matrix to soil may be taken up by some plants (e.g. lettuce)⁴⁷. In another study, while partial sulfidation appeared to decrease Ag toxicity to the soil nematode *C. elegans* in liquid media, toxicity was still observed that did not strongly correlate with the Ag/S ratio.¹⁸ In consideration of these results, three mechanisms can be envisaged as possible routes of toxicity: i) despite the extremely low solubility of Ag₂S, the trace amounts of dissolved Ag released may still be sufficient to cause toxicity; ii) root exudates or certain biomolecules that may be released by the plants or soil organisms could enhance the dissolution of Ag₂S and this may be followed by plant uptake; or iii) Ag₂S-NPs may be directly absorbed into the plant/organism as has been observed in some studies with Ag-NPs.⁴⁸ In the case of ii) and iii), the mechanisms are not necessarily mutually exclusive as exudates/biomolecules may form a surface layer (i.e. environmental corona)⁴⁹⁻⁵⁰ that may result in enhanced uptake without dissolution. At the same time, these processes may be insignificant if NPs are applied via biosolids since they would already be coated with a complex matrix that may alter or inhibit the interactions that free Ag₂S-NPs may experience. As it stands, while DGT is able to address case i), the other scenarios cannot be differentiated by XANES or DGT alone, but will require the use of EXAFS or additional considerations in the DGT experiment such as comparative studies using conventional DGT and nano-DGT, or time-resolved experiments.⁵¹⁻⁵² Experiments to address these scenarios as well as tests incorporating more soil types and investigating plants and microbial contributions will lead towards a more comprehensive fate and risk assessment of Ag-NPs along this release pathway.

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Supporting Information

Elemental analyses of amended soils are shown in Table S1. Ag K-edge XANES spectra of Ag standards (Figure S1) and LCF results for all treatments are shown in Tables S2 – S4. XRD patterns of AgCl- and Ag₂S-NPs (Figure S2), comparisons of Ag₂S-NPs in soil in k-space (Figure S3), C_{DGT} of Mn and Fe

at 0, 2 and 4 weeks (Figure S4) are also included in the supporting information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Table 1. Sizes of Ag nanomaterials used in this experiment. The DLS data are given as both intensity and number-weighted modal diameters, the DCS data as absorption and number-weighted modal diameters and the SEM diameters on a number-weighted basis. The reported d_{SEM} is the equivalent circular diameter. The standard deviations of the DLS and DCS results are based on repeat measurements, while the standard deviation for the SEM results represents the width of the measured number-based distribution. A detailed discussion is included in the SI.

	Coating	d_{DLS} (nm) Intensity, modal	d_{DLS} (nm) Number, modal	d_{DCS} (nm) Absorption, modal	d_{DCS} (nm) Number, modal	d_{SEM} (nm)
Ag-NPs	Citrate	47 ± 3	27 ± 4	23 ± 1	19 ± 1	21 ± 5
Ag ₂ S-NPs	PVP	80 ± 5	77 ± 3	61 ± 1 163 ± 2	61 ± 1	61 ± 16
AgCl-NPs	PVP	186 ± 8	166 ± 8	57 ± 2 204 ± 5	51 ± 3 182 ± 3	96 ± 36

Table 2. Results of the linear combination fitting of the Ag K-edge XANES spectra using the standards listed; as percentage composition of the total Ag.

			Components (Standards)* ¹									
Ag form	pH	Age	Ag ₂ CO ₃	Ag-NP	AgCl	Ag ₂ S * ²	Ag-cysteine * ²	Ag-cystine * ²	Ag-HA	Ag-ferrhydrite	R-factor * ³	S-bound * ² (%)
AgNO ₃	5.7	Fresh	11		77		14				0.00054	14
		3 mth		1	75		21			4	0.00005	21
	6.9	Fresh	34		32		35				0.00030	35
		3 mth	19	12			70				0.00042	70
	7.8	Fresh	25		42		21			12	0.00006	21
		3 mth		9		57	26				0.00020	83
Ag-NPs	5.7	Fresh		8	72		21				0.00009	21
		3 mth		3	70		21				0.00009	21
	6.9	Fresh		73		27					0.00107	27
		3 mth		19	24	19	38				0.00004	57
	7.8	Fresh		88		7		5			0.00010	12
		3 mth		11			72		17		0.00007	72
AgCl-NPs	5.7	Fresh			100						0.00009	0
		3 mth		5	69		22			4	0.00006	22
	6.9	Fresh			97		1			2	0.00006	1
		3 mth		18	38	26	19				0.00009	45
	7.8	Fresh			95		4			1	0.00008	4
		3 mth		12	40		49				0.00016	49
Ag ₂ S-NPs	5.7	Fresh		1		90		9			0.00005	99
		3 mth			5	39	24	33			0.00006	95
		7 mth				94		6			0.00012	100
	6.9	Fresh		2		92		6			0.00006	98
		3 mth	11		3	87					0.00012	87
		7 mth		12			90				0.00018	90
	7.8	Fresh				100		1			0.00006	100
		3 mth				66	12	22			0.00007	100
		7 mth				87		13			0.00029	100

*¹ The sum of components were not forced to equal 100 % in the LCF analyses.

*² S-bound percentage is the sum of Ag₂S, Ag-cysteine and Ag-cystine components.

*³ The R-factor indicates the quality of the fit.

Figure Captions

Figure 1. SEM secondary electron (SE) images of Ag-NPS (left), Ag₂S-NPs (centre) and AgCl-NPs (right). Scale bars indicate 1 μ m.

Figure 2. Ag K-edge XANES spectra of a) AgNO₃, b) Ag-NPs and c) AgCl-NPs aged in soil for 3 months. The spectrum of the starting Ag form is shown, with the corresponding XANES of its aged forms at the 3 different pH values (5.7, 6.9 and 7.8).

Figure 3. Ageing of Ag-NPs in soil of pH 6.9 over 3 months illustrated by XANES spectra. The initially metallic character of Ag-NPs ($t = 0$) is observed to gradually decrease throughout the incubation from $t = 2$ w to 4 w and 3 m. The table inset shows the percentage sulfidation of Ag-NPs and AgNO₃ in soil (inclusive of sulfides and thiol-bound components).

Figure 4. Comparison of Ag K-edge XANES spectra from soils spiked with Ag₂S-NPs sampled at $t = 0$ (no ageing) and after ageing for 3 months ($t = 3$ m) and 7 months ($t = 7$ m). All spectra are representative of Ag₂S as the dominant component of Ag species in the soils, indicating that Ag₂S are stable in soils under these conditions. *Spectrum acquired in transmission mode.

Figure 5. Labile concentrations of Ag in soil as measured by DGT (C_{DGT}) as a function of pH and ageing time for AgNO₃, Ag-NPs and Ag₂S-NPs (4 weeks only).

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5