Silver nanoparticles entering soils via the wastewater-sludge-soil pathway pose low risk to plants but elevated Cl concentrations increase Ag bioavailability

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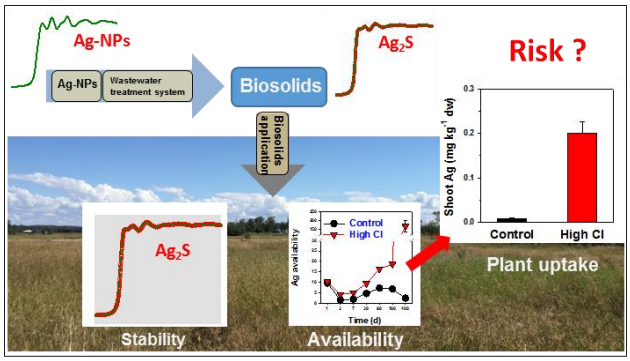
Abstract

The widespread use of silver nanoparticles (Ag-NPs) results in their movement into wastewater treatment facilities and subsequently to agricultural soils via application of contaminated sludge. On-route, the chemical properties of Ag may change and further alterations are possible upon entry to soil. In the present study, we examined the long-term stability and (bio)availability of Ag along the 'wastewater-sludge-soil' pathway. Synchrotron-based X-ray absorption spectroscopy (XAS) revealed that ca. 99% of Ag added to the sludge reactors as either Ag-NPs or $AgNO_3$ was retained in sludge, with ≥ 79% of this being transformed to Ag_2S, with the majority (≥ 87%) remaining in this form even after introduction to soils at various pH values and Cl concentrations for up to 400 d. Diffusive gradients in thin films (DGT), chemical extraction, and plant uptake experiments indicated that the potential (bio)availability of Ag in soil was low but increased markedly in soils with elevated Cl, likely due to the formation of soluble $AgCl_x$ complexes in the soil solution. Although high Cl concentrations increased the bioavailability of Ag markedly, plant growth was not reduced in any treatment. Our results indicate that Ag-NPs entering soils through the 'wastewater-sludge-soil' pathway pose low risk to plants due to their conversion to Ag_2S in the wastewater treatment process, although bioavailability may increase in saline soils or when irrigated with high-Cl water.

TOC/Abstract



INTRODUCTION

Silver nanoparticles (NPs, <100 nm size range) exhibit strong antimicrobial properties and are used extensively in a wide-range of products from detergents, textiles and home appliances, to socks, toothpastes, air filters, and nutritional supplements[1]. According to nanoproduct databases from the USA[1] and Europe[2], silver is the most commonly identified element in consumer nanotechnology products. Indeed, the Nanotechnology Consumer Product Inventory of the Woodrow Wilson Institute (2016) lists more than 350 manufacturer-identified products containing silver NPs[1] – this being more than for any other nanomaterial.[1, 2] As a result, their widespread production, utilization and disposal have raised substantial concerns regarding the risks of silver NPs upon their subsequent release into the broader environment.

The main pathway by which silver NPs enter the environment is via the application of biosolids from wastewater treatment plants (WWTPs).[3-5] This has led to increasing concern regarding their accumulation in soils given that biosolids from wastewater treatment facilities are often applied to agricultural lands and rangelands.[3, 6] Metallic (pristine) silver NPs are transformed within WWTPs and converted to silver sulfide (Ag_2S).[7-10] Unfortunately, many studies investigating Ag in soils have utilized the pristine forms of Ag that are not actually found in treated biosolids (c.f. transformed form, Ag_2S).[11-13] For example, Colman et al.[11] used soils amended with sludge that had been freshly spiked with metallic silver NPs (Ag-NPs) and Settimio et al.[12] spiked soils with $AgNO_3$ at rate of 100-800 mg Ag kg^{-1} soil. More recently, a limited number of studies have investigated the stability and (bio)availability of the transformed Ag_2S in soils[14], however, these studies have generally only examined changes in Ag stability and (bio)availability in the short-term. For example, del Real et al.[14] applied Ag-containing sludge to soils at a 1/10 ratio (sludge/soil) before incubation for four weeks, finding that Ag_2S was the main species remaining in these sludge-applied soils. Furthermore, whilst Sekine et al.[15] found that Ag_2S was quite stable over a seven month aging period, these authors

spiked the soils directly with Ag_2S-NPs rather than applying the NPs within the biosolids. Thus, whilst some progress has been made in understanding the fate and subsequent risk of Ag in the wastewater-sludge-soil pathway, we are unaware of any long-term studies that have examined Ag speciation and availability along the complete wastewater-sludge-soil-plant pathway. In the present study, we have also given consideration to the effect of soil pH and Cl concentration – Cl is a ubiquitous ligand with a strong affinity for oxidized Ag^+ and is often present at high concentrations in saline soils or soils irrigated with poor quality water.

The aims of the present study were to (i) determine the speciation of Ag along the wastewater-sludge-soil pathway, (ii) examine the stability of Ag species in soils during a 400 d incubation period, and (iii) assess the effects of soil pH and Cl on the stability and bioavailability of Ag. We have examined environmentally-relevant doses using a realistic exposure route, with speciation and availability assessed using novel approaches, including *in situ* synchrotron-based techniques. These data are crucial for the development of appropriate risk assessment frameworks for the production, usage, and disposal of silver NPs via the wastewater-sludge-soil pathway.

MATERIALS AND METHODS

Sludge

The sludges used in the present study were produced from three individual sequencing batch reactors (R_A, R_B, and R_C) (Figure S1, Supporting Information), each with a working volume of 10 L and an initial mixed liquor suspended solid concentration of 4 ± 0.2 g L^{-1}. These reactors were prepared as described by Doolette, et al. [16], and each received a different Ag-treatment; Ag-NPs (R_A), $AgNO_3$ (R_B) and Control (i.e. no Ag, R_C) (see Supporting Information for more details). The treatment to which the $AgNO_3$ was added serves as an additional Control by enabling a comparison of the effects of nanoparticles to the effects of

Ag^+. After 28 d operation, the sludge from each of the three reactors (R_A, R_B, and R_C) was collected for subsequent experiments. Subsamples were immediately frozen (-20 ºC) and later analysed using synchrotron-based X-ray absorption spectroscopy (XAS).

According to a large survey of 74 large Publicly Owned Treatment Works in the USA,[17] Ag concentrations in sewage sludge ranged from 2 to 195 mg Ag kg^{-1} (excluding one outlier at 856 mg Ag kg^{-1}), with the upper 95th confidence interval being 57 mg Ag kg^{-1}. Thus, in the present study, the sludges from R_A and R_B were then thoroughly mixed with control sludge from R_C (denoted as **Sludge C**) at two rates as required to achieve one of two final Ag concentrations: either 57 or 570 mg kg^{-1} (hereafter referred to as **Sludge A** to which Ag-NPs had been initially added, and as **Sludge B** to which $AgNO_3$ had been initially added). In addition, in order to examine the impact of the exposure pathway, subsamples of Sludge C were directly sprayed with appropriate volumes of Ag_2S-NP (Supporting Information), Ag-NP, or $AgNO_3$ stock solutions and mixed thoroughly to obtain final Ag concentrations of either 57 or 570 mg kg^{-1} (**Sludge D**, **E**, and **F**, respectively). Thus, Sludge A and B contained Ag-treatments that were added during processing within the batch reactor (hereafter termed 'pre-loaded' sludge), whilst Sludge D, E, and F contained the same Ag-treatments but were added after processing in the batch reactor had been completed (but immediately prior to the addition of sludge to the soils, hereafter referred to as 'post-loaded' sludge).

Soils

The soil used in the study was an Ultisol (US Soil Taxonomy), collected at a depth of 0-20 cm from an agricultural production system in Queensland, Australia (see Table S1 in Supporting Information for further information). The soil was air-dried and sieved to <2 mm. The initial, unadjusted soil pH (1:5 soil: water suspension) was 5.4 (**Soil 1**). Two different rates of $CaCO_3$ (3.3 and 36.3 g kg^{-1}) were added to increase pH to 6.4 **(Soil 2**) and 7.1 (**Soil 3**) to

examine the effect of pH. In addition, in order to examine the effect of Cl, two different levels of NaCl (1 and 2 g kg^{-1})[18] were added to increase soil salinity, which resulted in the electrical conductivity (EC) soil solution increasing to 3 dS m^{-1} (**Soil 4**, pH 5.5) or 6 dS m^{-1} (**Soil 5**, pH 5.6). Following addition of $CaCO_3$ and NaCl, deionized water was added to achieve 60% field capacity and allowed to equilibrate for one month.

Soil Incubation Experiments

A total of 25 experimental treatments were examined (Table 1), consisting of sludges that varied both in the form of Ag added (Ag-NPs, $AgNO_3$, or Control) and the time of Ag-addition (pre-loaded or post-loaded), as well as soils varied in pH (5.4, 6.4, or 7.1) and added Cl (0, 1, or 2 g NaCl kg^{-1}). The mass of sludge applied to each soil was equivalent to the rate reported by the US EPA (870 g m^{-2})[19], calculated as being 17.5 g kg^{-1} (assuming 8 cm depth of incorporation of soil with a density of 1.25 g cm^{-3} and two applications per year[20]). Thus the sludges, which had one of two Ag concentrations (either 57 or 570 mg/kg), were applied at a constant mass across all treatments (17.5 g kg^{-1}), resulting in soils with final Ag concentrations of either 1 or 10 mg kg^{-1}. Given that the 95th confidence interval for sludge from the USA was 57 mg Ag kg^{-1}, the two concentrations of Ag in soil (1 and 10 mg/kg) represent the addition of high-Ag sludge (i.e. 'worst-case scenario') to soils for 1 or 10 years (with an equivalent Ag accumulation of ca. 1 mg kg^{-1} y^{-1}). Soils to which Sludge C was applied had an Ag concentration of 0.02 mg kg^{-1} soil, serving as a Control. In order to examine the effect of sludge on the behaviour of Ag_2S-NPs in soils, appropriate volumes of Ag_2S-NPs were also added directly to Soil 1 (i.e. with no sludge), with final Ag concentrations of either 1 or 10 mg kg^{-1} soil (Table 1).

Treatments were prepared in 1 L plastic containers with 500 g of soil, with each treatment replicated three times yielding a total of 75 experimental units. Deionized water was

added to each container to maintain the water content at 60% of field capacity during the entire experimental period. The containers were covered with lids with several outlets to allow for gas exchange. Soils were incubated in the dark (to avoid effects of light) at 25 ± 1 ºC for up to 400 d. Samples collected after 1 d were digested in concentrated HNO_3 and analyzed for total Ag by ICP-MS. After aging for 1, 3, 7, 30, 90, 180, and 400 d, subsamples were collected for assessment of soluble Ag (see below). Furthermore, additional subsamples were collected after 1, 7 and 90 d for *in situ* speciation analyses using XAS. These subsamples were immediately frozen in liquid nitrogen, temporarily stored at –20 ºC, and freeze-dried.

Soluble Ag in Soil Extractions

Soils (n=3) were weighed (2.0 ± 0.02 g) into 50 mL polypropylene centrifuge tubes, mixed with 20 mL of 0.01 M $Ca(NO_3)_2$ (pH 6.0), shaken for 2 h and centrifuged at 14,000 ×g for 20 min. The supernatant was acidified with concentrated HNO_3 and analysed for Ag using triple quadrupole inductively-coupled plasma mass spectrometry (Agilent 8800 Triple Quadrupole ICP-MS). It was noted that the concentration of Ag in almost all solutions was <1 µg L^{-1}, but the Triple Quad ICP-MS used in the study offered a detection limit of 0.08 µg L^{-1} for Ag (calculated as being equal to three times of standard deviation of a blank), with a 96-105% recovery of Ag spiked samples.

Silver Speciation by X-ray Absorption Spectroscopy (XAS)

The speciation of Ag was assessed *in situ* using Ag K-edge (25,514 eV) X-ray absorption near edge structure (XANES) spectroscopy. The XANES spectra were collected using a liquid helium cryostat at the XAS Beamline at the Australian Synchrotron, Melbourne (see ref [21] for more details). Eleven Ag standard compounds were also examined, being metallic Ag, $AgNO_3$, nano-Ag_2S, bulk Ag_2S, Ag_2CO_3, AgCl, Ag_2O, Ag-cysteine, Ag-humic

acid, Ag_3PO_4, and Ag-histidine. The average of two to three scans was normalized using the Athena software package.[22] Principal component analysis (PCA) and target transformation (TT) were undertaken using SixPack software,[23] while linear combination fitting (LCF) analysis was performed using Athena software[24] (See Supporting Information for more details). The fitting range was -30 to +100 eV relative to the Ag K-edge.

Plant Growth Experiment

At the conclusion of the soil incubation experiment (i.e. after 400 d), the soils were used to conduct a plant growth experiment. Soil moisture content was maintained at ca. 60% field capacity during the entire experimental period. Seeds of wheat (*Triticum aestivum* L. cv. Sunbrook) were germinated for 2 d and five seedlings transferred to each container. After growth in soils for two weeks, shoots were cut at 1 cm above the soil surface, rinsed with deionized water, blotted dry with filter paper, and fresh biomass recorded. The shoots were oven-dried, digested in concentrated HNO_3 and analyzed for total Ag by ICP-MS.

Labile Ag Measured with Diffusive Gradients in Thin Film (DGT)

Upon plant harvest, soil samples were adjusted to saturation and mixed thoroughly to make a smooth slurry. The soils were allowed to equilibrate for 24 h before the DGT devices (DGT Research Ltd, Lancaster, UK)[25] were deployed to measure the labile concentration of Ag (C_{DGT}) in soils. After 48 h, the devices were retrieved, rinsed with deionized water, disassembled, and the binding layer (resin) were eluted in 1 mL of 1 M HNO_3 for 24 h before being analysed by ICP-MS.

Statistical Analysis

Treatment-differences were tested for significance ($p < 0.05$) using a one-way analysis of variance (ANOVA) performed with IBM SPSS Statistics 20.

RESULTS

Speciation of Ag in Sludge

The addition of either Ag-NPs or $AgNO_3$ to the reactor resulted in an increase in the Ag concentration of the mixed liquor, with the initial increase being approximately linear before peaking at ca. 10-15 d (Figure S2). The XANES spectra obtained from the sludges from both R_A (pre-loaded with Ag-NPs) and R_B (pre-loaded with $AgNO_3$) did not match those of the initial pristine Ag forms (i.e. Ag-NPs or $AgNO_3$) (Figure 1A and Figure S3). Instead, the spectra obtained from these pre-loaded sludges were visually similar to the spectrum of Ag_2S-NPs (Figure 1A and Figure S3). Indeed, LCF analysis confirmed that in sludge pre-loaded with Ag-NPs, no Ag was present as pristine Ag-NPs, rather, 87% was present as Ag_2S and 13% as AgCl (Figure 1B). Similarly, in sludge pre-loaded with $AgNO_3$, Ag_2S was predicted to account for 81% of the total Ag with 19% present as AgCl (Figure 1B). These results indicate that the majority of Ag in these sludges pre-loaded with either Ag-NPs or $AgNO_3$ was converted to Ag_2S.

Speciation of Ag in Sludge-Amended Soils

Across all aging periods examined, the XANES spectra of the various sludge-amended soils were similar to that of Ag_2S-NPs regardless of pH, Cl, and sludge form (Figure 2). These results indicate that not only is Ag converted to Ag_2S during processing (Figure 1A), but that this Ag_2S is also stable in soils across a range of conditions. Indeed, LCF analyses indicate that, in all cases, the dominant Ag species was Ag_2S (87-100%), with the remaining Ag present as Ag_3PO_3 (1-10%), AgCl (2-11%) or metallic Ag (4-13%) (Table 2). Interestingly, even when

soil was amended with sludge post-loaded with Ag-NPs ('Sludge E') or $AgNO_3$ (Sludge F'), ≥ 92% of the total Ag in the sludge-amended soil was present as Ag_2S after only 1 d incubation, suggesting that these forms of Ag were quickly transformed to Ag_2S either in the sludge or soil. It should be noted, however, that the XAS technique employed in this study is not without some limitations and uncertainty. In particular, XAS analyses are insufficiently sensitive to identify small (e.g. <5%) contributions of species of the target element. Therefore, it is difficult to determine if the difference is significant between post-added Ag treatments and pre-loaded Ag treatment. For this reason, changes in speciation are not discussed if their difference is less than 10%.

Extractable Ag in Soils

Regardless of soil or sludge, $Ca(NO_3)_2$-extractable Ag was low in all treatments, ranging from 0.82 to 167 µg Ag kg^{-1} soil (equivalent to 0.008% to 1.7% of the total Ag in soil, respectively) (Figure 3). Nevertheless, some important differences were observed between treatments. Firstly, comparing the effect of soil properties, it was found that increasing Cl tended to increase concentrations of extractable Ag (Figure 3A). This effect was not immediate, with elevated Cl increasing extractable Ag concentrations after ca. 30 d. Unlike Cl, variations in pH had no clear impact on extractable Ag concentrations within the range of pH 5.4 to 7.1. Secondly, it was noted that soils amended with pre-treated sludge (i.e. Sludge A, B) had lower concentrations of extractable Ag than did soils amended with post-treated sludge (i.e. Sludge D-F), although the magnitude of this difference decreased as the period of incubation increased (Figure 3B). Nevertheless, regardless of the form in which the Ag was supplied or the timing at which it was added to the reactor (i.e. pre-treated or post-treated), extractable Ag concentrations after 400 d were less than? ca. 2.9 µg kg^{-1} in all treatments

except those with elevated Cl. Similar trends were observed for sludge-amended soils containing 1 mg Ag kg^{-1} (Figure S4).

Labile Ag in Soils

Labile Ag in soils was assessed using DGT. This not only provides a measure of soluble Ag in pore water but also for the Ag that can be resupplied from the solid phase or other ligands over the measurement period, thereby improving the correlation to plant Ag uptake.[25] For soils amended with sludge containing 10 mg Ag kg^{-1}, DGT-Ag ranged from 1.2 to 3.6 µg L^{-1} after a 400 d aging period (Figure 4). As observed for $Ca(NO_3)_2$-extractable Ag (Figure 3), although soil pH had no significant effect on DGT-Ag, increasing concentrations of Cl resulted in substantial increases in DGT-Ag. Indeed, DGT-Ag increased from 1.6 µg L^{-1} in the control soil to 2.6 µg L^{-1} in the low Cl treatment and to 3.6 µg L^{-1} in the high Cl treatment (Figure 4A). Again, as observed for $Ca(NO_3)_2$-extractable Ag, DGT-Ag concentrations after 400 d incubation were similar irrespective of the form in which the Ag was supplied or the timing to which it was added to the reactor (Figure 4B).

Concentration of Ag in Plant Shoot Tissues

After plant growth in sludge-amended soils (10 mg/kg, 400 d aging) for two weeks, the concentration of Ag in wheat shoot tissues ranged from 0.007 to 0.20 mg kg^{-1} dry weight (dw) basis (Figure 5A,B). As with extractable and labile soil Ag, it was observed that elevated soil Cl concentrations resulted in increased Ag accumulation in the shoot tissues, with the shoot tissue Ag at high Cl (0.20 mg kg^{-1} dw) being 11.5 times higher than that in 'Soil 1' (0.017 mg kg^{-1} dw). On the other hand, differences in pH (from 5.4 to 7.1) and differences in the sludge treatments had no influence on concentrations of Ag in shoot tissues. Although elevated soil Cl

resulted in an increased shoot tissue Ag concentration, this Ag was not phytotoxic, with the mass of shoot tissue not differing significantly ($P > 0.05$) in any treatment (Figure 5C,D).

DISCUSSION

In the present study, we initially investigated the effect of the wastewater treatment process itself on the transformation of Ag when added as Ag-NPs or $AgNO_3$. Our results show that during wastewater treatment, the majority of the Ag was transformed to Ag_2S (Figure 1). This finding confirms previous reports that have demonstrated that Ag_2S is the dominant form of Ag in sludge.[7-9, 16, 26, 27] For example, analytical high-resolution transmission electron microscopy (TEM) has been used previously to identify nano-sized particles of Ag_2S in sewage sludge materials.[9, 27] Similarly, synchrotron-based XAS analyses revealed that after ≥10 d wastewater treatment, the majority of Ag (more than 83% of the total Ag) was converted to Ag_2S, regardless of the form in which Ag was added.[7, 8, 16] Finally, in a study using archived, stockpiled, and contemporary biosolids from the UK, USA, and Australia, Donner et al.[28] reported that ≥64% of the total Ag was present as Ag_2S in a wide range of materials, ranging from freshly-produced sludges to biosolids weathered under ambient environmental conditions for ≥50 years.

After examining its transformations within the wastewater treatment process, next we investigated the subsequent stability and (bio)availability of Ag in sludge-amended soils when incubated for up to 400 d (14 months). Our results clearly demonstrate that Ag_2S was markedly stable in soil in all treatments, with ≥87% of Ag in soils present as Ag_2S, regardless of pH (5.4 to 7.1) and Cl (Figure 2 and Table 2). Interestingly, even for post-loaded sludge (i.e. where Ag-NPs or $AgNO_3$ were added to sludge after removal from the reactor), ≥91% of the Ag was present as Ag_2S after only 1 d incubation in soil (Table 2). These results regarding the stability of Ag_2S in soils are in agreement with those reported previously.[14, 15] For example, in a study

using soils spiked with Ag_2S-NPs, Sekine et al.[15] showed that ≥90% of the Ag remained as Ag_2S over a seven month aging period. More recently, del Real et al.[14] applied Ag-containing sludge to soils at a 1/10 ratio (sludge/soil) followed by an incubation for four weeks, and confirmed that Ag_2S was the main species in these sludge-applied soils.

Whilst our findings regarding the long-term importance of Ag_2S in soil are in agreement with previous short-term studies, our findings regarding the lack of effect of pH (from pH 5.4 to 7.1) on the transformation of Ag_2S differs somewhat from previous studies spiking soils with other Ag forms. For example, Sekine et al.[15] found that when soils varying in pH were freshly spiked with $AgNO_3$, Ag-NPs or AgCl-NPs (in the absence of biosolids), acidic conditions favoured the formation of AgCl, while Ag_2S dominated in neutral or alkaline conditions. In that study,[15] interestingly, when soils were freshly spiked with Ag_2S-NPs, the majority of Ag (>98%) was still present as Ag_2S, regardless of soil pH. In contrast to Sekine et al.[15], Settimio et al.[12] spiked soils with $AgNO_3$ at dose of 100-800 mg Ag kg^{-1} soil and found that in alkaline soils (pH 8.5-8.9) the majority of Ag added as $AgNO_3$ was reduced to metallic Ag. Therefore, it seems that upon direct addition of Ag^+, AgCl-NPs, or Ag-NPs to soils, the transformation of Ag depends on experimental conditions and soil properties (such as pH and organic matter). However, we have shown that upon the addition of Ag_2S to soils (either directly or within sludge), the majority of Ag remains as Ag_2S and does not change over time regardless of soil properties (Figure 2 and Table 2). It has been shown that when soil *pe* + pH < 4.29, α-Ag_2S becomes the most stable mineral.[29] It is unclear if the soil redox potential has an important role in the transformation of Ag_2S, apart from pH.

Thus, Ag is converted to Ag_2S within the wastewater treatment process and this Ag_2S is stable in soils, but what is the subsequent Ag (bio)availability from these soil-Ag systems? In the present study, we have assessed the potential availability using three approaches: $Ca(NO_3)_2$ extraction, DGT, and a bioassay using wheat. Other than for treatments with elevated Cl, the

availability of Ag was similarly low irrespective of treatment, with availability decreasing slowly over time. For example, after 400 d incubation, the $Ca(NO_3)_2$-extractable Ag concentration was 2.87 µg Ag kg^{-1} soil, only accounting for 0.29% of the total Ag in soil (Figure 3) – this being in agreement with previous findings.[14, 15, 28] This low availability is unsurprising given the stability (i.e. low solubility) of Ag_2S. At the same time, although availability was generally low, it was noted that the addition of up to 2 g kg^{-1} of NaCl resulted in a ca. 2.3- to 160-fold increase in the availability of Ag (Figures 3-5). This difference in the apparent increase in Ag availability (2.3- to 160-fold) varied depending upon the method of assessment, with DGT predicting a 2.3-fold increase and $Ca(NO_3)_2$ extraction predicting a 160-fold increase (DGT uses an ion-exchange resin which acts as a sink for Ag^+ and labile Ag, whilst $Ca(NO_3)_2$ provides a measure of soluble Ag, including Ag^+ and colloidal Ag). Regardless, the increase in Ag availability in the high-Cl treatments did not alter the speciation of Ag in the bulk soils, with Ag_2S still the dominant form (Table 2) (given that extractable Ag accounted for ≤ 1.7% of the total Ag in soils, it must be noted that XANES would be insufficiently sensitive to identify changes in speciation within the extractable Ag pool itself). Similarly, these changes in extractable Ag at elevated Cl did not result from changes in soil pH (nor would changes in pH be expected to alter availability, Figure 3), with soil pH being relatively stable over the entire experimental period (Figure S5). Although we are unaware of any studies reporting the effect of Cl on the bioavailability of Ag in soils, Cl is widely known to increase Cd uptake by plants from soils.[30, 31] Indeed, in saline soils with elevated Cd, Cl complexes are known to be of importance, including $CdCl^+$, $CdCl_2^0$, $CdCl_3^-$ and $CdCl_4^{2-}$, with log values of their stepwise formation constants being 2.0, 2.6, 2.4, and 2.5 respectively.[29] High concentrations of Cl increased plant uptake of Cd either by enhancing mass transport of Cd or by uptake of the $CdCl_x$ by plant roots.[32] Similarly, Cl has a strong affinity for oxidized Ag^+ and tends to form $AgCl^0$ $AgCl_2^-$, $AgCl_3^{2-}$, or $AgCl_4^{3-}$ complexes, with similar step formation

constants (i.e. 3.3, 5.3, 6.4, and 6.1 respectively).[29] Therefore, we propose that the increased availability of Ag at elevated Cl resulted from the formation of these $AgCl_x$ complexes in soluble or colloidal forms. As a result, higher Cl concentrations increased mass transport of Ag and labile Ag, and consequently increased Ag uptake by plants. However, further work is required to clarify the chemical species within the soil solution.

Environmental Implications

Silver NPs are used in more consumer products than any other type of nanomaterial.[1] Once silver NPs are released, they enter municipal sewers and WWTPs, in which Ag is converted to insoluble Ag_2S, regardless of the form in which it is added.[8-10] Previous studies have shown that Ag_2S is very stable in sludge for long periods of time and will therefore be the primary form added to the soil through this pathway.[24] Upon entry into soils through sludge application, we have shown in the present study that this Ag_2S has low availability, and that the availability is not influenced by pH. Furthermore, the availability of Ag in soils decreases over time, subsequently decreasing the risk of Ag in soils. Importantly, although wheat shoot growth was not reduced in any treatment, elevated Cl significantly increased the availability of Ag. Thus, we contend that whilst the overall risk is low and is likely to decrease further over time, consideration should be given to this effect in saline soils or soils irrigated with high Cl water.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. Characteristics of silver nanoparticles, sludge production, X-ray absorption spectroscopy (XAS) analyses, XANES spectra of Ag standards, characteristics of the soil, the sequencing batch reactor, silver concentrations in the mixed liquor and effluent, effect of aging on

extractable Ag concentrations in soils, and effect of aging on measured pH in extractions of soils.

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Notes

The authors declare no competing financial interest.

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Table 1. Treatments used to investigate the effect of soil properties and sludge type on Ag behavior followed incubated for up to 400 d.

Treatment No.	Soil	Sludge applied[a]	Soil Ag (mg kg^{-1})
1, 2	Soil 1 (pH 5.4)	Sludge A	1 or 10
3	Soil 1 (pH 5.4)	Sludge C	0.02[b]
4, 5	Soil 2 (pH 6.4)	Sludge A	1 or 10
6	Soil 2 (pH 6.4)	Sludge C	0.02[b]
7, 8	Soil 3 (pH 7.1)	Sludge A	1 or 10
9	Soil 3 (pH 7.1)	Sludge C	0.02[b]
10, 11	Soil 4 (Low Cl)	Sludge A	1 or 10
12	Soil 4 (Low Cl)	Sludge C	0.02[b]
13, 14	Soil 5 (High Cl)	Sludge A	1 or 10
15	Soil 5 (High Cl)	Sludge C	0.02[b]
16, 17	Soil 1 (pH 5.4)	Sludge B	1 or 10
18, 19	Soil 1 (pH 5.4)	Sludge D	1 or 10
20, 21	Soil 1 (pH 5.4)	Sludge E	1 or 10
22, 23	Soil 1 (pH 5.4)	Sludge F	1 or 10
24, 25	Soil 1 (pH 5.4)	No sludge (only Ag_2S-NPs)	1 or 10

[a]: Sludge A, B and C refer to the pre-loaded sludges from the reactors daily spiked with Ag-NPs (Sludge A), $AgNO_3$ (Sludge B), and deionized water (Sludge C); Sludge D, E, and F refer to post-loaded sludge spiked with Ag_2S-NPs (Sludge D), Ag-NPs (Sludge E), and $AgNO_3$ (Sludge F).
[b]: Background

Table 2. Results of the linear combination fitting of the Ag K-edge XANES spectra of various soils to which various sludges had been applied (Table 1) before being incubated for various times.

Treatment No.	Age (d)	Ag standards				R-factor
		Ag_2S	AgCl	Ag_3PO_4	Metallic Ag	
2	1	90 (1.4)		10 (1.4)		0.00014
	7	94 (1.5)		6 (1.5)		0.00017
	90	87 (1.4)			13 (1.3)	0.00029
5	1	91 (0.9)	9 (0.9)			0.00011
	7	99 (1.4)	2 (1.4)			0.00025
	90	89 (0.9)	7 (0.8)	4 (1.2)		0.00018
8	1	95 (1.7)	6 (1.6)			0.00037
	7	97 (1.5)		3 (1.5)		0.00018
	90	97 (1.2)		3 (1.7)		0.00011
14	1	96 (0.9)	4 (0.9)			0.00011
	7	99 (1.4)	2 (1.4)			0.00027
	90	89 (0.8)	11 (0.8)			0.00008
17	1	96 (1.4)		4 (1.3)		0.00014
	7	96 (1.2)			4 (1.2)	0.00019
	90	95 (0.9)			5 (0.9)	0.00012
19	1	93 (0.8)			7 (0.8)	0.00008
	7	94 (1.5)	6 (1.4)			0.00029
	90	95 (0.9)			5 (0.9)	0.00012
21	1	92 (1.6)	8 (1.5)			0.00027
	7	93 (1.0)		7 (1.0)		0.00009
	90	99 (0.9)		1 (0.9)		0.00008
23	1	92 (2.6)		2 (2.9)	6 (1.4)	0.00023
	7	93 (1.2)		7 (1.2)		0.00011
	90	95 (1.2)			5 (1.2)	0.00020
25	1	100 (--)				0.00017
	7	91 (1.7)			9 (1.7)	0.00039
	90	91 (0.9)			9 (0.9)	0.00012

The values in brackets show the percentage variation in the calculated values. The goodness of fit is indicated by the R-factor. R factor = $\sum_i(\text{experimental-fit})^2/\sum_i(\text{experimental})^2$, where the sums are over the data points in the fitting region.

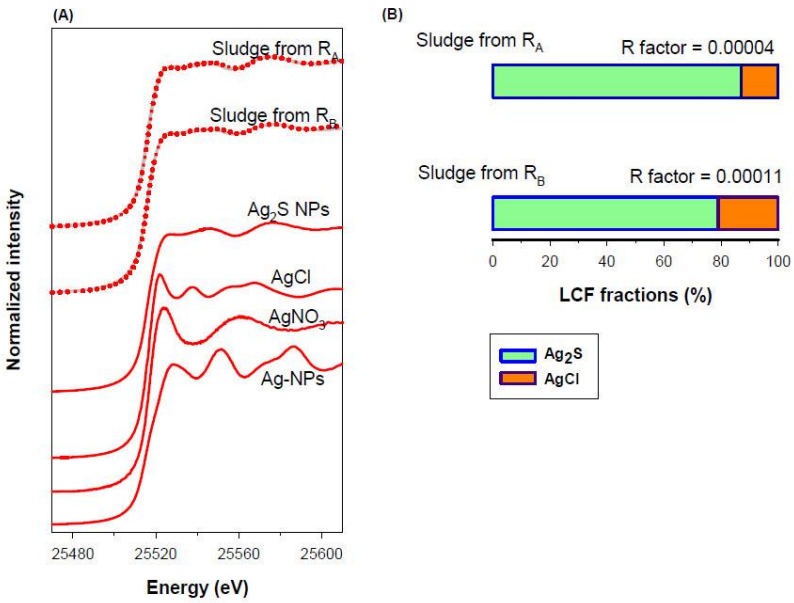

Figure 1. Normalized Ag K-edge XANES spectra (A) for the sludges pre-loaded with either Ag-NPs or $AgNO_3$. (B) The horizontal bars display the proportion of each reference compound fitted in the linear combination fitting (LCF) analysis. In (A), data are also presented for the four standard compounds predicted by LCF with dashed lines showing the fit predicted by LCF.

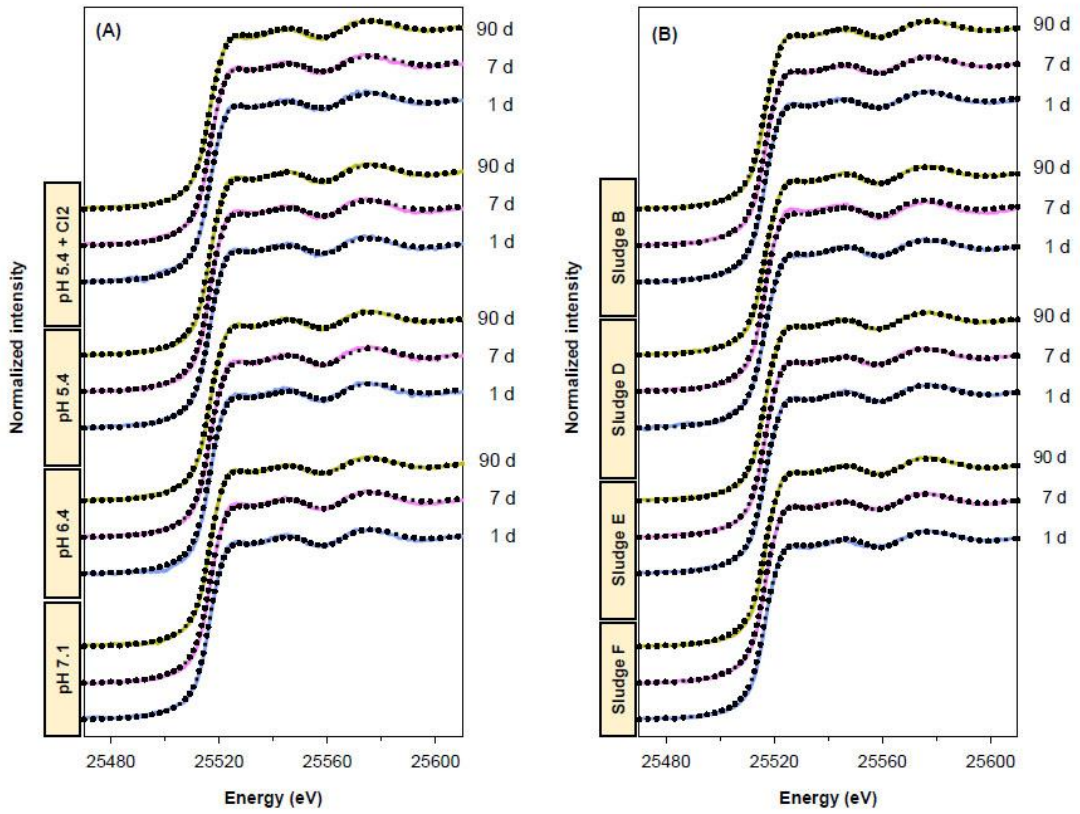

Figure 2. Ag K-edge XANES spectra of various sludge-amended soils that were incubated for 1, 7, or 90 d. (A) "Sludge A' was applied to soils varying with pH and Cl. (B) Various sludges (Sludges B, D, E, and F) was applied to 'Soil 1'. See Table 1 for details about treatments.

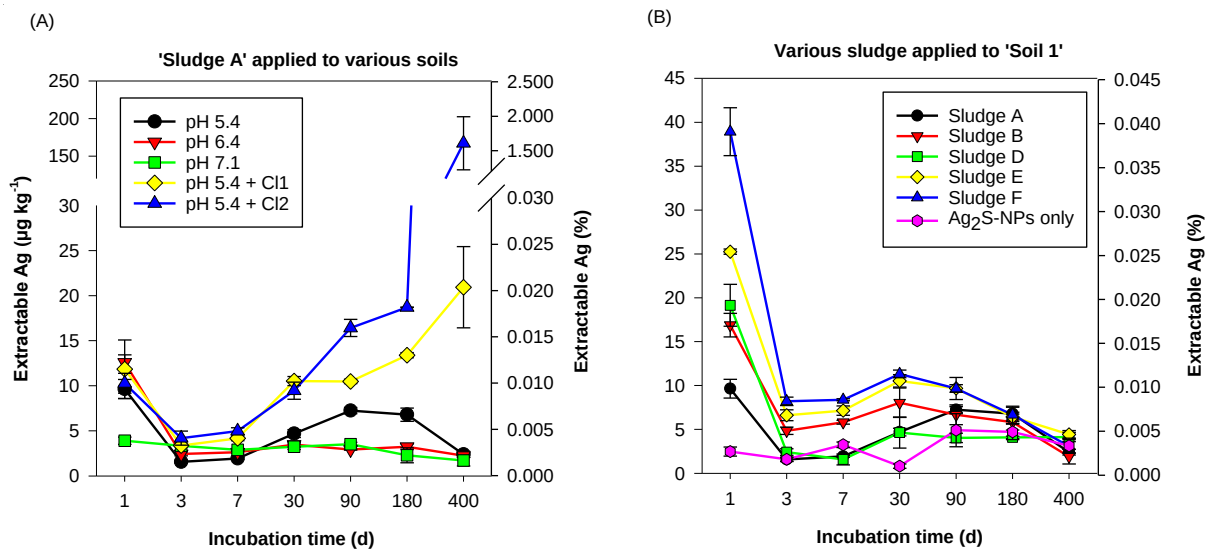

Figure 3. Effect of aging on extractable Ag concentrations in soils containing 10 mg Ag kg^{-1}. (A) Soils with three pH (5.4, 6.4, and 7.1) and two NaCl concentrations (1 and 2 g kg^{-1} soil) were applied with the pre-loaded sludge with Ag-NPs ('Sludge A', see Table 1 for more details). (B) 'Soil 1' (pH 5.4 with no $CaCO_3$ and NaCl added) was amended with Ag_2S-NPs alone, or with one of five post-loaded sludges.

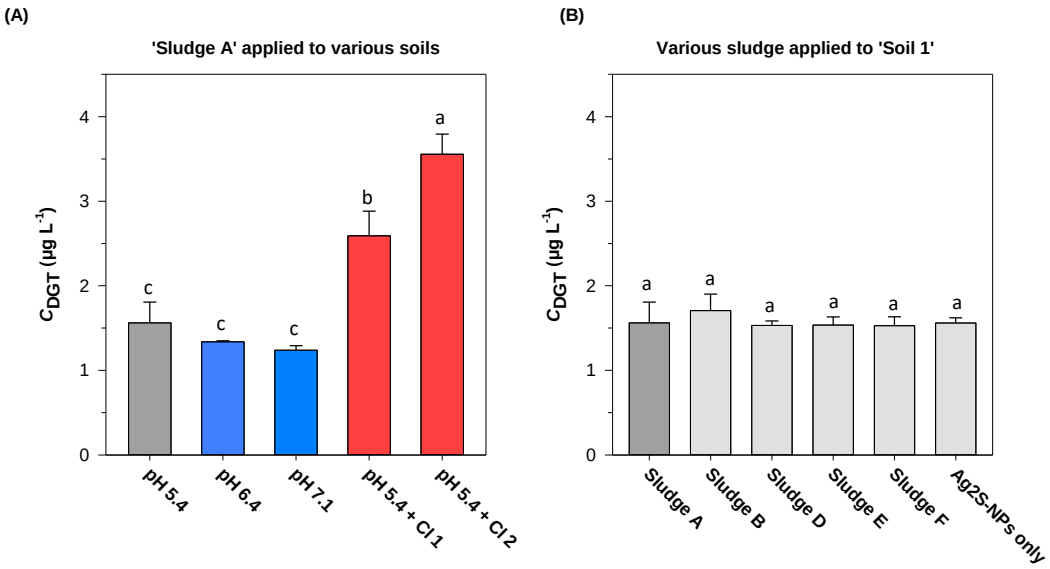

Figure 4. Labile Ag concentrations as measured by DGT (C_{DGT}) in soils containing 10 mg Ag kg^{-1} and aged for 400 d. (A) 'Sludge A' was applied to soils varying in pH and Cl. (B) Various sludges were applied to 'Soil 1' (Table 1). Significant differences between the treatments are indicated by different lowercase letters ($p < 0.05$).

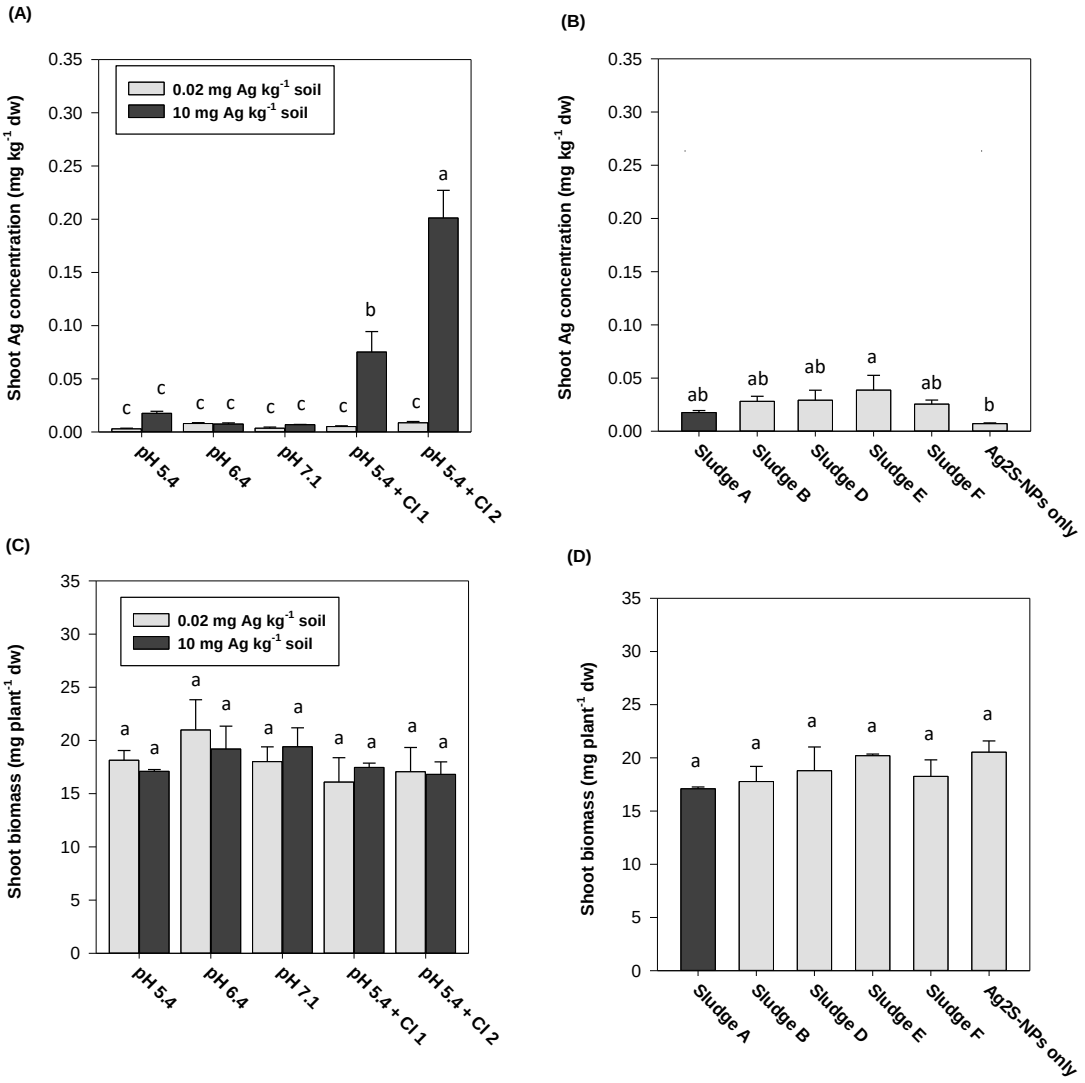

Figure 5. Effect of sludge-amendment on concentrations of Ag in shoot tissues (A and B) and the shoot mass (C and D) of wheat grown in soils containing 0.02 or 10 mg Ag kg^{-1}. (A and C) 'Sludge A' and 'Sludge C' (control sludge) were applied to soils varying in pH and Cl. (B and D) Various sludges were applied to 'Soil 1' (Table 1). Significant differences between the treatments are indicated by different lowercase letters ($p < 0.05$).