# Remobilisation of silver and silver sulphide nanoparticles in soils

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## ARTICLE INFO

## ABSTRACT

Manufactured nanoparticles (NPs) present in consumer products could enter soils through re-use of biosolids. Among these NPs are those based on silver (Ag), which are found sulphidised (e.g. silver sulphide, $Ag_2S$) in biosolids. Herein, our aim was to examine the release of retained $Ag^0$ and $Ag_2S$ NPs in soils and biosolids as facilitated by environmentally and agriculturally relevant ligands. Under natural soil conditions, exemplified by potassium nitrate and humic acid experiments, release of Ag retained in soil was limited. The highest total Ag release was facilitated by ligands that simulated root exudates (citrate) or fertilisers (thiosulphate). Released Ag was predominantly present in the colloidal phase (>3 kDa–< 0.45 μm); intact NPs only identified in $Ag_2S$-NP extracts. For biosolids containing nanoparticulate-Ag-S, release was also enhanced by thiosulphate, though mostly as colloidal-Ag — not intact NPs. These results suggest that exposure to NPs as a result of its release from soils or biosolids will be low.

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## 1. Introduction

Silver nanoparticles ($Ag^0$NPs) are the most common materials incorporated in many nanotechnology-enabled products (Project On Emerging Nanotechnologies, 2013), typically as a broad-spectrum non-specific antimicrobial (Kim et al., 2007). The antimicrobial properties of $Ag^0$NPs have been mostly related to dissolution of the NPs and direct toxicity of silver ($Ag^+$) ions (Reidy et al., 2013). Over time, $Ag^0$NPs (intact or transformed) could be released from products (Benn and Westerhoff, 2008; Farkas et al., 2011), thus inevitably entering the environment. Although $Ag^+$ is not highly toxic to humans, it could have detrimental effects to soil and aquatic organisms (Reidy et al., 2013).

Manufactured NPs from consumer products will likely enter sewer and wastewater treatment plant (WWTP) systems where NPs are removed from the wastewaters via flocculation and sorption to sludge (Kaegi et al., 2011; Kiser et al., 2009). Detailed investigations reveal that $Ag^0$NPs undergo a series of transformations, which involve oxidation ($Ag^0 \rightarrow Ag^+$) followed by sulphidation (Doolette

et al., 2013; Kaegi et al., 2011; Liu et al., 2011). This process has been confirmed to occur regardless of the Ag starting material (Doolette et al., 2013; Kim et al., 2010; Lombi et al., 2013). Indeed, Ag-species found in the biosolids are predominantly present as nano-particulate Ag–S — speciation that does not significantly change with aging and only partially converts to elemental Ag ($Ag^0$) with incineration (Impellitteri et al., 2013). Hence, with the practice of using treated biosolids as fertilizers and soil amendments, the main exposure of organisms to Ag through this route will mostly occur as nanoparticulate silver sulphide ($Ag_2S$). It must be noted that with the expansion of nanotechnology in the agriculture sector, soils may also be directly exposed to $Ag^0$NPs (Gogos et al., 2012; Jo et al., 2009).

In the soil environment, strong retention of $Ag^+$ and $Ag^0$NPs could be expected for soils with high clay and/or high organic carbon contents (Cornelis et al., 2012, 2010). Interactions with both clay and soil organic matter control the fate, bioavailability, and potential toxicity of $Ag^+$ and $Ag^0$NPs to soil organisms. Consistent to this, toxicity of $Ag^0$NPs to soil organisms (e.g. earthworms) has been found in soils where free $Ag^+$ are present in the soil pore water (Schlich et al., 2013; Shoults-Wilson et al., 2011), though more recently, NP-specific toxicity toward microbial communities has been reported (Colman et al., 2013). For $Ag_2S$, a lower risk is typically expected in the soil environment due to its low solubility

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(Hirsch, 1998). The perceived lower risk of Ag—S species found in biosolids is based on the assumption that agronomic practices or environmental conditions are unable to remobilise $Ag_2S$ as soluble $Ag^+$ or as NPs in soils.

Studies on release or remobilisation of NPs retained in soils and sediments have been limited. Remobilisation is a means by which NPs can become mobile and potentially bioavailable. For NPs, whose retention properties are primarily dictated by their surface chemistry, interactions with different ligands (derived from soil microorganisms, plant roots, or introduced to soil) may be particularly important. These ligands are capable of interacting with NPs — potentially stabilising the NPs in suspension. For example, phosphates have been reported to decrease retention (increase mobility) of (uncoated) ceria NPs in soils by imparting a highly negative surface charge to the NPs (Cornelis et al., 2011). Similarly, organic ligands like citrate (found in the rhizosphere) and thio-sulphates (used as fertilizers) may interact with NP surfaces to form stable suspensions and facilitate remobilisation of ionic or nano-particulate Ag [$K_{f(Ag-mono/dicarboxylates)} \approx 10^2 - 10^4$ (Sillen and Martell, 1964); $K_{f(Ag-thiols)} \approx 10^{13}$ (Bjerrum et al., 1958)]. The presence of these ligands will have significant implications on the fate, bioavailability, and toxicity of Ag-based NPs and/or their dissolution products following biosolids application to soil.

The aim of this study was to examine the effect of environmentally and agriculturally relevant ligands on remobilisation of $Ag^+$, $Ag^0$NPs, $Ag_2S$ NPs, and $Ag_2S$-bulk in soils and biosolids. This information will provide further insights into the fate and potential risks associated with $Ag^+$, $Ag^0$NPs, $Ag_2S$ NPs, and $Ag_2S$-bulk in soil environments.

## 2. Materials and methods

### 2.1. General

All salts used in this study, namely: silver nitrate ($AgNO_3$), silver sulphide ($Ag_2S$) solids (powder/chunks), sodium borohydride ($NaBH_4$), potassium nitrate ($KNO_3$), trisodium citrate dihydrate, sodium thiosulphate pentahydrate, L-cysteine, and 3-mercaptopropionic acid, were from Sigma—Aldrich. Ultrapure water used to prepare all solutions/suspensions was from a Millipore Milli-Q Plus water purification system (18.2 MΩ/cm resistivity).

### 2.2. Preparation of NP suspensions

Suspensions of $Ag^0$NPs coated with citrate (Ag-NP-cit) were prepared by reduction of $Ag^+$ ($AgNO_3$) with $NaBH_4$ followed by stabilization in citrate (Liu and Hurt, 2010). Suspensions of $Ag^0$NPs coated with humic acids (Ag-NP-ha) were prepared by reduction of $Ag^+$ ($AgNO_3$), with Suwannee River humic acid solution (50 mg/L SRHA, International Humic Substances Society, USA) acting as a reducing and stabilizing agent (Akaighe et al., 2011). Both suspensions were filtered using a 3 kDa Amicon-Ultra centrifugal filter (Millipore) to remove unbound ligands and $Ag^+$ from the suspension (see supporting information, SI, for detailed procedure). Ag-NP-pvp suspensions were prepared by sonication of AgNP-pvp powder (Nano-Amor) in water (100 mg in 50 mL) using a probe sonicator (3 min at 1500 W/L; Misonix). Similarly, $Ag_2S$-NP-cys suspensions were prepared by sonication of $Ag_2S$-NP-cys powder in 5 μg/mL SRHA solution (16 mg powder in 50 mL). $Ag_2S$-NP-cys powder was prepared following the method by Xiang et al. (2008) which involves the reaction of $AgNO_3$ (in AR-grade ethanol, Fischer Scientific) with L-cysteine in an autoclave reactor (180 °C, 10 h). Details on the synthesis and characterisation are in the SI. Ag-NP-pvp and $Ag_2S$-NP-cys suspensions were centrifuged (2800 g, 15 min) to remove larger/undispersed particulates; only the top 35 mL were recovered and

used. All NP suspensions were protected from light to prevent potential photooxidation, particularly of the $Ag^0$NPs (Grillet et al., 2013).

### 2.3. Silver nanoparticle retention and release in soils

Retention experiments were performed in soils where retention of Ag (ions and NPs) was found to be high, based on previous work done by Cornelis et al. (2012). The physicochemical properties of the two Australian soils used in this study are described in Table 1.

Silver was added to the soils in different NP forms: Ag-NP-cit and Ag-NP-pvp, as the most commonly used forms in fate and toxicity testing; Ag-NP-ha, as the environmentally-relevant $Ag^0$NP; $Ag_2S$-NP-cys, as the Ag-transformation product in biosolids. Nanoparticle treatments were compared to soluble-$Ag^+$ ($AgNO_3$) and bulk-sized $Ag_2S$ treatments ($Ag_2S$-bulk; particle size>200 nm).

Batch retention of different Ag forms in two soils were performed by mixing dry soil (2 g) with Ag solutions/suspensions (1 μg/mL Ag in 20 mL 2 mM $KNO_3$; for $Ag_2S$-bulk, $Ag_2S$ solids were directly weighed into the soil to achieve 10 mg/kg concentration) in a 50-mL polypropylene centrifuge tube (Greiner). Ag concentration of 1 μg/mL was chosen to be as low as possible to be environmentally-relevant and high enough for instrument detection limits. A soil:solution ratio of 1:10 (m/v) was selected due to its use in previous retention studies on manufactured NPs in soils (Cornelis et al., 2012, 2010) and common use in sorption-desorption studies of chemicals in soils. Future studies may need to be performed to examine the influence of soil:solution ratio on the release of manufactured NPs in soils. The soil solution mixtures were shaken end-over-end (24 h, dark) then centrifuged (2600 g, 30 min). The supernatant was removed and collected in a separate container. Soil residues were used in the subsequent experiments.

Batch release experiments were performed by adding individual release solutions (20 mL) containing 2 mM $KNO_3$ (weak ionic exchange solution) and 2 mM $KNO_3$ plus environmentally/agriculturally-relevant ligands to the soils recovered from the retention experiments. The ligands, namely, humic acids, citrate, thiosulphate, cysteine, and mercaptopropionic acid, were selected based on environmental relevance (source and occurrence), recognized interaction with $Ag^+$ or NPs (i.e. used in NP preparation/synthesis), and the different active groups (-SH, -COOH, -NH$_2$, -R, etc.) (Table S1). All ligands are envisaged to facilitate the release of NPs via interaction with the core NP surface and/or surface coatings. The concentration of the release solutions introduced were 1000x the concentration of Ag used (1 μg/mL ≈ 9 μM): 10 mM citrate/thiosulphate/cysteine/mercaptopropionic acid in 2 mM $KNO_3$ and adjusted to neutral pH. These concentrations are higher than what would be expected to be present in soil solutions and is considered a worst case scenario of exposure to these ligands (Table S1). Organic acids may reach mM concentrations in the rhizosphere (Jones, 1998) while spatial/temporal increase in thiosulphate concentration will occur when used as fertiliser. Note that the SRHA concentration used (20 μg/mL; 10 μgC/mL) is within the normal range of dissolved organic carbon concentration (DOC) found in soil solutions (Table S1). The soil solution mixtures were shaken end-over-end (24 h, dark) then centrifuged (2600 g, 30 min). The supernatant was removed and collected in a separate container. The experimental schematic is summarised in Figure S1.

Treatments without soil were also prepared to determine stability of the NP suspensions during the 24-h retention experiment. Soils were also incubated in the absence of Ag as controls. All samples were prepared in triplicate.

#### 2.3.1. Calculations

For typical solutes/chemicals ($Ag^+$ and $Ag_2S$-bulk), partitioning was expressed as a $K_d$ value (OECD, 2000). As $K_d$ values are considered inappropriate for metal-containing NPs that may dissolve in environmental media (Kuhnel and Nickel, 2014), for Ag NPs, retention was expressed as a $K_r$ value (Cornelis et al., 2010). $K_r$ values account for potential dissolution processes of metallic NPs. This method allows the ranking of Ag NPs with each other, soluble and bulk forms of Ag, and other possible soil contaminants (Cornelis et al., 2010). For all Ag-retained soils, Ag release was expressed as percentage Ag released (ionic and particulate) relative to the amount of Ag retained in the soil. See SI for calculation details.

### 2.4. Release of Ag in biosolids

Biosolids prepared from laboratory-based batch reactors and sampled from a local wastewater treatment plant (WWTP) were also studied for Ag release,

**Table 1**
Soils used for investigating the potential remobilization of Ag-based NPs.

| Soil | EC* | pH* | Total | CEC | Oxalate extractable | | Particle size analysis | | | MWHC (%) |
| | | 2 mM | C | | Al | Fe | Clay | Silt | Sand | |
| | mS/cm | KNO$_3$ | % | cmol$^+$/kg | mg/kg | mg/kg | % | % | % | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.04 | 4.21 | 1.6 | 3.4 | 240 | 300 | 14 | 10 | 75 | 29.4 |
| 2 | 0.1 | 6.41 | 0.9 | 65.7 | 2530 | 1800 | 59 | 14 | 27 | 64.4 |

Acronyms: EC = electrical conductivity; Total C = Total Carbon; CEC = cation exchange capacity; MWHC = maximum water holding capacity.
*For pH and EC, samples were analysed on a 1:5 soil:solution ratio in duplicate. Reported values are based on the average. Further details are described in the SI.