

Behaviour and fate of soluble silver added to soils

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ABSTRACT

Silver (Ag) and silver nanoparticles (AgNPs) are increasingly being used in solar energy production, water purification, medicine and applications of nanotechnology. The antibacterial properties of Ag and AgNPs are the key reason for their addition to a range of consumer products. But these properties also represent a potential risk when Ag and or AgNPs are released into the environment by loss during mining processes, disposal of consumer and industrial products to wastewater, the residues of which (biosolids) are directly applied to land, and through application of Ag containing pesticides. Once in the soil environment, Ag (including Ag released from AgNP) may partition itself between the solid and liquid phases. But only a fraction of the total Ag concentration in soil may be in a labile or available form. The speciation and fate of Ag in soils will also be important for understanding what influences the amount of labile metal available for uptake by soil dwelling organisms.

This thesis presents the partitioning and lability of Ag added to a wide range of soils by measurement of the partition coefficient (K_d value) and concentration of labile metal (E -value) using an isotope dilution method. This initial method development, which was optimised for Ag, provided the basis for further evaluation of the long term aging of Ag and its impact on the labile metal pool, speciation of Ag over time and the significant interactions between Ag and dissolved organic matter (DOM).

An isotopic dilution method to simultaneously measure the K_d -value and E -value of Ag in soils was developed using the ^{110m}Ag radioisotope. E -values were corrected for possible interferences from non-isotopically exchangeable Ag associated with soil colloids in suspension (E_r -value). Measurement of K_d values for Ag in a range of soils indicated strong partitioning to the solid phase is positively associated with soil cation-

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exchange capacity or total organic carbon and pH. Measurements of labile Ag after a two week incubation period indicated rapid conversion of soluble Ag into non-isotopically exchangeable forms, either irreversibly adsorbed or precipitated in the soil. This indicated that measurements of labile Ag will be important to estimate toxicity risks to soil organisms or to predict bioaccumulation through the food chain.

The fate and lability of added soluble Ag in soils over time was examined by isotopic dilution and the solid-phase speciation of Ag by X-ray absorption near edge structure (XANES) spectroscopy. After two weeks of ageing the *E*-values for Ag decreased by 20-90% with a further decrease of 10-40% after six months. The overall decrease in labile Ag for all soils after the six month ageing period was 50-100%. The ageing was more rapid and pronounced in the alkaline soils. XANES results for Ag in soils indicated that for the majority of soils the added Ag⁺ was reduced to metallic Ag over time, and associations with Fe-oxohydroxides and reduced S groups in organic matter also decreased Ag lability. Strong positive correlations were found between metallic Ag and non-labile Ag and between organic carbon and Ag bonded with S species.

There remains uncertainty in important aspects of Ag speciation in soils. In particular, the complexation of Ag⁺ with dissolved organic matter (DOM) appears complicated. It is not known whether DOM quality or quantity is more important. Complexed Ag across a wide range of soil water extracts with natural varying concentrations of dissolved organic carbon (DOC) was measured and was normalized to DOC concentration in order to observe differences in DOM affinity or strength of complexation with Ag⁺. Characterization of the DOM in the soil water extracts, was also carried out by size fractionation and elemental analysis, and spectroscopic analysis

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using UV-Visible, fluorescence and mid infra-red. Then using statistical analysis these qualities of DOM were used to explain the amount of complexed Ag measured in the soil water extracts. Overall the results indicated that up to 70% of Ag was strongly complexed in solution. The variability in Ag⁺ binding by DOM across different soils was most closely related ($R^2= 0.8$) to the MIR spectra of the extracts. The normalised Ag complexation values indicated that humic content and overall aromaticity increased Ag's affinity to DOM. Spectroscopic information from UV-Visible, mid infra-red and fluorescence spectroscopy was also shown to potentially be more useful in measuring and characterizing DOM than only chemical measurements. Silver's demonstrated affinity and ability to complex with DOM could result in increased mobilization of this metal in the soil environment.

DECLARATION

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Date

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STRUCTURE OF THIS THESIS

This thesis is presented as a combination of papers that have been published or submitted for publication.

Chapter 1 provides an overview of the literature on environmental chemistry of silver, reactivity and behaviour in soils and methods for the determination of available silver. This chapter also includes the proposed objectives of the research presented in this thesis.

Chapter 2 comprises a paper published in *Environmental Chemistry*. It describes the development of an isotope dilution method for the measurement of partitioning and lability of silver in soils.

Chapter 3 comprises a paper published in *Environmental Pollution*. It describes an ageing experiment to determine the long term fate and availability of silver in soils. It combines chemical lability measurements with solid phase speciation data collected by X-ray absorption near edge structure spectroscopy.

Chapter 4 comprises a paper that has been submitted for publication in *Environmental Pollution*. It describes an isotope labelling and cation exchange resin experiment to examine the complexation between complexed silver ions and dissolved organic matter in soil solutions.

Chapter 5 provides a synthesis of the findings contained in this thesis and includes recommendations for future work.

PUBLICATIONS ARISING FROM THIS THESIS

Settimio, L., McLaughlin, M.J., Kirby, J.K., Langdon, K.A., 2014. A method to determine silver partitioning and lability in soils. *Environmental Chemistry* **11**, 63-71.

Settimio, L., McLaughlin, M.J., Kirby, J.K., Langdon, K.A., Lombi, E., Donner, E., Scheckel, K.G., 2014. *Environmental Pollution* **191**, 151-157.

CHAPTER 1

REVIEW OF THE LITERATURE

1. Introduction

Silver (Ag) is a precious metal that is actively mined to supply 75% of the total world demand for Ag (30,000 t) (Thomson Reuters GFMS 2013). The highest demand is for industrial applications, a direct result of the desirable properties of Ag: strength, malleability and ductility, electrical and thermal conductivity, sensitivity to and high reflectance of light and the ability to endure extreme temperature ranges.

The strong antibacterial properties of Ag have resulted in its increasing addition to a range of readily available consumer products, as both ionic Ag (Ag^+) and in the form of Ag nanoparticles (AgNPs) (Morones et al. 2005; Benn et al. 2010). Examples of consumer products containing Ag^+ and AgNPs include personal hygiene products, textiles, plastics, and electrical items. The antibacterial properties of Ag^+ , and Ag^+ released from AgNPs, may pose a risk to the terrestrial environment. The release of Ag to the terrestrial environment can occur during mining processes but the major contamination pathway is from urban areas (e.g. consumer products and industries) through wastewater treatment plants (WWTPs), predominately *via* land application of biosolids (Johnson et al. 2005; Oliver et al. 2005).

The presence of Ag^+ in the terrestrial environment is of concern due to its potential toxicity to a range of biota such as microorganisms, plants and invertebrates living in the soil (Ratte 1999). It is well established for metal contaminants in soils that the total metal concentration is often a poor measure of the bioavailable metal fraction (Hamon et al. 2008). In soils, there are many processes or transformations of metals that can take place, for example sorption, partitioning and ageing, that can result in changes in metal bioavailability and toxicity (Degryse et al. 2009; Tack 2010). Although these

processes have been well studied for many other metals (e.g. cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn)), to date there is little known about how these processes affect the bioavailability of Ag in soils.

Previous studies of sorption and desorption of Ag in soils found the controlling factors to be soil organic matter (SOM) or humus materials via complexation or exchange mechanisms (Jacobson et al. 2005). There is limited information on partitioning of Ag in soils, but one recent study found that it was strongly influenced by pH, clay content, organic matter content and chloride concentration (Cornelis et al. 2012). The effect of SOM on partitioning was further highlighted when the concentration of free Ag^+ ion in soil solution was found to be lower for a soil with high organic matter content compared to a soil with lower organic matter content (Benoit et al. 2013). The conclusions from these studies investigating Ag^+ have been further supported by studies investigating the behaviour of AgNPs in soils. A comparison of different AgNP coatings (citrate stabilised and uncoated) and soluble Ag (as AgNO_3), found all Ag types examined to be more mobile in a mineral soil compared to an organic matter rich soil (Coutris et al. 2012). In this study, after two days, only trace concentrations of Ag were extractable from the soil. After five weeks for the uncoated AgNPs, the bioaccessible form was eight to nine times greater than the AgNO_3 and citrate-stabilised AgNP treatments. These results indicate that longer term ageing reactions are taking place over time and that differences in nanoparticle coating may also influence the fate of Ag, aside from soil properties. In another study, ageing for Ag and AgNPs was found to increase in soils following additions of sewage sludge, which tends to be relatively high in SOM (Whitley et al. 2013).

To fully understand the behaviour and fate of Ag in the terrestrial environment, its chemical nature needs to be understood. This review summarises the environmental chemistry of Ag relevant to soils, the exposure routes of Ag to the terrestrial environment, the biological effect of Ag to soil dwelling organisms, the soil processes, mechanisms and reactions that control the behaviour and fate of Ag in soil. In addition, it outlines the methods available to measure the bioavailable fraction of Ag in soils as well as characterising the speciation and interactions of Ag with various soil components.

2. Environmental chemistry of silver

2.1 Geochemical occurrence

Silver is a rare, naturally occurring element found in the earth's crust (Evans and Barabash 2010) with average concentrations ranging from 0.1 to 0.2 mg kg⁻¹ (Petering and McClain 1991; Kabata-Pendias and Mukherjee 2007). Silver is often found with compounds containing arsenic (As), bismuth (Bi), sulfur (S), antimony (Sb), selenium (Se), tellurium (Te) and is widely distributed as minerals such as argentite (Ag₂S), cerargyrite (AgCl), Ag arsenide (Ag₃As), pyrargyrite (Ag₃SbS₃), proustite (Ag₃AsS) and native metallic Ag (Petering and McClain 1991; Evans and Barabash 2010). Such compounds often have a metallic lustre, are semi-conductors and can be found as impurities in ores of Cu, Pb and Zn. Trace concentrations may be present in sulfide minerals such as sphalerite (ZnS), pyrite (FeS₂), chalcopyrite (CuFeS₂) and galena (PbS). In soils, the geochemical occurrence of Ag is between <0.01 to 5 mg kg⁻¹ with most reported values below 0.1 mg kg⁻¹ (Purcell and Peters 1998; Evans and Barabash 2010). Silver has two naturally occurring stable isotopes ¹⁰⁷Ag and ¹⁰⁹Ag with abundances of 48% and 52% respectively (Petering and McClain 1991).

2.2 Chemical properties and reactivity

Silver is a group 11 element in the periodic table with gold (Au) and Cu. Silver has an atomic mass of 107.87 M, an electronic configuration of $[\text{Kr}]4d^{10}5s^1$, a density of 10.5 g cm^{-3} , a melting point of $960 \text{ }^\circ\text{C}$ and a boiling point of $2170 \text{ }^\circ\text{C}$ (Petering and McClain 1991; Elvers et al. 1993). As a transition metal, Ag has coordinating properties, commonly forming linear complexes with a coordination number of two (Petering and McClain 1991; Evans and Barabash 2010). There are four oxidation states of Ag; 0, +1, +2 and +3, with 0 and +1 the most common valences found in nature (Lindsay 1979; Etris 1997).

The Ag ion is a soft metal cation and tends to complex soft bases (Evans and Barabash 2010). Soft and hard acid and base theory can predict metal bond formation and is useful for understanding the complexation of metal ions in soils (Wolt 1994; Evangelou 1998). As explained by Pearson (1963) the characterization of soft, hard or borderline ‘Lewis acids or bases’ is decided by ion electronegativity, ion polarizability and oxidation potential. For Ag^+ , a soft ‘Lewis acid’, it is characterised by its relatively small size, low electronegativity and high polarizability. Hence, Ag is highly chalcophilic and has a strong affinity for S. Silver also forms strong multihalide complexes at relatively low halide concentrations which predominate over a large pH range (Evans and Barabash 2010). These multihalide complexes can be soluble e.g. $[\text{AgCl}_4]^{3-}$ (Jacobson et al. 2005).

Silver will bind strongly with inorganic and organic S groups with high formation constants (log K) for inorganic sulfides ranging from 14 to 21 and organic S from 12 to 15 (Evans and Barabash 2010). With such a strong affinity for S, Ag should

outcompete most other metals for the available sulfide ions (mercury $(\text{Hg})^{2+} > \text{Ag}^+ > \text{Cu}^+ > \text{Pb}^+ > \text{Zn}^{2+}$) (Adams and Kramer 1998). As a result (with the exceptions of Cu^+ and Hg^{2+}), Ag can rapidly sorb onto and into colloidal or particulate sulfides even at low nanomolar concentrations to form $\text{Ag}_2\text{S}_{(s)}$ (Bell and Kramer 1999). With sufficient Ag^+ and S present, $\text{Ag}_2\text{S}_{(s)}$ will be the ultimate product formed since other transition metals will be displaced. Silver and inorganic S chemistry is described by Bell and Kramer (1999) as being mononuclear (AgL_n) or polynuclear (Ag_mL_n) where $L = \text{HS}^-$, S^{2-} and HS . In this model, under the usually low concentration of Ag^+ in natural solutions, the predominance of various complexes increases from simple (mononuclear) to polynuclear with increasing concentrations of Ag^+ and S^{2-} . Adams and Kramer (1998) also demonstrate Ag's affinity for S showing the ability for Ag^+ to adsorb rapidly onto to amorphous FeS.

Precipitates formed by Ag include $\text{AgCl}_{(s)}$ and $\text{Ag}_2\text{S}_{(s)}$ which are common in oxidising and anaerobic environments, respectively (Evans and Barabash 2010). The formation of these precipitates will be dependent on the relative amounts of chloride and sulfide in the given system. The Ag^+ ion is well known to form a range of insoluble compounds with the halides (Br^- , Cl^- , F^- , I^-) as well as sulfates and sulfides as previously mentioned (Jacobson et al. 2005). In some cases, the concentrations of these halides and sulfates may be too low for such complexes to precipitate from solution. Alternatively, at very high concentrations of Cl^- e.g. during aqua regia soil digestion, the opposite occurs, where Ag remains solubilised as multihalide complexes: AgCl_2^- , AgCl_3^{2-} or AgCl_4^{3-} .

Redox chemistry is highly relevant to the behaviour and reactivity of Ag. Given the common oxidation states of Ag are +1 and 0 in most natural waters and soil, the standard electrode potential for the Ag^+/Ag^0 couple is calculated as 0.799 V (Evans and Barabash 2010). This can be compared to other important electrochemical reactions occurring in soil and their corresponding redox potentials such as $\text{O}_2/\text{H}_2\text{O}$, NO_3/NO_2 , $\text{MnO}_2/\text{Mn}^{2+}$ and $\text{Fe}(\text{OH})_3/\text{Fe}^{2+}$ with E_h of 0.82, 0.54, 0.41 and 0.17 V, respectively. Given the highly positive value of the redox potential for Ag^+/Ag^0 in comparison to these other redox couples, in aerobic soil environments, Ag would be found present in soils solutions as Ag^+ (Figure 1). The pH of the soil solution and redox potential (E_h) directly and indirectly influence all chemical processes in soil (Tack 2010), consequently influencing the chemical behaviour of trace elements such as Ag. Figure 1 shows an E_h -pH diagram for the Ag-S-O-H system and demonstrates that in the presence of sulfide ions, Ag can precipitate as $\text{Ag}_2\text{S}_{(s)}$, especially in anoxic systems where sulfides may control speciation, at $\text{pH} < 6$. Metallic $\text{Ag}_{(s)}$ dominates a large portion of the diagram under reducing conditions at $\text{pH} > 6$. In aerobic environments (high E_h values) Ag oxidises to Ag^+ in the pH range commonly found in most soils (e.g. pH 4-8).

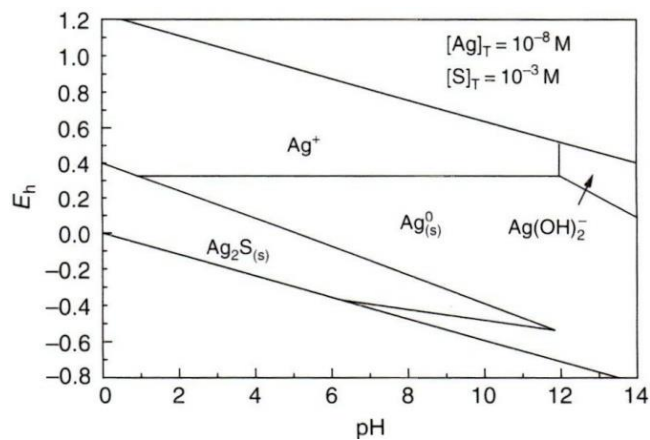


Figure 1. E_h -pH diagram for the system Ag-S-O-H (Evans and Barabash 2010).

2.3 Sources of silver into the environment

The unique properties of Ag include strength, malleability and ductility, electrical and thermal conductivity, sensitivity to and high reflectance of light, the ability to endure extreme temperature ranges and antimicrobial activity. As a result, applications of Ag range from traditional (coinage, silverware), industrial (bearings, catalysts), medicinal (wound dressings), environmental (solar energy, water purifications) and agricultural (biocides and pesticides) (Elvers et al. 1993; The Silver Institute 2011; Innovotech 2014). Silver and increasingly AgNPs are now minor or major constituents in a range of consumer products including textiles, electrical appliances, personal hygiene products, cosmetics and plastics (Nowack et al. 2011).

2.3.1 Mining

Silver is actively mined with the most recent World Silver Survey reporting mine supply was 22,000 t in 2012 (Thomson Reuters GFMS 2013). The top two producing countries are Mexico and Peru, a result of Pb/Zn operations. During the mining process, specific losses of Ag to the environment occur via tailings, slag, smelting, dust and leachates (Eckelman and Graedel 2007). It is estimated by Eckelman and Graedel (2007) that the total global losses of Ag to the environment were more than 1.3×10^7 kg annually with tailings and landfills accounting for three-quarters of the total amount.

During Ag extraction, it is estimated that 20% of Ag is returned as tailings to the lithosphere (Johnson et al. 2005). This is unfavourable because wind and water can transport contaminated material into the soil environment. Mine tailings can have high concentrations of metals, low concentrations of plant nutrients and unusual pH values (Cano-Resendiz et al. 2011). Silver is commonly mined as a by-product from the

extraction of Au, Cu, Pb, tin (Sn) and Zn ores (Petering and McClain 1991; Elvers et al. 1993) and this contributes to the high percentage of Ag in mine tailings.

During the mining and milling process there is a fraction of Ag that can become airborne as dust particles, which contribute to the source of Ag air emissions (Eckelman and Graedel 2007). Other sources of Ag air emissions are primarily particulates formed during ore processing and burning of fossil fuels, especially coal (Purcell and Peters 1998). The methods of Ag separation after extraction results in Ag losses as follows: 10% for tank leaching, 11% for thiosulfate leaching, 63% for heap leaching, 0.12% for smelting and 0.02% lost in slag during the refining process (Johnson et al. 2005).

2.3.2 Silver scrap waste

Approximately one fifth of Ag production comes from recycling of used and unwanted Ag scrap including silverware, jewellery, photographic chemicals, and computers (Evans and Barabash 2010). Silver waste is generated from commercial uses such as photographic processing and is often discharged to urban treatment plants. The photographic industry contributed 26% of the total Ag disposed to land in 1978 (Purcell and Peters 1998). This has now almost ceased due to developments and widespread use of digital photography.

2.3.3 Wastewater treatment

In urban areas, there is the potential for Ag to enter wastewater treatment plants (WWTPs) *via* the sewage system, as a result of the use and disposal of consumer products containing Ag. An additional input of Ag into WWTPs is through inputs of

industrial wastes from facilities producing such consumer products. Urban wastewaters are treated at WWTPs to remove impurities and contaminants so that the remaining liquid fraction, referred to as effluent, can be reused (e.g. through irrigation) or released into surface waters (Di Bonito 2008). During the wastewater treatment process, the solid fraction (sewage sludge) is separated from the liquid fraction and treated by a combination of physical, chemical and biological processes. The term biosolids refers to sewage sludge that has been treated *via* these processes to reduce pathogens and volatile organic matter. Biosolids tend to have high organic carbon content and high nutrient loads and therefore can be applied to land as a fertiliser. During wastewater treatment processes, a large proportion of the Ag^+ or AgNPs present is likely to be associated with the solid fraction. Silver can become concentrated in biosolids due to its strong affinity to S and organic carbon and under the reduced and S-rich environment, results in the formation of Ag sulfides (Kim et al. 2010). As a result, these biosolids may contain elevated concentrations of Ag from 1 to 6 mg kg^{-1} (Gottschalk et al. 2009) and are a direct route of transfer for Ag into the soil environment.

3. Biological reactions and effects of silver

Silver when in its ionic form (Ag^+) is one of the most toxic metal contaminants, surpassed only by mercury (Hg) (Ratte 1999). This is attributed to the antibacterial mechanism of the Ag^+ ion. Aside from bacteria, Ag^+ has also been found to exhibit toxicity to other microorganisms, plants and invertebrates. Although Ag^+ has been found to show a high degree of toxicity to organisms, transformations of this ion by reactions with various soil components may alter its bioavailability and toxicity to terrestrial organisms.

3.1 Antibacterial mechanism

The singly charged cation, Ag^+ , is directly responsible for the antibacterial, antifungal and antimicrobial action of Ag. There may however be differences between these mechanisms for Ag and AgNPs since the nanometer size may interact with the cell differently to Ag^+ . The main mechanism is thought to be the interaction of Ag^+ with the thiol groups of vital enzymes rendering them inactive (Percival et al. 2005). The Ag^+ ion may also target other SH groups within the cell. Evidence suggests that bacterial DNA loses its ability to replicate upon exposure to Ag^+ . Structural changes in cell membranes and formation of small electron-dense granules of Ag and S also contribute to the bactericidal mechanism of Ag (Russell and Hugo 1994). Overall it is the surface binding and damage to the membrane that are fundamental to the toxic action (Percival et al. 2005).

The mechanism of toxicity of AgNPs to gram-negative bacteria was found to occur by 1-10 nm size NPs attaching to the cell membrane and disrupting permeability and respiration. The NPs are able to penetrate into the cell where they interact with S- and P-containing compounds like DNA and finally by release of Ag^+ (Morones et al. 2005). A recent study also attributed the release of Ag^+ (responsible for the toxic effect on *Arthrobacter globiformis* bacteria) from AgNPs to particle size, surface area or whether the NPs were in metallic or oxide form (Engelke et al. 2014). The EC50 values for inhibition of *Arthrobacter globiformis* dehydrogenase activity with and without soil are reported in Table 1.

3.2 Toxicity and Uptake

Aside from bacteria, the Ag^+ ion is highly toxic to a range of organisms, including other microorganisms, plants and invertebrates (Ratte 1999). In soil environments, the ionic form of Ag is expected to interact with the surrounding matrix altering its speciation and potentially making it less bioavailable and therefore less toxic. The toxicity of a metal must be assessed experimentally usually by acute, chronic or long-term toxicity tests. To quantify toxicity, an effect threshold that corresponds to the percentage of a population that is affected for a given endpoint (e.g. mortality or reproduction) is often determined. Common measures of toxicity include the LC50 (lethal concentration for 50% of test organisms) and EC50 (effective concentration for 50% of test organisms). Toxicity can also be quantified by using hypothesis-based testing, for example the no observable effect concentrations (NOEC), which is equal to the highest test concentration that produces a response that is not significantly different from the control, and the lowest observable effect concentrations (LOEC), which is the lowest concentration that produces a response that is significantly different from the control.

3.2.1 Microorganisms

Assessing the effects of trace metals on soil microorganisms can include a combination of microbial activities, population measurements and a consideration of kinetics of the investigated process (Johansson et al. 1998). In soils there is evidence for Ag's inhibitory effects on nitrification, denitrification, respiration and activities of soil exoenzymes (Johansson et al. 1998; Throback et al. 2007; Langdon et al. 2012; Shin et al. 2012). In particular, the toxicity of Ag^+ to nitrification processes across a range of soils was found to vary by several orders of magnitude depending on soil type, with a

stimulatory response observed at lower concentrations (Langdon et al. 2014). Soil pH and organic carbon were the most influential properties mitigating toxicity changes in these properties and were found to account for approximately 90% of the variability in the toxicity data. The EC50 values are reported in Table 1.

3.2.2 Plants

Plants are known to absorb nutrients through their roots from the soil solution and can take up metals in the same way. The uptake mechanisms for many metals are poorly understood. It is unlikely that plants have developed specific uptake mechanisms for non-essential metals such as Ag (McLaughlin et al. 2000). The uptake mechanism for non-essential metal contaminants would be across the root membrane as a substitute for nutrients using the same transport mechanisms or through breaks in the endodermal barrier at sites of lateral root initiation or root apices (McLaughlin et al. 2000).

Silver uptake has been investigated for a variety of higher plant species using soluble Ag and AgNPs treatments. In some cases, plants have been shown to internally transform soluble Ag up taken from roots into metallic AgNPs, for example the uptake of Ag⁺ by *Brassica juncea* grown hydroponically resulted in reduction of Ag⁺ to AgNPs at sites of high reducing sugars (fructose and glucose) in plant chloroplasts (Beattie and Haverkamp 2011). Another study showed that alfalfa sprouts were able to uptake Ag(0) NPs from an agar medium (Gardea-Torresdey et al. 2003). The effects of soluble Ag and AgNPs with different coatings have been assessed for *Lolium multiflorum* and other common wetland plant species (Yin et al. 2011; Yin et al. 2012). Overall there is reduced growth from both forms of Ag but this varies for species and the effect of AgNP properties like coating or size. Toxicity endpoints that are

commonly measured for plants include inhibition of germination, root growth and overall decrease in biomass. Roots are generally more sensitive than shoots. The uptake of soluble Ag (as AgNO_3 and AgSO_4) has been shown to decrease biomass of wheat seedlings and inhibit rhizosphere proteins and uptake of nutrients and trace elements (Shtangeeva et al. 2011).

Toxicity will be greatly influenced by other interactions in the soil environment as demonstrated by the influence of humic and fulvic acids on Ag uptake by perennial rye grass, which have been shown to alleviate toxicity (Jones and Peterson 1986). Plant toxicity has been measured in a variety of microflora and higher plants. Microflora includes marine and freshwater algal species. Some EC50 and NOEC values are reported in Table 1 for green algae, *Selenastrum capricornutum* and *Gymnodium sp.* The toxicity of Ag was higher (lower EC50 values) when applied in a soluble Ag form (e.g. AgNO_3 or NaAgS_2O) compared with the less soluble forms such as Ag_2S or AgCl . The toxicity of Ag to cowpea seedlings determined by solution culture experiments are also reported in Table 1 as EC50 values (Blamey et al. 2010). These results showed that Ag was rapidly rhizotoxic to the cowpea seedlings after four hours of exposure showing a similar effect as other trace metals: Cu and Al. Other terrestrial plants tested include lettuce, ryegrass, radish, marigold and maize and their NOEC values are reported in Table 1 showing lettuce to be the most sensitive and ryegrass and marigold to be the most tolerant (Ewell et al. 1993).

3.2.3 Invertebrates

In comparison with plants and microbes, soil invertebrates have uptake routes through the skin, the gut wall (soil ingestion), *via* drinking or specific organs such as the ventral

tubulus in springtails (Peijnenburg et al. 1997). Uptake depends largely on a particular species, taking into account its preference for certain soil layers, whether it be weak bodied and able to absorb through its skin or hard bodied and surface active with uptake through the gut wall. Earthworms are considered to be a suitable test organism and studies with Ag and AgNPs are common.

The toxicity of soluble Ag and AgNPs in an earthworm reproduction test using *Eisenia andrei* was measured along with the uptake and accumulation of Ag by adult earthworms (Schlich et al. 2013). Similar toxicities were found between soluble Ag and AgNP treatments in the earthworm reproduction test (EC50 values are reported in Table 1). This study found uptake of Ag from AgNPs was slightly higher than the uptake of Ag from AgNO₃. Spiked soils showed a concentration-dependent effect on reproduction, but there was no concentration-dependent increase in the amount of Ag in earthworm tissues. An important finding was that the inhibition seemed to reflect Ag released into the soil pore water, which affected cocoons and juveniles in the soil rather than uptake by the earthworms.

Another study compared the effects of soluble Ag and AgNPs on the earthworm, *Eisenia fetida* (Shoults-Wilson et al. 2011). The soluble Ag treatment caused significant reduction of growth and reproduction at 7.41 ± 0.01 mg Ag kg⁻¹ in the sandy loam, where reproduction was less sensitive at a concentration of 94 ± 3 mg Ag kg⁻¹ in an artificial soil (relatively higher CEC, organic matter and clay content than the sandy loam). In the artificial soil, significant reproductive toxicity was only observed in organisms exposed to the AgNPs at concentrations approximately eight times higher than those at which the effects from Ag⁺ were observed. *Eisenia fetida* exposed to

either AgNO₃ or AgNPs in the sandy loam accumulated higher concentrations of Ag than those exposed in the artificial soil and had higher bioaccumulation factors. These results suggest Ag was more bioavailable in the sandy loam soil compared to the artificial soil due to its lower CEC, organic matter and clay content. Earthworms exposed to soluble Ag also accumulated significantly higher concentrations of Ag than those exposed to AgNPs. It was suggested that Ag⁺ was responsible for the effects on growth and development caused by exposure to AgNPs and overall their results also suggest that soil type is a more important determinant of Ag accumulation than the particle size of AgNPs.

Table 1. Examples of silver toxicity data for bacteria, microbes, plants and soil invertebrates

Test organism	Medium	EC50	NOEC	Reference
<u>Bacteria/Microbes</u>				
Soil nitrification processes	Soil	0.43 - >640 mg kg ⁻¹		Langdon et al (2014)
Basal respiration	Soil	61 mg kg ⁻¹	15 mg kg ⁻¹	Johansson et al (1998)
<i>Arthrobacter globiformis</i> (dehydrogenase activity)	Soil Solution culture	7.9 mg kg ⁻¹ 1.4 mg kg ⁻¹		Engelke et al (2014)
<u>Microflora</u>				
Marine algae (<i>Gymnodium sp.</i>)	Solution culture		0.002-0.01 mg L ⁻¹	Wilson and Freeberg (1980)
Freshwater algae (<i>Selenastrum capricornutum</i>)	Solution culture	>0.13 mg L ⁻¹	10 mg L ⁻¹	Schmittschmitt et al (1996), Ewell et al (1993)
<u>Terrestrial plants</u>				
<i>Vigna unguiculata</i> [L.] Walp. cv. <i>Caloona</i> (Cowpea)	Solution culture	0.0011-0.0023 mg L ⁻¹		Blamey et al (2010)
<i>Lactuca sativa</i> (Lettuce)	Soil		0.75-771 mg L ⁻¹ (germination)	Ewell et al (1993)
<i>Lolium perenne</i> (Ryegrass)	Soil		75-771 mg L ⁻¹ (germination)	Ewell et al (1993)
<i>Rhaphanus sativas</i> (Radish)	Soil		7.5-771 mg L ⁻¹ (germination)	Ewell et al (1993)
<i>Tagetes patula</i> (Marigold)	Soil		75-771 mg L ⁻¹ (growth)	Ewell et al (1993)
<i>Zea mays</i> (Maize)	Soil		7.5-771 mg L ⁻¹ (growth)	Ewell et al (1993)
<u>Soil Invertebrates</u>				
Earth worm (<i>Eisenia andrei</i>)	Soil	15-84 mg kg ⁻¹	<15 mg kg ⁻¹	Schlich et al (2013)

4. Behaviour and fate of soluble silver in soil

There are a number of reactions or processes that soluble Ag may undergo once it is introduced into the soil environment. Transformations of Ag^+ in the soil may heavily impact the bioavailability of this contaminant. Importantly a measure of the total exchangeable metal fraction in soils is desirable to quantitatively assess the availability of Ag and how this may change with time. The speciation of Ag in soil and interactions with soil components like dissolved organic matter (DOM) also provide valuable information on what controls Ag^+ availability in soils.

4.1. Sorption

Sorption is the general term for the partitioning of a solute from an aqueous solution to solid phases and for soils this is the accumulation of matter at a surface without formation of a three dimensional structure (Tack 2010). Sorption encompasses both adsorption (solute clings to solid surfaces) and absorption (solute diffuses into a porous solid and clings to interior surfaces) processes. For metals, sorption is important in controlling the ion activity in the solution phase of soils. In addition, precipitation reactions may also remove ions from the soil solution by formation of a three dimensional structure. Precipitates commonly include oxides, oxyhydroxides, hydroxides, carbonates, phosphates and silicates (Tack 2010). As previously mentioned, $\text{AgCl}_{(s)}$ and $\text{Ag}_2\text{S}_{(s)}$ are the most commonly formed precipitates, where formation is highly dependent on concentrations of chloride and sulfide, respectively (Evans and Barabash 2010). Other factors contributing to their precipitation will be the pH and solubility products of these precipitates.

Sorption reactions generally control the ion activity in the solution phase. For instance, sorption curves of Cd, Co, Cu, Ni, Pb and Zn in four soils suggested that solubility of these metals was controlled by sorption and complexation processes, rather than precipitation (Welp and Brummer 1999). Similarly, Cd, Cu, Pb and Zn solubilities in moderately contaminated soils were found to be controlled by sorption reactions (McBride et al. 1997). Silver sorption in soils has been previously modelled with Freundlich isotherms, a soil adsorption model. Specifically the adsorption of soluble Ag by various contaminated and uncontaminated soils were modelled (Jones et al. 1986). Evidence was found for a strong association between SOM and Ag, suggesting that humus may control the partitioning and availability of Ag in the short term. Silver sorption at trace levels has also been evaluated using short-term and long-term sorption isotherms generated under batch conditions for three illite-rich mineral soils (silt loam and fine sandy loam), a peaty-muck soil drained for agricultural use, and soil minerals (ferrihydrite and birnessite) (Jacobson et al. 2005). Silver was more strongly sorbed to the peaty-muck soil than the mineral soils, and Ag sorption to soils was found to be dominated by SOM either through exchange or complexation reactions.

Desorption of Ag has also been measured using illite-rich mineral soils with differing textures and an organic peaty-muck soil (Jacobson et al. 2005). A strong cation exchange resin (Duolite G-73) containing thiol functional groups was used to desorb Ag from the soils. The study monitored desorption over time (one hour to four weeks) from soils previously equilibrated with Ag⁺ for 24 h and up to one year. Silver could not be effectively recovered from the resin. However, qualitatively the study found that more Ag was desorbed after the 24 h sorption period than after a one-year period. More Ag desorbed from the sandy soil than from the peaty-muck soil or the mineral soils

with higher clay contents. These results suggest Ag sorption may be a reversible process, and that soil properties and time will play an important role in the release of Ag.

4.2 Partitioning

The behaviour and fate of metals in soils can be described by their solid-liquid partitioning, which is a measure of the degree of sorption. While sorption is a mechanistic process, solid-liquid partitioning, describes the distribution of an added metal between the soil solid phase and the soil solution. This partitioning greatly affects metal mobility and availability in soils. The partitioning of metals in soils can be quantified by the distribution coefficient, K_d , expressing distribution of an element between the solid phase and solution phase (Degryse et al. 2009):

$$K_d = M_{\text{solid}} / [M] \quad (1)$$

Where, M_{solid} is the solid phase concentration, expressed on a soil-weight basis (mg kg^{-1}), and $[M]$ the (total or free ion) concentration in solution, expressed on a solution-volume basis (mg L^{-1}). As a result, the units for K_d are normally L kg^{-1} .

The different mechanisms of metal partitioning in soils between solid and liquid phases are illustrated in Figure 2, adapted from Degryse et al. (2009). Figures 2a-c are in order of increasing mechanistic complexity. In Figure 2a, the most simple K_d^{tot} is a measure of the distribution of metal between solid (M_{solid}) and solution (M_{sol}) phases. Figure 2b divides the solid phase (M_{solid}) into M_{inert} (cannot be released into solution, non-exchangeable) and M_{labile} (adsorbed on solid phase but exchangeable with metal in soil solution). In this scenario, partitioning occurs between the solid and solution phases by exchange between M_{labile} and M_{sol} , described by the K_d^{lab} . In Figure 2c, the solution

phase is now separated into the free metal ion (M^{n+}) and metal complex (ML) in soil solutions, showing that the free ion may form complexes with other soil solution ligands and this complexation is reversible. The $K_d^{lab/free}$ represents similar partitioning to Figure 2b, but the M^{n+} concentration may change with M^{n+} in equilibrium with ML. Overall, the distribution coefficients represent the concentration ratio of total metal on the solid phase to total metal in solution (K_d^{tot}), or the concentration ratios of labile metal on the solid phase to the total metal in solution (K_d^{lab}) or the free metal ion ($K_d^{lab/free}$). It is important to note that while K_d values imply an equilibrium, they are operationally defined (by a set time frame and procedure) and can change over time for example with ageing the K_d values can increase.

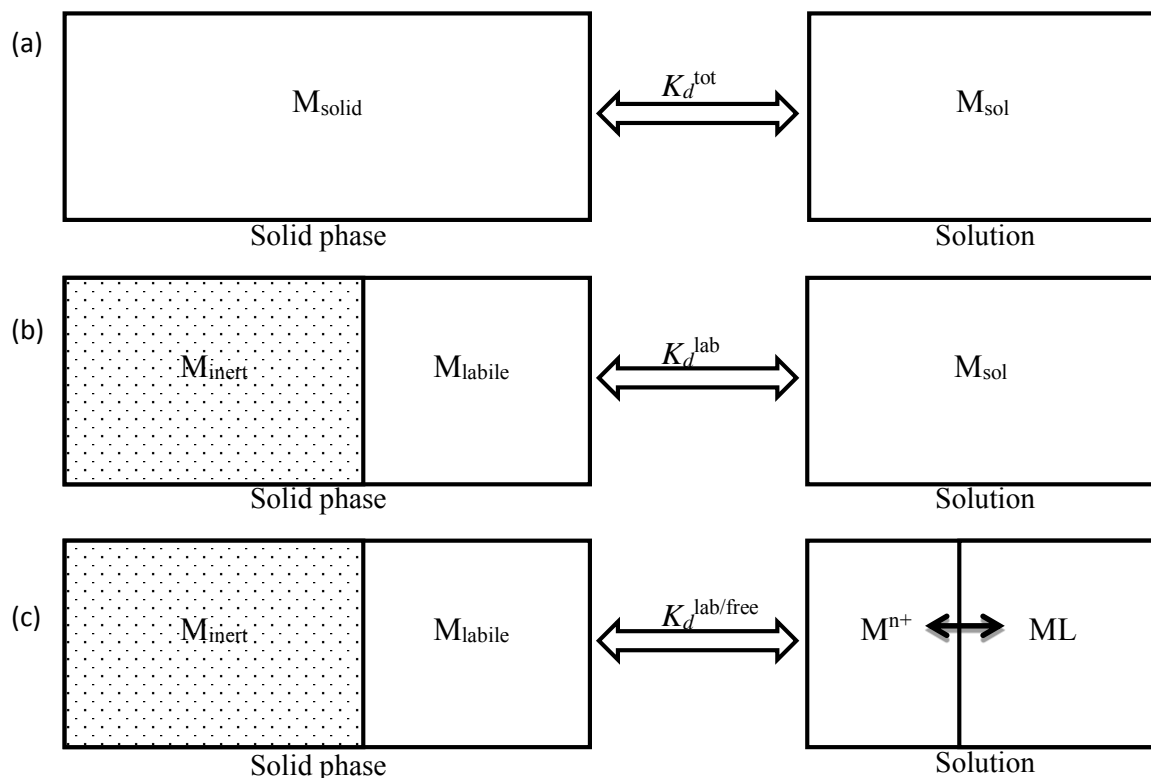


Figure 2. Schematic representation of the partitioning of metals in soil between the solid phase and solution, in increasing order of complexity, (a) metal on solid phase (M_{solid}) and metal in solution (M_{sol}), (b) exchangeable metal (M_{labile}) and M_{sol} and (c) M_{labile} and the free metal ion (M^{n+}) in solution (assumed to be in equilibrium with complexed metal (ML)) from Degryse et al. (2009).

K_d values can vary by several orders of magnitude for the same metal depending on soil type and metal loading. For metals Cd, Cu, Pb, Ni and Zn, the K_d values have been compiled from over 70 studies (Sauvé et al. 2000). The relationships between the reported values to variations in soil solution pH, SOM, and the total soil metal concentrations were investigated. Multiple linear regressions showed that the best prediction of K_d values was achieved using empirical linear regressions with pH (R^2 values of 0.29-0.58) or pH and log of SOM or log of total metal (R^2 values of 0.42-0.76). The best tool, however, was a semi-mechanistic model based on competitive adsorption of metal and H^+ , where there is dependence on pH, total metal content and log(SOM) where R^2 values increased to 0.61-0.88 (except Pb: 0.35). The median K_d values for Cd, Cu, Pb, Ni and Zn reported by Sauvé et al. (2000) were 390, 2120, 102410, 2333 and 1731 L kg⁻¹, respectively.

Previous reports of Ag partitioning in the literature are limited. In soils, Allison and Allison (2005) reported K_d values to range from 10-32000 L kg⁻¹ but they do not link these results to specific peer reviewed literature. Another study reports K_d values for Ag in geogenic and soluble Ag treatments for 5 different soil types to be 48-212 L kg⁻¹ and 35-1816 L kg⁻¹, respectively (Cornelis et al. 2010). These data indicate that there is large variability of K_d values across different soil types. This partitioning of Ag in soil was thought to be strongly influenced by pH, clay content, organic matter content and chloride concentration. Another recent study examining Ag partitioning in soils found less free Ag⁺ ion concentrations in soil solution for soils with high organic carbon (OC) content compared to a soils with low OC content (Benoit et al. 2013).

4.3 Lability

Lability refers to the metal fraction that is in rapid exchange on solid phases and in solution (Young et al. 2005). The total 'labile' or 'exchangeable' metal fraction consists of M_{labile} adsorbed on solid phase plus the M^{n+} and ML of the solution phase (Figure 2). An *E*-value is a measure of this total exchangeable metal fraction in soil and is generally considered to be the concentration of metal that is "available" and therefore potentially taken up by soil dwelling organisms (Young et al. 2005; Hamon et al. 2008). In risk assessments for metals this is the most important metal concentration that should be measured in the soil. The importance of *E*-values is that they provide information on the availability of metals, hence they can be used to investigate various management options like *in situ* remediation using soil amendments (Hamon et al. 2002; Lombi et al. 2003) and to investigate the ageing process for soil metals such as Ag (Nakhone and Young 1993; Crout et al. 2006; Ma et al. 2006; Ma et al. 2006).

E-values are commonly expressed as a percentage of the total metal concentration of the soil, assisting in comparison between soils, various metals and different metal loadings of soils. An *E*-value can be presented as an E% value, defined as the percentage of labile metal relative to the total concentration in the soil. The E% values reported in the literature vary greatly and for a given metal can range from 0-100%. For Cd, the labile fraction has been measured for a wide range of contaminated soils (Nakhone and Young 1993). It was found that labile Cd was pH dependent and at pH > 5 approximately 5% of Cd was labile. Another study on Cd and Zn found that the E% values ranged from 9% to 92% (mean: 61%) and from 3% to 72% (mean: 33%), respectively (Degryse et al. 2004). For Cd there was no correlation between soil properties and E% but for Zn, E% was negatively correlated with soil pH. This study

also found that the source of the contamination may control the exchangeable metal fractions of Cd and Zn.

4.4 Ageing

When soluble metals are added to soils there are initial fast sorption reactions which remove metals from soil solution (adsorption and precipitation), followed by slower reactions that continue to remove metals from the exchangeable fraction. The decrease in metal lability that occurs with time after metal addition to soil is thought to occur through the following general mechanisms: micropore diffusion, cavity entrapment, occlusion in solid phases by co-precipitation and co-flocculation and surface precipitation (Kabata-Pendias and Pendias 2000; Fendorf et al. 2004; Ma et al. 2006). These processes are generally referred to as “ageing”. As a consequence the bioavailability and toxicity of the metal to soil organisms may decrease with time as the bioavailability decreases.

The ageing of a range of soluble cationic and anionic metals in soils has been studied (e.g. As, Cd, Cu, Mo, Ni and Zn) by measuring metal lability after various periods of time up to 28 months (Fendorf et al. 2004; Crout et al. 2006; Ma et al. 2006; Ma et al. 2006; Buekers et al. 2007; Kirby et al. 2012). These studies have shown that the extent of ageing is dependent on the soil type, soil physical and chemical properties, source (e.g. soluble, oxides, etc.) and concentration of the metal, as well as environmental conditions such as temperature (Barrow 1998). A study over an ageing period of 850 days was conducted with 28 soils to assess the role of iron-oxyhydroxides and carbonates on fixation of Cu, Cd, Zn and Ni (Buekers et al. 2007). After 850 days of ageing, the labile fraction of added metal (by calculating *E*-values) was averaged over

28 soils, with the results as follows: 57% for Cu, 59% for Zn, 59% for Ni and 72% for Cd. The ageing observed was most pronounced in alkaline soils or in soils with elevated concentrations of iron oxyhydroxides.

To date, little is known of the extent of ageing of soluble Ag in soils. There is a lack of understanding regarding the solid phase speciation and lability of Ag and its ageing in natural soils. There are a few recent studies that have examined the fate of Ag over time but without measurement of the exchangeable metal fraction in soils. One study compared the behaviour of three forms of Ag: soluble Ag (as AgNO₃), citrate stabilised AgNPs and uncoated AgNPs, in two soils with contrasting organic matter content (Coutris et al. 2012). They measured binding strength over time periods of two hours, two days, five weeks and ten weeks, followed by a sequential extraction method. A ^{110m}Ag radio-tracer was used to measure Ag through the different sequential extraction fractions. The authors found all types of Ag to be more mobile in the mineral soil compared to the organic matter rich soil, indicating that the organic matter content is highly influential to Ag mobility. After two days, over 20% of the citrate stabilised AgNPs were extractable with water for both soils (compared to 1-3% for soluble Ag and uncoated AgNPs treatments) however, this decreased to trace levels thereafter. For the uncoated AgNPs the extractable fraction was eight to nine times greater than the other Ag treatments after 70 days.

In another study, soluble Ag and AgNPs (citrate and PVP coated) treatments, were aged in un-amended and sewage sludge amended soil (1% and 3% w/w) for up to six months. The concentrations of dissolved Ag in extracted soil pore waters in each treatment were then determined (Whitley et al. 2013). For the soluble Ag treatment,

there was no significant decrease in Ag concentrations in pore water over time in the unamended soil, but a significant decrease was observed in both the 1% and 3% sewage sludge amended soils attributed to the formation of Ag₂S. For both nanoparticle treatments there were significant decreases in Ag concentration in soil pore water by six months for the 1% and 3% sludge amended soils. The PVP-coated AgNP treatment exhibited an increase in Ag concentration in the unamended soil's pore water after six months, while the Ag concentration in the pore water of the citrate-coated treatment remained constant.

4.5 Complexation between Ag⁺ and soil DOM

K_d values and *E*-values can be used to provide important information on the partitioning and lability of metals in soils. However, metal uptake and toxicity may still be dependent of the concentration and speciation of the metal in soil solution (Parker and Pedler 1997). In solution, metal ions are in equilibrium with other soil solution ligands and these interactions may play a pivotal role in assessing potential metal uptake and toxicity to soil dwelling organisms (Figure 2c). In particular, the complexation of Ag⁺ with dissolved organic matter (DOM) in soil solution may occur and there is a lack of understanding of the strength of binding and underlying mechanisms. Indeed other aquatic studies have shown that Ag⁺ forms complexes with DOM, with humic and fulvic acids and there is a strong emphasis placed specifically on the reduced S binding sites of DOM (Adams and Kramer 1998; Bell and Kramer 1999; Chen et al. 2012; Chen et al. 2013). This also occurred in wastewater effluents where Ag⁺ was shown to be strongly complexed with S²⁻ (Adams and Kramer 1999). There is, however, a limited understanding of this interaction when considering DOM in soil solution. The only other study from the literature simply stated that the free ion

concentration of Ag^+ was significantly lower in an organic matter rich soil compared to a low organic matter soil (Benoit et al. 2013).

Defining DOM in soils is difficult due to its complex and heterogeneous nature. The distinction between DOM and particulate organic matter (POM) is not clear-cut in a chemical sense, although the usual operational definition is that DOM is the organic matter fraction less than $0.45 \mu\text{m}$ (Bolan et al. 2011). In addition, the concentration of DOM is not usually directly quantified, but rather its concentration inferred through measurement of dissolved organic carbon (DOC), which usually represents the bulk of its elemental composition (~67%) (Bolan et al. 2011). Dissolved organic matter generally consists of a mixture of carbohydrates, carboxylic acids, amino acids, hydrocarbons and potentially other unknown compounds (~20%) as well as humic and fulvic substances which are residues of plant materials (~80%) (Ravichandran 2004). Although DOM represents a small proportion of the total OM in soils, it is considered to be the most mobile and actively cycling fraction and as a result is highly significant to a range of biogeochemical processes in the soil environment.

When considering complexation, it is the functional groups of DOM that are the reactive sites that may bind metal cations like Ag^+ and therefore influence their behaviour and fate in the soil environment. The transport and bioavailability of metals can be influenced by their ability to form soluble or insoluble complexes with soil DOM (Bolan et al. 2011). Metals including As, Cu, Cd, Hg and Pb have all shown affinity for DOM, although this affinity is stronger for some than for others (McBride et al. 1997; Weng et al. 2002). Copper in particular has been extensively studied and it is well established that DOM has the ability to mobilise Cu in soils, which is

specifically based on the DOM quality, aromatic carbon content (Amery et al. 2008).

The humic/fulvic content of DOM is another quality measure and contains a significant amount of aromatic carbon, carboxylic acid, alcohol, sulfhydryl or amine functional groups that are influential to metal complexation (Chen et al. 1978; Weng et al. 2002).

5. Methods for assessing the availability, speciation and fate of silver in soils

It is well established that the total metal concentration of the soil is not an appropriate measure of the potentially bioavailable metal concentration. Two relatively common approaches to characterising metal availability are chemical extraction and isotopic dilution. Chemical extraction procedures are classified as single or sequential, implementing one or multiple chemical extractants, respectively, to release available metals from soil. Isotopic dilution can be used to measure an *E*-value which represents the isotopically exchangeable metal fraction and therefore the fraction that is potentially bioavailable to organisms. In addition to the concentration of exchangeable metal, the speciation of Ag in solid or solution phases is important in understanding the processes that control the exchangeable metal fraction. The speciation technique, X-ray absorption near edge structure spectroscopy (XANES) is appropriate for this and provides information on the oxidation state and coordination environment.

The presence of DOM in the soil solution is potentially very important in understanding how the concentration of free Ag⁺ varies between soils therefore characterisation of the DOM from soils should also be considered. Spectroscopic techniques like UV-visible, fluorescence and infrared spectroscopy are commonly employed techniques and can provide information on structure and functional groups of DOM.

5.1 Chemical extraction techniques

Chemical extraction procedures using a single or sequential approach have been widely applied to characterising the availability of metals in soils, sludges and sediments. The extractants used in chemical extraction are classified as weak (e.g. water, calcium chloride), reductive (e.g. sodium ascorbate), weak acids (e.g. diluted acetic acid), strong complexing agents (e.g. ethylenediamine tetra-acetate (EDTA)), combined salt-acid (e.g. ammonium oxalate-oxalic acid) and dilute or concentrated strong acids (e.g. nitric or hydrochloric) (Peijnenburg et al. 2007).

For single extraction the end result is separation of the sorbed phase into labile and non-labile fractions. Single extraction methods commonly use dilute calcium solutions to remove weakly complexed metals from the surface of solid phases. As a result studies using 0.1 M CaCl_2 , NaNO_3 and NH_4NO_3 have often been found to have good relationships between the extractable metal concentration and plant content of metals, independent of soil characteristics (Novozamsky et al. 1993). In the case of estimating Cu bioavailability to maize in soils, four extractants were used : EDTA, diethylene triamine pentaacetic acid (DTPA), ammonium acetate and 0.01 CaCl_2 . In this case the metal concentrations in the stronger chelating and ammonium acetate extractants from soils correlated better to plants metal concentrations than using CaCl_2 (Brun et al. 2001). In single extraction procedures, careful choice must be made for selecting the right extractant at the right concentration to measure the availability of a given metal. The use of these tests is limited to a general assessment of potentially available or soluble metal fraction in the soil.

Sequential extraction procedures (SEPs) are used to examine metals associated with various soil components. They can involve a number of steps and elaborate extraction schemes isolating up to 10 identifiable elemental fractions. In particular, the procedure proposed by Tessier *et al.* (1979) is widely applied and often with various modifications. This method fractionates metals into exchangeable, carbonate bound, Fe and Mn oxide bound, organic matter bound and residual (bound to primary mineral structure) fractions (Tessier *et al.* 1979). This SEP has been developed to investigate the partitioning of trace metals: Cd, Co, Cu, Ni, Pb, Zn, Fe and Mn. Another example is the BCR fractionation procedure employing three-steps that fractionates metals into three operationally defined fractions: CH_3COOH , $\text{NH}_2\text{OH}\cdot\text{HCl}$ and $\text{H}_2\text{O}_2/\text{CH}_3\text{COONH}_4$ exchangeable plus a residual fraction. This technique is standardised by the European Community Bureau of Reference in Brussels (Peijnenburg *et al.* 1997).

Sequential extraction procedures can become complex and tedious, employing a range of harsh chemical extractants. The main concerns with SEP procedures are in their lack of selectivity and that the amount of metal obtained is still considered to be “operationally defined” (Peijnenburg *et al.* 1997). In addition, dissolution of other soil components may occur, metals liberated can become resorbed among the remaining solids or speciation of metals can be altered during handling prior to extraction (e.g. oxidation of reduced species in anoxic soils) (Peijnenburg *et al.* 1997; Young *et al.* 2005; Hooda 2010). Unfortunately all operational aspects of the procedure can be a potential source of variability, including but not limited to soil preparation, solid-solution ratio, extraction time and temperature, concentration of extractants and shaking duration. Particularly in the case of Ag, a redox sensitive metal, the reducing

extractants that release metals bound to Fe-Mn oxides may be of particular concern, as Ag^+ will likely become reduced to Ag^0 .

5.2 Isotopic dilution

Isotopic dilution is a widely applied technique to examine the exchangeability of metals in soils, in particular to investigate the fate and availability of different elements in soils (Hamon et al. 2002; Hooda 2010). Isotopic dilution is a method that reflects the isotopically exchangeable fraction of metals in soil and is applied with minimal disturbance to solid phases. It was first used in 1947 to study reactions, fixation and residual availability of fertilizer nutrients with the radioisotopes of Ca, K and P (McAuliffe et al. 1947). Isotopic dilution was then applied to trace elements or potentially toxic metals like As, Cd, Hg, Ni and Zn. The benefit of radioisotopes is their ability to examine the trace elements' partitioning into isotopically and non-isotopically exchangeable fractions, labile and non-labile, respectively. The elements in the isotopically exchangeable fraction are considered potentially available to organisms and their reactions or activities.

One approach to isotopic dilution is the *E*-value method. This tracks depletion of an isotope from solution in a soil suspension to determine the amount of isotopically exchangeable element at a given time following the addition of an isotope (Hamon et al. 2002). When the isotope of interest is introduced to the soil, it readily redistributes itself among the solution and exchangeable phases, which are in dynamic equilibrium, as would other isotopes of the same element:

$$\frac{r_{sol}^*}{r_{exch}^*} = \frac{M_{sol}}{M_{exch}}$$

Where, sol and exch represent the radioactivity (r^*) of the isotope, or the concentration of the metal (M), in solution phase and exchangeable metals (M_{exch}) adsorbed on the solid phase, respectively.

The amount of depletion of isotope from solution relative to the quantity of element at a given time can be used to determine specific elemental compartments in the soil. The E -value can be used to determine the size of the isotopically exchangeable and hence the potentially available metal fraction (Hamon et al. 2002). The E -value can be calculated with the following equation adapted from Hamon et al (2008):

$$E = \frac{S}{r^*} \times IR \times \frac{v}{m}$$

Where, S is the solution Ag concentration (mg L^{-1}), r^* is the $^{110\text{m}}\text{Ag}$ solution radioactivity after equilibration (Bq), IR is the initial spiked $^{110\text{m}}\text{Ag}$ radioactivity (Bq), v is the solution volume (L) and m is the sample mass (kg). As a result the units for E -value are mg kg^{-1} .

According to Hamon et al (2008) the isotopic dilution method and measurement of the E -value are only valid if certain conditions are satisfied. Firstly, the isotope introduced into the system (usually as a small amount) must behave exactly as the natural element in soil without disrupting the equilibrium of the system. Secondly, the metal species measured in solution (M_{sol}) should all be isotopically exchangeable; as a result the introduced isotope will be physically mixed with all isotopically exchangeable metal in the labile fraction. The time allowed to reach this equilibrium is also important, since it is time dependent for the introduced isotope to mix uniformly with the entire soil metal pool. This time frame is operationally limited to one to three days (Hamon et al. 2008). Thirdly, any change to the conditions of the soil such as pH or salinity may cause

differences to or exchange between the labile and non-labile fractions. Therefore these conditions must remain constant during the experiment e.g. before or after isotope addition, and the suspension matrix should not cause any chemical interference e.g. precipitation of metal of interest.

There is also a potential error in *E*-value determinations that can occur as a result of the presence of non-isotopically exchangeable elements associated with colloidal material in soil suspensions for example with Cu (Ma et al. 2006). This potential error can be corrected by a resin purification step, commonly with Ca-form Chelex 100, to bind all free metal cations in solution, thus removing non-exchangeable forms from the *E*-value calculation (Hamon et al. 2002).

In isotopic dilution experiments, radioactive isotopes have typically been used. Suitability of the radioisotope depends on the half-life. When a half-life is very short e.g. ^{64}Cu ($t_{1/2} = 12.7$ hours) the use in longer term studies is not possible (Hamon et al. 2008). Alternatively, the half-life can be relatively long e.g. ^{63}Ni ($t_{1/2} = 100$ years) causing issues with disposal upon completion of the study. Enriched stable isotopes are now readily utilised due to the increasing availability of sensitive analytical techniques (Tongtavee et al. 2005). Despite the use of stable isotopes being analytically more demanding, they have an indefinite “shelf-life” and can prove useful when the radioisotopes required are unavailable, decay rapidly, hazardous, or unaffordable (Hamon et al. 2008). Isotopic dilution methods have great potential when coupled with speciation techniques such as high performance liquid chromatography-inductively coupled plasma mass spectrometry and X-ray absorption spectroscopy to understand

the fate and availability of metals in complex soil environments (Hill et al. 2004; Young et al. 2005).

Specific examples of isotopic dilution experiments with stable or radioactive Ag isotopes, ^{109}Ag (48% natural abundance) or $^{110\text{m}}\text{Ag}$ ($t_{1/2}=250$ days) respectively, in the literature are limited. Reported applications of Ag isotopes include: Ag isotope fractionation, ratios or measurement thereof in geologic materials coupled with MC-ICP-MS (Schonbachler et al. 2007; Yang et al. 2009; Luo et al. 2010), nature of $^{110\text{m}}\text{Ag}$ binding to soils as a result of Chernobyl (Szabo et al. 1993), Ag uptake experiments with $^{110\text{m}}\text{Ag}$ in a green alga (Fortin and Campbell 2000) and recently a study with AgNPs used the $^{110\text{m}}\text{Ag}$ tracer to follow the partitioning of $^{110\text{m}}\text{Ag}$ across different sequential extraction soil fractions (Coutris et al. 2012). There has been no reported use of isotope dilution methods to examine the exchangeability of Ag in soils.

5.2 Solid state speciation in soils by X-ray absorption near edge structure spectroscopy

X-ray absorption spectroscopy (XAS) and in particular XANES spectroscopy using synchrotron radiation can be used to provide information on the oxidation state and coordination environment (hereby referred to as solid phase speciation) of metal contaminants in complex matrices such as soil, generally by analysing the resulting spectra using principle components analysis (PCA) and linear combination fitting (LCF) (Lombi and Susini 2009). Many studies have already demonstrated the ability of this technique to provide solid phase speciation data for a range of metal contaminants in soils e.g. As, Co, Cr, Cu, Mn, Ni, Pb (Nolan et al. 2003; Lombi and Susini 2009).

Examples of XANES spectroscopy applied to Ag can be found for a variety of media but has most commonly been used to assess the fate of AgNPs in a WWTP scenario and subsequent speciation in biosolids (Kaegi et al. 2011; Lombi et al. 2013; Whitley et al. 2013; Ma et al. 2014). Many of these studies have shown that soluble Ag and AgNPs are commonly converted to Ag₂S or other Ag-S species in WWTPs. Other examples involving XANES spectroscopy include Ag speciation in liver tissue of marine mammals (Nakazawa et al. 2011), ageing of AgNPs in kaolin suspensions (Scheckel et al. 2010), reduction of Ag⁺ to metallic Ag in plants (Beattie and Haverkamp 2011) and speciation of AgNPs added to microcosms with freshwater sediments (Bone et al. 2012). Overall these studies have shown that there is a tendency for Ag⁺ or AgNPs (following dissolution) to form Ag-S bonded species or to be converted to metallic Ag.

5.3 Spectroscopic characterisation of soil DOM

Many techniques have been applied to characterise DOM, including nuclear magnetic resonance (NMR), UV-visible absorption, infrared (IR), fluorescence and X-ray absorption spectroscopy (Preston 1996; Ellerbrock and Gerke 2013; Nebbioso and Piccolo 2013). These techniques all provide information on the composition and functional groups of DOM. While NMR and synchrotron powered X-ray absorption spectroscopy can provide detailed information, they are also relatively time consuming, inaccessible and costly. On the other hand, recent advances in IR spectroscopy and fluorescence spectroscopy mean these techniques can provide efficient, sensitive and effective characterisation of DOM (Janik et al. 2007; Andrade-Eiroa et al. 2013).

Excitation-emission matrix (EEM) fluorescence coupled with parallel factor analysis (PARAFAC) of the spectra has been used to characterise DOM from aquatic systems (Al-Reasi et al. 2011), soils (Fellman et al. 2008; Ohno et al. 2008) and wastewaters (Cohen et al. 2014). The EEMs collected are used to visualise fluorophores or fluorescent components as a function of excitation-emission pairs of wavelengths. Handling the EEMs with PARAFAC has improved the identification of the components and their abundance and contribution to the total fluorescence. Fluorescent components identified in this way are normally classified as proteinaceous or humic/fulvic-like and the end result is a fluorescent “fingerprint” made up of the various components for each DOM sample.

Infrared spectra of soils contain extensive information on the molecular composition of DOM (Gholizadeh et al. 2013). There are different IR techniques for recording spectra, of which attenuated total reflectance (ATR) spectroscopy is best suited for DOM characterisation (Soriano-Disla et al. 2014). The FTIR-ATR technique has been successfully applied as a screening tool for DOM quality in rainwater (Santos et al. 2009) and regenerated peat soils (Artz et al. 2008) but has many more applications for environmental samples (Jarusutthirak and Amy 2006; Sharma et al. 2010). FTIR-ATR spectra can be related to sample properties and therefore is useful for extracting relevant components from large data sets (Janik et al. 2007; Janik et al. 2010; Gholizadeh et al. 2013).

6. Objectives of this research

The use of Ag (and AgNPs) in consumer products and industrial applications continues to increase. Once in the terrestrial environment, there is potential for Ag^+ and Ag^+ released from AgNPs or other Ag compounds to pose a risk to soil-dwelling organisms. At present, there is a limited understanding of the behaviour and fate of Ag in soils. A comprehensive understanding of how this metal contaminant behaves in soils and a method for measuring the potentially bioavailable metal fraction is required. This involves development of a quantitative method to measure the concentration of exchangeable metal in soils, solution and solid-phase speciation techniques to examine reactions, processes and transformations of Ag in soils and soil solutions, soil physical and chemical properties on fate and availability of Ag and an understanding of the effect of ageing on Ag exchangeability in soils.

Generating new information in this field will improve our current understanding on the behaviour and fate of Ag in soils. The main objectives of this research were therefore to:

- 1) Develop and optimise a sensitive method for measuring the partitioning (K_d -value) and exchangeable (labile) metal fraction (E -value) of Ag in soils;
- 2) Assess the effect of long term ageing of soluble Ag added to soils by monitoring E -values and speciation with time; and
- 3) Determine the effect of DOM quantity and quality on Ag complexation in soil water extracts by relating complexation to various chemical and spectroscopic measurements.

7. References

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CHAPTER 2

A METHOD TO DETERMINE SILVER PARTITIONING AND LABILITY IN SOILS

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CHAPTER 3

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Fate and lability of silver in soils: Effect of ageing



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ABSTRACT

The fate and lability of added soluble Ag in soils over time was examined by measurement of labile metal (E-value) by isotopic dilution using the ^{110m}Ag radioactive isotope and the solid-phase speciation of Ag by X-ray absorption near edge structure (XANES) spectroscopy. After two weeks of ageing the E-values for Ag decreased by 20–90% with a further decrease of 10–40% after six months. The overall decrease in labile Ag for all soils after the 6 month ageing period was 50–100%. The ageing was more rapid and pronounced in the alkaline soils. XANES results for Ag in soils indicated that for the majority of soils the added Ag⁺ was reduced to metallic Ag over time, and associations with Fe-oxohydroxides and reduced S groups in organic matter also decreased Ag lability. Strong positive correlations were found between metallic Ag and non-labile Ag and between organic carbon and Ag bonded with S species.

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1. Introduction

The use of silver (Ag) and silver nanoparticles (AgNPs) in applications such as solar energy, water purification, medicine and nanotechnology is rapidly increasing (Elvers et al., 1993). Commonly exploited for their antibacterial properties, Ag and increasingly AgNPs are being used in a range of consumer products but this may also lead to environmental risks following their potential release into the environment (Morones et al., 2005; Benn et al., 2010).

Apart from mining processes, the exposure pathways for Ag into the environment from urban areas (e.g. consumer products and industry) will be from wastewater treatment plants (WWTPs), predominantly via land application of biosolids (Johnson et al., 2005; Oliver et al., 2005) and through agriculture by application of Ag containing pesticides such as Agress[®] (Marques et al., 2011; Innovotech Products ~ Agr). As a result of these exposure pathways, soil environments may be one of the major sinks for Ag in the environment. Elevated concentrations of Ag may pose a risk to soil environments due to the potential toxicity of Ag to a range of organisms such as plants, invertebrates, microbes and bacteria living

in soil (Ratte, 1999; Roh et al., 2009; Blamey et al., 2010; Throckmole et al., 2007; Murata et al., 2005).

When soluble metals are added to soils there are initial fast reactions which remove metals from soil solution (adsorption and precipitation), followed by slower reactions that continue to remove metals from the labile (i.e. exchangeable) pool. The decrease in metal lability that occurs with time after metal addition to soil is thought to occur through the following general mechanisms: micropore diffusion, cavity entrapment, occlusion in solid phases by co-precipitation and co-flocculation and surface precipitation (Ma et al., 2006a; Fendorf et al., 2004; Kabata-Pendias and Pendias, 2000). These processes are generally referred to as “aging”. As a consequence the bioavailability and toxicity of the metal to soil organisms may decrease with time as the actual exposure concentration decreases. The ageing of other metals in soils has been studied (e.g. As, Cu, Mo, and Zn) by measuring metal lability after various periods of time up to 27 months (Ma et al., 2006a; Fendorf et al., 2004; Ma et al., 2006b; Crout et al., 2006; Kirby et al., 2012). These studies have shown that the extent of ageing is dependent on the soil type and chemical properties of the metal, as well as environmental conditions such as temperature (Barrow, 1998). To date, little is known of the extent of ageing of soluble Ag in soils and with the predicted increase in Ag concentrations in the environment (due to increased use of Ag- and AgNP-containing products), it is important to examine the fate and potential bioavailability (lability) of soluble Ag in soils over time.

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Table 1
Selected physical and chemical properties of the soils.

Soil location	pH _{water}	Total organic carbon	DOC ^b	CEC ^c	Oxalate extractable		Particle size analysis			Water-extractable Cl	Water holding capacity	Total added Ag
					Al	Fe	Clay	Silt	Sand			
		%	mg L ⁻¹	cmol _c kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	%	%	%	mg L ⁻¹	%	mg kg ⁻¹
Kingaroy	6.1	0.9	3	13	2500	2300	60	17	19	0.6	37	100 ± 7
Charleston	6.0	6.9	65	12	410	2500	14	12	63	3.1	32	190 ± 1
Balaklava	8.5	1.9	21	27	1200	650	30	20	47	3.0	35	180 ± 9
Port Kenny ^a	8.8	1.8	36	13	480	300	12	4.2	21	2.3	26	200 ± 30
Millicent	6.9	11.8	75	42	2300	1500	19	5.2	48	8.1	39	390 ± 20
Inman Valley	6.0	5.3	110	25	1500	3700	42	22	26	5.0	48	770 ± 10

^a Note that particle size distribution for Port Kenny is equal to 37.2% due to the high concentration of CaCO₃ that is present in this soil (60%).

^b Dissolved organic carbon.

^c Cation exchange capacity.

The total concentration of metals in soils is known to be a poor indicator of the potential bioavailable fraction (McLaughlin et al., 2000). Isotopic dilution (ID) is an established method used to measure the total labile metal fraction in soils (in soil solution and on solid phases in rapid exchange with soil solution) and it is determined by calculating an *E*-value (Young et al., 2005; Nolan et al., 2004; Wendling et al., 2009). An ID method for Ag was recently developed to measure *E*-values for soils with a range of chemical and physical properties and added Ag concentrations (Settimo et al., 2014). This initial study found that not all soluble Ag added to the soil remained in the labile pool and significant ageing occurred after a relatively short period of time (two weeks). A similar result was also observed when AgNO₃ was added to soils and analysed by sequential extraction whereby soluble Ag was found to be rapidly immobilised in soil (Coutris et al., 2012). Although the amount of labile metal can be determined at various ageing periods using the ID method, there is still a lack of understanding in the chemistry associated with the labile and non-labile metal pools within the soil solid phase.

X-ray absorption spectroscopy (XAS) and in particular X-ray absorption near edge structure (XANES) spectroscopy using synchrotron radiation can be used to provide information on the oxidation state and coordination environment (hereby referred to as solid phase speciation) of metal contaminants in complex matrices such as soil, generally by analyzing the resulting spectra using principle components analysis (PCA) and linear combination fitting (LCF) (Lombi and Susini, 2009). Many studies have already demonstrated the ability of this technique to provide solid phase speciation data for a range of metal contaminants in soils e.g. As, Co, Cr, Cu, Mn, Ni, Pb (Lombi and Susini, 2009; Nolan et al., 2003). Examples of XANES spectroscopy applied to Ag can be found for a variety of media but has most commonly been used to assess the fate of AgNPs in a WWTP scenario and subsequent speciation in biosolids (Kaegi et al., 2011; Lombi et al., 2013; Ma et al., 2014; Whitley et al., 2013). Many of these studies have shown that AgNO₃ and AgNPs are commonly converted to Ag₂S or other Ag–S species in WWTPs. Other examples involving XANES spectroscopy include Ag speciation in liver tissue of marine mammals (Nakazawa et al., 2011), ageing of AgNPs in kaolin suspensions (Scheckel et al., 2010), reduction of Ag⁺ to metallic Ag in plants (Beattie et al., 2011) and speciation of AgNPs added to microcosms with freshwater sediments (Bone et al., 2012). Overall these studies have shown that there is a tendency for Ag⁺ or AgNPs (following dissolution) to form Ag–S bonded species or to be converted to metallic Ag.

There is a lack of understanding regarding the solid phase speciation and lability of Ag and its ageing in natural soils. Therefore the aim of this study was to examine the fate and lability of added soluble Ag over time by measuring labile Ag (*E*-value) concentrations and the solid-phase speciation of Ag (XANES) in soils. This will provide an insight into the long-term fate of Ag⁺ ion added to soils and the mechanisms or interactions with soil components that can affect its lability and bioavailability to soil organisms.

2. Materials and methods

2.1. Soil sampling and characterisation

This study used soils that were sourced from six locations in Queensland and South Australia, Australia: Kingaroy, Charleston, Balaklava, Port Kenny, Millicent and Inman Valley. The soils were collected from 0 to 10 cm depth, air-dried, homogenised and sieved to <2 mm. Selected physical and chemical properties for the experimental soils can be found in Table 1. Soil pH was measured using a soil:solution ratio of 1:5 (m/v), with ultrapure deionised water (Milli-Q, Millipore) and shaking for 1 h. Total organic carbon (TOC) content, cation exchange capacity (CEC), particle size and oxalate-extractable aluminium (Al_{ox}) and iron (Fe_{ox}) were determined according to standard methods (Gee and Bauder, 1986; Rayment and Higginson, 1992). Concentrations of dissolved organic carbon (DOC) (Shimadzu TOC-V_{CSH/CSN} + TNM-1) and chloride (Cl⁻) (Dionex ICS-2500) were determined

using a 1:5 soil:solution ratio (m/v) with ultrapure deionised water (Milli-Q) and shaking for 1 h, with the suspension filtered through 0.2- μm cellulose acetate filter (Sartorius, Germany). Total Ag concentrations in soils were determined using a microwave-assisted *aqua regia* (US-EPA 3051A) procedure. This was conducted by weighing 0.25 g soil into Teflon™ vessels and adding 10 ml of nitric (HNO_3) and hydrochloric (HCl) acid at a ratio of 1:3 (v/v). The soils were open vessel digested at room temperature for 12 h, then sealed and heated for 45 min at 175 °C (after a 10 min ramp period) in a microwave oven (Mars Express, 1600 W). After digestion, samples were filtered through 0.2- μm cellulose acetate filters (Sartorius, Germany) and Ag concentrations determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Spectro ARCOS) or ICP-mass spectroscopy (ICP-MS) (Agilent 7500ce). The limit of Ag detection of the method was 0.04 mg kg⁻¹. A digest blank and a certified reference material (NRC-CNRC PACS-2) with an Ag concentration of 1.22 ± 0.14 mg kg⁻¹ were included for quality control in all sample batches. Blank solutions were consistently <0.03 $\mu\text{g Ag L}^{-1}$ and the measured concentration of Ag in the certified reference material was 1.15 ± 0.16 mg kg⁻¹ in close agreement with the certified value.

2.2. Soil spiking with soluble silver and incubation conditions for ageing

Soils were spiked to the desired Ag concentration using AgNO_3 (Sigma–Aldrich, ≥99%) in ultrapure deionised water (Milli-Q). The nominal soil Ag concentrations used in this study were 100 mg kg⁻¹ for the Kingaroy soil, 200 mg kg⁻¹ for the Charleston, Balaklava and Port Kenny soils, 400 mg kg⁻¹ for the Millicent soil and 800 mg kg⁻¹ for the Inman Valley soil. Soils were spiked with soluble Ag at effective concentrations determined during preliminary experiments to inhibit barley growth by 50% (EC50). The Ag spiking solutions in 20 ml of ultrapure deionised water were aspirated onto dry soils (400 g) using a glass nebulizer. The soils were then leached using artificial rainwater (Oorts et al., 2007), air dried and sieved < 2 mm. The water content of soils was then increased to 50% of field capacity (FC) (determined at pF 2.0 using a tension plate technique (Marshall et al., 1979)) (Table 1) and incubated at 22 °C in the dark, with daily aeration. The samples were maintained at 50% FC by weight for two weeks and six months prior to measuring the labile Ag concentrations and determining solid state speciation of Ag in the samples. Total Ag concentrations in the spiked soils after leaching were determined using the microwave assisted *aqua regia* digest method outlined above (Table 1).

2.3. Isotopically exchangeable silver (E-value) concentrations in soils with time

E-values for Ag in soils (after soil spiking and incubation) were measured according to the method published by Settimio et al. (Settimio et al., 2014). Soils ($N = 5$) were weighed (4.0 ± 0.02 g) into 50 mL polypropylene centrifuge tubes (LabServ) and mixed end-over-end for 48 h with 40 mL of 0.01 M calcium nitrate ($\text{Ca(NO}_3)_2$). Following this initial equilibration period, each sample was spiked with 100 kBq of ^{110m}Ag in 200 μL carrier-free solution (in the form of ^{110m}AgNO₃ ($t_{1/2} = 250$ days)). The ^{110m}Ag spiked soil suspensions were mixed on an end-over-end shaker for a further 72 h, followed by centrifugation at 1200 g for 20 min and the supernatants filtered through 0.2- μm cellulose acetate filters (Sartorius, Germany). The pH of soil suspensions was determined before ^{110m}Ag addition and after the spike equilibration period (no significant changes in pH were observed). A 30-mL aliquot of the <0.2 μm soil suspensions was transferred into 50 ml centrifuge tubes, with approximately 0.15 g of Chelex 100 resin (Bio-Rad Laboratories). The resin had previously been converted from a sodium to a calcium form using $\text{Ca(NO}_3)_2$ (Sigma–Aldrich). The 0.2 μm filtered samples with resin were mixed end-over-end for 12 h, centrifuged at 1200 g for 20 min and the supernatants discarded. The resins were rinsed twice with ultrapure deionised water (Milli-Q) and eluted with 6 ml of 1 M HNO_3 . The eluted solutions were analyzed for ^{110m}Ag activity by gamma spectroscopy and total Ag concentrations by ICP-OES or ICP-MS. The E-values accounting for the possible presence of non-exchangeable Ag in soil solutions (E-value) was determined using the following equation:

$$E - \text{value} = \frac{S}{r^*} \times \text{IR} \times \frac{v}{m}$$

where, E-value = resin-corrected isotopically labile Ag concentration in the soil in mg kg⁻¹; S = solution Ag concentration in resin eluate in mg L⁻¹; r* = ^{110m}Ag solution radioactivity in resin eluate in Bq; IR = initial spiked ^{110m}Ag radioactivity in Bq; v = solution volume in L; and m = sample mass in kg.

The change in lability of added soluble metals usually requires consideration of the lability of background metals in the native soil, but in all the soils used in this study the background concentrations of Ag were below instrumental detection limits (<0.04 mg kg⁻¹). Hence the lability of added soluble Ag⁺ was calculated as a percentage of total Ag in soil (E%).

The time zero E-values for Ag were determined in soils after simultaneously spiking soil suspension, following initial equilibration with 0.01 M $\text{Ca(NO}_3)_2$, with 200 μL of the ^{110m}Ag spike and 200 μL of the required soluble Ag⁺ concentration. The Ag concentration of the spiking solution for each soil was measured by ICP-OES to confirm total Ag spike concentrations in soils at time zero.

2.4. Solid phase speciation of Ag in soils by X-ray absorption spectroscopy (XAS)

In this study we used the specific signature of various Ag species as assessed by XANES spectroscopy to investigate the behaviour and transformation of added soluble Ag in soils. The XANES data were collected at the Materials Research Collaborative Access Team (MRCAT) beamline 10-ID, Sector 10 located at the Advanced Photon Source (APS), Argonne National Laboratory (ANL), Argonne, IL. (Segre et al., 2000) The electron storage ring was operating at 7 GeV in top-up mode. A liquid N₂ cooled double crystal Si(111) monochromator was used to select incident photon energies and a platinum-coated mirror was used for harmonic rejection. The monochromator was calibrated by assigning the first derivative inflection point of the absorption K-edge of Ag metal at 25,514 eV and an Ag metal foil spectrum was collected congruently with each sample scan. Samples were pressed into pellets and three XANES scans were collected for each sample in transmission and fluorescence mode using a Ge multielement detector (Canberra). The possibility of beam-induced photo-reduction was investigated by repeating three scans at the same location in selected samples. No indication of an increase in metallic Ag was observed; nevertheless repeat scans were collected at different locations of the samples to reduce this risk of photo-reduction. The Ag standards analysed included: Ag carbonate (Ag_2CO_3), Ag oxide (Ag_2O), Ag sulfide (Ag_2S), Ag chloride (AgCl), metallic Ag, Ag sulphate (Ag_2SO_4), Ag glutathione (Ag-GSH), Ag-humic acid and Ag sorbed by ferrihydrite, cystine and acetate. The standard materials were analysed as finely ground powders (diluted in boron nitride to approximately 1000 mg kg⁻¹). Principal component analysis (PCA) of the normalised sample spectra was used to estimate the likely number of species contained in the samples, whilst target transformation (TT) was used to identify relevant standards for linear combination fitting (LCF) of the sample spectra (Malinowski, 1991). The PCA and TT were performed using SixPack (Webb, 2005) while data normalisation and LCF were performed using Athena (Ravel et al., 2005). Fitting range was -30 to +100 eV relative to the Ag K-edge. For each sample, the combination of standards with the lowest residual parameter was chosen as the most likely set of components.

2.5. Statistical analysis

Analyses of variance (ANOVA) were conducted using Genstat 11 to determine if significant differences in labile Ag concentrations were present between samples aged for different durations of time. All statistical analyses were conducted at a significance level of 0.05.

3. Results and discussion

3.1. Changes in labile Ag (E%) concentrations in soils with time

The labile concentration of Ag in the soils was found to significantly decrease after the two week ageing period by approximately 20–90%, followed by an additional significant decrease of 10–40% after the six month ageing period (Fig. 1). The overall decrease in labile Ag in soils after 6 month ageing was approximately 50–100%. After two weeks the decrease in labile Ag was similar for the Kingaroy, Charleston and Inman Valley soils where approximately 80% of the total added Ag was labile in soils. The Balaklava and Port Kenny soils experienced the most rapid ageing after two weeks

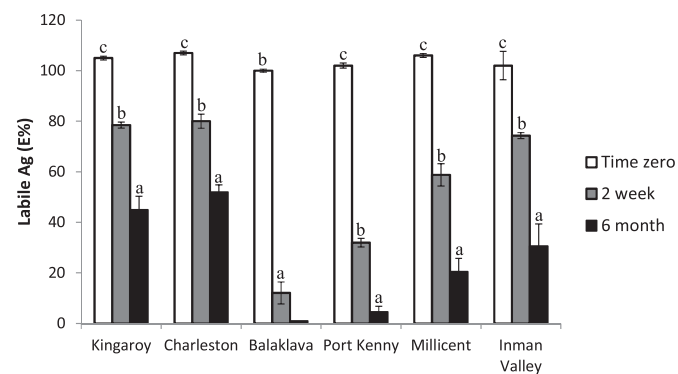


Fig. 1. Labile Ag represented as a percentage of total Ag added to soils (E%) (mean ± standard deviation of 5 replicates) at time zero, two week and six month ageing periods. E% values with different letters within soils are significantly different at $P < 0.05$.

with the greatest decreases in labile Ag (E% was 12% and 32%, respectively). After six months ageing the Kingaroy and Charleston soils had similar decreases in labile Ag where approximately half the added Ag was non-labile, while in the Millicent and Inman Valley soils approximately three quarters of the added Ag was non-labile. The decrease in Ag lability was the most pronounced in the Balaklava and Port Kenny soils, where greater than 90% of the added Ag was removed into non-labile forms in these soils (Fig. 1).

The rapid ageing observed after two weeks in this study confirms previous findings that suggested rapid ageing of soluble Ag in soils (Settimo et al., 2014). The six month ageing results suggest slower processes and reactions are taking place in the soils removing Ag further from the labile pool over a longer time period. The Balaklava and Port Kenny soils, which showed the most rapid ageing throughout the experiment, are distinguishable from the other soils tested due to their high pH (8.5–8.8) (Table 1). These results are similar to those found for other metals (i.e. Co, (Wendling et al., 2009) Cu¹³ and Ni (Ma et al., 2013)) which also experienced a rapid and largely pH-dependent initial ageing (2–3 weeks) in soils followed by longer term ageing (≥ 6 months) at slower rates.

3.2. Solid-phase speciation of Ag in soils with time

Of the standards examined, TT identified six standard Ag species with SPOIL values < 3 (Malinowski, 1979), and thus considered suitable for LCF of Ag speciation in the soil samples: Ag adsorbed on ferrihydrite, AgCl, metallic Ag, Ag₂S, Ag-cystine and Ag-GSH (Fig. 2). Furthermore, PCA showed that the majority of variability in the spectra could be explained by a mixture of four components. Therefore, a maximum combination of up to four standards was selected for LCF analysis. Examination of the XANES spectra of the standards (Fig. 2) showed that the metallic Ag, AgCl and Ag sorbed on ferrihydrite have characteristic spectra that could be easily distinguished from the Ag bonded with S standards and each other. The Ag-cystine, Ag-sulfide and Ag-GSH spectra were similar so their percentage contributions to the sample model fits were combined (Table 2).

The Ag K-edge XANES spectra of all soil samples and time periods are shown in Fig. 3, where it is also apparent that the LCF analysis provided good fits to all experimental data. The speciation data are summarised in Table 2, showing the percentage contribution of each Ag species for each soil and time period. A visual inspection of the spectra for samples reported in Fig. 3 immediately suggests that metallic Ag was a dominant species in the Balaklava, Port Kenny and Inman Valley soils at the six month time period. There is an obvious (e.g. Balaklava) or more subtle (e.g. Charleston) change in spectra from time zero to six months for all soils that would suggest some formation of metallic Ag over time. In fact, analysis of the LCF results indicates that metallic Ag formation increased over time for all soils except Kingaroy and Millicent (Table 2). The metallic Ag observed in the Kingaroy soil at time zero is likely a result of rapid reduction of soluble Ag in this soil that occurred prior to XAS analysis during the two hour delay between the soil samples being spiked with the concentrated Ag solution and collection of the spectra. In addition to the metallic Ag formation, there was a significant amount of Ag bonded with S species and this was consistent across all soils. The presence of AgCl was also observed but mostly at the time zero or two week period with the exception of Millicent. Silver sorbed on ferrihydrite (a model for a variety of iron oxhydroxide minerals) was most dominant in the Kingaroy soil but was also present in the Charleston and Balaklava soils at two weeks and in the Port Kenny and Inman Valley soils at two weeks and six months.

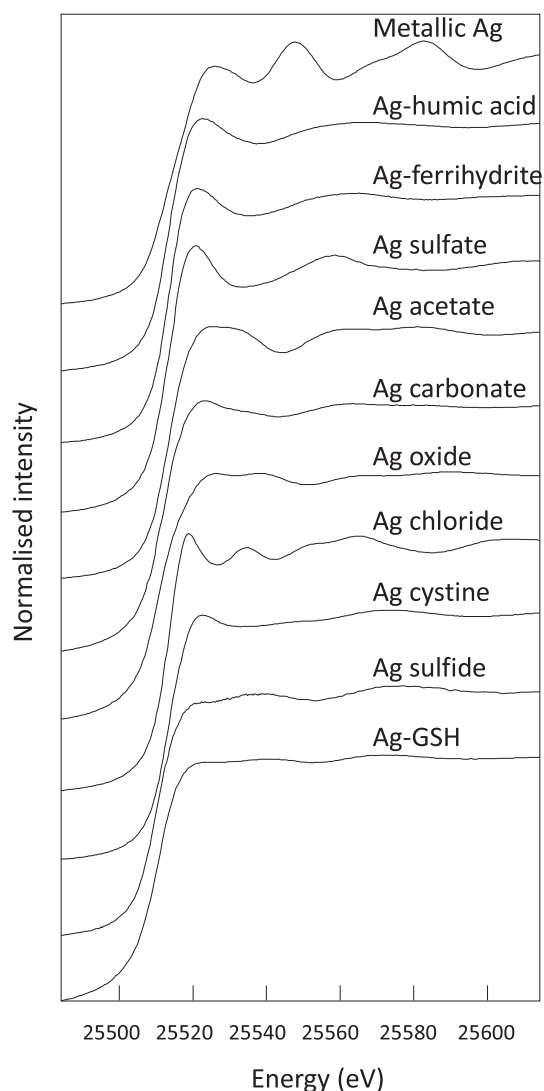


Fig. 2. Normalised Ag K-edge XANES spectra of the standards investigated.

3.3. Relating labile Ag concentrations and solid phase speciation of Ag in soils with time

Of the standards that contributed to the Ag speciation in these samples (Table 2) it was assumed that metallic Ag and AgCl would be non-labile forms of Ag (i.e. not readily isotopically exchangeable), with the remainder (Ag bonded with S species) thought to be a mixture of labile and non-labile Ag.

It is evident that immediately after spiking the soils with soluble Ag, the predominant form of Ag in the samples was Ag bound to reduced S groups; most likely, given the aerobic conditions of the soils, those found associated with organic matter (represented here by the Ag-cystine and Ag-GSH standards). The exception to this finding was the Millicent soil, which was found to contain a higher percentage of AgCl, possibly due to its higher water-soluble Cl concentrations and precipitation following addition of soluble Ag to the surface of this soil (Table 1). The strong association of Ag with soil organic matter has been known for some time (Jones et al., 1986; Szabo et al., 1995). Over time the percentage of Ag bonded with S species decreased in all soils except Charleston and Millicent (higher organic matter soils) where Ag bonded with S species increased with time (Table 2). The percentage of metallic Ag

Table 2

Silver solid phase speciation as identified by LCF of K-edge XANES spectra. Silver species abundances are presented as percentages and the values in brackets show the percentage variation in the calculated values. Goodness of fit is indicated by the R-factor.

Sample	Ag sorbed on Fe-oxohydroxides	Ag bonded with S species	AgCl	Metallic Ag	R-factor × 1000
Kingaroy					
Time zero (+2 h)	18 (3)	38 (4)	29 (2)	16 (6)	0.10
2 weeks	41 (5)	30 (6)	23 (3)	6 (8)	0.20
6 months	59 (3)	0 (5)	13 (3)	29 (1)	0.19
Charleston					
Time zero (+2 h)		75 (14)	25 (2)		0.14
2 weeks	22 (2)	55 (3)	13 (2)	11 (1)	0.07
6 months		72 (11)		29 (1)	0.07
Balaklava					
Time zero (+2 h)		79 (15)	21 (2)		0.17
2 weeks	17 (3)	60 (13)		23 (1)	0.07
6 months	1 (0)	11 (0)		88 (0)	0.15
Port Kenny					
Time zero (+2 h)		72 (15)	29 (2)		0.17
2 weeks	21 (3)	58 (13)		21 (1)	0.07
6 months	13 (2)	20 (9)		67 (1)	0.13
Millicent					
Time zero (+2 h)	3 (5)	40 (3)	57 (3)		0.25
2 weeks		71 (12)		30 (1)	0.09
6 months		72 (7)	15 (2)	14 (1)	0.08
Inman Valley					
Time zero (+2 h)	16 (4)	52 (9)	31 (3)		0.18
2 weeks	11 (3)	73 (7)	17 (2)		0.09
6 months	12 (3)	35 (15)		53 (1)	0.06

increased in all soils with time, except the Millicent soils, with the highest amount occurring in the alkaline pH soils (Balaklava and Port Kenny) (Table 2).

Sulfidisation of Ag^+ ion with reduced S groups or reductive precipitation to Ag^0 (metallic Ag) has not been reported in natural soils previously but Ag^0 has been reported to form upon titration of Ag^+ with a purified humic acid from soil (Maurer et al., 2012) and Ag_2S has been observed to form in wastewater treatment plants (WWTPs) where biosolids can contain Ag_2S nanoparticles (Kim et al., 2010). Conditions in WWTPs are commonly anaerobic (for denitrification to occur and for production of methane) so there are often high concentrations of reduced S in WWTP liquors. In aerobic soils, the presence of reduced S is unlikely except in anaerobic microsites and Ag^+ ion will combine readily with this reduced S to precipitate as Ag_2S . Soils rich in organic matter are also likely to have higher levels of microbial respiration and hence a greater likelihood of having reduced S in anaerobic microsites (van der Lee et al., 1999). Reductive precipitation of Ag^+ ion in soils is highly likely, given the standard electrode potential for the Ag^+/Ag^0 couple is 0.799 V (Evans et al., 2010). In the soil with the lowest content of organic matter and the highest clay content (Kingaroy), iron oxohydroxide sorbed Ag was the predominant form. The two soils with the greatest decreases in labile Ag over the 6-month ageing period as determined by ID (Fig. 1: Balaklava and Port Kenny) had the highest amount of metallic Ag formed as determined by XAS. The metallic Ag would not be readily isotopically exchangeable unless it could be oxidised during the measurement of labile Ag and it is evident from the 6 month aged samples from Port Kenny and Balaklava that this did not occur – there was therefore reasonable agreement between the lability as determined by ID and the solid-phase speciation of Ag as determined by XAS.

An examination of the lability and solid phase speciation results shows that generally there was an increase in the percentage of metallic Ag as labile Ag concentrations decreased in soils (Fig. 4a). It is important to note that the percentage of metallic Ag in each soil does not account for all non-labile Ag. The summary of the proposed transformations of Ag from labile to non-labile pools in soils is summarised in Fig. 5. Based on the labile Ag measured (Fig. 1) and

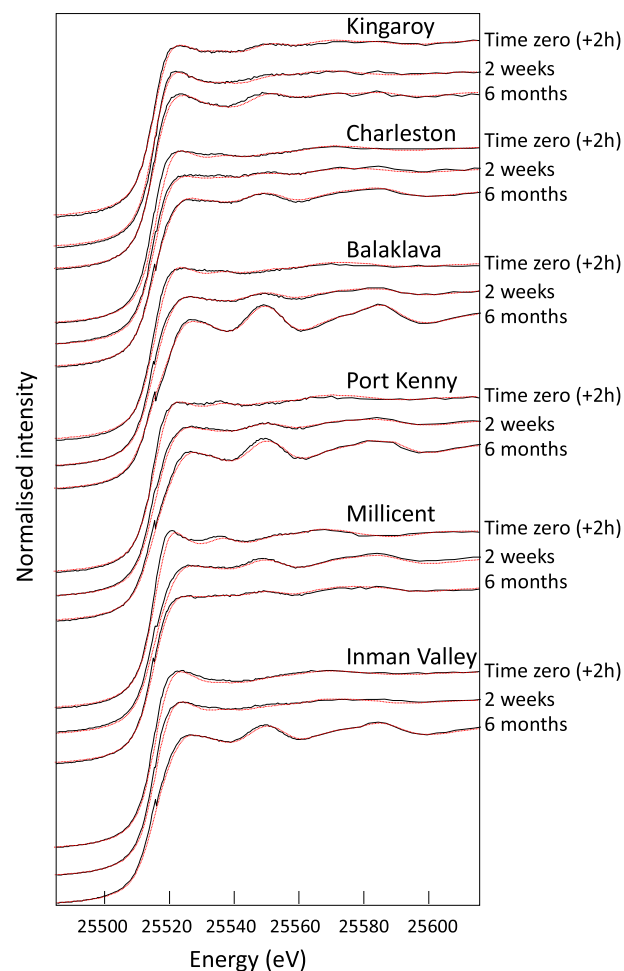


Fig. 3. Normalised Ag K-edge XANES spectra of the soils at time zero (+2 h), 2 week and 6 month ageing periods (50% FC). The red lines show the best 4-component linear combination fit of standard spectra. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

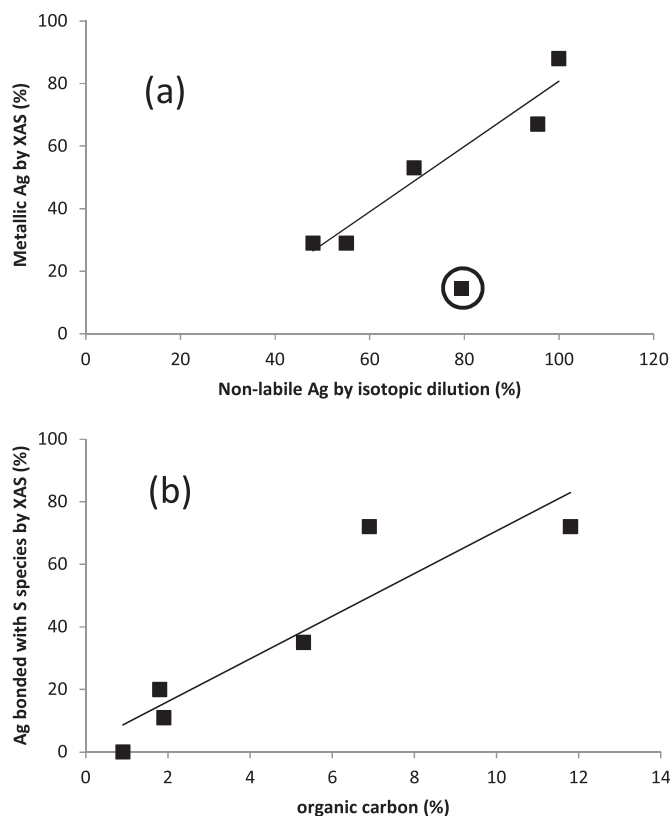


Fig. 4. Relationship between (a) the percentage of non-labile Ag in soils determined by isotope dilution and the percentage of metallic Ag present in soil determined by XAS. For the fitted line: $R^2 = 0.93$. The point circled in (a) is the Millicent soil with very high organic matter content (excluded from the regression); and (b) the percentage of organic carbon in soils and the percentage of Ag bonded with S species formed 6 months after addition of soluble Ag^+ to soils. For the fitted line: $R^2 = 0.84$.

the percentage contributions of each species (Table 2), when metallic Ag does not account for all non-labile Ag, there must be a contribution from the other species. This means that some iron oxohydroxide sorbed Ag may be irreversibly sorbed or surface precipitated, it may also be fixed within crystal lattices of oxides (Fig. 5). Although iron oxohydroxide sorbed Ag is not as strongly

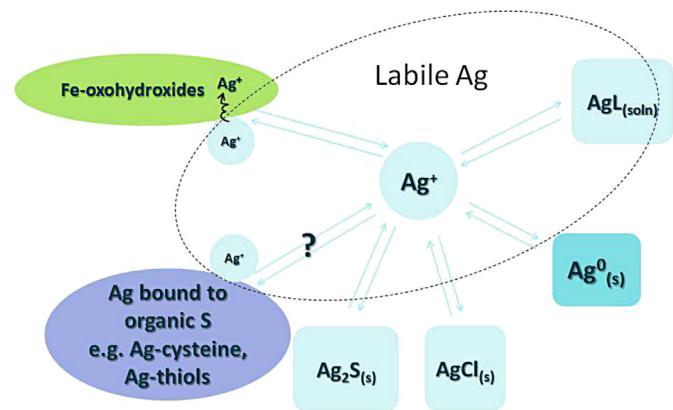


Fig. 5. Summary schematic of the possible transformations of labile Ag to non-labile Ag fractions in soils according to speciation data collected by XANES. The dotted oval represents all labile forms of Ag: Ag^+ , reversibly sorbed Ag^+ to Fe-oxohydroxides and organic S of organic matter, Ag^+ weakly complexed with other soil solution ligands (L). Non-labile Ag is solids: metallic Ag, AgCl and Ag_2S and Ag irreversibly bound to organic S and Fe-oxohydroxides (surface precipitated or fixed within crystal lattices).

sorbed as Ag to soil organic matter, it is thought that over time oxides are important for retaining Ag in soils (Jacobson et al., 2005). For Ag bonded with S species, it is likely that the Ag_2S is non-labile due to its extremely low solubility (Levard et al., 2012), but there could also be non-labile Ag associated with the Ag-cystine and Ag-GSH if Ag is strongly bound rather than reversibly sorbed to solid phase organic matter (thiols and disulfides) (Fig. 5). There was a positive correlation ($R^2 = 0.84$) found between the organic carbon content in the soils and the percentage of the sum of all Ag bonded with S species (Fig. 4b). It is well established that Ag is strongly associated with S groups, (Smith et al., 2002; Kramer et al., 2007; Bell and Kramer, 1999) so this relationship shows that the association of Ag with soil organic carbon is likely through the organic S in soil organic matter. Further work is required to establish the extent of these Ag–S interactions in soil and their effect on Ag lability. When considering the non-labile forms of Ag: metallic Ag, Ag_2S and AgCl (Fig. 5), it is important to remember that the formation of these solid phases is still a reversible reaction, even though their dissolution may be minimal and slow especially for metallic Ag and Ag_2S ($K_{\text{sp}} = 5.92 \times 10^{-51}$) but more likely for AgCl ($K_{\text{sp}} = 1.77 \times 10^{-10}$) (Levard et al., 2012). The data presented here suggests that all these species to some degree are capable of these reverse reactions in the time frame between two weeks to six months based on the speciation data collected for soils: Charleston, Balaklava, Port Kenny and Millicent (Table 2). It is unknown if Ag complexed with soil solution ligands such as DOC (Adams and Kramer, 1999) or polysulfide complexes (Fig. 5) are labile (Bell and Kramer, 1999). Further work is required to establish whether Ag complexed with soil solution ligands can be regarded as labile and potentially available for uptake by soil organisms. It is also important to consider that formation of metallic Ag may be a secondary reaction occurring once all sites of the soil organic matter (SOM) are saturated and this may be more prominent in soils with higher Ag exposure concentrations or lower SOM.

This study has shown that there is a significant decrease in labile Ag over time for all soils. There was rapid ageing within the first two weeks of addition of soluble Ag, suggesting that Ag ageing needs to be considered in any risk assessments for Ag in the terrestrial environment where this is likely to be a relevant source species. Speciation data showed that Ag^+ is reduced to metallic Ag over time in soils and associations with S and iron oxides in soil can also lead to reductions in Ag lability.

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CHAPTER 4

COMPLEXATION OF SILVER AND DISSOLVED ORGANIC MATTER IN SOIL WATER EXTRACTS

The work contained in this chapter has been submitted to *Environmental Pollution*.

Statement of Authorship

Title of Paper	Complexation of silver and dissolved organic matter in soil water extracts
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Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Lara Settimio		
Contribution to the Paper	Experimental development, performed analysis on all samples, data analysis and critical interpretation, wrote manuscript.		
Signature		Date	

Name of Co-Author	Mike McLaughlin		
Contribution to the Paper	Supervised development of work, data analysis and interpretation, reviewed manuscript.		
Signature		Date	20-10-2014

Name of Co-Author	Jason Kirby		
Contribution to the Paper	Supervised development of work, data analysis and interpretation, reviewed manuscript.		
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Name of Co-Author	Kate Langdon		
Contribution to the Paper	Supervised development of work, data analysis and interpretation, reviewed manuscript.		
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Statement of Authorship

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Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Lara Settimio		
Contribution to the Paper	Experimental development, performed analysis on all samples, data analysis and critical interpretation, wrote manuscript.		
Signature		Date	

Name of Co-Author	Les Janik		
Contribution to the Paper	Supervised development of work, data analysis and interpretation, reviewed manuscript.		
Signature		Date	22/10/2014

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Signature		Date	08/10/2014

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Contribution to the Paper			
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Abstract

There is increasing interest in understanding the behaviour and fate of silver (Ag) in soils due to an increased use of this metal in consumer and industrial products. In soil solutions and leachates, dissolved organic matter (DOM) and other soil colloids will play a critical role in the availability of the Ag⁺ ion, but little is known about the complexation and strength of binding between Ag⁺ and DOM. This study examined and modelled the complexation and strength of binding of Ag⁺ with DOM in soil extracts based on a range of chemical and quality DOM measurements. Silver ion binding was measured by addition of soil water extracts spiked with the ^{110m}Ag radioisotope to a cation exchange resin which was used to bind free and rapidly exchangeable ^{110m}Ag⁺ in solutions. Up to 70% of Ag was found to be strongly complexed in solution and therefore is not readily bioavailable. Characterization of the DOM in the soil water extracts was carried out by size fractionation and elemental analysis, and spectroscopic analysis using ultraviolet/visible (UV/Vis), fluorescence and mid infrared (MIR) spectroscopy. This was followed by the development of a partial least-squares regression model to predict Ag complexation with DOM in soil water extracts. The variability in Ag⁺ binding by DOM across different soils was closely related ($R^2= 0.8$) to the MIR spectra of the extracts. The affinity of Ag⁺ for DOM was stronger in solutions containing DOM with a greater content of humic species and aromatic structures. The demonstrated affinity and ability of Ag⁺ to complex with DOM could result in increased mobilisation of this metal in the soil environment.

Introduction

Silver (Ag) and Ag nanoparticles (AgNPs) are used in solar energy production, water purification, medicine and commercial products (e.g. socks and washing machines).^[1]

The antibacterial properties of Ag and AgNPs are the key reason for their addition to a range of consumer products and also represent a potential risk if released to the environment.^[2, 3] Exposure pathways include releases during mining and processing, disposal of consumer and industrial products to wastewater, land application of biosolids and through application of Ag-containing agricultural products, such as Agress®, used for crop protection as a seed treatment and foliar spray to target bacterial and fungal infestations.

While most studies on the environmental effects of Ag have focused on the aquatic environment,^[4-7] there is increasing need for information on the impacts of Ag in terrestrial environments. Recent studies have shown that soluble Ag⁺ is rapidly immobilised in soils (50% in 14d) and then undergoes slower long term aging, that further reduces its lability, influenced by soil properties such as cation exchange capacity, organic carbon and pH.^[8-10] Solid phase speciation using X-ray absorption near edge structure (XANES) spectroscopy showed that soluble Ag⁺ is reduced to metallic Ag over time in soils and that this transformation, as well as associations of Ag⁺ with Fe-oxohydroxides and reduced sulfur groups in organic matter, decrease Ag lability.^[11]

The Ag present in soil solutions is considered the most bioavailable fraction. In soil solutions, Ag can be present as free Ag⁺ ions or inorganic and organic complexes.^[12] Settimo et al. (2014) found that the fraction of total Ag present in soil solutions as soluble Ag⁺ (bound by a cation exchange resin) decreased (100% to 10%) with increasing dissolved organic carbon (DOC) concentrations of 10 to 140 mg C L⁻¹. The authors suggested that this finding supported the hypothesis of strong binding of Ag by

DOC in soil water extracts (defined by the ability of Chelex resin to complex Ag^+ with a logK value of 7^[6]). There is little information available in the literature on the strength of binding and complexation of Ag^+ with dissolved organic matter (DOM) in soils. In aquatic environments, Ag^+ can form complexes with DOM, especially with humic and fulvic acids^[12-15] but there is still limited understanding of this interaction in soils.

The characterisation of DOM in soils is difficult due to its complex and heterogeneous nature. The usual operational definition is that DOM is the organic matter fraction in solution with a diameter less than 0.45 μm . Dissolved organic matter concentrations in soil solutions are not usually directly quantified, but rather its concentration inferred through measurement of DOC, which usually represents the bulk of its elemental composition (~67%).^[16] DOM consists of a mixture of carbohydrates, carboxylic acids, amino acids, hydrocarbons and other unknown organic compounds (~20%) as well as humic and fulvic substances (~80%).^[17] Although DOM represents a small proportion of the total organic matter content in soils it is considered to be the most mobile and actively cycling fraction and as a result is highly significant to a range of biogeochemical processes in the soil environment.^[16]

Techniques used to characterize the DOM fraction present in soil solutions, include nuclear magnetic resonance (NMR), ultraviolet/visible (UV/Vis) absorption, mid infrared (MIR), fluorescence and X-ray absorption spectroscopy.^[18-20] These techniques provide information on the composition and functional groups of DOM. Techniques such as NMR and synchrotron based X-ray absorption spectroscopy can provide detailed characterisation information, however they are often inaccessible, costly,

require experienced operators and data collection or analysis can be slow. On the other hand, IR, fluorescence and UV-Vis absorption instruments are commonly found in soil chemistry laboratories and can provide rapid, efficient, sensitive and inexpensive techniques for the characterisation of DOM in soil solutions.^[21, 22]

Excitation-emission matrix (EEM) fluorescence coupled with parallel factor analysis (PARAFAC) is increasingly being used as a powerful tool to characterise DOM from aquatic systems,^[23] soils^[24, 25] and wastewater.^[26] The EEMs collected are depicted as three dimensional contour plots that visualize fluorophores or fluorescent components as a function of excitation-emission pairs of wavelengths. Data handling of EEMs with PARAFAC analysis has improved the identification of fluorescent DOM components and their abundance and contribution to the total fluorescence. Fluorescent components identified in this way are normally classified as proteinaceous or humic/fulvic-like.^[23]

Mid infrared analysis can be used to provide information on the composition of DOM in soil solutions.^[27] Attenuated total reflectance (ATR) spectroscopy, which utilizes the absorption of radiation from the interface between a high refractive index, reflecting crystal (e.g. diamond) or fibre and the sample surface, in conjunction with IR spectroscopy, has successfully been used for DOM characterisation.^[28] The MIR-ATR technique has been successfully applied for the characterisation of DOM in rainwater^[29], regenerated peat soils^[30] and environmental samples.^[31, 32] The MIR-ATR spectra can be related to sample properties by partial least squares regression (PLSR), a multivariate method, commonly used to predict sample properties (e.g. pH, cation exchange capacity) from multivariate data (including spectra).^[21, 27, 33]

The transport and bioavailability of metals can be influenced by their ability to form soluble or insoluble complexes with soil DOM.^[16] Metals including Cu, Cd, Pb, Hg and As have all been shown to have a strong affinity for DOM.^[34, 35] Copper in particular has been extensively studied and it is well established that DOM has the ability to mobilise Cu in soils which is based on the DOM composition or quality (principally aromatic carbon content).^[36] Aromaticity of DOM can be measured by specific UV-Visible absorbance at 254 nm (SUVA₂₅₄) and has been shown to be significantly positively correlated with the Cu-mobilising potential of DOM.³⁶ The humic/fulvic component of DOM is another quality measure and contains aromatic carbon, carboxylic acid, alcohol, sulfhydryl or amine functional groups that are influential to metal complexation.^[34, 37]

The aims of this study are to develop a better understanding of the complexation of Ag and DOM in soil water extracts using rapid, accessible and sensitive laboratory methods (e.g. UV, fluorescence and IR spectroscopy and elemental analysis) and the development of a partial least-squares regression model to predict Ag complexation with DOM in soil water extracts. These findings will enable a better understanding of the mobility and bioavailability of Ag in soil environments.

Materials and Methods

Soil sampling and general characterisation

This study used 18 Australian soils and two soils from Europe. The soils were collected from a depth of 0-10 cm, air-dried, homogenized and sieved to <2 mm. Selected physical and chemical properties for the experimental soils can be found in Table 1.

Soil pH was measured using a soil:solution ratio of 1:5 (m v⁻¹), with ultrapure deionized water (Milli-Q, Millipore) and shaking for 1 h. Total organic carbon (TOC) content, cation exchange capacity (CEC), particle size and oxalate-extractable aluminium (Al_{ox}) and iron (Fe_{ox}) were determined according to standard methods.^[38, 39] Background Ag concentrations in soils were determined by inductively coupled plasma-mass spectrometry (ICPMS) (Agilent 7700) following a microwave-assisted *aqua regia* (US-EPA 3051A) digestion procedure. After digestion, samples were filtered through 0.2- μ m cellulose acetate filters (Sartorius, Germany) and Ag concentrations determined by ICP-MS. The quantification limit of Ag in soils on a soil basis was 0.04 mg kg⁻¹. A certified reference material (NRC-CNRC PACS-2) was included for quality control in all sample batches. The measured concentration of Ag in the certified reference material was 1.15 \pm 0.16 mg kg⁻¹ (n=6), which was in close agreement with the certified value of 1.22 \pm 0.14 mg kg⁻¹.

Table 1. General soil properties.

Soil	Soil number	pH _{water}	Total organic carbon %	CEC [§] cmol _c kg ⁻¹	Oxalate Extractable			Clay (< 2µm) %	Silt (2-20 µm) %	Sand (20-2000 µm) %	Geoegenic Ag mg kg ⁻¹
					Al mg kg ⁻¹	Fe mg kg ⁻¹					
Charleston	1	6.0	6.9	12	410	2500	14	12	63	bd	
Port Kenny	2	7.8	1.8	13	483	295	12	4	21	bd	
Inman Valley	3	6.1	5.3	25	1460	3680	42	22	26	bd	
Millicent	4	6.9	12	42	2300	1500	19	5	48	bd	
Tepko	5	6.8	1.0	5	180	200	8	3	89	bd	
Thorpedale	6	4.9	4.6	14	4800	6600	50	34	16	0.05	
Siekmann Trial	7	4.8	4.3	5	2200	3500	23	10	67	bd	
Clarke Hill	8	5.4	4.9	15	2180	3500	28	40	32	bd	
Mintaro	9	5.7	2.6	20	7850	8300	41	22	37	bd	
Ingham	10	4.7	1.7	7	920	1400	30	26	44	bd	
Bordeaux	11	5.4	1.9	6	910	400	3	2	93	bd	
Houthalen	12	4.8	1.5	5	483	762	1	2	93	bd	
Kingaroy	13	7.3	0.9	13	2350	2500	60	17	19	0.10	
Balaklava	14	7.1	1.9	27	1240	648	30	20	47	bd	
Berrigan	15	6.1	0.4	3	230	300	7	5	88	bd	
Emerald Black	16	7.4	0.9	66	2500	1800	59	14	27	bd	
Mimimpa	17	7.4	0.2	2	110	100	1	<1	99	bd	
Pinnaroo	18	8.1	0.7	14	670	400	19	4	77	bd	
Tully	19	4.9	2.7	5	6300	700	32	11	57	0.05	
Bute	20	6.9	0.5	4	190	200	5	2	92	bd	

*Note that particle size distribution for Port Kenny is equal to 37.2% due to the high concentration of CaCO₃ that is present in this soil (60%);

§Cation exchange capacity; bd indicates soils where total Ag concentrations were below the detection limit: <0.04 mg kg⁻¹

Determination of complexed Ag in soil water extracts

The percentage of Ag complexed by DOM in soil water extracts was determined using the $^{110\text{m}}\text{Ag}$ radioactive tracer and a cation exchange resin procedure. Soil water extracts ($n=3$) for 20 test soils were obtained using a 1:5 soil:solution ratio (m v^{-1}) with ultrapure deionized water (Milli-Q, Millipore), shaking end-over-end for 1 h and the resulting suspensions filtered through a 0.2- μm polyethersulfone membrane (Sartorius, Germany). Following filtration (~ 50 ml), 15 mL triplicate samples from each soil water extract were transferred into 50 mL polypropylene centrifuge tubes (LabServ) and 50 μL of a soluble stock Ag^+ solution (as AgNO_3) was added to each sample to achieve a final Ag^+ concentration of 20 $\mu\text{g L}^{-1}$ (to simulate an environmentally relevant soil pore water concentration). Simultaneously with the soluble Ag spike, 12 kBq of $^{110\text{m}}\text{Ag}$ in 50 μL of carrier-free solution (in the form of $^{110\text{m}}\text{AgNO}_3$ ($t_{1/2} = 250$ days)) was also added to each sample. The $^{110\text{m}}\text{Ag}/\text{Ag}$ spiked soil suspensions were mixed on an end-over-end shaker for 12 h. The $^{110\text{m}}\text{Ag}$ activity in the samples (4 ml) was then determined by gamma spectroscopy (WIZARD², Perkin Elmer) without any further treatment.

The strength of complexation of Ag in soil water extracts was examined using a strong cation exchange resin procedure. A 10-mL aliquot from each 0.2 μm filtered sample was transferred into 50 ml centrifuge tubes, with approximately 0.1 g of Ca-form Chelex 100 resin (Bio-Rad Laboratories). The extracts with the resin were mixed end-over-end for 12 h to allow the free Ag^+ and weakly complexed Ag in samples to bind to the resin resulting in only the strong or irreversible 'complexed Ag' remaining in solutions. The strong complexed Ag is defined by the Chelex resin's ability over 12 h to complex Ag^+ ($\log K$ value of 7),^[6] outcompeting all complexes that are weaker e.g.

N, O interactions ($\log K < 6$) but not stronger e.g. inorganic sulfide interactions ($\log K \approx 13$).^[40] The samples were then centrifuged at 1200 g for 20 min, supernatant removed and the ^{110m}Ag activity in 4 ml of supernatant sample measured by gamma spectroscopy. Blank solutions of ultrapure deionized water spiked with 12 kBq of ^{110m}Ag were also measured to ensure that the resin effectively bound all free $^{110m}\text{Ag}^+$ in solution. The resin was found to bind 98 ± 0.03 % of the $^{110m}\text{Ag}^+$ spiked into solution in the absence of DOC.

The percentage of strong or irreversible complexed Ag in soil water extracts was determined using the following equation:

$$\% \text{ complexed Ag} = r/R \times 100 \quad \text{Equation 1}$$

where, % complexed Ag is the fraction of total Ag in soil water extracts that did not bind to the resin in 12 h; $r = ^{110m}\text{Ag}$ radioactivity in solutions after resin extraction for 12 h in Bq; and $R =$ initial spiked ^{110m}Ag radioactivity (total ^{110m}Ag added to the sample) in Bq.

Characterisation of 3 kDa to 0.2 μm size fractions in soil water extracts

Soil water extracts ($n=3$) were split into duplicates and analyzed independently to characterize the 3 kDa to 0.2 μm colloidal size fraction in soil water extracts. The first duplicate was filtered through a 0.2- μm polyethersulfone membrane (Sartorius, Germany) and the second duplicate filtered through a 3 kDa centrifugal device with Omega membrane (modified polyethersulfone) (Pall, USA) for 30 minutes at 2000 g. The 0.2 μm and 3 kDa filtered extracts were analyzed in triplicate as three independent samples, for total Al, Fe, P and S (ICP-OES) (Spectro ARCOS), dissolved organic

carbon (DOC) (Shimadzu TOC-V_{CSH/CSN}+ TNM-1) and anion (Br⁻, Cl⁻, F⁻, NO₃⁻, SO₄²⁻) (Dionex ICS-2500) concentrations.

Following analysis of the 3 kDa and 0.2 μm filtrates, colloidal concentrations were calculated by subtraction of the 3 kDa concentrations from the 0.2 μm concentrations. The elemental concentrations for Al, Fe and P are reported in Table S1, supplementary information. The S calculated from sulfate anion concentrations was subtracted from the total elemental S in the soil extracts to calculate “reduced S” values shown in Table S2, supplementary information (note that there may be other S species remaining e.g. disulfides). The total S and DOC concentrations were used to calculate a molar C:S ratio of the 3 kDa-0.2 μm colloidal material (Table 2).

All further data analyses conducted to explain complexed Ag in the soil water extracts were carried out with colloidal concentrations since Ag was only detected in this size fraction (3 kDa to 0.2 μm fraction).

Table 2. Colloidal concentration of dissolved organic carbon (representing dissolved organic matter concentration) and sulfur (S) and the molar C:S ratio with standard deviation in brackets.

Soil	DOC (mg L ⁻¹)	Total S (mg L ⁻¹)	C:S ratio
1	39(4)	0.85(0.05)	123(9)
2	13(3)	0.33(0.10)	110(17)
3	43(9)	1.26(0.08)	90(13)
4	28(1)	1.21(0.25)	61(2)
5	22(4)	0.60(0.06)	100(4)
6	33(1)	0.75(0.03)	127(47)
7	59(4)	0.90(0.22)	175(2)
8	33(4)	0.49(0.06)	180(27)
9	31(4)	0.97(0.01)	88(18)
10	20(5)	0.56(0.01)	98(32)
11	3(1)	0.03(0.03)	262(103)
12	6(0)	0.07(0.04)	234(38)
13	0(0)	<0.03	-
14	6(1)	0.29(0.04)	51(8)
15	7(1)	<0.03	-
16	3(1)	0.10(0.04)	94(44)
17	2(1)	<0.03	-
18	11(1)	0.17(0.01)	172(22)
19	12(1)	0.15(0.09)	154(16)
20	12(4)	0.14(0.01)	148(32)

Spectroscopic characterisation of DOM in soil water extracts

Characterisation of the DOM in the 0.2 µm filtered soil water extracts was carried out with independent triplicate samples. Absorbance (UV), fluorescence and mid infrared measurements were performed to measure the quality parameters of DOM such as aromatic carbon content, fluorescent components and functional groups.

To measure absorbance and fluorescence, the soil water extracts were diluted using ultrapure deionized water to DOC concentrations of 10 mg C L⁻¹ or below to prevent the need for an inner filter correction of the samples during PARAFAC analysis of

data. All absorbance and fluorescence scans of the extracts were performed in a 1-cm quartz cuvette (Helma Canada Ltd., Concord, ON, Canada). The cuvette was pre-rinsed thoroughly with ultrapure deionized water (Milli-Q), followed by washing with the extract to be measured. For each extract, absorbance and fluorescence scans were conducted using a Varian Cary 40 UV/visible spectrophotometer and Varian ECLIPSE Cary fluorescence spectrophotometer (Varian Incorporation, Old Oak, NJ, USA), respectively. The absorbance of the extracts was determined at a wavelength range of 200-800 nm. Specific UV absorbance at 254 nm, defined as: $SUVA_{254} (L\ g^{-1}cm^{-1})$, was measured ($SUVA_{254}=Abs_{254}/DOC$) to estimate the aromaticity of the DOM.

The fluorescence of DOM in samples was measured using excitation wavelengths between 200 and 450 nm, with 10-nm increments, and emission intensities were collected for wavelengths of 250-600 nm every 1-nm increment. To conduct the excitation-emission fluorescence measurements, ultrapure deionized water, standard solutions of tryptophan (0.5 μM) and tyrosine (1.0 μM) (Sigma Aldrich, St. Louis, MO, USA) and a mixed solution (10 mg C L⁻¹ of a well characterised DOM isolate plus 0.5 μM tryptophan and 1.0 μM tyrosine) were scanned along with the samples to check for instrument drift, which did not occur during the collection of spectra.

Excitation-emission matrices (EEMs) obtained from fluorescence measurements were summarised into contour plots to identify the fluorescent components or peaks present in each sample. DOM fluorescence is characterised by broad intensities when excitation occurs between 200 and 450 nm.^[41] Fulvic-like and humic-like components are characterised by longer emission wavelengths (400-550 nm) whereas tryptophan-like and tyrosine-like components are characterised by shorter emission wavelengths

(300-400 nm).^[42] In order to quantify the relative abundance of each component for every sample, the EEMs were modelled using PARAFAC as implemented in the PLS toolbox (Eigenvectors Research Inc. WA, USA), which resulted in estimates of the relative concentrations of five fluorescent components.

The MIR spectroscopy of the extracts was performed using a Varian Excalibur 3100 FTIR spectrometer (Agilent, Santa Clara, CA, USA) equipped with a 9 reflection ZnSe crystal multiple attenuated total reflectance (MATR) accessory. For the triplicate liquid extracts, spectra were scanned for 60 seconds over a spectral range of 7500-375 cm^{-1} , at a resolution of 8 cm^{-1} . The 1.5 mL aliquots of each extract were spread onto the ATR crystal plate with even distribution and dried at 60°C for 12 minutes, resulting in a thin film. This procedure was repeated for each sample in order to have sufficient thickness of DOM film on the ATR plate. Prior to addition of each liquid extract, a background reference spectrum was collected from the clean ZnSe crystal without any sample.

Statistical Analysis

The EEM spectra were analysed with the PLS toolbox (Eigenvectors Research Inc.) to determine the relative fluorescent concentration of each component. A 5 component model was selected as it explained 99% of the the variance in the EEM spectra. The MIR spectra were analysed by partial least squares regression (PLSR) using Unscrambler multivariate analysis software (Camo Trondheim, Norway). The PLSR regression models were derived by combining the ATR spectra and laboratory analysis values and analysing these models with the PLSR software to determine the contribution of each input variable to the total variance in Ag binding. Analyses of variance (ANOVA), correlation matrices and principle component biplot analysis were

conducted using Genstat 15 (VSN International Ltd) to determine significant statistical differences and correlations between samples and or variables (i.e. for the biplot analysis). A significance level of 0.05 was implemented in all ANOVA and correlation matrix analysis.

Results and Discussion

Complexed Ag in soil water extracts

The proportion of strong complexed Ag in the 0.2 μm filtered soil water extracts from 20 soils ranged from 2 – 67% over a DOC concentration range of 0 – 59 mg C L^{-1} (Fig. 1). There was a significant positive ($p < 0.001$) relationship between the proportion of complexed Ag (%) in the soil water extracts and the DOC concentration (Fig. 1).

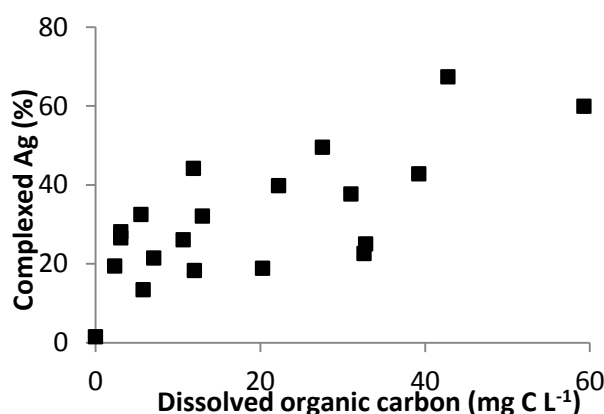


Figure 1. Variation in the percentage of complexed Ag measured and the concentration of dissolved organic carbon in the 20 soil water extracts ($n=3$).

Normalisation of the complexed Ag (%) data to the concentration of DOC allows for comparisons (complexed Ag % per mg C L^{-1}) between soils (Fig. 2). The normalized data varied considerably across the 20 soils, indicating that DOC concentration alone could not explain the proportion of Ag complexation in the soil water extracts. For example, soil 7 had the highest DOC concentration, however similar complexed Ag % per mg C L^{-1} to other soils with significantly lower DOC concentrations (e.g. soils 9

and 19). Moreover for soils with similar DOC concentrations in soil water extracts there were significant differences in the proportion of Ag complexation (e.g. soils 11, 12 and 16) (range = 0.9 to 2.2 complexed Ag % per mg C L⁻¹). This finding suggests an influence of DOM composition or quality on complexation capacity with Ag⁺ in soil water extracts. Soils with the highest normalised complexed Ag values are thought to have DOM that is optimal for Ag⁺ complexation. These results indicate that DOM from soils 11, 14 and 16 has the greatest affinity for Ag⁺ with values of 2.1, 1.8 and 1.8 complexed Ag % per mg C L⁻¹, respectively, which are greater than the mean value of 0.8 for the data set.

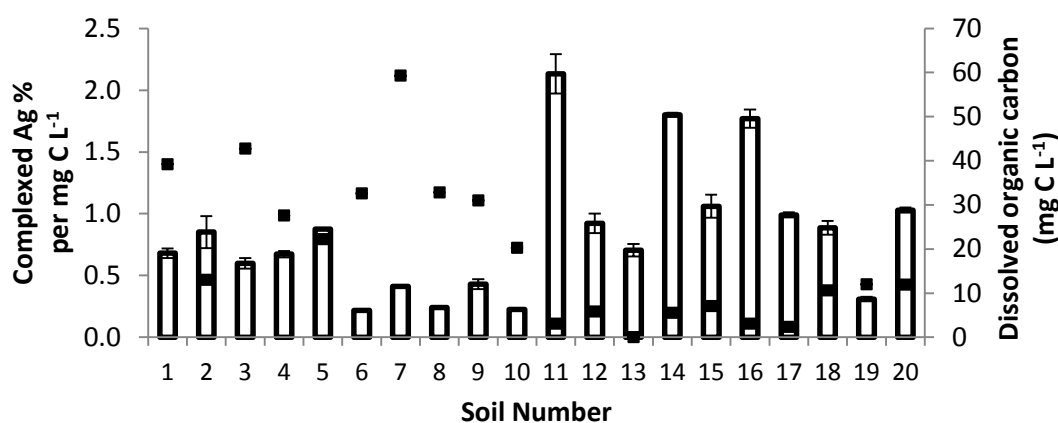


Figure 2. Comparison between complexed Ag % per mg C L⁻¹ (unfilled vertical bars) to the concentration of DOC for the soil water extracts of the 20 soils (filled squares), error bars represent standard deviation of 3 replicates.

Spectroscopic characterisation of the soil water extracts

The SUVA₂₅₄ values determined from soil water extracts ranged from 7 to 40 L g⁻¹ cm⁻¹ representing a diverse range of aromaticity in the DOM across these soils (Fig.3).

These values are consistent with previous reported values for DOM from soils and at the higher end comparable to that of Suwanee River Fulvic Acid (37 L g⁻¹ cm⁻¹).^[36]

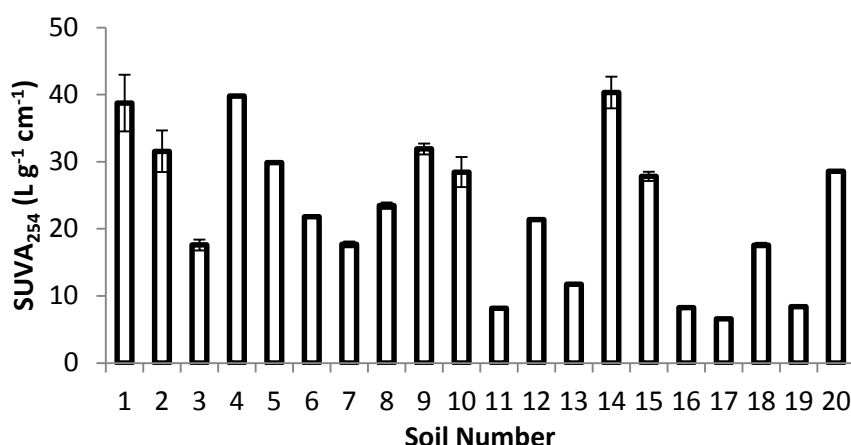


Figure 3. SUVA_{254nm} (L g⁻¹ cm⁻¹) values across the 20 soils, error bars represent standard deviation of 3 replicates.

Five unique fluorescent components were identified using PARAFAC analysis on EEM spectra for soil water extracts (Fig. 4). The proteinaceous components tryptophan (Trp) and tyrosine (Tyr) are identified by their emission peaks at ~350 – 375 nm and 300 nm, respectively. There are three humic-like components, HS1, HS2 and HS3, distinguished by their excitation/emission peaks. Component HS1 has a primary excitation peak at ~250 nm and a secondary peak at ~325 nm. This component also has a broad emission peak from 375 to 500 nm. Component HS2 has a primary excitation peak at just below 450 nm and a secondary peak around 250-300 nm. Its emission is similar at a wavelength of 400–500 nm. Component HS3 has a primary excitation peak between 250-300 nm and a secondary peak between 350-400 nm. It has a very broad emission peak at a longer wavelength than HS1 and HS2 from 450-550 nm. These humic-like components that emit over slightly different emission wavelengths may reflect differences in molecular weights, where larger molecular weight humic substances emit at longer wavelengths (i.e. the HS3 component). These excitation-emission wavelength pairs are similar to those previously reported.^[43-45]

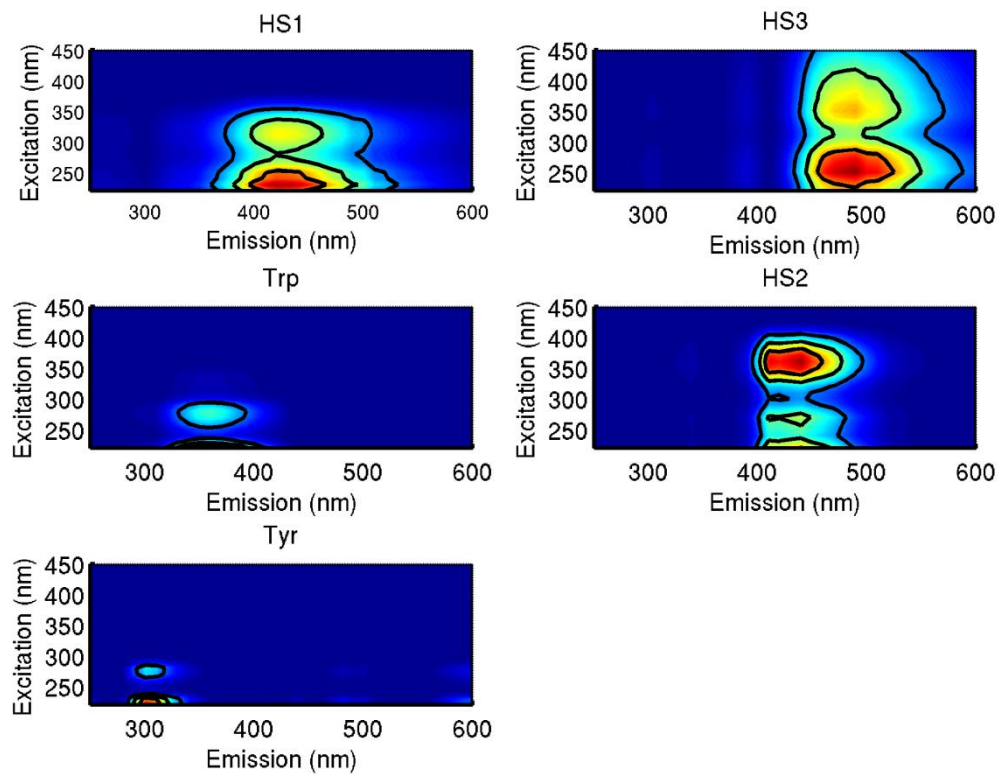


Figure 4. Parallel factor analysis (PARAFAC) components HS1, HS2, HS3, Trp and Tyr identified from the excitation–emission matrix (EEM) fluorescence spectra of DOM from all soil water extracts. Contour lines represent increasing fluorescence intensity in arbitrary units.

The relative content of these fluorescent components differed greatly among the DOM from the soil water extracts (Fig. 5). The humic-like components dominated (75-95%) the soil water extracts, while the proteinaceous components (Trp and Tyr) represented approximately 2-25% of the total fluorescence in samples. The component with the most variation across the 20 soil water extracts is HS3, suggesting that the larger molecular weight humic substances were most variable across soils. Out of the three humic components, the HS1 component was the most dominant (42-60%), accounting for the majority of humic substances present.

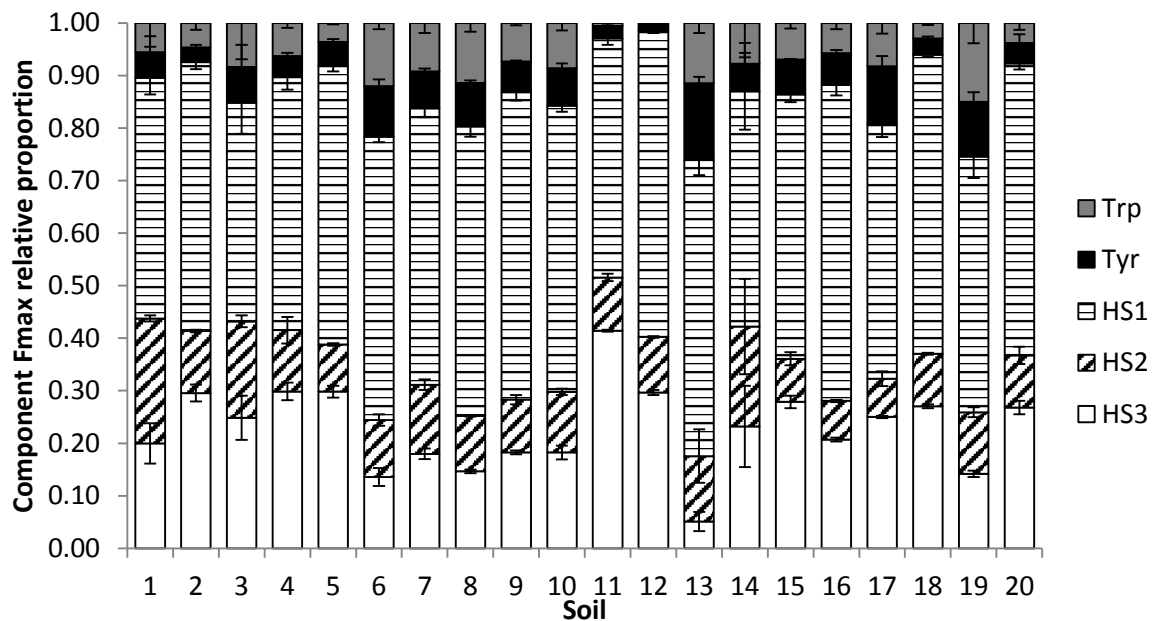


Figure 5. Proportion of maximum fluorescence (F_{max}) of PARAFAC components trp, tyr, HS1, HS2 and HS3 relative to the total component fluorescence for soil water extracts of the 20 soils where error bars represent the standard deviation ($n=3$).

The MIR analysis of samples showed significant variation across the soil spectra (Fig. 6). This variation was indicative of differences in functional group compositions as well as total DOM concentration in samples. Typical characteristic organic matter spectral peaks^[28] were observed across these soils' spectra for alkyls ($2930-2850\text{ cm}^{-1}$), amines/alcohols ($\sim 3300\text{ cm}^{-1}$), carboxylic acids (1720 cm^{-1}), carboxylate anions (1600 and 1400 cm^{-1}), aromatics ($1600-1570\text{ cm}^{-1}$) and carbohydrates (1050 cm^{-1}). Quartz minerals can also be present at $1000-1100\text{ cm}^{-1}$ and clay minerals such as kaolinite were visible in low concentration for some spectra in the region of $3620-3690\text{ cm}^{-1}$.

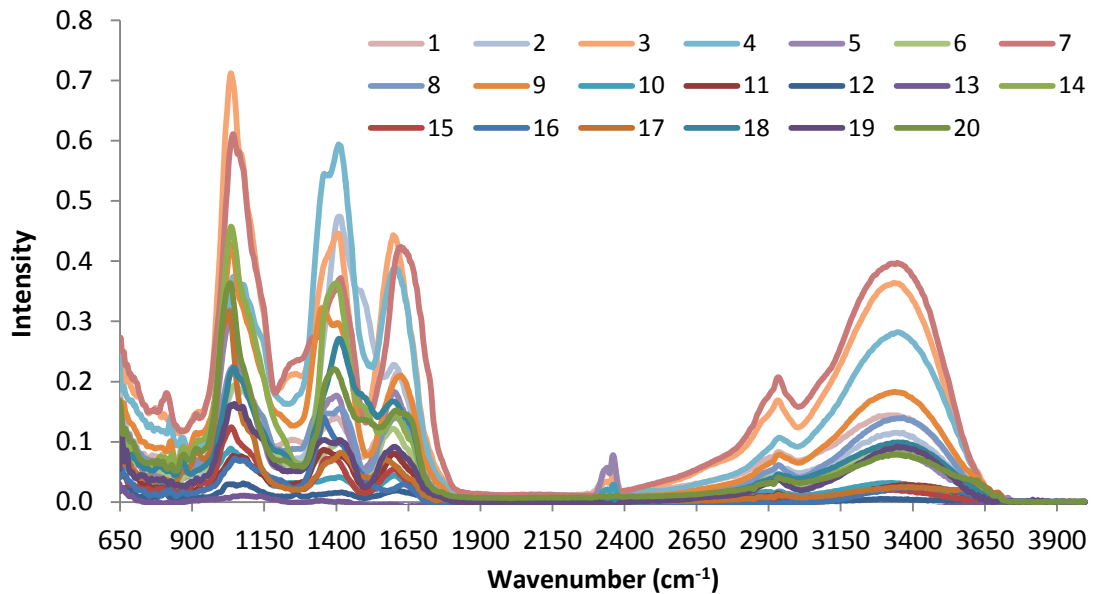


Figure 6. Mid infrared spectra collected with the ATR accessory for the 20 soil water extracts, showing one replicate per soil.

Relationship between complexed Ag and DOM properties

The amount of complexed Ag (%), was significantly positively ($p < 0.001$) correlated with both colloidal total S and “reduced S” and DOC concentrations in soil water extracts (total S results are shown in Table 2 and reduced S in Table S2 of Supplementary Information). Reduced S concentrations were calculated for most soils except in the case of some colloidal concentrations when there was no S remaining after subtraction of the 3 kDa fraction from the 0.2 μm . In all cases the reduced S concentrations were less than the total S concentrations across the soil water extracts. These findings are in agreement with the strong affinity Ag^+ has for ligands with S(II-) groups reported by Bell et al.^[12] While these relationships highlight the importance of DOC and S in complexation of Ag in soil water extracts, they do not provide an overall explanation of the variation observed in the data across all soil extracts.

Partial least-squares regression (PLSR) analysis is a multivariate method commonly used to predict sample properties from multivariate data, including infrared spectra.

The resulting PLSR models of regression coefficients and PLS loadings (weighted with respect to the analyte concentrations) for each of the analytes can, in principle, be used for the subsequent prediction of complexed Ag (%) in the soil water extracts.^[21] The reference analytical data used for the first PLSR model included colloidal DOC concentrations, colloidal elemental concentrations, colloidal C:S ratios, fluorescence data and SUVA₂₅₄ values in order to assess their relative significance toward understanding complexed Ag (%) in these soil water extracts. It is important to note that, initially, a range of soil physicochemical properties were analysed, including pH. They were not found to be of significant weighting in the prediction of complexed Ag in soil solution. The main focus of this paper was to investigate the properties of DOM that may control the complexation of Ag. We acknowledge that while pH is not discussed further, it is still an important property in controlling the amount of DOM in solution and the presence of complexation sites within DOM. The second PLSR model included only the MIR spectra since it was found that combining them with the previous model did not improve the result of the analysis, and overall, MIR spectra alone are very suitable as analytical data for a PLSR model.

The PLS score plot (PC1 versus PC2) and the corresponding loading weights of the first and second PCs are shown in Figs. 7a and 7b, respectively. In Fig 7a, the relative distance between the symbols is indicative of how similar the samples were. For example, the relative distance of the symbols for soils 12 and 17, suggest that these two samples were similar to each other. In contrast, the symbols for soils 7 and 13 were further apart indicating they were positively and negatively correlated with complexed Ag (%) i.e. soil 7 has a high amount of complexed Ag (%) and soil 13 low complexed Ag (%) measured in solution. The different symbols in Fig. 7a represent the three

replicates of each of the 20 soil water extracts analysed. Fig. 7b shows that DOC concentration, total fluorescence, HS1, HS3 and C:S ratio had the most significant contribution to complexed Ag (%) according to the first component explaining 44% of the variation. Thus, soil extracts with higher DOM, fluorescence, HS1, HS3 and C:S ratio will have a greater percentage of complexed Ag.

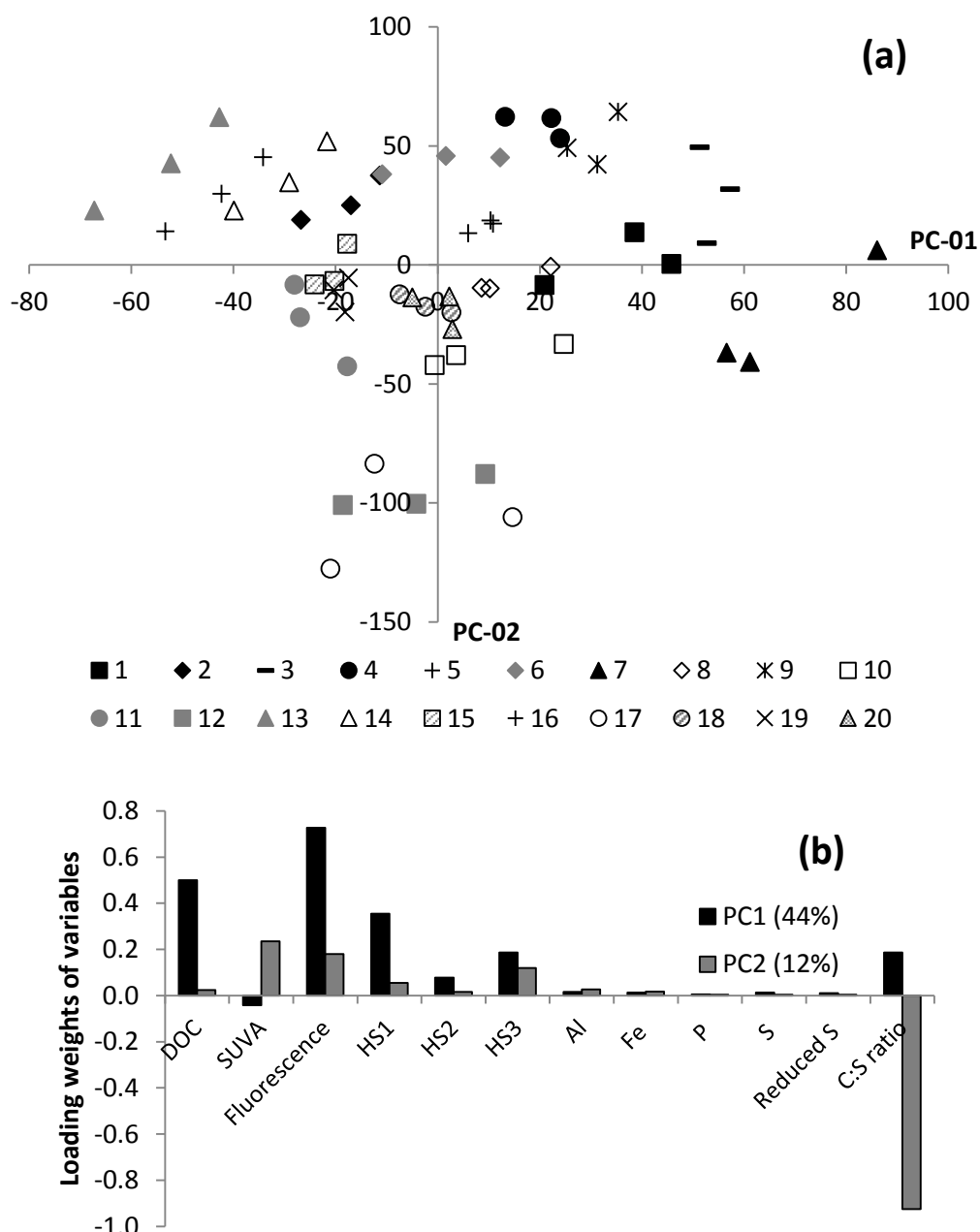


Figure 7. Partial least squares regression analysis results **(a)** PCA score-2 versus score-1 plot for complexed Ag modelled with test variables: DOC, SUVA, fluorescence, elemental concentrations and C:S ratio where each soil extract in triplicate is represented by a different symbol and **(b)** the loadings of the first and second principal component scores which explain 44% and 12% of the variation respectively.

Considering the PC-1 axis in Fig. 7a, which separates soils based on the correlation between analyte concentrations and complexed Ag (i.e. mainly fluorescence, DOM, HS1 and HS3 in decreasing importance), soil extracts in the positive region of this axis had high positive contribution to loading weight 1, while those in the negative region of the axis were negatively correlated with loading weight 1. Principal component 2 which explained 12% of the variation, weights C:S ratio and SUVA as the most significant. While SUVA had a positive contribution to PC-2, C:S was strongly negative and thus negatively correlated with complexed Ag and this strong negative contribution in effect cancels out what was observed in PC-1. The concentrations of Al, Fe, P, S and reduced colloidal S did not contribute significantly to the variation in complexed Ag.

Each replicate was analysed by PLSR separately and then an average (N=3) was taken. The final regression plot (Fig. 8) shows measured complexed Ag (%) versus predicted complexed Ag (%) with an R^2 value equal to 0.71 as determined by PLSR cross-validation. The significance of fluorescence, aromaticity and humic components in the regression has shown that humic acids can complex Ag^+ in soil solution through N and S groups.^[46,47] Despite no significant weighting of S or reduced S in the regression, it does not imply that Ag^+ complexation will not proceed via other S-containing functional groups. Sulfur speciation by sulfur K-edge XANES has found that the fraction of reduced S in humic substances (oxidation state <1) can range from 20 - 60% and comprises thiol groups, thioethers and organic disulfides.^[48] In addition, the strong negative correlation with C:S ratio indicated that as the ratio increases (i.e. less S per mole of DOC) there is less Ag complexation. The “reduced S” measurements in this study may also contain a significant amount of disulfides (which cannot bind Ag^+)

which may explain why this property did not have a significant weighting in the regression.

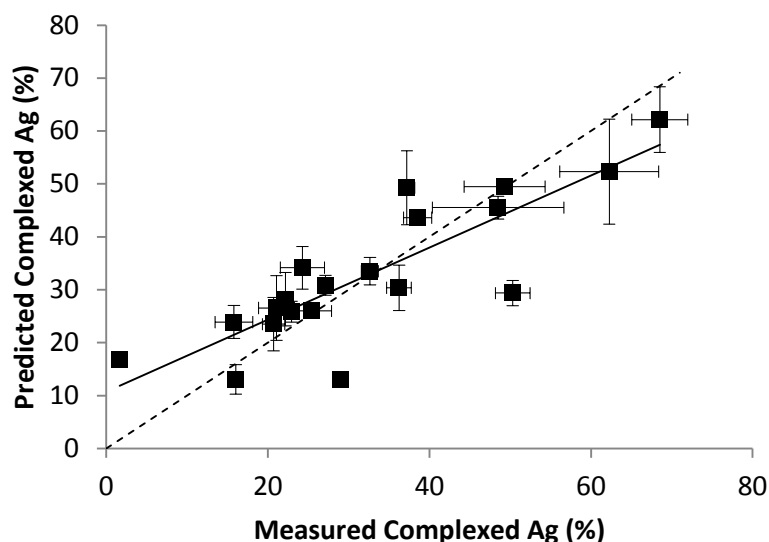


Figure 8. Partial least squares regression plot for the prediction of complexed Ag (%) in soil water extracts. The predictor variables are DOC concentration, fluorescence data, C:S ratio and SUVA₂₅₄. For the fitted line R^2 is 0.71, with the dashed 1:1 line also shown. Error bars represent the standard deviation ($n=3$) of the measured (horizontal) and predicted (vertical) percentages of complexed Ag in soil water extracts which was modelled separately for each replicate then averaged.

The results of PLSR cross-validation of Ag complexation in soil water extracts, using only the MIR spectra as predictor variables, are illustrated in Figs. 9a and 9b. The PCA plot (Fig. 9a) showed more clustering of soils than was observed in Fig 7a. In particular soils 3 and 7 were close together along PC-1 and far from the rest of the soils. There was also a cluster near the origin (0,0) for soils 1, 5, 6, 8 and 20, and then in the negative region of PC-1 for soils 10,11,12,13 and 15. The remainder fell close to these clusters, except for soils 2 and 4 which were far from each other and separated from the rest of the soils. Samples 2 and 4, and samples 3 and 7, may have a strong influence on the PLSR loading weight 1 calculation. In such cases, the PLSR calibration may actually derive a PC based on just these samples, rather than the rest of the population.

The analysis was performed again in the absence of these soil samples and found to have no significant effect on the PLSR calibration. Hence the soils were not excluded from the data set.

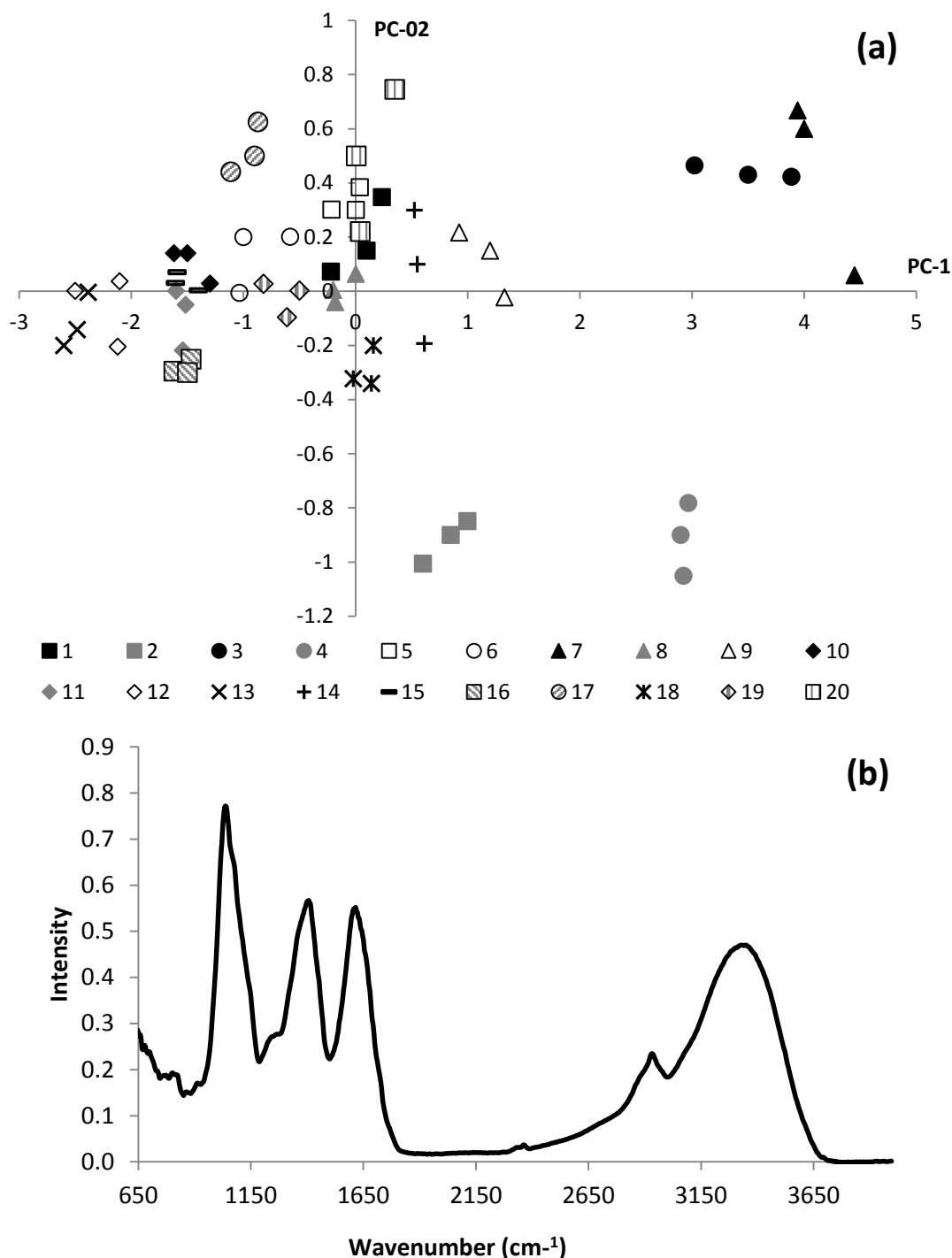


Figure 9. Partial least squares regression analysis results (a) PCA score-2 versus score-1 plot for complexed Ag modelled using the MIR spectra of the 20 soil water extracts in triplicate as the test variables and (b) the spectral loading of the first principal component scores explaining 74% of the variation.

The most significant spectral loading, PC-1, explained 74% of the variation in Ag complexation followed by PC-2 which only explained 4%. Soils 2 and 4 were distanced from the other soils as a result of PC-2 which only accounted for 4% of the variation in Ag complexation. For this set of spectral data, the most important features in the spectra resulted from PC-1 (Fig 9b) and were primarily due to absorption peaks at 1050 and 1080 cm^{-1} (carbohydrates), 1400 and 1600 cm^{-1} (together account for the carboxylate anion as well as aromatic groups), 2900 cm^{-1} (alkyl groups) and 3325 cm^{-1} (amine or alcohol groups).^[21, 28] These peak assignments highlight the important spectral features that relate to Ag complexation in the soil water extracts. However, overall the MIR spectra are DOM concentration dependent. The final regression plot showing measured complexed Ag (%) versus predicted complexed Ag (%) has an R^2 value of 0.80 (Fig.10) which was modelled separately for each replicate and then averaged.

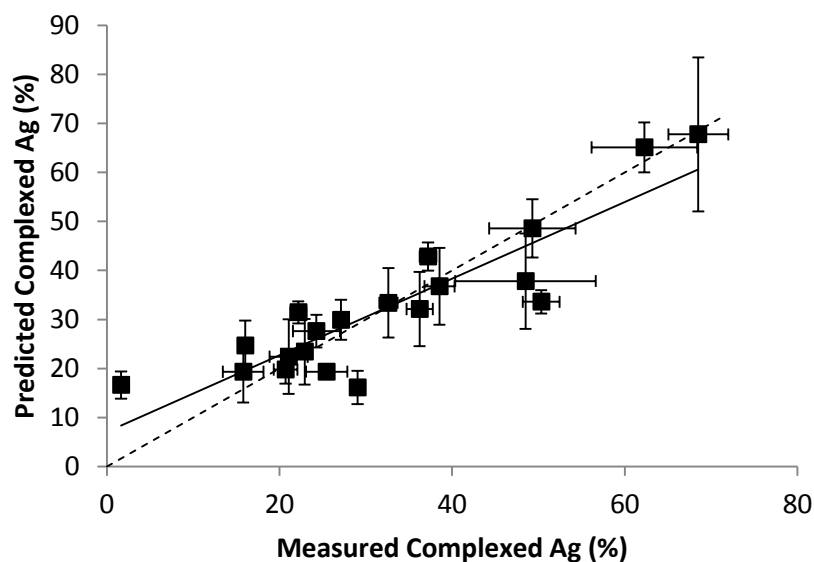


Figure 10. Partial least squares regression plot for the prediction of complexed Ag (%) in soil water extracts. The predictor variables are the MIR spectra. For the fitted line R^2 is 0.80, with the dashed 1:1 line also shown. Error bars represent the standard deviation ($n=3$) of the measured (horizontal) and predicted (vertical) percentages of complexed Ag in soil water extracts which was modelled separately for each replicate then averaged.

Finally, considering the potential for describing a relationship between the normalised complexed Ag values (complexed Ag % per mg C L⁻¹), with the quality of DOM measured, no highly significant model could be generated using the PLSR method. Instead a principal components biplot analysis (see supporting information) which accounted for 60% of the variation (from the first and second principle components) in the data set showed that the normalised complexed Ag is strongly correlated with the HS3 component, total fluorescence, and SUVA₂₅₄ values. This finding suggests that the strength of Ag complexation is influenced by the aromatic properties of the DOM which could directly result from humic substances. Silver complexation was also found to be negatively correlated to C:S ratio, an indication that, as the ratio increases (i.e. less S per mole of DOC) the strength of Ag complexation is weaker. This suggests that complexation of Ag could still occur through the S- groups that are present within aromatic and humic substances.

Conclusions

This study highlighted the significance of quantity and quality of soil DOM to Ag complexation in soil water extracts. While DOC concentration alone gave an indication of Ag complexation ($R^2=0.5$), the model was strengthened by adding quality information through fluorescence, UV-Visible, and C:S ratio data to explain 71% of the variability in the data. The PLSR model using MIR data alone highlighted that carbohydrate, carboxylate, aromatic, amine and alcohol functional groups were important for Ag complexation to DOM in soil water extracts with an overall improved model explaining 80% of the data variability.

In conclusion, DOM is important for the strong/irreversible complexation of Ag in soil water extracts. The normalised Ag complexation values point towards larger molecular weight humic substances and overall aromaticity increasing the affinity of Ag to DOM with the underlying mechanism being that this could still occur via S-groups present on the aromatic and humic substances. Spectroscopic information from UV-Visible, MIR and fluorescence spectroscopy may be more useful in measuring and characterising DOM than only chemical measurements. Knowledge of the affinity of Ag to complex with DOM could have implications for the mobility and bioavailability of this metal in the soil environment where up to 70% of Ag was found to be strongly complexed. While this complexed Ag is not considered to be bioavailable, there is potential through mobilisation in the terrestrial environment for dissociation to occur upon changing conditions of the surrounding environmental matrix and this should be considered in future studies.

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Supporting information

Table S1. Analysis of 0.2 μm and 3 kDa filtered soil water extracts and the calculated colloidal fraction. All concentrations are reported in mg L^{-1} .

Soil	Al			Fe			P		
	0.2 μm	3 kDa	Colloidal	0.2 μm	3 kDa	Colloidal	0.2 μm	3 kDa	Colloidal
1	0.40	0.23	0.17	0.68	0.10	0.58	1.85	1.50	0.35
2	0.21	0.20	0.01	0.14	0.01	0.13	0.39	0.21	0.18
3	2.48	0.36	2.12	1.34	0.09	1.25	1.31	0.82	0.48
4	0.77	0.19	0.58	0.43	0.01	0.42	0.49	0.26	0.23
5	2.49	0.24	2.24	1.90	0.05	1.85	1.29	0.97	0.32
6	0.56	0.39	0.17	0.33	0.06	0.27	0.26	0.22	0.03
7	3.68	3.03	0.65	1.21	0.36	0.85	0.23	0.15	0.08
8	0.52	0.25	0.27	0.24	0.03	0.21	0.38	0.30	0.08
9	2.94	0.21	2.73	1.67	0.03	1.64	0.65	0.42	0.23
10	1.96	0.44	1.52	0.53	0.13	0.41	0.31	0.25	0.06
11	0.32	0.22	0.10	0.08	0.05	0.03	0.98	0.99	0.04
12	0.20	0.12	0.09	0.16	0.03	0.13	0.07	0.07	0.07
13	0.15	0.13	0.02	0.01	0.01	-	0.01	0.04	-
14	2.61	0.15	2.47	1.61	0.01	1.59	0.95	0.80	0.16
15	2.88	0.29	2.60	1.45	0.08	1.37	1.24	1.23	0.01
16	1.15	0.13	1.02	0.49	0.01	0.48	0.14	0.13	0.01
17	1.89	0.18	1.71	1.41	0.02	1.39	0.25	0.28	-
18	0.67	0.16	0.51	0.48	0.01	0.47	0.78	0.68	0.10
19	0.44	0.29	0.16	0.03	0.02	0.01	0.05	0.09	-
20	3.71	0.18	3.52	2.25	0.01	2.24	1.20	1.00	0.20

- no colloidal concentration could be calculated when 3 kDa concentrations were equal to 0.2 μm concentrations

Table S2. Analysis of 0.2 µm and 3 kDa filtered soil water extracts and the calculated colloidal fraction for total S, sulfate, S in sulfate and reduced for S. All concentrations are reported in mg L⁻¹.

Soil	0.2 µm				3 kDa				Colloidal			
	Total S	Sulfate (SO ₄ ²⁻)	S in (SO ₄ ²⁻)	Reduced S [†]	Total S	Sulfate (SO ₄ ²⁻)	S in (SO ₄ ²⁻)	Reduced S [†]	Total S	Sulfate (SO ₄ ²⁻)	S in (SO ₄ ²⁻)	Reduced S [†]
1	1.54	2.13	0.71	0.83	0.69	1.19	0.40	0.30	0.85	0.94	0.31	0.54
2	1.93	4.40	1.47	0.47	1.61	4.00	1.34	0.27	0.33	0.40	0.13	0.19
3	3.10	4.47	1.49	1.61	1.84	3.47	1.16	0.69	1.26	1.00	0.33	0.92
4	4.78	11.33	3.78	0.99	3.57	9.20	3.07	0.50	1.21	2.13	0.71	0.50
5	1.47	2.63	0.88	0.59	0.87	1.85	0.62	0.25	0.60	0.78	0.26	0.34
6	6.45	20.00	6.45	0.00	5.70	17.07	5.70	0.01	0.75	2.93	0.76	-
7	2.26	3.50	1.17	1.09	1.36	2.60	0.87	0.49	0.90	0.90	0.30	0.60
8	2.39	5.37	1.79	0.60	1.90	4.87	1.62	0.28	0.49	0.50	0.17	0.32
9	4.04	8.37	2.79	1.25	3.07	7.60	2.54	0.54	0.97	0.77	0.26	0.71
10	1.43	2.33	0.78	0.65	0.87	1.87	0.63	0.24	0.56	0.46	0.15	0.41
11	0.36	0.89	0.30	0.06	0.32	0.85	0.28	0.04	0.03	0.04	0.01	0.02
12	0.18	0.48	0.16	0.02	0.11	0.18	0.06	0.05	0.07	0.29	0.10	-
13	1.54	4.60	1.54	0.01	1.64	4.83	1.61	0.02	-	-	-	-
14	1.59	4.77	1.59	0.00	1.30	3.80	1.27	0.03	0.29	0.97	0.32	-
15	0.65	1.93	0.65	0.01	0.63	1.78	0.59	0.03	0.03	0.15	0.05	-
16	1.40	4.20	1.40	0.00	1.30	3.87	1.29	0.01	0.10	0.33	0.11	-
17	0.23	0.62	0.21	0.02	0.30	0.70	0.23	0.07	-	-	-	-
18	0.82	0.95	0.32	0.50	0.65	0.97	0.32	0.33	0.17	-	-	0.17
19	1.35	4.00	1.34	0.02	1.20	3.53	1.18	0.02	0.15	0.47	0.16	-
20	0.83	1.20	0.40	0.43	0.70	1.11	0.37	0.32	0.14	0.09	0.03	0.11

[†]Concentration of S remaining after subtraction of S in sulfate from total S.

- no colloidal concentration could be calculated when 3 kDa concentrations were equal to 0.2 µm concentrations

Table S3 pH values of soil water extracts

Soil	pH of soil water extracts
1	5.4
2	7.8
3	6.1
4	7.3
5	6.0
6	5.1
7	4.6
8	5.4
9	5.7
10	4.7
11	5.2
12	4.8
13	7.3
14	7.1
15	6.2
16	5.4
17	6.0
18	7.1
19	5.1
20	6.9

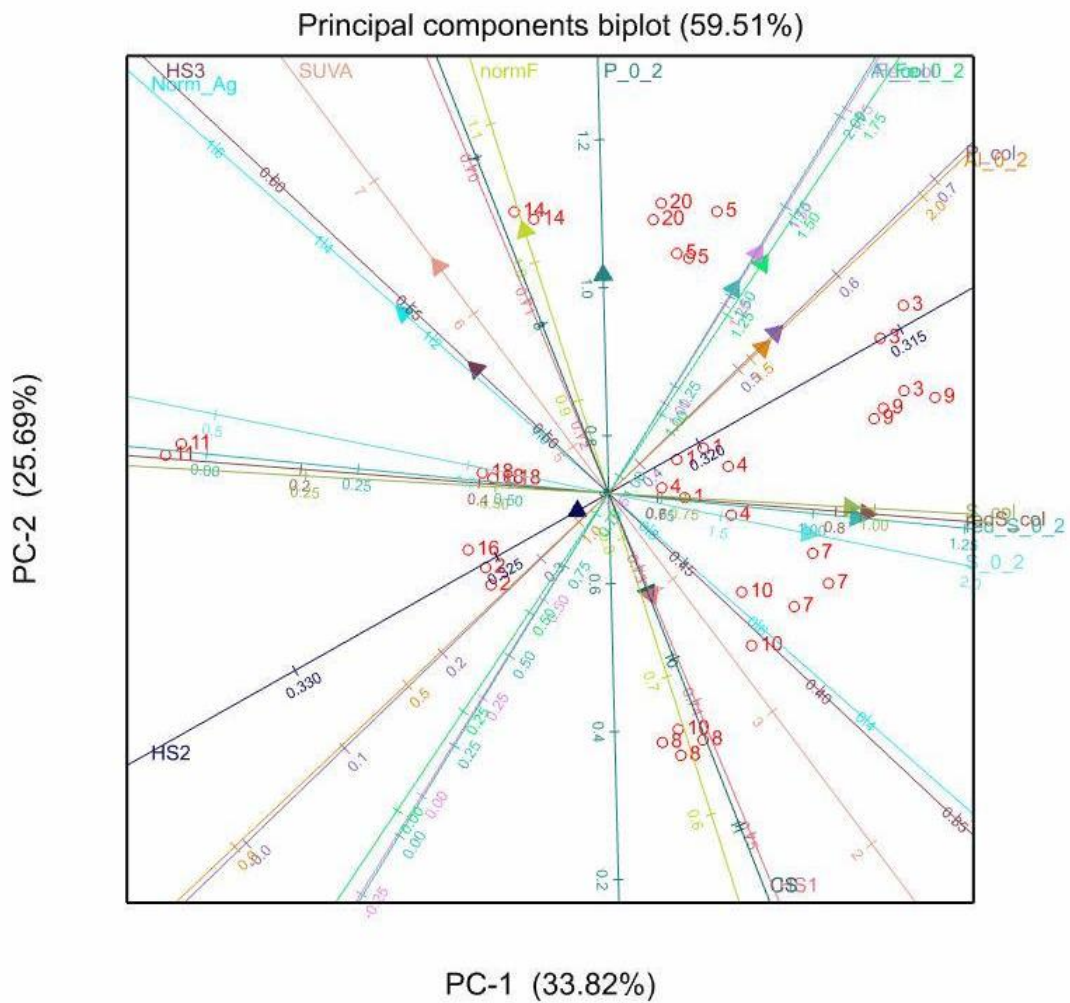


Figure S1. Principle components biplot showing the correlations between normalised complexed Ag Values and the various parameters measured in the study. Normalised Ag is most significantly and positively correlated with HS3, SUVA254 and normalised fluorescence.

CHAPTER 5

SUMMARY, CONCLUSIONS AND FUTURE RESEARCH
DIRECTIONS

Metal contaminants, including silver (Ag), that are released to the environment may pose a threat to soil dwelling organisms including microorganisms, plants and soil invertebrates. Silver may enter the environment as the soluble ionic Ag^+ species or as nanoparticles that can release soluble Ag^+ . The transformations that Ag^+ may undergo once in the soil can alter its chemical form and therefore potential uptake and toxicity. Measuring the partitioning, lability and speciation of Ag in soils, provides a better understanding of its potential bioavailability, compared to measuring total Ag concentrations in soil alone. To fully understand the availability of Ag in soils, there are various factors to be taken into consideration such as an understanding of the chemical nature and behaviour of Ag in soil and the influence of varying soil properties on the transformations and reactions of Ag^+ .

The work presented in this thesis was conducted to gain a greater understanding of the transformations and reactions of Ag in soil. This research provides further information in understanding the risks of Ag in soil environments through an understanding of its fate and potential availability. This includes an initial study to develop a sensitive method to measure the partitioning and exchangeability of Ag in soils using isotope dilution and the $^{110\text{m}}\text{Ag}$ radioisotope. The subsequent study utilised this isotope dilution method in combination with solid phase speciation using X-ray absorption near edge structure (XANES) spectroscopy to investigate the effect of ageing reactions and transformations on Ag exchangeability in soils. The final study used a range of rapid, accessible and sensitive laboratory methods (e.g. UV, fluorescence and IR spectroscopy and elemental analysis) with partial least-squares regression to examine the complexation of Ag^+ by DOM in soil solutions. These findings will enable a better understanding of the mobility and bioavailability of Ag in soil environments.

Development of a method to determine partitioning and lability of silver in soils

This chapter developed a sensitive method to measure the partitioning and exchangeability of Ag in soils using an isotope dilution technique. To develop this method, initially a suitable equilibration solution was identified by comparing water, CaCl₂ and Ca(NO₃)₂. Through this process, Ca(NO₃)₂ was highlighted as the most suitable equilibration solution. The exclusion of CaCl₂ as an equilibration solution was due to variable results caused by precipitation of AgCl which is not desirable as it compromises the assumptions of the isotope dilution principle by disrupting the equilibrium of the system (Hamon et al. 2008). Calcium nitrate was chosen over water because it provides an ionic strength similar to that of a soil solution and should minimise the influence of colloids. Following this, the influence of Ca²⁺ concentration in the equilibration solution on the partitioning and exchangeability of Ag was investigated. The outcomes from this research indicated that 10 mM Ca(NO₃)₂ provided the most consistent results and should therefore be used in future work.

The final component of this research involved examining the effect of non-exchangeable Ag colloids in soil suspensions on accurate measurements of Ag exchangeability (*E*-value). This was investigated through use of a cation exchange resin to correct for these potential colloidal interferences. While the use of Ca(NO₃)₂ as the equilibration solution minimises the presence of colloids in solution, it was still found that a resin clean up step was required to correct *E*-values for non-labile forms of Ag in soil solutions. The need to correct for isotopically non-exchangeable Ag in soils solutions during *E*-value determinations was found to differ between soil types and for the Ag spiking concentration applied. The presence of non-isotopically exchangeable colloids observed for Ag is similar to that for Cu. The isotopically non-exchangeable

Cu in the soil extracts led to an overestimation of the E -value, a result of the colloidal Cu complexes (Ma et al. 2006). This also compromises another assumption of the isotope dilution principle where the metal species measured in solution should all be isotopically exchangeable. The finalised method provides a powerful tool to examine the exchangeability and hence the potential availability of Ag in a range of soils with varying physical and chemical properties.

The research in this chapter using the optimised isotope dilution method highlighted some important findings related to the fate and behaviour of Ag in soils. The presence of Ca^{2+} in the equilibration solution revealed the significant impact on K_d and E -values for Ag in soils with low and high DOC concentrations. This difference was attributed to the ability of Ca^{2+} to suppress the mobilisation of dissolved organic carbon (and Ag). The flocculation of DOC in soil solution occurs through divalent Ca^{2+} forming bridges between the negative sites of DOC structures, resulting in Ag (in a similar way to Cu) getting trapped in these structures thus decreasing its soil solution concentration (Romkens et al. 1996). The solid-solution partitioning of TOC was found to be positively correlated to the Ag- K_d values in soils. Therefore, when the TOC- K_d was low (higher solubility of TOC, more DOC in solution), Ag- K_d values were also low (more Ag in soil solution). Hence, the TOC- K_d was always higher when Ca^{2+} ions were present, compared to no Ca^{2+} ions present (i.e. water) and therefore, the Ag- K_d was also high. This finding highlights the importance of DOC in the mobility and availability of Ag in soils.

In addition to Ag partitioning being positively correlated with TOC, it is likely there will be complexation of Ag with DOC, resulting in increased Ag being found in the

solution phase of the soil suspensions with higher DOC. It was found that as the concentration of DOC in solution increased, the concentration of free Ag^+ in solution decreased. This result suggested that there was complexation of Ag with DOC, which was the subject of further research in Chapter 4.

Soil properties such as CEC or TOC concentrations with pH were found to be the best predictors of Ag partitioning in soils, supported by another study that found TOC concentrations and pH to be important to the partitioning of Ag in soils (Cornelis et al. 2010). The K_d values for Ag in soils from the method development study (median K_d value of 1400 L kg^{-1}) are comparable to that observed for As (1800 L kg^{-1}) and Zn (1700 L kg^{-1}) in soils (Sauvé et al. 2000). The mean E -value as a percent of the total Ag added to soils ($E\%$), was $51 \pm 19\%$, indicating that Ag can be rapidly immobilised in the soil after two weeks. This is difficult to compare to other metals as the range in the literature is from 0 – 100 % across various metals, incubation times and soils. A comparison can be made with added soluble Cu to soils that were also incubated for two weeks, where an average $E\%$ was $76 \pm 19\%$ (Ma et al. 2006). This comparison suggests rapid reactions in soils decrease the exchangeability of Ag in soils to a similar extent as soluble Cu. The concentrations of geogenic exchangeable Ag in soils were below detection limits, indicating occlusion of the Ag within poorly soluble solid phases. These results indicate that measurement of exchangeable Ag is an important consideration when estimating the potential risks associated with Ag in soil. The conclusions drawn suggested that further work was necessary to assess the changes in Ag lability over a longer term, which was the focus of Chapter 3.

Effect of ageing on the fate and lability of silver in soils

The findings in Chapter 3 showed that exchangeable concentrations of added soluble Ag decreased over time for all of the soils investigated. After a six month ageing period, the proportion of added soluble Ag that was exchangeable ranged from <10 to 50% (mean $E\% = 26 \pm 21\%$). The most rapid ageing was observed in soils that had high pH (8.5-8.8). This study demonstrated that while there is an initial rapid ageing of Ag in soil, this is followed by slower processes and reactions, further removing Ag from exchangeable pools over a longer period. The removal of Ag into non-exchangeable pools with age was found to be greater than other soluble metals such as Cd, Cu, Ni and Zn) over longer aging time periods of 850 days ($E\% = 57-72\%$) (Buekers et al. 2007). These authors also found ageing of metals was more prominent for soils with increased alkalinity, which is consistent with the findings in this study.

Synchrotron based XANES analysis of Ag in soils indicated that for the majority of soils, the added soluble Ag was reduced to metallic Ag over time, and associations with Fe-oxohydroxides and reduced S groups in organic matter also decreased Ag lability. Strong positive correlations were found between metallic Ag and non-labile Ag and between organic carbon and Ag bonded with S species. The amount of metallic Ag formed did not account for all non-labile Ag in the soils, indicating that other Ag species can contribute to the non-labile pool. These other species may include Ag irreversibly sorbed to Fe-oxohydroxide and Ag-S species (including Ag_2S and Ag strongly bound to cysteine and thiol groups). There was also a significant and strong positive relationship between the percentages of Ag-S bonded species present in soils to the organic carbon content. This demonstrates that the association of Ag with organic matter is likely through the S groups of organic matter. Other studies have

demonstrated that conversion of Ag^+ to Ag-S species is certain to occur through wastewater treatment processes and through formation of biosolids (Kaegi et al. 2013).

These findings highlighted the importance of organic matter to the solid phase speciation of Ag in soils. Overall, there was a significant decrease in exchangeable Ag over time for all soils. Rapid ageing within the first two weeks of addition of soluble Ag was found, suggesting that Ag ageing needs to be considered when evaluating the potential risks associated with Ag in the terrestrial environment. Speciation data showed that Ag^+ is reduced to metallic Ag over time in soils and associations with S and iron oxides in soil can also lead to reductions in Ag lability. The results in this study suggest the risk of Ag in soil environments will be significantly reduced due to aging reactions and changes in speciation that will move soluble Ag into non-exchangeable pools in soils.

Complexation of silver and dissolved organic matter in soil water extracts

The research findings in Chapter 2 found that free Ag^+ concentrations decreased as DOC concentrations increased in soil solutions. This finding highlighted the importance of DOC in soil solutions on the mobility and potential availability of Ag in soils. A final study was performed to examine the effects of quantity and quality of DOM on Ag complexation in soil solutions. The study involved measuring the amount of complexed Ag in soil water extracts across a range of soils with natural varying concentrations of DOM. The complexed Ag was then considered in relation to the concentration of DOM to provide an understanding of variations in strength of binding between soils. The soil water extracts were also characterised by size fractionation and elemental analysis, and spectroscopic analysis using UV-Visible, fluorescence and mid

infra-red (MIR) spectroscopy. The chemical and spectroscopic measurements were then used to model and predict complexed Ag in solution.

The proportion of complexed Ag using a resin technique in the soil water extracts was found to vary considerably between the different soils tested. There was a positive relationship found between Ag complexation and DOM concentrations, where soil solutions with the highest DOM concentrations having the greatest proportion of complexed Ag. Comparison of the complexed Ag results after normalisation to DOM concentration (complexed Ag per mg C) showed differences in strength of binding or affinity of the DOM to Ag. Indeed soils with similar DOM concentrations did not have the same amount of complexed Ag in solution. This indicated that differences in DOM composition or quality may be responsible for differences between complexation capacities with Ag⁺.

A range of chemical and spectroscopic measurements were performed on soil water extracts to better understand the complexation of Ag and develop partial least-squares regression models to predict Ag complexation and DOM in soil water extracts. The results from this study found that: (i) DOM concentration was significant in the complexation of Ag in soil water extracts, and quality information through the addition of fluorescence, SUVA₂₅₄ and C:S ratio data strengthens the DOM complexation model (accounting for 71% of the data variability) and (ii) complexed Ag in soil water extracts could be well predicted by MIR analysis alone, accounting for 80% of the data variability. The fluorescence and SUVA₂₅₄ data are representative of the humic and aromatic groups that represent a significant proportion of DOM composition and can contain up to 60% S-groups. The MIR spectra suggested that carbohydrate,

carboxylate, aromatic and amine or alcohol functional groups in DOM were responsible for the amount of complexed Ag in soil water extracts.

The findings examining complexed Ag % per mg C, found Ag complexation to be strongly and positively correlated to the larger molecular weight humic substances and aromatic groups, but also negatively correlated to the molar C:S ratio. The negative correlation with C:S ratios, indicated that when there was less S per mole of C, the strength of complexed Ag was weaker. This also supported the negative correlation between C:S ratio and the amount of complexed Ag from the PLSR model. Overall, these results are complimentary to those in the aquatic environment that indicate S groups on DOM and humic/fulvic acids are important for the complexation of Ag⁺ and that such complexation alleviates toxicity of Ag to biota since the complexed Ag is not in a bioavailable form (Bell and Kramer 1999; Chen et al. 2013).

Overall this study demonstrated the critical role of DOM in soil water extracts for complexation with Ag. It has also shown that spectroscopic techniques such as MIR are very effective and efficient in predicting complexed Ag. The implications of this study are that since Ag⁺ in soil solution has a very strong interaction with DOM it is not likely that Ag⁺ will be available when high DOM concentrations are present. This strong interaction is also comparable to the strong affinity of Cu²⁺ to DOM where the aromatic carbon content was found to significantly impact the Cu mobilising potential in soils (Amery et al. 2008). As a result, the complexation of Ag with DOM and its relationship to DOM quality could cause significant leaching of Ag-DOM complexes, which should be considered in assessing potential mobility of Ag in the terrestrial environment.

Conclusions

1. Silver partitions strongly to the solid phase in soils, which is positively correlated with cation exchange capacity, total organic carbon and pH. In addition, a rapid decrease in Ag exchangeability was observed after a short two week incubation.
2. Long term ageing of soluble Ag in soils revealed that the concentration of exchangeable Ag continued to decrease and this was consistent with an increased formation of metallic Ag as well as associations with Fe-oxohydroxides and reduced S groups of organic matter. After six months of ageing, the majority of added Ag was found to be in non-exchangeable pools in the soils examined.
3. Dissolved organic matter plays a critical role in the complexation of Ag in soil solutions. This is influenced by both DOM quantity and quality where the aromatic and humic groups and C:S ratio are the most influential to the strength of binding.

As a consequence of these main outcomes, the research contained in this thesis highlights the importance of measuring isotopically exchangeable metal concentrations in soil rather than total metal concentrations alone. This technique provides environmentally relevant information on the behaviour of Ag in soils as it allows for a greater understanding of the fate and potential availability of Ag in soils. The strong interaction between Ag^+ and DOM in soils suggests Ag^+ will not be bioavailable when high DOM concentrations are present in soil solutions. In contrast, the strong interaction could cause significant leaching of Ag-DOM complexes which should be

considered in assessing potential mobility of Ag in the terrestrial environment.

Speciation data showed that Ag^+ is reduced to metallic Ag over time in soils and associations with S and iron oxides in soil can also lead to reductions in Ag lability.

The results in this study suggest the risk of Ag in soil environments will be significantly reduced due to aging reactions and changes in speciation that will remove soluble Ag into non-exchangeable pools in soils.

Future research directions

To address some of the issues raised in this study, and in order to further develop our understanding of the behaviour and fate of Ag in soils, further research in the following areas is recommended:

1. This study presented an isotopic dilution method for measuring the partitioning (K_d -value) and lability (E -value) of Ag in soils. The results showed great variation in both K_d and E -values across soils, which was heavily dependent on soil properties and the concentration of added metal. Assessment of field-contaminated soils will enable a greater understanding of the partitioning and lability of Ag in the environment following exposure to various Ag sources (e.g. AgNPs, biosolids, effluents, mine tailings). Applying the E -value method to soils amended with these various Ag sources will provide a measure of the available fraction in soils as a result of Ag pollution to the soil environment.
2. Following addition of soluble metals, initial ageing reactions were observed over a short period of time, but over a longer term these ageing processes slowed. There are many seasonal changes that could occur in the environment that may change the conditions of the soil. As a result, this could alter the

chemical speciation of the metal. Silver is a redox sensitive metal, therefore changes in soil water content, salinity or pH will heavily influence the amount of exchangeable metal. In order to assess such effects on the behaviour and fate of Ag in soil, further research should include monitoring *E*-values through wetting and drying cycles, mimicking agricultural practices (e.g. application of biosolids) and over a range of salinities and pH values. While Ag is overall strongly partitioned to the solid phase of soils and over time most soluble Ag converts to non-exchangeable forms in soil, changes in environmental conditions could result in the re-mobilisation of this metal as well as potential for the dissolution of non-labile precipitates like AgCl or Ag₂S to occur slowly over time.

3. The solid-phase speciation and solution complexation findings in this study highlighted the importance of soil organic matter on the availability of Ag in soils. Through both solid phase interaction and the complexation of Ag with DOM in solution, it is apparent that these interactions decrease the amount of labile and hence potentially available Ag⁺. The solid-phase speciation data highlighted the significance of S-groups whereas the solution phase complexed Ag results indicated humic and aromatic groups were most influential along with the C:S ratio. Further characterisation of the DOM by specifically measuring the concentration of reduced S groups present in solution DOM would be insightful to assess whether these groups are specifically responsible for the affinity of DOM for Ag⁺ in soil solutions. Further investigation of the complexation between Ag and DOM could also include a quantitative measure of the complexation capacity of DOM with Ag⁺.

The characterisation of DOM by fluorescence and MIR could also be carried out with the presence of Ag^+ in the solution, across a range of concentrations, in order to make spectral comparisons with and without Ag. Changes in spectral features could perhaps be used to precisely identify the characteristics of DOM responsible and the mechanism behind Ag complexation in soil solution.

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