Non-labile silver species in biosolids remain stable throughout 50 years of weathering and ageing.

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Abstract

Increasing commercial use of nanosilver has focussed attention on the fate of silver (Ag) in the wastewater release pathway. This paper reports the speciation and lability of Ag in archived, stockpiled, and contemporary biosolids from the UK, USA and Australia, and indicates that biosolids Ag concentrations have decreased significantly over recent decades. XANES revealed the importance of reduced-sulfur binding environments for Ag speciation in materials ranging from freshly produced sludge to biosolids weathered under ambient environmental conditions for more than 50 years. Isotopic dilution with 110mAg showed that Ag was predominantly non-labile in both fresh and aged biosolids (13.7% mean lability), with E-values ranging from 0.3 to 60 mg/kg and 5 mM CaNO3 extractable Ag from 1.2 to 609 µg/kg (0.002 - 3.4% of the total Ag). This study indicates that at the time of soil application, biosolids Ag will be predominantly Ag-sulfides and characterised by low isotopic lability.

Capsule

Analysis of historic and contemporary biosolids from three continents indicated decreasing wastewater silver releases, and non-labile, extremely stable silver speciation.

Keywords

Silver, biosolids, XANES, speciation, isotopic dilution, E-values

Introduction

Silver (Ag) has been used by humans for millennia; not only as a precious metal and symbol of wealth, but also in applications as diverse as sanitation (Oyanedel-Craver and Smith, 2008), photography (Bergthaller, 1996), industrial catalysis (Serafin et al., 1998), dentistry (Lorenz et al., 2012), and healthcare (Sambhy et al., 2006; Silver, 2003). In recent decades, efforts to reduce environmental pollution have brought about numerous resource recovery and effluent treatment guidelines and discharge limits; including some which specifically target key Ag emitting industries such as the photographic sector (e.g. (DEP, 2007; EPA, 1981) These instruments, together with voluntary industry action, have successfully lowered wastewater Ag loads from major commercial and industrial sources (Council, 1997; EPA, 1999), and in some cases, market changes have further compounded this trend. For example, in 1990, the photographic uses of silver in the USA, Japan, and Western Europe accounted for 40% of the total global Ag demand (GFMS, 1990) but this declined significantly due to the subsequent rise of digital photography. In fact, recent market analysis shows that between 2003 and 2013 the annual use of Ag for photographic applications fell from 6002 tonnes to 1555 tonnes (193 to 50 Moz.); a steady decrease from 25 % of the global Ag demand in 2000 to only 5 % of the forecasted demand in 2015 (GFMS, 2011, 2013, 2014). Material use patterns are never static however, and the declining use of Ag in the photographic sector has been accompanied by an increase in other uses; including some which are likely to result in inadvertent wastewater release. One trend in particular, namely the growing use of nanosilver (e.g. silver nanoparticles) in consumer products (Kim et al., 2010; Levard et al., 2011), is currently focussing significant attention on the fate and effects of Ag in the wastewater-biosolids-soil environmental release pathway (Impellitteri et al., 2013; Kaegi et al., 2013). Although the size of this market is not easily determined, recent reports indicate that up to 500 tons/year of Ag-NPs are currently produced and used worldwide (Sun et al. 2014), and

the sector continues to grow as evidenced by ongoing additions to nanoproduct inventories such as the Woodrow Wilson Institute's Project on Emerging Nanotechnologies (http://www.nanotechproject.org/cpi) and the Danish nanodatabase (http://nanodb.dk).

Nanosilver itself is not a new material. Colloidal (nanoscale) Ag has been sold commercially for more than a millenium (Nowack et al., 2011). However, Ag nanoparticles/nanomaterials are increasingly surface functionalised for specialist purposes (e.g. as the active agents in antibacterial textiles), and the range and number of products in which nanosilver features have grown exponentially in recent years (GFMS, 2013; WWI, 2014). The commercial drivers for this trend include both the functional value of silver as an antibacterial substance (Nowack et al., 2011), and the marketing value of antibacterial products comprising 'advanced technology' (i.e. nanotechnology). Many of these products include silver as an active ingredient and are used dispersively (e.g. in personal care products and cleaning products) or in applications directly linked to the production of wastewater (e.g. textiles and washing machines) (Benn and Westerhoff, 2008; Kaegi et al., 2013; WWI, 2014). The direct measurement of engineered nanoparticles in the environment is extremely challenging (Sekine et al., 2013; von der Kammer et al., 2012), but attempts to determine major nanosilver release pathways and loads via probabilistic material flow analysis have identified the wastewater network as a key environmental release pathway for nanosilver and its derivatives (Gottschalk et al., 2009; Gottschalk et al., 2013; Sun et al., 2015). These recent developments have generated renewed interest in the role of Ag as a wastewater pollutant and highlighted the need for further risk assessment of Ag in the "wastewater - biosolids - soil" pathway.

Different jurisdictions have developed different regulations and guidelines for the land application of biosolids, and metal contents commonly play a key role in determining biosolids quality grading, application rates, and uses. For example, the US EPA regulates land application on the basis of pollutant concentration limits, cumulative pollutant loading limits, and annual pollutant loading limits for key contaminants such as As, Cd, Cr, Cu, Pb, Hg, Ni, Se, and Zn (EPA, 1993). Other jurisdictions such as Australia, New Zealand, and European Union member states monitor a similar suite of metals (Ministry for the Environment/ NZ WWA, 2003; European Communities, 1986). Silver limits for biosolids are not stipulated in these regulations/guidelines, and as a result, Ag has not been routinely monitored. Consequently, there are very little data documenting Ag concentrations in biosolids, with the notable exception of the most recent US Targeted National Sewage Sludge Survey (US TNSSS) (US EPA, 2009) in which Ag was particularly selected as a non-routine analyte requiring further study. In view of this overall lack of data and the recent concerns about the increasing use and release of nanosilver, there is a need to determine the baseline concentrations and chemistry of Ag in biosolids, and to investigate how biosolids Ag chemistry changes with time and as a function of post-processing treatments (e.g. composting).

This paper presents an overview of biosolids Ag chemistry in historic and contemporary biosolids sourced from the UK, USA and Australia from the 1950s until today by drawing on a unique collection of archived, stockpiled and contemporary biosolids samples. Characteristics of biosolids Ag chemistry determined in this study included total Ag measurement by neutron activation analysis (NAA); the assessment of Ag lability by ^{110m}Ag isotopic dilution (E-values); and Ag speciation by X-ray Absorption Near Edge Spectroscopy (XANES). Two major questions were addressed: 1) whether the speciation and lability of Ag

in biosolids from different sources and production eras differs, and 2) whether Ag chemistry in biosolids changes significantly upon weathering and ageing.

Materials and methods

Biosolids samples

The 22 biosolids samples examined in this study were sourced from Australia, the USA, and the UK (Table 1). The samples were chosen to cover a range of municipal wastewater treatment systems and a production span of more than 60 years. Treatments systems included in this study ranged from simple lagoon systems to activated sludge treatment plants with aerobic and anaerobic digestion, Most of the historic materials were obtained from pre-existing archive collections. The Perry Oaks (London, UK) biosolids from the 1950s, 1960s, and 1990s were originally used in long-term biosolids field-trials (Le Riche, 1968). These samples were air-dried and stored under ambient conditions and atmosphere in sealed glass jars from the time of production and initial experimental use (e.g. 1960s and 1990s) until subsampled for this study. These jars have occasionally been opened under ambient conditions for the extraction of sample material. The 2006 USA biosolids samples were archived as part of the US TNSSS (EPA, 2009). These samples were initially stored frozen, then freeze-dried and maintained under ambient conditions in sample jars. The contemporary biosolids samples were sourced directly from wastewater treatment plants in the UK and Australia in 2009, air dried and stored in sealed glass jars in the same manner as the other samples. These jars were also opened periodically for sample removal. Some of the contemporary samples were sourced directly from the belt press/dewatering stage prior to drying, whereas others had been stabilising in open-air stockpiles in preparation for land application (a standard practice in Australia, see Figure S1). These details have been documented in Table 1. The 1970 to 2009 time series of Australian (Melbourne) biosolids

was sampled from a biosolids storage facility containing stockpiled samples from a single large-scale treatment plant. Biosolids stored on this site are maintained in large open-air stockpiles and were subjected to natural weathering processes from the time of production until analysis. At the time of sampling (Australian summer) the materials were dry. Together, these samples provide a window to the past that allowed us to investigate historic trends in biosolids silver concentrations, speciation and lability.

General chemical characterisation

Electrical conductivity (EC) was measured in a 1:5 solid:H_2O solution, and pH was measured in 0.01 M $CaCl_2$ solution (1:5 solid:solution). Total C and Total S were measured by combustion in a LECO CNS elemental analyser.

Neutron Activation Analysis

Total Ag concentration was measured by neutron activation analysis (NAA) using the OPAL Research Reactor, Lucas Heights, NSW, Australia. The k0-method of standardisation was used (k0-NAA), employing certified reference material IRMM-530R as the comparator (Bennett 2008; Popelka-Filcoff, et al. 2011). Two sub-samples of 50 mg were taken from each biosolid sample. One sub-sample was irradiated for 30 seconds in the short residence time (SRT) facility and the other was irradiated for 9 hours in the long residence time (LRT) facility. After the end of the SRT irradiation two gamma-ray energy spectra were collected for 3 minutes after a decay of around 5 minutes and for 12 minutes after a decay of 18 minutes. After the LRT irradiation two gamma-ray energy spectra were collected for 30 minutes after a decay of around 4 days and for 4 hours after a decay of 2 weeks. Analysis of the data using commercial software HyperLab and Kayzero for Windows enabled a total of over 50 elements per sample to be quantified.

Silver speciation by X-ray Absorption Near Edge Spectroscopy (XANES)

The majority of biosolids K-edge Ag XANES spectra were collected on the Materials Research Collaborative Access Team (MRCAT) beamline 10-ID at the Advanced Photon Source, Argonne National Laboratory, IL, USA. Some additional spectra were collected on the XAS beamline at the Australian Synchrotron. An elemental Ag foil was used for energy calibration (Ag K-edge at 25514 eV) and collected in transmission at the same time as the sample spectra. Sample spectra were collected at room temperature in fluorescence mode. At the APS, the electron storage ring was operating at 7 GeV in top-up mode. A liquid nitrogen cooled double crystal Si(111) monochromator was used to select the incident photon energies during scanning. The fluorescence detector was a 13-element silicon drift detector. At the Australian Synchrotron, the electron storage ring was operating at 3 GeV in top-up mode. The XAS beamline setup used a liquid nitrogen cooled Si (311) crystal monochromator and the fluorescence data were collected using a 100 element Ge fluorescence detector. Sample spectra (average of 3 scans) were normalized using the Athena software (Ravel and Newville, 2005). Standard spectra were collected at both beamlines and used to fit the spectra of the relevant biosolids samples. Materials used to collect standard spectra included silver-oxide (Ag_2O), -sulfide (Ag_2S), -chloride (AgCl), -sulfate (Ag_2SO_4), and -carbonate (Ag_2CO_3), as well as silver nanoparticles, and Ag sorbed to humic acids (Sigma Aldrich), cysteine, cystine, glutathione, ferrihydrite and acetate. Detailed information on the use of XANES for metal speciation analysis in environmental samples can be found in (Graefe et al., 2014). All spectra were normalized over the range from −80 to +250 eV relative to the K-edge prior to Linear Combination Fitting (LCF) analysis. Principal component analysis (PCA) of the sample spectra was used to estimate the number of standards needed to account for the majority of spectral variance. This is a common step used in XAS LCF (Gräefe et al., 2014).

Standard spectra used for LCF were selected on the basis of low target transformation SPOIL values (Malinowski, 1991) and relevant published literature (e.g. (Impellitteri et al., 2013; Kaegi et al., 2011; Lombi et al., 2013). PCA and TT were undertaken using SixPack (Webb, 2005). LCF was performed using Athena to fit the spectra in the range -25 to 100 eV. This procedure uses stepwise comparison of different combinations of standard spectra to compute the fraction of standard species that sum together to yield the best fit for the sample spectrum. For each sample, the LCF combination of standards with the lowest residual parameter was chosen as the most likely set of components (Kirpichtchikova et al., 2006) and provides an indication of the average speciation of Ag in the biosolids.

^{110m}Ag isotopic dilution (E-values)

Aside from two samples for which insufficient sample masses were available, isotopic dilution with ^{110m}Ag was used to investigate the proportion of labile or potentially available Ag in the biosolids. For detailed information about the derivation and interpretation of 'E-values' using isotopic dilution see Hamon et al. (2008). Triplicate samples of the biosolids (1.2g) were equilibrated in 12 ml of 5 mM $Ca(NO_3)_2$ for 24 hours. The suspensions were then spiked with 120 KBq ^{110m}Ag per sample and equilibrated for 24 hours before being centrifuged (45 minutes at 2500 g) and filtered through a 0.22 µm pore size syringe filter. Samples were then acidified to < pH 2 with concentrated nitric acid. All samples and aliquots were kept in the dark prior to analysis. A 4 ml aliquot from each sample was used for ^{110}Ag radio-assay using a Perkin Elmer WIZARD[2] Gamma Counter, and the remainder of the sample was used to determine the concentration of Ag in solution with a triple quadrupole ICP-MS (Agilent 8800 ICP Triple Quad (ICP-QQQ)). The ICP MS E-value results were also used to calculate the (1:10 solid:solution) 5 mM $Ca(NO_3)_2$ extractable Ag for these samples. The detection limit for this analysis using the triple quadrupole ICP in He mode was 0.0034

μg l^{-1}. E-values were calculated according to Equation 1, where Csol is the concentration of Ag in solution (μg ml^{-1}), C*sol is the radioactivity of the isotope ^{110m}Ag remaining in solution after equilibration (Bq ml^{-1}), R is the total amount of radioisotope added to each sample (Bq ml^{-1}), and V/W is the ratio of volume of solution (ml) to mass of sample (g).

$$E\text{-}value = \frac{Csol}{C*sol} \cdot R \cdot \frac{V}{W} \qquad (1)$$

The percentage of labile Ag was calculated according to Equation 2.

$$Labile\ Ag\ (\%) = \frac{E-value\ (mg.kg^{-1})}{Total\ Ag\ (mg.kg^{-1})} \times 100 \qquad (2)$$

Results and Discussion

Chemical characterisation of the selected biosolids

Some key characteristics of the 22 biosolids examined are presented in Table 1. Their pH values varied considerably, with the major differences corresponding to the mode of storage since production. The aged samples taken from outdoor stockpiles were acidic (pH 4 - 4.5), whereas the freshly produced and archived samples had neutral to acidic pH values (pH 5.8 - 7). This likely reflects the weathering, leaching, and nitrification the stockpiled samples have undergone in the decades since production. The weathering effect is also reflected in the comparatively low EC values measured in the stockpiled samples, which ranged from 0.9 - 3 mS/m. The highest EC value, 5.8 mS/m, was that of a freshly digested sludge sampled straight from the belt press of an Australian treatment plant. Total C ranged from 10 - 37 % dry mass, with an average of 27.2 %. Interestingly, the total C was not particularly low in the outdoor stockpiled samples from the 1990s and earlier. Circumspectly, lower values might be

expected in these samples if the organic matter had degraded over time as a result of ageing as suggested by the 'time-bomb' hypothesis (Beckett, 1979). Total S ranged from 0.4 - 2.7 %, with an average of 1.1 %, and showed no particular trend in relation to biosolids provenance, processing or storage method. Linear regression plots for comparisons discussed above are presented in Figure S6.

Comparison of total silver concentrations in contemporary and historic biosolids

Silver, sourced both from traditional industrial sources and products containing nanosilver, partitions predominantly with the solid phase during wastewater treatment (Benn and Westerhoff, 2008; Kaegi et al., 2013; Lytle, 1984; Shafer et al., 1998). Consequently, total biosolids Ag concentrations are an effective indicator of the cumulative amount of Ag released to municipal wastewater from household, commercial and industrial uses, and can be used to determine whether changing Ag uses and waste treatment practices are translating into increasing or decreasing wastewater pollutant loads.

The results in Table 1 show that total Ag concentrations in these biosolids ranged from 4.3 to 332 mg kg^{-1}, and were generally higher in the older samples. This is in keeping with the widespread trend of decreasing contaminant metal concentrations and increasing biosolids quality observed over recent decades (Stehouwer et al., 2000); a positive outcome of increasingly stringent wastewater permitting procedures and resource recovery activities. However, as the contemporary samples used in this study were selected specifically on the basis of their Ag contents (as higher concentration samples produce better quality XANES spectra), a review of national sludge surveys and published literature was conducted to further investigate this apparent trend. The available data are presented in Table 2, and, whilst limited, do support the finding that Ag concentrations in biosolids have generally decreased

over recent decades. Nevertheless, as indicated by the upper outlier values from the last US EPA national biosolids survey, in which 3 of the 78 samples contained > 125 mg kg^{-1} Ag (EPA, 2009), it appears that Ag concentrations in some contemporary samples are still notably high.

Silver speciation measured using X-ray Absorption Near Edge Spectroscopy (XANES)

Bulk Ag K-edge XANES spectra were collected for 19 biosolids samples sourced from three different continents over a 60 year period. The spectra of standard Ag species identified by target transformation as likely sample components are provided in the Supplementary Information (Figure S2), together with the complete set of sample spectra (Figure S3). All standards (from a collection of 18 standard materials) giving SPOIL values < 6 were used for LCF (Malinowski, 1991), and 3 additional standards which had higher SPOIL values but were considered likely to occur on the basis of other literature data were also included. On this basis the following standards were used (SPOIL values are given in brackets): Ag_2S (2.3), Ag-cysteine (3.3), elemental Ag (3.6), AgCl (5.4), Ag-ferrihydrite (9.4), Ag_2PO_4 (9.7), and Ag-humic acid (11.8). The best-fit Ag speciation results identified by XANES LCF (limited to a maximum of 4 component species on the basis of PCA as discussed in Gräfe et al., 2014) are given in Table 3. The goodness of fit is indicated by the R-factor (low = good, Table 3) and is also evident upon visual inspection of the experimental and fitted spectra (Figure S3). Together, these XANES spectra and LCF results indicated major similarities in Ag speciation across this diverse collection of biosolids, despite the large differences in their respective Ag concentrations, source catchment characteristics, wastewater treatment regimes, and biosolids production era and storage conditions. In all samples, reduced sulfur ligands (represented by silver sulphide (Ag_2S) and thiol-bound Ag (Ag-cysteine) for LCF), were determined to form the dominant Ag binding environment (i.e. 60-92%). As these two

reference spectra are very similar when plotted on the basis of normalised intensity (see Figure S2) it was not possible to discriminate between them for the purposes of species apportionment via XANES LCF; hence the contribution of these two species to the overall fit were combined in Table 3. However, as shown in Figure S4, when plotted in k-space, there is a difference in the position of the Ag_2S and Ag-cysteine oscillations. Although these sample spectra are quite noisy (even after smoothing) when viewed in k-space, and not suitable for EXAFS LCF analysis; comparison of the biosolids sample spectra with the Ag_2S and Ag-cysteine spectra (Figure S4) strongly suggests that Ag_2S plays a more significant role than Ag-cysteine in the overall Ag speciation in these samples. While reduced Ag-sulfur binding environments clearly dominate across the full range of samples, a variety of minor species are also indicated to be present (Table 3). Of these, $Ag-PO_4$ is the next most common species, forming part of the LCF fit in the vast majority of samples examined and accounting for up to 22% of the total Ag. This is not surprising given that biosolids comprise on average about 1% P (PSD, 2009). Among the likely forms of Ag present in biosolids, $AgPO_4$ is also the second least soluble form, with a Ksp of 8.89×10^{-17}, as compared for example with a Ksp of 1.77×10^{-10} for AgCl. This supports the finding that Ag_2PO_4 is likely to remain in biosolids as a long-term species. Other minor species identified here included elemental Ag, and Ag bound to organic ligands (i.e. humic acid). It should be noted, however, that as uncertainty in species apportionment is estimated to be approximately 10% of the total amount of the target element, values less than 10% should be considered with caution (Manceau et al., 2002; Kirpichtchikova et al., 2006).

It has already been demonstrated several times in the literature that when Ag is introduced into the wastewater stream, it is likely to undergo rapid and extensive sulfidation (irrespective of the source and form of the Ag entering the wastewater) (Kaegi et al., 2013; Kaegi et al.,

2011; Lombi et al., 2013). However, the fate of Ag after biosolids ageing, post-processing, and/or land application is less well understood. Recently, Impellitteri et al. (2013) showed that different disposal pathways for biosolids can induce different changes in Ag speciation. They found that $AgNO_3$ and Ag-NPs were converted to reduced Ag-sulfur species in fresh biosolids and remained as such after a 1-month ageing period, but showed that a significant proportion of the reduced Ag-sulfur species (30 – 50%) in both fresh and aged biosolids converted to elemental Ag and Ag_2SO_4 upon incineration at 850 °C. These types of changes can have major implications in terms of Ag toxicity, mobility, and bioavailability; making it extremely important that the speciation of Ag throughout the whole wastewater-biosolids lifecycle is well understood. In some countries (e.g. Australia, Canada), biosolids used for agricultural application are typically composted/stockpiled for periods up to several years prior to land application. Previous studies have shown major changes in biosolids metal (e.g. copper, zinc) speciation as a result of this ageing process (Donner et al., 2013; Donner et al., 2011; Lombi et al., 2012), however Ag-sulfides have been found to be much more recalcitrant than other metals to oxidation and subsequent changes in speciation. For example, Lombi et al. (2013) showed that even after a six-month ageing period with wetting and drying cycles under ambient atmospheric conditions, reduced Ag-sulfur species were still dominant in biosolids produced from the anaerobic digestion of sewage sludge spiked with 50 mg kg^{-1} Ag as either $AgNO_3$ or Ag-NPs. This is explained by the extremely low solubility product of Ag_2S (K_{sp} = 5.02 x 10^{-51}) as well as the relative stability of Ag bound to reactive organic sulfur groups such as cysteine (log k_f = 11.9) ((Levard et al., 2012). The existing literature reporting on the environmental chemistry and fate of Ag strongly indicates that the oxidation of reduced sulfide species is not thermodynamically/kinetically favourable under relevant environmental conditions (e.g. Elechiguerra et al., 2005; Levard 2011, 2012, 2013; Sekine et al., 2015; Carbonaro et al., 2005 etc). For example, in their review of the structural

chemistry and geochemistry of silver-sulfur compounds, Bell and Kramer (1999) noted that Ag(I) binds strongly with sulfur(II-) in inorganic and organic species, and Ag(I) thiolates react rapidly with H_2S or HS_2 to form Ag2S, but the reverse process is poor and this reaction thus represents a possible final fate for Ag(I) thiolate and Ag(I)-S(II-) species. In a very comprehensive study investigating metal release from metal-spiked sediments, Carbonaro et al (2005) also demonstrated the significant recalcitrance of silver sulfide to oxidation relative to other metal sulfides, and in fact, the affinity of Ag for S is so great that it even has a tendency to replace other metals in sulfide minerals when they are oxidising (Chen and Lin (2009),

In Figure 1, speciation data for a time series of 6 biosolids sourced from a single municipal wastewater treatment plant and aged in outdoor stockpiles for up to 5 decades is presented. These results clearly demonstrate that the reduced-sulfur-Ag species in biosolids are extremely stable over time, with LCF indicating this form to account for at least 77 % of the average Ag speciation in all 6 samples, despite them having been exposed to extended weathering. Interestingly, the older samples, which had been ageing since the 1970s, showed the highest percentage of reduced sulfur-Ag species (88 - 92 %). Aside from confirming their long term stability, this could also indicate that Ag was progressively added to this recalcitrant pool as part of the ageing process, and/or that some of the more labile minor species had solubilised and been (partly) leached from the materials during stockpiling. As the initial mass of fresh biosolids and corresponding Ag concentrations for these long-term stockpiled samples are unknown, it is no longer possible to determine this directly, but it is worth noting that in complex environmental media such as biosolids, bulk XANES analysis is very useful for directly identifying major chemical species but is insensitive to small changes in minor species (e.g. free Ag^+) which may nevertheless have important influences on the

bioavailable fraction. Thus, to further investigate the lability and potential bioavailability of Ag in these samples, the ^{110m}Ag isotopically exchangeable fraction and 5 mM $CaNO_3$ extractable fractions were also determined.

^{110m}Ag isotopically exchangeable silver and 5 mM $CaNO_3$ extractable silver

The size of the labile pool, as determined by isotopic dilution (i.e. E-value), has major implications for element mobility and bioavailability. An element pool containing a large proportion of highly insoluble, stable species such as Ag_2S is expected to result in low lability, however E-values reflect more than just the effects of element speciation (Degryse et al., 2011). For example, Ag ions bound internally within a mineral lattice are expected to be less isotopically exchangeable than Ag ions bound to the lattice edges of the same mineral, while Ag ions occluded within particles of organic matter should likewise be less labile than ions adsorbed to the external surfaces. In the samples examined here, isotopic dilution with ^{110m}Ag showed that, in all cases, in both fresh and aged biosolids, the Ag was predominantly in a non-labile form, with E-values ranging from 0.3 to 60 mg kg^{-1} (Table 1). Taking the total Ag concentration into account and expressing these results as the percentage of total Ag that is readily labile (as per Equation 2), the maximum lability was found to be 30.6 %, with an average of 13.7 % (n=20). In comparison with the lability of other contaminant metals in aged biosolids, this is relatively low. For example, Cu and Cd lability in aged biosolids have been shown to increase to > 50 % over just 3 months of ageing under laboratory conditions (Donner et al., 2013).

Interestingly, Ag lability was lowest in the stockpiled samples which had been subject to extended outdoor weathering (median lability of 1.3 % in samples that had been weathering

for at least 10 years). Previous research has shown that changes in metal speciation (e.g. oxidation of metal sulfide minerals) associated with biosolids ageing/composting may be accompanied by a significant increase in lability (Donner et al., 2013), but this was not observed in the extended time series of samples examined here. The persistent low lability is in keeping with the XANES LCF results, which indicated that insoluble and recalcitrant Ag species continued to dominate in these samples even after 50 years of weathering and ageing. Furthermore, as shown in Figure S5, there is a correlation between Ag lability and the proportion of biosolids Ag bound with reduced sulfur species (which are presumably non-labile), with lability tending to be lower in biosolids containing greater proportions of reduced sulfur bound Ag. As noted above, it is possible that ageing reactions (e.g. diffusion) during stockpiling contributed to the observed reduction in the labile Ag pool as a function of stockpiling time, and/or that a proportion of the labile Ag was solubilised and leached from the weathering biosolids during stockpiling leaving the less soluble Ag_2S species behind. Decreasing metal lability as a function of ageing is a commonly recognised phenomenon and has recently been observed in soils spiked with ionic Ag (Settimio et al., 2014), while the leaching hypothesis is loosely supported by the relatively low pH of the stockpiled samples, which could reflect a combination of base cation leaching and H^+-producing metal hydrolysis reactions. Even so, due to its highly reactive nature and propensity to form thermodynamically stable species (e.g. Ag_2S, $AgPO_4$, $AgCl$), the proportion of free Ag in most environmental samples is expected to be very low. Biosolids are very complex matrices which contain a myriad of reactive mineral and organic phases with highly reactive binding sites, and any free Ag^+ is likely to rapidly react in these materials. For example, in an incubation study where $AgNO_3$ was added to 6 soils, Settimio et al. (2014) found that ^{110m}Ag isotopic lability decreased rapidly, with up to 90 % of the added Ag becoming non-labile within the first 2 weeks after spiking. In view of these findings, it seems unlikely that

significant quantities of Ag will have leached from the biosolids during stockpiling, but further research is needed to investigate this directly, along with the potential for release following soil application.

Given the extremely high toxicity of Ag^+ to microorganisms and the common use of biosolids in agriculture, it is also worth considering the amount of 5 mM $CaNO_3$ extractable Ag (Table 1). These data are generally in keeping with the other results presented, with relatively low total percentages of extractable Ag when compared against total Ag concentrations. For instance, the mean 5 mM $CaNO_3$ extractable Ag ranged from 1.2 to 609 µg kg^{-1}, or from 0.002 to 3.4 % of the total Ag. Only two of the samples had 5 mM extractable Ag corresponding to more than 1.1% of the total Ag concentration. Although these concentrations may seem low, it must be recalled that Ag is extremely toxic to microorganisms. For example, published reports of minimum inhibitory concentrations (MICs) for susceptible bacteria in axenic liquid cultures have been reported to be in the range of 0.3 µg Ag^+ ml^{-1} (Lok et al., 2008), 0.05 – 0.2 (Radzig et al., 2009), and 0.06 – 0.2 (Radzig et al., 2013), while the EC_{50} of Ag on the soil nitrification process has been reported to be as low as 0.43 mg Ag kg^{-1} (Langdon et al., 2014). This does vary substantially however on the basis of soil type, with EC50 > 640 mg Ag kg-1 also reported for some soils in that study

Implications for research and management

As noted previously, much of the recent interest in the environmental fate and effects of Ag is due to the increasing use of nanosilver as an antimicrobial agent. Although the total mass of Ag used in this manner is relatively small in comparison with other industrial and commercial uses, many nanosilver applications (e.g. in personal care products and textiles) are dispersive in nature (Donner, 2010), and wastewater is the principal receiving pathway. This contrasts

with other major uses where resource capture and recovery are used to both economic and environmental advantage. Moreover, with concentrations in some contemporary biosolids reportedly in the order of hundreds of mg kg^{-1} (EPA, 2009) the need for ongoing biosolids Ag monitoring is apparent, particularly in countries where biosolids are used in agriculture. In addition, the capacity of soils to accommodate Ag loads without detrimental effects on soil health and productivity still remains to be determined.

Despite the wide range of dispersive commercial and industrial uses that contribute to wastewater Ag loads, and the variety of biosolids processing and storage scenarios, the presented data strongly support previous reports that Ag undergoes strong sulfidation reactions in wastewater; and uniquely, these data also confirm that the reduced sulfur species formed during treatment remain stable despite extensive weathering over multiple decades. In fact, Ag$_2$S is thermodynamically favoured under relevant environmental conditions (Levard et al., 2012, 2014) and is recognised as one of the most insoluble inorganic compounds known (Lytle, 1984). The dominance and persistence of this form of Ag in biosolids has important implications for subsequent ecotoxicity and selective pressure on microbial communities in biosolids-amended soils, as sulfidation of silver (both Ag+ and AgNPs) has been reported to reduce effective silver toxicity (Reinsch et al., 2012; Leblanc et al., 1984), and may therefore mitigate the risks associated with AgNPs (Levard et al., 2012, 2013).

On the basis of the results of this and other recent studies it is clear that future ecotoxicological studies should include experiments focussed directly on Ag as it occurs in biosolids, as well as on major component species such as Ag$_2$S, as these are the forms most relevant to the wastewater-biosolids-soil pathway. Recent studies moving in this direction have shown that Ag$_2$S nanoparticles added directly to soil are also stable for extended

periods. Sekine et al (2015) showed that Ag_2S nanoparticles added to soil remained stable over a seven month period, but although labile Ag assessed by diffusive gradient in thin film (DGT) was very low at the end of the incubation period, it was still detectable. In another study, a small but detectable remobilisation of Ag_2S-NPs was observed over 24 h in a batch experiment in the presence of environmentally relevant ligands (Navarro et al., 2014). Therefore, further consideration should be given to the lability of sulfidised Ag species and its relevance for soil health and productivity.

Acknowledgments

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Figure 1. Silver K-edge XANES spectra (solid lines) and linear combination fits (dotted lines) for a time series of Australian biosolids samples from outdoor stockpiles at a single municipal wastewater treatment plant (left). Bar chart showing the relative proportions of standard materials identified as major spectral components by linear combination fitting (right).

Table 1. Provenance and key chemical characteristics of biosolids samples examined in this study. For pH (0.01 M CaCl$_2$), EC, total C, total S, and total Ag, n=1. For 5mM Ca(NO$_3$)$_2$ extractable Ag, ^{110}Ag E-values, and labile Ag, n=3. For n=3, the reported values are mean values with the standard error given in brackets.

Sample Name	Production year(s)	Source of sample*	WWTP Location	pH (H$_2$O)	pH (0.01M CaCl2)	EC (mS/m)	C (%)	S (%)	Total Ag (mg/kg)	5 mM Ca(NO$_3$)$_2$ extractable Ag (µg/kg)	5 mM Ca(NO$_3$)$_2$ Extractable Ag (%)	^{110}Ag E-val (mg/kg)	Labile Ag (%)
Macquarie Point	2009	Sludge belt press	Australia	6.3	6.1	5.8	30.4	1.0	14.1	32.0 (10)	0.2 (0.1)	2.1 (0.3)	15.2 (1.9)
Merrimac	2009	Sludge belt press	Australia	6.0	5.7	3.7	34.0	0.8	4.3	6.3 (1.0)	0.1 (0.0)	0.3 (0.0)	6.3 (0.7)
Melbourne A	2009	Outdoor stockpile	Australia	6.6	6.2	2.0	18.7	0.8	17.5	4.9 (0.0)	0.03 (0.0)	0.7 (0.0)	4.2 (0.1)
Katherine	2009	Drained lagoon (3 Month)	Australia	5.9	5.9	3.9	27.7	1.5	38.7	13.8 (0.7)	0.04 (0.0)	2.2 (0.0)	5.7 (0.1)
Rushmoor	2009	Archived sample	UK	6.8	6.7	4.7	27.9	1.1	18.4	202 (12)	1.1 (0.1)	2.5 (0.1)	13.6 (0.6)
Gleadthorpe	2009	Archived sample	UK	6.9	6.8	4.8	31.3	1.1	18.1	609 (69)	3.4 (0.4)	4.4 (0.4)	24.6 (2.5)
EPA 355	2006	Archived sample	USA	7.7	7.0	1.0	27.4	0.8	10.7†	48.8 (2.1)	0.5 (0.0)	1.2 (0.0)	11.5 (0.3)
EPA 361	2006	Archived sample	USA	7.3	6.7	0.8	34.7	1.3	31.6†	ND	ND	ND	ND
EPA 371	2006	Archived sample	USA	7.2	7.0	3.6	32.9	2.2	18.8†	216 (10)	1.1 (0.1)	5.8 (0.2)	30.6 (1.1)
EPA 336	2006	Archived sample	USA	7.4	7.2	2.2	33.2	2.7	125†	ND	ND	ND	ND
Melbourne B	1990s/2000s	Outdoor stockpile	Australia	4.7	4.5	2.1	22.8	0.7	58.3	1.3 (0.0)	0.002 (0.0)	3.7 (0.2)	6.4 (0.3)
Melbourne C	1980s/2000s	Outdoor stockpile	Australia	6.1	5.6	2.1	32.9	1.2	35.4	13.2 (0.2)	0.04 (0.0)	1.0 (0.0)	2.8 (0.1)
Melbourne D	1980s/1990s	Outdoor stockpile	Australia	4.8	4.7	2.6	24.5	0.8	74.0	2.1 (0.1)	0.003 (0.0)	3.5 (0.1)	4.7 (0.2)
Banbury	1996	Archived sample	UK	6.0	5.9	4.0	37.4	1.3	22.2	299 (50)	1.3 (0.2)	5.0 (0.2)	22.5 (1.0)
Colesworth	1996	Archived sample	UK	6.0	6.0	4.8	31.9	1.2	59.9	120 (4.4)	0.2 (0.0)	16.2 (0.3)	27.0 (0.6)
Perry Oaks A	1996	Archived sample	UK	6.1	6.0	3.0	10.8	0.4	96.7	14.4 (0.4)	0.01 (0.0)	19.9 (1.1)	20.6 (1.1)

Melbourne E	1970s/1980s	Outdoor stockpile	Australia	4.3	4.1	3.1	22.0	1.1	58.6	1.2 (0.0)	0.002 (0.0)	2.2 (0.2)	3.7 (0.3)
Melbourne F	1970s & earlier	Outdoor stockpile	Australia	4.7	4.3	0.9	27.9	0.7	54.6	1.3 (0.0)	0.002 (0.0)	1.6 (0.1)	2.9 (0.2)
Nu-Earth	1970s	Archived sample	USA		5.8	ND	18.0	0.8	61.5	14.4 (0.1)	0.02 (0.0)	7.1 (0.2)	11.5 (0.3)
Perry Oaks B	1961	Archived sample	UK	5.8	5.9	ND	21.5	0.8	264.5	225 (65)	0.1 (0.0)	60.1 (6.2)	22.7 (2.3)
Perry Oaks C	1955	Archived sample	UK	5.8	5.6	4.3	26.4	0.9	331.6	157 (1.4)	0.05 (0.0)	52.5 (0.4)	15.8 (0.1)
Perry Oaks D	1950	Archived sample	UK	5.6	5.8ha	4.1	24.7	0.7	246.1	453 (42)	0.2 (0.0)	52.0 (1.4)	21.1 (0.6)

* Sample source is classified according to the manner of sample storage prior to analysis for this study. Most were stored in glass archive bottles from the time of production (archived samples), but some were stored in outdoor stockpiles and exposed to the weather for several decades from the time of production until sampling, drying and archiving in 2009 (outdoor stockpile samples). Others were sampled fresh, directly from the wastewater treatment plant and air dried prior to analysis (sludge belt press samples; drained lagoon samples).

[†] Total Ag values for these samples are as reported in the US EPA TNSSS (2009). Samples could not be reanalysed for Total Ag as insufficient sample remained.

ND = Not determined. Insufficient sample mass available for analysis.

Table 2: A summary of the available literature data and survey data reporting total silver (Ag) concentrations in biosolids. This table shows the median Ag concentration, mean Ag concentration, minimum Ag concentration, maximum Ag concentration, and the analytical technique used to measure total Ag in a wide range of samples

Sample Provenance and Year(s) of Production	WWTP Information	Median Ag (mg kg⁻¹)	Mean Ag (mg kg-1)	Min. Ag (mg kg⁻¹)	Max. Ag (mg kg⁻¹)	SE (mg kg-1)	No. of samples	Analytical technique	Reference
Australia: 2009	29 municipal WWTPs spread across all states and territories of Australia.	7.4	10.3	1.5	61.5	2.3	29	Neutron Activation Analysis	Unpublished data collected for this study
UK: 2009	6 municipal WWTPs in England	10.3	11.0	3.4	18.4	2.5	6	Neutron Activation Analysis	Unpublished data collected for this study
USA: 2006-2007	Sewage sludge from 74 randomly selected publicly owned WWTPs in 35 states.	13.6	31.0	1.9	856 (outlier); only 3 values > 125, and 1 value > 195	11.1	78	Acid digest and ICP AES	US EPA TNSSS (2009)
UK: 1996	Sewage sludge from 5 municipal WWTPs in England and Scotland. Banbury, Carterton, Coleshill, Perry Oaks, Selkirk.	22.2	39.2	8.2	96.7	13.6	5	Neutron Activation Analysis	Unpublished data collected for this study
Australia: 1970s-1990s	All 5 samples were derived from the same large municipal WWTP plant.	58.3	56.2	35.4	74.0	6.2	5	Neutron Activation Analysis	Unpublished data collected for this study
USA: 1988-1989	208 WWTPs with at least secondary wastewater treatment were selected from across the USA to represent a national probability sample.	25.5	48.2	Not known	852	112	176 above detection limit (but DL not reported)	Acid digest and ICP AES	Appendix F US EPA TNSSS, (2009) Table 4-8 2009 Stat Report Doc
USA: 1983	6 treatment plants chosen specifically to cover a range of different silver sources in the wastewater catchment	88.8	4612	15 (no major industrial Ag sources in catchment)	27000 (photoprocessing source); next highest value was 450 (also from a photoprocessing source)	4478	6	Acid digest and ICP AES	Lytle, 1984
USA: 1970s	2 biosolids produced in Chicago	46.7	46.7	31.9	61.5	-	2	Neutron Activation Analysis	Unpublished data collected for this study
UK: 1950-1961	3 biosolids produced in London	265	281	246	332	26	3	Neutron Activation	Unpublished data collected for this

								Analysis	study

Table 3. Best fit Ag speciation as identified by Linear Combination Fitting (LCF) of k-edge Ag X-ray Absorption Near Edge Spectroscopy (XANES) spectra (fitting range -25 to 100 eV). Species apportionments 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 ID	Production Year	Ag_2S-NPs/ Ag-cysteine	Ag_3PO_4	Elemental Ag	Ag-Humic acid	R-factor
Australian biosolids time series data presented in Figure 2						
Melbourne A	2009	80 (1.3)	20 (1.3)			0.0001
Melbourne B	1990s/2000s	82 (1.6)	16 (1.2)	2 (1.0)		0.0001
Melbourne C	1980s/2000s	80 (11)	20 (1.7)			0.0002
Melbourne D	1980s/1990s	77 (1.9)	20 (1.4)	4 (1.4)		0.0001
Melbourne E	1970s/1980s	88 (1.4)	11 (1.1)	1 (1.0)		0.0001
Melbourne F	1970s	92 (1.7)	7 (1.3)	1 (1.1)		0.0001
UK biosolids time series data						
Perry Oaks A	1996	77 (0.0)		6 (2.5)	17 (4.1)	0.0003
Perry Oaks B	1961	78 (1.6)	19 (1.2)	3 (1.1)		0.0001
Perry Oaks C	1955	85 (0.0)			16 (0.0)	0.0007
Perry oaks D	1950	81 (1.7)	15 (1.3)	4 (1.1)		0.0001
Other samples analysed from the UK and USA						
Rushmoor	2009	77 (2.8)	22 (3.8)	1 (3.6)		0.0006
Gleadthorpe	2009	60 (1.9)		27 (2.3)	13 (2.7)	0.0002
EPA 355	2006	68 (3.7)	14 (2.7)	18 (2.7)		0.0004
EPA 361	2006	88 (8.0)		4 (1.0)	8 (1.2)	0.0001
EPA 371	2006	64 (8.0)	19 (5.8)	18 (7.0)		0.0002
EPA 336	2006	86 (7.9)		2 (1.0)	12 (11)	0.0001
Banbury	1996	79 (1.6)	16 (2.1)	6 (2.1)		0.0002
Colesworth	1996	79 (2.4)		7 (1.3)	15 (2.1)	0.0001
Nu-Earth	1970s	74 (2.2)	22 (2.1)	4 (1.9)		0.0002

Figure 1. Silver K-edge XANES spectra (solid lines) and linear combination fits (dotted lines) for a time series of Australian biosolids samples from outdoor stockpiles at a single municipal wastewater treatment plant (left). Bar chart showing the relative proportions of standard materials identified as major spectral components by linear combination fitting (right).
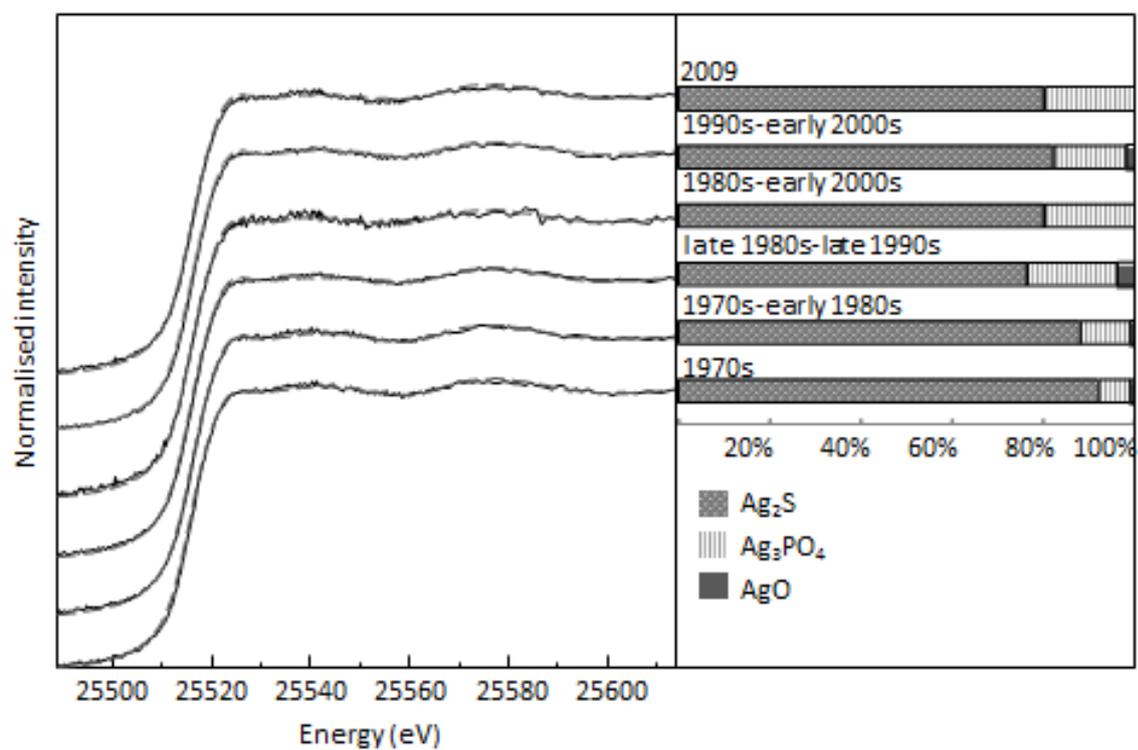
Supplementary Information

Figure S1a. Post-processing of biosolids in South Australia. Biosolids are dried in open-air stockpiles under ambient environmental conditions for up to 3 years with occasional turning prior to application in agriculture.

Figure S1b. Biosolids examined in this experiment had all been air-dried and stored in sealed glass jars as shown here.

Figure S2. Normalised Ag K-edge XANES spectra of standard reference materials identified as likely components during Linear Combination Fitting of the 19 biosolids samples for which XANES spectra were collected (solid lines).
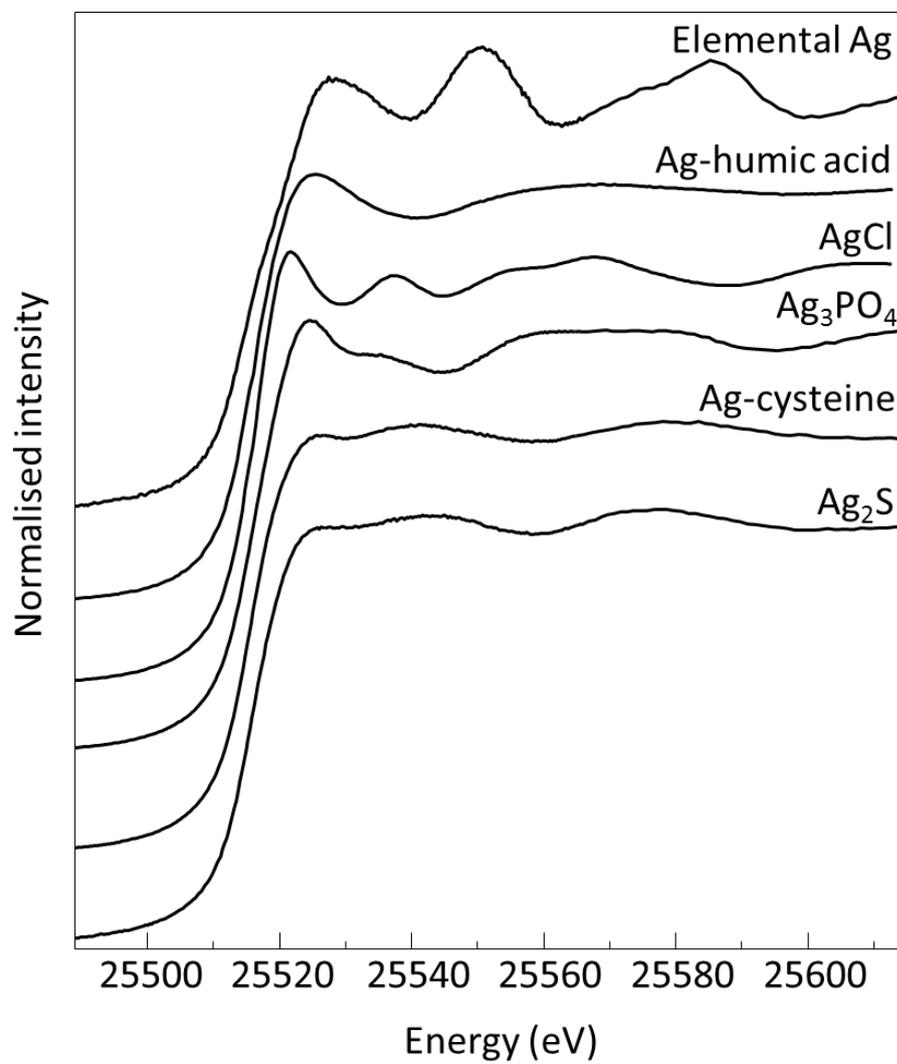
Figure S3. Normalised Ag K-edge XANES spectra of all 19 biosolids samples examined using X-ray absorption spectroscopy (in black), together with the best matching linear combination fit of reference spectra (in red) as documented in Table 3.
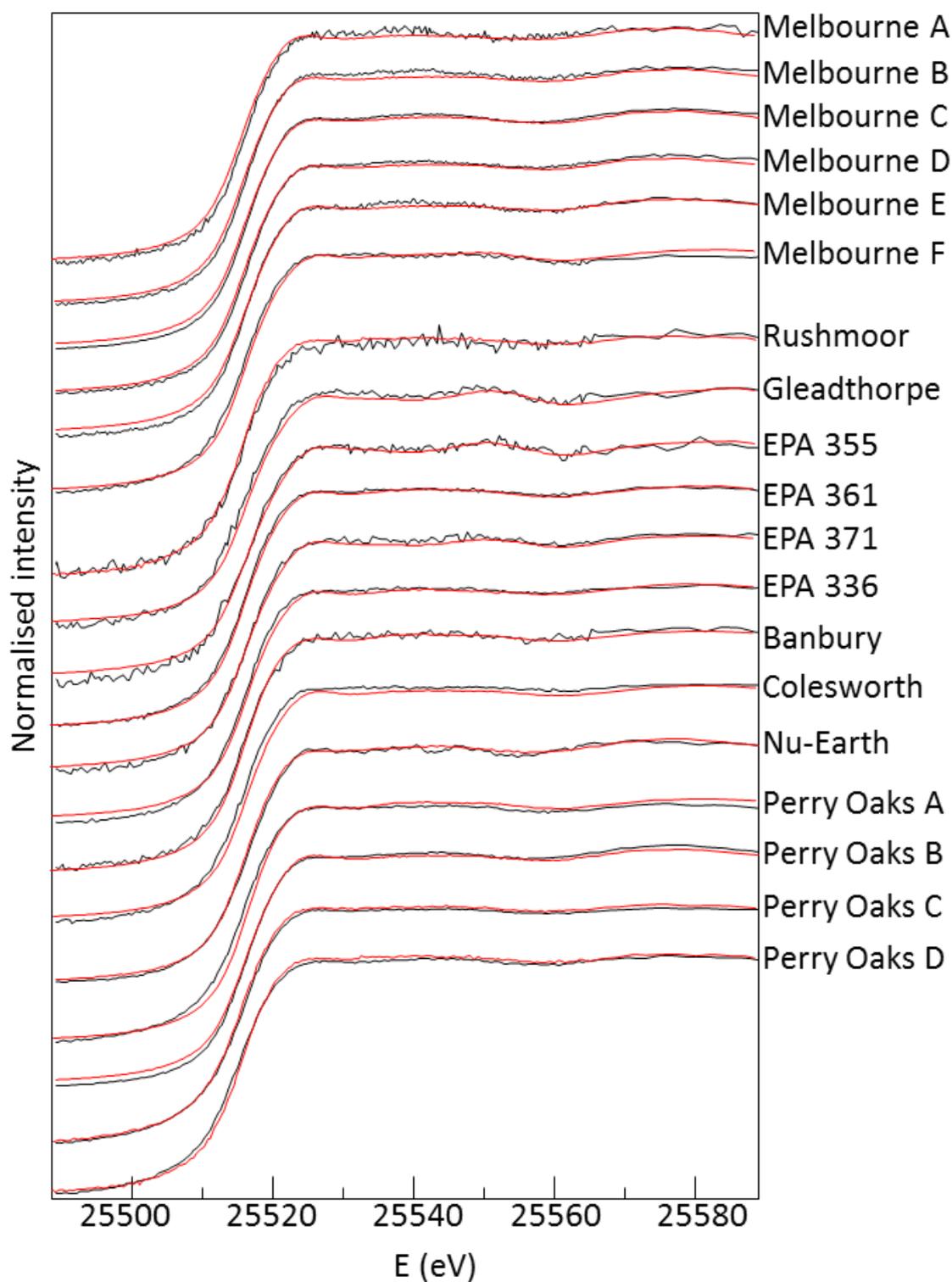
Figure S4. Silver K-edge extended XANES spectra plotted in k-space for 8 biosolids samples. A smoothing factor of 3 has been applied to these spectra (the remaining sample spectra have not been presented as they were too noisy to be meaningfully interpreted). For comparison, Ag-sulfide standard spectra are shown in red and Ag-cysteine standard spectra are shown in blue. Vertical lines indicate the characteristic differences in the oscillations for sulfide (ref) and cysteine (blue) that can be used to distinguish between these species in k-space.
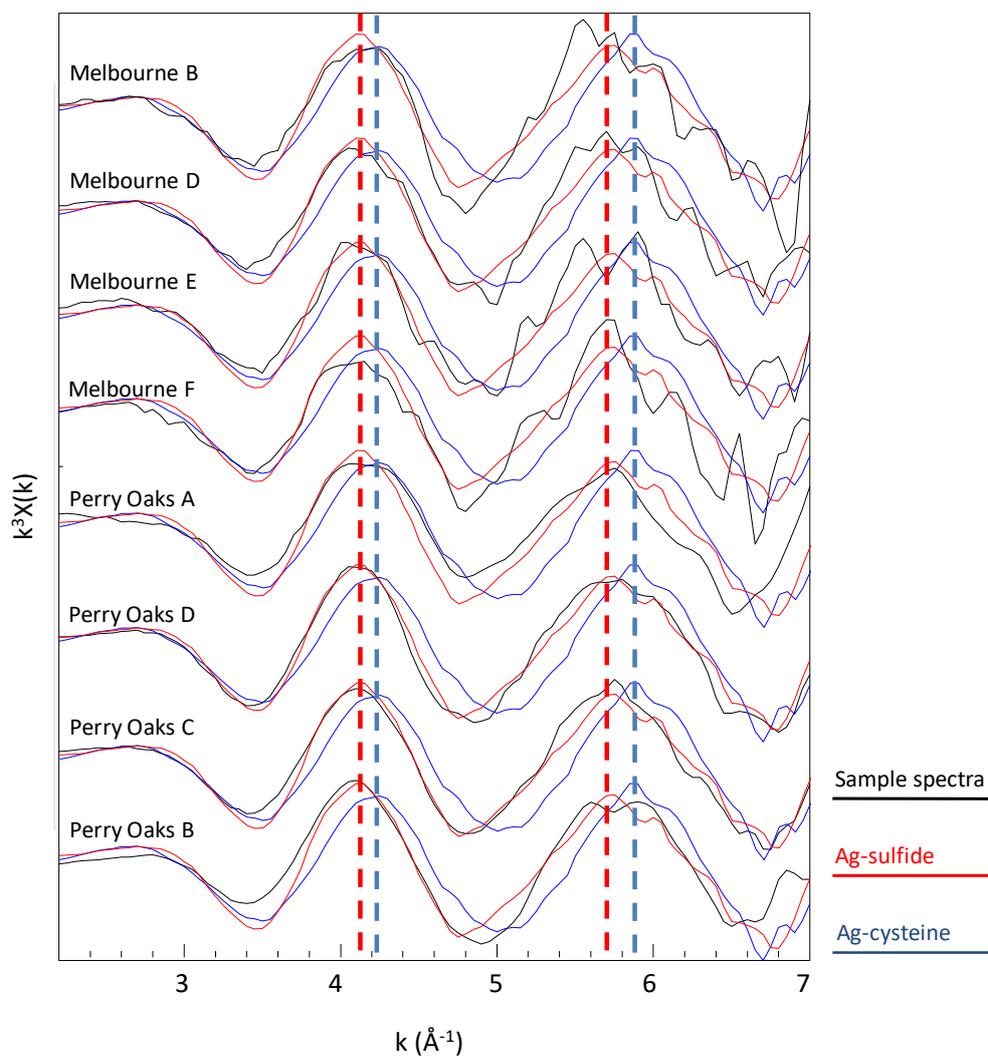
Figure S5. Scatter plot showing the relationship between labile silver in biosolids and the proportion of biosolids silver present in reduced sulfur binding environments (as determined by linear combination fitting of Ag K-edge XANES spectra).
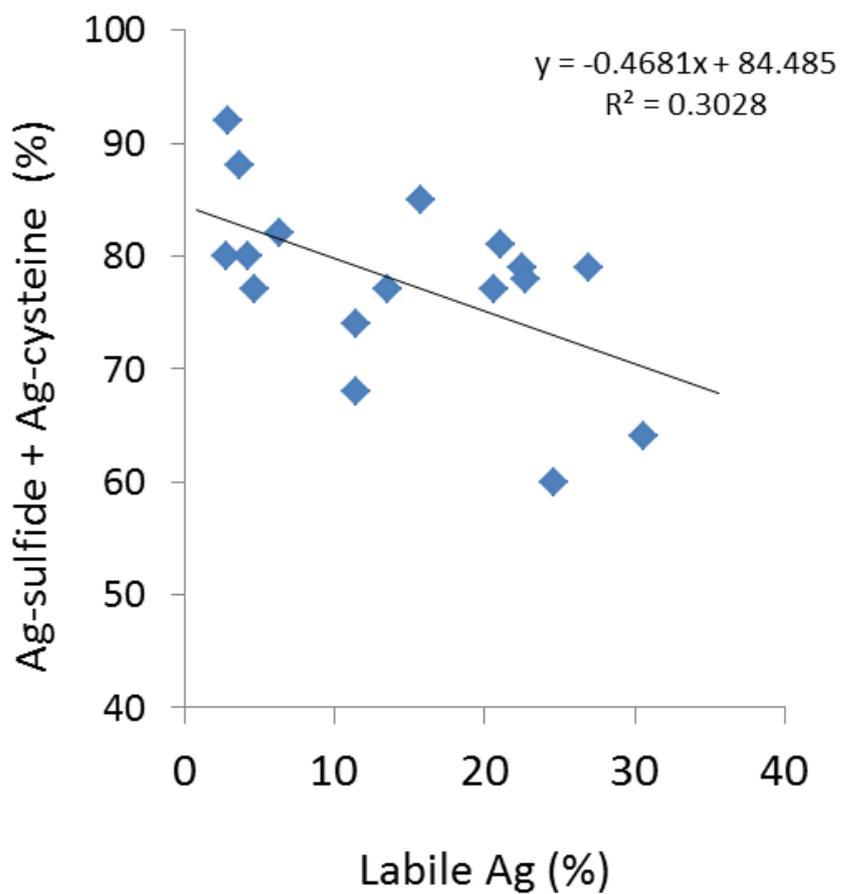

$y = -0.4681x + 84.485$
$R^2 = 0.3028$

Figure S6. Linear regression analysis of key variables, including the year of production, pH, 5 mM CaCl2 extractable Ag, isotopically exchangeable Ag (labile Ag), total Ag, and total S.