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Developing Understanding of the Fate and Behaviour of Silver in Fresh Waters and Waste Waters

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Abstract

The Windermere Humic Aqueous Model (WHAM) is often used for risk assessment of metals; WHAM can be used to estimate the potential bioavailability of dissolved metals, where metals complexed to dissolved organic matter (DOM) are expected to be less toxic than ionic forms. Silver is a potential metal of concern but WHAM has not been rigorously tested against experimental measurements. This study compares WHAM predictions to measured ionic silver during fixed pH (4, 8 or 10) argentometric titrations of DOM from diverse origins. There were almost two orders of magnitude variation in free silver between sources but, within model uncertainty, WHAM captured this variability. This agreement, between measurements and models, suggests that WHAM is an appropriate tool for silver risk assessment in surface receiving waters when DOM is predominantly in the form of humic/fulvic acids. In sewage samples WHAM dramatically underestimated silver binding by approximately 3 orders of magnitude. Simulations with additional specific sulphide-like binding sites could explain Ag binding at low loadings, but not at higher loadings. This suggests the presence of additional intermediate strength binding sites. These additional ligands would represent components of the raw sewage largely absent in natural waters unimpacted by sewage effluents. A revised empirical model was proposed to account for these sewage-specific binding sites. Further, it is suspected that as sewage organic matter is degraded, either by natural attenuation or by engineered treatment, that sewage organic matter will degrade to a form more readily modelled by WHAM; i.e., humic-like substances. These ageing experiments were performed starting from raw sewage, and the material did in fact become more humic-like, but even after 30 days of aerobic incubation still showed greater Ag^+ binding than WHAM predictions. In these incubation experiments it was found that silver (up to 1000 µg/L) had minimal impact on ammonia oxidation kinetics.

Keywords: silver, Windermere Humic Aqueous Model (WHAM), ion selective electrode, sulphide binding sites, risk assessment, metal bioavailability

Introduction

Exposures to ionic silver are known to result in adverse effects in laboratory test organisms at concentrations in the sub microgram per litre range (Hogstrand et al., 1996; Peters et al., 2011). Yet, environmental exposures are rarely in the ionic form and so are likely to be less ecotoxicologically potent (Johnson et al., 2014). Silver can bind to inorganic ligands such as chloride and sulphide, as well as binding to organic ligands such as dissolved organic matter (DOM). An understanding of the chemical form, or speciation, of environmental exposures of silver is critical in assessing the potential risks from silver uses. A developed understanding of the chemical behaviour in an environmental matrix is critical in predicting potential bioavailability (e.g. Brauner and Wood (2002)). It is widely recognised that bioavailability, as a reflection of the exposure of a metal that the organism experiences, delivers the most biologically relevant metric by which to assess potential risk in soils, sediments and waters. Metals, such as nickel and lead, have European wide “bioavailability” based environmental quality standards (EQS)(European Commission (EC), 2013, 2018) and for copper and zinc national bioavailability-based EQS are also available in some countries (e.g. Environment Agency (2009)). It has long been established that water chemistry and especially dissolved organic carbon (DOC) and sulphur compounds in freshwater and effluents can bind with silver, mitigating ecotoxicity and influencing its behaviour and fate (Di Toro et al., 2001; Hiriart-Baer et al., 2006). Despite recognising the importance of water chemistry upon silver in freshwaters regulatory effrts to incorporate this understanding into EQS setting are lacking (Sahlin and Ågerstrand, 2018).

Silver has been identified as a potential substance for prioritisation as a potential EU-wide EQS. One of the lines of evidence used to facilitate this risk-based prioritisation is the consideration of measured national silver exposures. Unfortunately, these data are diffiCult to interpret, as measured silver data in fresh and marine waters are often below detection; for example, in Carvalho et al. (2016), less than 9% of the more than 13,000 samples were quantifiable. Trying to handle “non-detects”, renders the summary statistics used for the risk-based approach extremely uncertain (Hites, 2019).

Ionic silver is known to have antimicrobial properties and is utilized in biocides, medical applications and commercial personal-care products for this purpose (Wakshlak et al., 2015); for similar reasons the use of silver nanoparticles has also increased, such as in treated clothing (Emam et al., 2013). Thus, there is potential for silver input to the environment, e.g., via municipal wastewater treatment efflents. With the increased discharge of silver to the environment, it is necessary to have in place methods to assess the risk of silver in aquatic environments.

There are many approaches to assessing the risks posed by discharges of silver into the environment. Bioavailability based approaches take the reactivity of different chemical forms of an element into account; the most bioavailable forms are expected to have greater toxicity. Such approaches have been useful in setting appropriate and protective regulatory guidelines for some other metals, such as aluminum, cadmium, cobalt, copper, nickel, lead and zinc (Adams et al., 2019). To properly accomplish this for silver, the greatest current data gap is a detailed understanding of the role of reduced sulfide in silver binding, and ultimately in toxicity mitigation by decreasing the concentrations of potentially toxic bioavailable forms of silver. It is well understood that silver binds strongly to reduced sulfide ligands but measuring sulfide is not part of routine analysis when characterizing samples for risk assessment purposes. Even with sulfide data, there do not currently exist validated computer models to estimate bioavailability from measured water chemistry (i.e., measurements such as dissolved organic carbon (DOC), pH, major cations, major anions, inorganic sulfide and organic sulfide (thiol)). Thus, regulated industries do not yet have sufficient information or tools to make appropriate risk-based management decisions for Ag in the environment. There is a need to improve the understanding of the behaviour and fate of silver in environmental systems in order to ensure decisions in regard to potential risk and environmental stewardship are evidence-based.

It is the aim of the work described in this paper to determine the chemical availability of silver in natural freshwater and specifically improve the understanding of silver fate and behaviour to facilitate a scientific evidence driven approach to environmental risk assessment. Chemical speciation models, such as the Windermere Humic Aqueous Model (WHAM, (Tipping et al., 2011)), have been widely used in the derivation and implementation of water quality standards for metals such as copper, nickel and zinc in Europe (e.g. Merrington et al. (2016)). The silver data upon which WHAM is calibrated (see Supplementary Material associated with Tipping et al. (2011) available at http://www.publish.csiro.au/en/acc/EN11016/EN11016_AC.pdf) is limited to two studies (Sikora and

Stevenson, 1988; Rader et al., 2005); therefore, delivering a greater understanding of silver behaviour in freshwater requires improvement in the basis of the predictions, and subsequent validation of the model for silver. In addition, it is recognized that diffrent sources or dissolved organic matter may have different binding characteristics with metals (Al-Reasi et al., 2011b), thus a diversity of sources are studied here, including treated wastewater effluents. Finally, a range finder toxicity test was performed to determine the impact of silver on the natural attenuation treatment of wastewater; i.e., addressing whether or not silver impacts the kinetics of nitrogen oxidation in raw sewage.

2. Methods

2.1. Silver Ion Selective Electrode Titrations

The silver electrode is one of the most sensitive of the solid-state ion selective electrodes (ISE). There has been limited work titrating NOM samples using a silver ISE though (Sikora and Stevenson, 1988; Smith et al., 2004). Initial effrts for this study involved establishing best practices for silver ISE titrations and best operating conditions. Experimental methods requiring optimization included the calibration method (internal or external) as well as the sample exposure method (flow through or static). The titration curves in this report were all obtained using internal standard calibration and flow through ISE. This decision was based on best recovery of known [Ag^+] values for silver titration of EDTA (ethylenediaminetetraacetic acid). The EDTA titration data are presented in the Supplemental Material associated with this manuscript (Figure SI1).

A Cole Parmer Silver/Sulfide ISE was utilized for all experiments with an Orion double junction Ag/AgCl reference electrode (Model 900200, Boston, MA, USA). For flow through experiments a micro-Flowcell (FIAlab, Bellevue, WA) was utilized. Each electrode was connected to a potentiometer (Tanager, Model 9501, Ancaster, ON). For flow through experiments a valveless metering pump, the Cerampump FMI “Q” Pump (GQ6, Fluid Metering Inc., Syosset, NY) was used to deliver the test solution through the system. This type of pump was found not to interfere with the low current flow potential measurements of the electrode.

The potential (E) of the silver ion selective electrode responds to free silver ion concentration ([Ag^+]) according to the Nernst equation:

$$E = E_0 + m\log [Ag^+] \quad (1)$$

Equation 1 was used to calibrate the electrode in order to convert measured potentials into [Ag^+] values. At 25 _C, the Nernstian slope (m) typically has a value of 59.2 mV/decade of concentration for monovalent ions such as silver. For this study, an internal one-parameter calibration was used to determine E_0, the intercept of the calibration line; the theoretical Nernstian value for the slope in Equation (1) was assumed. All samples had intrinsic or added chloride ions, and once enough silver had been added during titration, the ion product for solid silver chloride would exceed the K_{sp} value and solid would precipitate. The presence of solid AgCl buffers the free silver ion and the electrode potential will remain constant. This constant mV response was used to solve for E_0 in Equation (1), again assuming a Nernstian slope. In order to perform this operation the [Ag^+] in this “bu_ered zone” must be determined.

This is accomplished assuming the only relevant species are $AgCl_{(s)}$, Ag^+ and Cl^-; using these species the mass balance and mass action equations can be written in terms of $[Ag^+]$ as the only variable:

$$[Ag^+]^2 + (Cl_T - Ag_T)[Ag^+] - K_{sp} = 0 \quad (2)$$

Equation 2 can be solved (e.g., by root finding) to determine $[Ag^+]$, using the measured total chloride in the sample (Cl_T), and the known total silver added at the end of the titration (Ag_T). The simplified speciation during the calibration is valid as long as the chloride concentration is much higher than other potential ligands (i.e., DOC) in the sample. A similar one-parameter internal standard method has been successfully utilized for a copper ion selective electrode in salt water (Tait et al., 2015).

2.2. Sample collection and characterization

Sample locations, and types of organic matter sources, utilized in this study are summarized in Table 1. Dissolved organic matter (DOM) was collected using a portable reverse osmosis (RO) unit. For details on reverse osmosis, see Sun et al. (1995), and previous publications using the same RO system and sample locations (Al-Reasi et al., 2011a). The prefilter on the RO unit was a 1 _m wound string filter. Dark, terrigenous (allochthonous) DOM was collected from Luther Marsh, while mixed autochthonous/allochthonous DOM was collected from Bannister Lake. Sewage-derived NOM samples were collected from the effluent of the Dundas Sewage Treatment Plant, as well as Burlington Bay, where effluent mixes with autochthonous carbon from Lake Ontario. Finally, raw sewage (i.e., not RO concentrates) from the University of Waterloo, and at Ashbridges Bay treatment plant in Toronto were collected. The University of Waterloo sewage is municipal sewage collected by means of a sump pump inside the sewer. Two reference NOM samples, Suwanee River and Nordic Reservoir, both obtained from the International Humic Substances Society (IHSS), were also used for silver titrations. All RO samples were stored in a cold room at 4 _C until use. Titrations were performed using approximately 100 mg C L^{-1}. The actual DOC concentration in titrated samples are indicated in Table 2, as well as measured total chloride concentration. For each sample at least one replicate titration was performed. Initial testing demonstrated that lower concentrations of DOC did not show sufficiently low detection limit to “probe” strong binding sites and higher concentrations resulted in lower binding site estimates per mg of carbon. It is suspected that at high DOC concentrations DOM-DOM interactions remove binding sites from availability towards silver, as has been observed before for lead and copper (Nadella et al., 2013; Cooper et al., 2014). It should be noted that the RO procedure concentrates all components of the initial sample so inorganic ligands (i.e.,

chloride) are elevated in addition to DOC. The RO concentrates are still representative of natural samples; although the ligand concentrations are elevated for analytical reasons, the carbon to chloride ratio is the same in the concentrate as in the original source water. To remove potential metal contamination, the reverse osmosis-derived NOM concentrates from each site were passed through a cation exchange resin (Amberlite IR-118H, Sigma), acidified (pH 2), and then stored in the dark, at 40^0C, in 4L polyethylene bottles until used in experiments or analysed (e.g. Schwartz et al. (2004); Winter et al. (2007)).

Table 1. Samples used in this study

Code	Name	Coordinates	Comment
SR	Suwannee River NOM	obtained from IHSS	reference NOM
NR	Nordic Reservoir NOM	obtained from IHSS	reference NOM
LM	Luther Marsh	$43^0$54’17.5’’N $0.0^0$24’34.9’’W	allocthonous
BL	Bannister Lake	$43^0$17’34.7’’N $080^0$23’14.0’’W	mixed autochthonous/allochthonous
BB	Burlington Bay	$43^0$18’03.3984’’N $079^0$50’33.8280’’W	mixed sewage/autochthonous
DC	Dundas Canal	$43^0$15’59.2308’’N $080^0$32’19.5576’’W	sewage effluent
UW	University of Waterloo	$43^0$28’14.5092’’N $080^0$32’19.5576’’W	raw sewage
AB	Ashbridges Bay	$43^0$39’25.6’’N $079^0$19’15.0’’W	raw sewage

Organic thiol was quantified using a fluorometric thiol assay kit (MAK151-1KT, Sigma Aldrich, Toronto, ON, Canada). Measurements were performed using a SpectraMAX Gemini XS microplate reader (Molecular Devices, Sunnyvale, CA, USA). Chromium reducible sulphide (CRS) was measured using the method originally developed by Bowles et al. (2003). The method was slightly modified in that Cr(II) was purchased directly from Sigma-Aldrich (Toronto, ON, Canada) instead of being generated from Cr(III) salt using a Jones Reductor column (i.e., mercury and zinc amalgam). To ensure high quality data several sulphide standards were run and 500, 1000 and 2000 nM standards gave 96.7, 97.7, 103 % recoveries respectively.

Absorbance and fluorescence spectroscopy have frequently been successfully employed to distinguish the molecular variability among natural samples from various sources, as well as between fulvic and humic acids from the same source. Optical properties such as the specific absorbance coefficient, SAC (estimated as 2.303 × absorbance at a specific

wavelength, often 340nm and, normalized to TOC) (Curtis and Schindler, 1997) and the fluorescence index (FI, determined as fluorescence intensity at 450 nm/fluorescence intensity at 500 nm; both taken at excitation wavelength of 370 nm) (McKnight et al., 2001) have been reported to distinguish NOM sources and composition. Fluorescence measurements were performed with a Cary Eclipse fluorescence spectrophotometer (Agilent, Toronto, ON, Canada) and absorbance spectra were measured using Cary 50 Bio spectrophotometer (Agilent, Toronto, ON, Canada). For all optical measurements 1 cm quartz cuvettes (Starna Cells, Inc. Atascadero, CA, USA, model 3-Q-10) were utilized.

The total DOC concentration was measured directly using a Shimadzu TOC-VCPH/CPN total organic carbon analyzer (Shimadzu Corporation, Kyoto, Japan). The reproducibility of the TOC analyzer using standard total carbon solutions of 5 and 10 mg L^{-1} (prepared from primary standard potassium hydrogen phthalate (KHP)) yielded 5.26 ± 0.66 mg C L^{-1} (n = 44) and 10.24 ± 0.43 mg C L^{-1} (n = 77). Chloride was determined using a chloride ion selective electrode (Chloride ionplus Sure-Flow Solid State Combination ISE, Thermo Scientific) calibrated using NaCl solutions in the range 1.00 µM to 0.0100 M.

2.3. Speciation Modelling

Speciation modelling was performed using WHAM7 (Tipping et al., 2011). Data analysis and plotting was performed utilizing Matlab R2019a (MathWorks , Natick, MA, USA) including the statistics toolbox for regression analysis.

2.4. Sewage Incubation Experiments

Raw sewage obtained from the University of Waterloo (UW) was used for two incubation experiments while the total chloride concentration was low enough to add sufficient silver to avoid AgCl precipitation. For unknown reasons, possibly due to road-salt additions, the UW source dramatically increased in chloride concentration, and Ashbridges Bay (AB) from Toronto was used for a third incubation experiment. These experiments were intended as “range finder” experiments to see if high concentrations of added silver would have any negative impacts on the potential treatment of sewage using natural attenuation process; in particular, the oxidation of more toxic ammonia to less toxic nitrate. The ammonia used in these experiments was just the ambient ammonia originally present in the sample. There was no spiking of additional ammonia. For each nominal dose of silver, 3 L of unfiltered raw wastewater was incubated in a fume hood, with aeration and constant temperature of 24 ± 2^0C for approximately 30 days. For each dose there were two replicate 4 L graduated glass bottles. The sewage samples were aerated using air pumps and air stones; the bubbles also provided mixing. Every day evaporative losses were replaced using deionized water. During the course of the experiment ammonia, nitrite and nitrate were measured in filtered samples

from the treatments, using Hach kits and a Hach spectrometer (DR3900 Hach Spectrophotometer). Subsamples were taken for DOC analysis (for method see above, including 0.45 µm filtration) as well as dissolved silver analysis. Dissolved silver was determined by graphite furnace atomic absorption spectroscopy (Perkin Elmer PinAAcle 900T AA). Experimental setup is shown in Figure SI2 and SI3 for clean water setup testing and actual sewage incubations, respectively.

3. Results and Discussion

3.1. Sample Characteristics

The samples span a range of quality parameters (Table 2). Chloride was highest in the BB and DC samples impacted by sewage effluent. This limited the upper end of the titration range, as AgCl(s) precipitation would begin at lower total silver concentrations. Chromium reducible sulfide normalized to DOC spanned more than an order of magnitude in concentration with values as low as 2.7 nmol CRS/mg C for the terrigenous NR sample and up to 95 for the UW sewage sample. Thiol concentrations spanned a much lower range with the sewage impacted BB sample actually being the lowest (44 nmol/mg C) and the highest being the other sewage impacted sample approximately 3X higher (138 nmol/mg C). The two estimates of reduced sulphur measure different things. CRS does not respond to orgainc thiol and measures only inorganic reduced S, such as in colloidal metal sulphides (Bowles et al., 2003). The thiol assay only measures the organic thiol group content. The ionic silver measured at the Ag_T/DOC *Ceriodaphnia dubia* EC50 value (Naddy et al., 2017) spans almost 4 orders of magnitude with UW showing the lowest value ($10^{-11.8}$ M) and NR the highest value ($10^{-8.12}$ M). In terms of metal complexation, as long as the complex is assumed to be 1:1, it is the metal to ligand ratio that determines the fraction of metal that is complexed by DOM. For this reason, the total added silver for each titration is presented normalized to the corresponding DOC value for that sample.

As expected, the SAC340 values were highest for the most terrigenous samples (LM, SR, NR) all with values near 30, and the more authochthonous and/or sewage samples (BL, BB, DC) with values closer to 10. Similarly the FI values clearly demarkated the same more authochthonous samples with the lowest values from the higher value autochthonous samples. The results of the absorbance and fluorescence optical characterizations are consistent with previous surveys of SAC340 and FI (Al-Reasi et al., 2011b). Original fluorescence excitation emission matrices (FEEMs) are presented in Figure SI4. These scans demonstrate the typical terrigenous shapes (two excitations around 250 and 350 with emission around 450 representing humic substances) for the NR and SR samples. The mixed source DC and BB and BL sources show more authochtonous characteristics with

shorter wavelength fluorescence peaks indicating proteinaceous material. The sewage sample (UW) showed the greatest proportion of protein-like fluorescence at these shorter wavelengths with the largest emission at wavelengths very similar to tryptophan (i.e., excitation around 240 nm and excitation at 350 nm). In the supplementary information table SI1 summarizes the usually reported parameters specific to the UW wastewater sample.

Table 2: Sample optical and chemical characterization. *a* DOC of titrated samples. *b* the logarithm of the silver ion concentration measured at the Ag_T:DOC ratio equivalent to the *Ceriodaphnia dubia* EC50 value measured by Naddy et al. (2017). Uncertainties reported as standard deviations on duplicate measurements. The "-" symbols indicate missing data where the samples were not measured.

Code	DOC[a] mg/L	Chloride µmol/mg C	CRS nmol/mg C	Thiol nmol/mg C	SAC_{340}	FI	$\log[Ag^+]^b$
SR	85	3.0	4.28±0.57	120±9	28.1	1.04	-8.89±0.52
NR	89	6.0	2.69±0.01	72±40	30.7	1.14	-8.12±0.20
LM	99	9.0	2.85±0.23	117±1	36.6	1.00	-8.87±0.23
BL	83	40	8.10±1.02	109±18	11.6	1.38	-9.07±0.01
BB	81	600	7.49±2.52	41±50	8.43	1.42	-9.45±0.12
DC	62	600	12.73±0.80	138±65	11.9	1.49	-9.51±0.13
UW	30	5.0	94.90±7.55	-	-	-	-11.80±0.09
AB	35	11.4	80.8	-	-	-	-11.78±

3.2. Fresh and wastewater DOM source titrations

As indicated in the methods section, all titrations were performed using internal standard calibration and flow through ISE. This decision was based on best recovery of known [Ag+] values for silver titration of EDTA (ethylenediaminetetraacetic acid). See supplemental information, Figure SI1 for EDTA model ligand titrations. Silver titration curves of samples are presented in Figure 1. Titration curves are shown with the total silver added normalized to DOC to correct for the slight di_erences between actual DOC concentrations in each titrated sample (Table 2). The various surface water samples cluster together with a range of approximately two orders of magnitude in free silver. The samples with the most wastewater character (DC and BB) tend to be at the low end of the free silver range and the more allochthonous samples (NR, SR and LM) tend towards the highest values for free silver. The raw wastewater sample shows much stronger silver binding than the other samples with free silver four orders of magnitude lower than for the allochthonous sources (Figure 1).

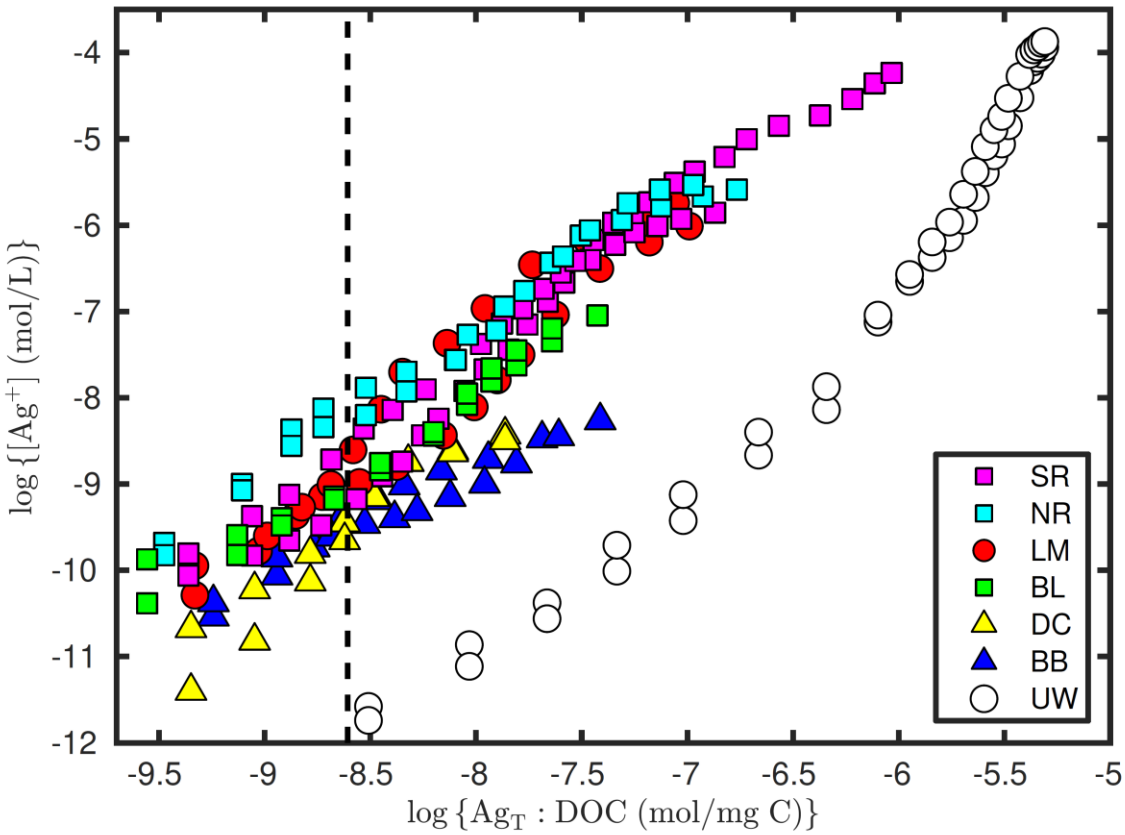

Figure 1: Silver titration data for DOM samples at pH 8.0 with 0.01 M ionic strength (KNO3). Colours and symbols for each sample code given in Table 1 are shown in the figure legend. The vertical dashed line corresponds to the *Ceriodaphnia dubia* EC50 value measured by Naddy et al. (2017)

The reproducibility of the individual titrations can be seen by comparison of the duplicate values for each source. The range of free silver spanned by all the samples is much greater than the range for each individual source (typically 0.2 orders of magnitude or less). The higher Cl samples (DC, BB, BL) were limited at the upper titration range due to AgCl(s) precipitation. Low ClT samples such as NR and SR could be titrated to much higher total silver concentrations. Despite being performed at high DOC, these titrations, with DOC normalization, span an environmentally reasonable range of concentrations given 202 that there are measured values above and below the sensitive, and regulatory relevant, effect concentration for the freshwater invertebrate Ceriodaphnia dubia (dashed line in Figure 1). This value corresponds to the lowest EC50 value measured in Naddy et al. (2017), 0.16 µg Ag/L at a DOC of 0.5 mg C/L. The range of silver speciation data is two orders of magnitude in free silver for the surface water samples and up to four orders of magnitude including the sewage sample. It should be possible to use a measure of quality to inform modelling to capture this variability. This has been done previously in copper biotic ligand modelling using

SAC340 or spectrally resolved fluorescence components to improve the estimates of %fulvic and %humic acid WHAM inputs (Al-Reasi et al., 2011b; De Schamphelare et al., 2004). To test whether various measures of organic matter quality correlate at all with measured silver speciation, the free silver ion measured at the DOC normalized *C. dubia* EC50 for each sample was regressed against each quality measure for the same samples. Results are reported in Table SI2 of the supplemental information. The only significant correlation was with the log (base 10) of DOC normalized CRS concentrations. These results are shown in Figure 2. The potential significance of CRS to help predict silver speciation is discussed further in the modelling section below.

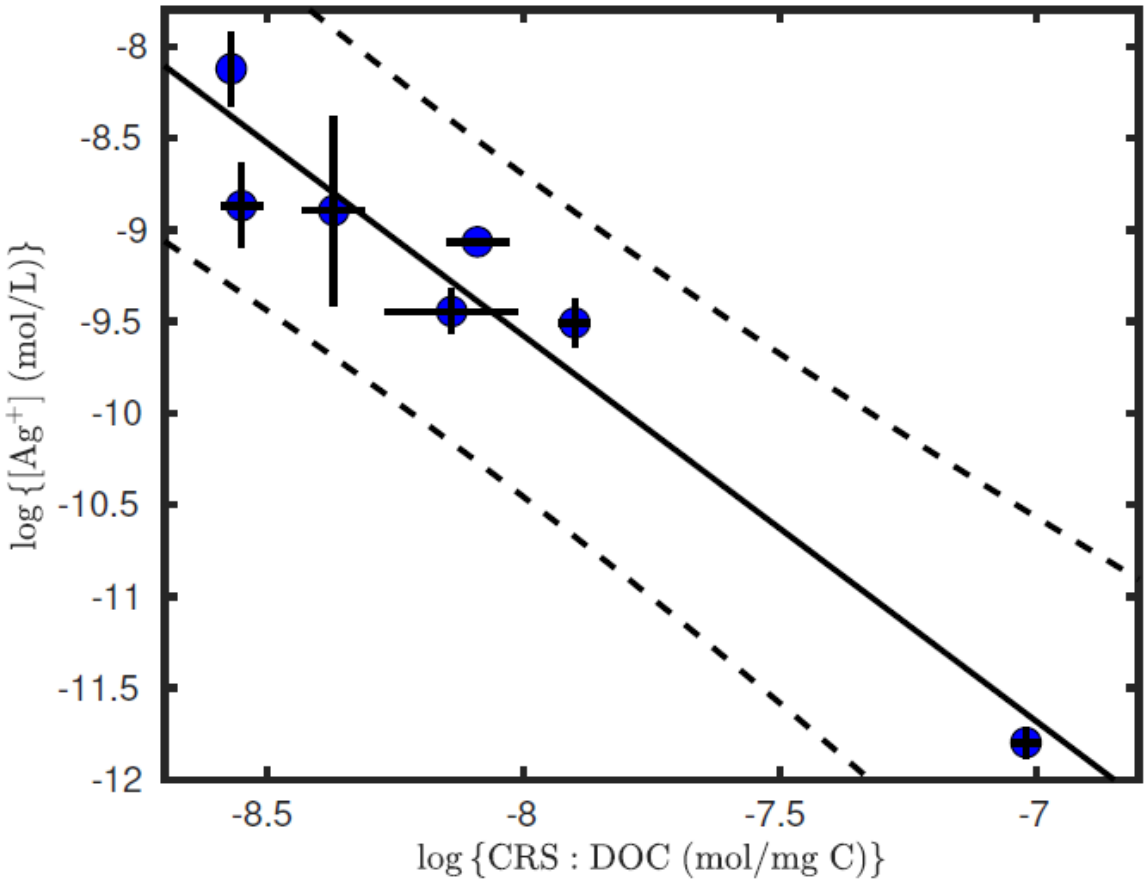

Figure 2: Relationship between CRS and free silver at *Ceriodaphnia dubia* 48 hour LC50 value (Naddy et al., 2017). The r^2 for the relationship with all 7 data points included is 0.936 (p value < 0.001), excluding the high CRS UW sample the r^2 value is 0.704 (p value 0.036). Error bars represent standard deviation on dublicate measurements.

3.3. WHAM modelling

Figure 3 shows WHAM predictions for all the DOM samples at pH 8.0, with the exception of the WS raw sewage sample, which is treated seperately below. For the SR, LM, NR and BL samples, the observed Ag complexation is within the range predicted by the WHAM model

when uncertainty is taken into account. WHAM prediction uncertainty was calculated using an absolute uncertainty of ±0.3 in the Ag binding constant ($\log K_{MA}$) (Lofts and Tipping, 2011; Ahmed et al., 2013). For the sewage effluent impacted, high chloride, samples BB and DC, the model predicts that most of the complexation is in fact due to the residual chloride present. Although, in both cases, the predictions with DOC present are better than those with only chloride present. In the case of DC, complexation at log Ag:DOC ratios below ~8.5 is greater than the range predicted by the model. Within the estimated uncertainty envelope,WHAM including DOC and chloride complexation, predicts silver speciation for a range of surface freshwater DOM samples, even for environments including some sewage effluent input. Figure 4 shows the corresponding WHAM predictions for samples SR, LM and DC at pH 4.0, 6.0 and 10.0. Predicted binding behaviour at pH 4.0 and pH 6.0 is reasonable (i.e., for the most part the data agrees with model predictions), although binding is somewhat underestimated for sample LM at pH 6.0. Binding at pH 10.0 differs for samples SR and LM; SR shows stronger binding than is predicted by WHAM, while LM binding shows a different trend to that predicted, being similar at low Ag loading, yet stronger than predicted by the model at high loading. Binding to sample DC is well predicted at all the pH values, due to the dominance of binding by the high concentration of chloride in the sample. Again, similar to pH 8 predictions, for surface water DOM sources WHAM predicts, within model uncertainty, silver speciation reasonably well. For high pH, outside the range of normal waters, the model does underpredict binding, but for risk assessment the underprediction of binding is conservative; thus, WHAM would still be an appropriate tool to help inform water management decisions.

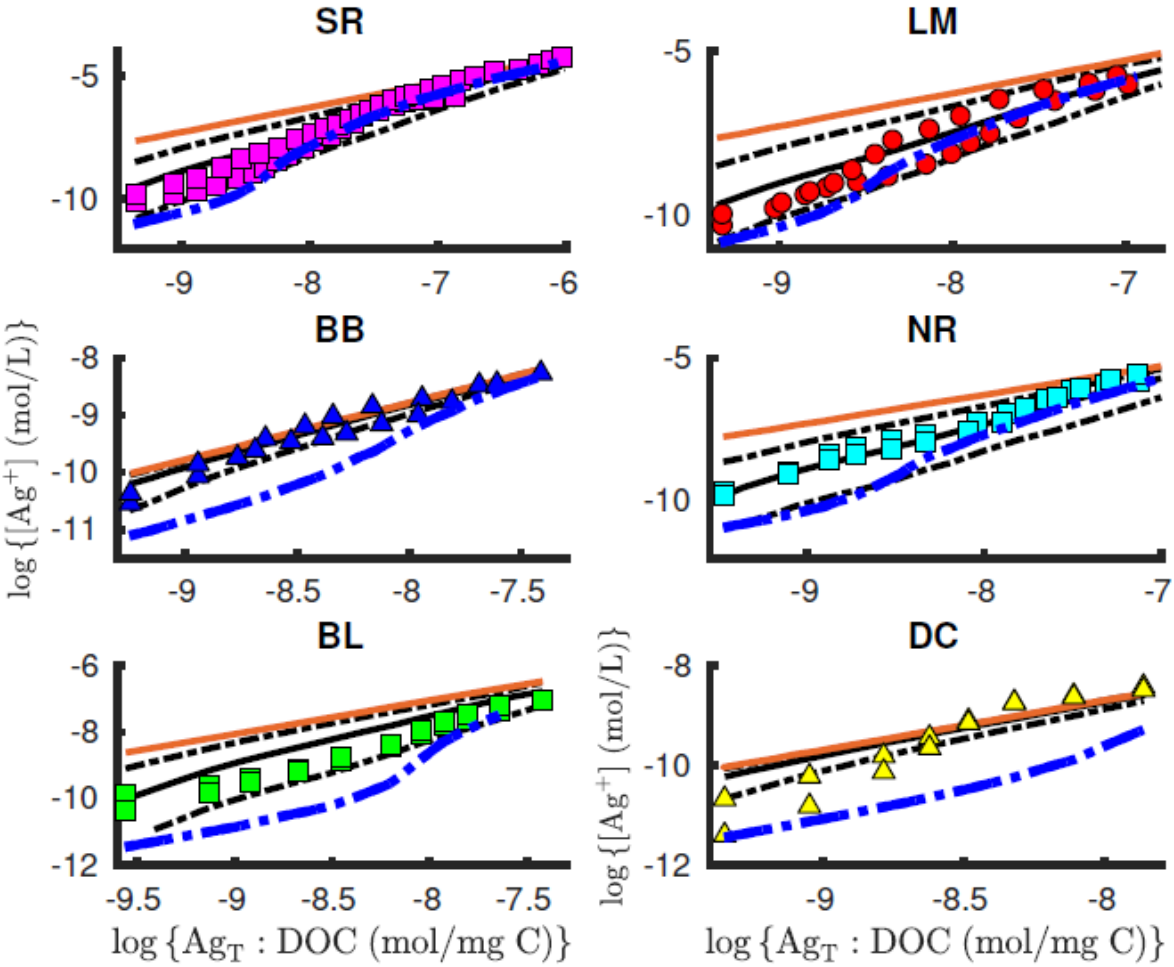

Figure 3: WHAM predictions of silver binding to the DOM samples at pH 8. Points are observations (sample legend is given in Figure 1). Black lines are WHAM predictions using the default assumption of DOM being 65% chemically active as fulvic acid, dashed lines show predictions with uncertainty of ±0.3 in the binding constant $\log K_{MA}$ for Ag^+ taken into account (Ahmed et al., 2013; Lofts and Tipping, 2011). Orange lines show the predicted free Ag in the absence of DOM, i.e. when complexation is due only to the presence of chloride. The blue dashed line is the calculated binding curve for cysteine-like sites, determined using measured CRS as the ligand concentration.

3.4. Silver Binding Model for Waste Water

Sulphur-based (thiol-type) groups in the samples may be important in binding Ag. To investigate whether this might be significant, we also ran simulations using cysteine as an analogue for S-binding moieties. Proton- and Ag binding constants for cysteine were obtained from the literature (Voronkov et al., 2002; Alekseev et al., 2012). The corresponding reactions and logK values are presented in Table 3.

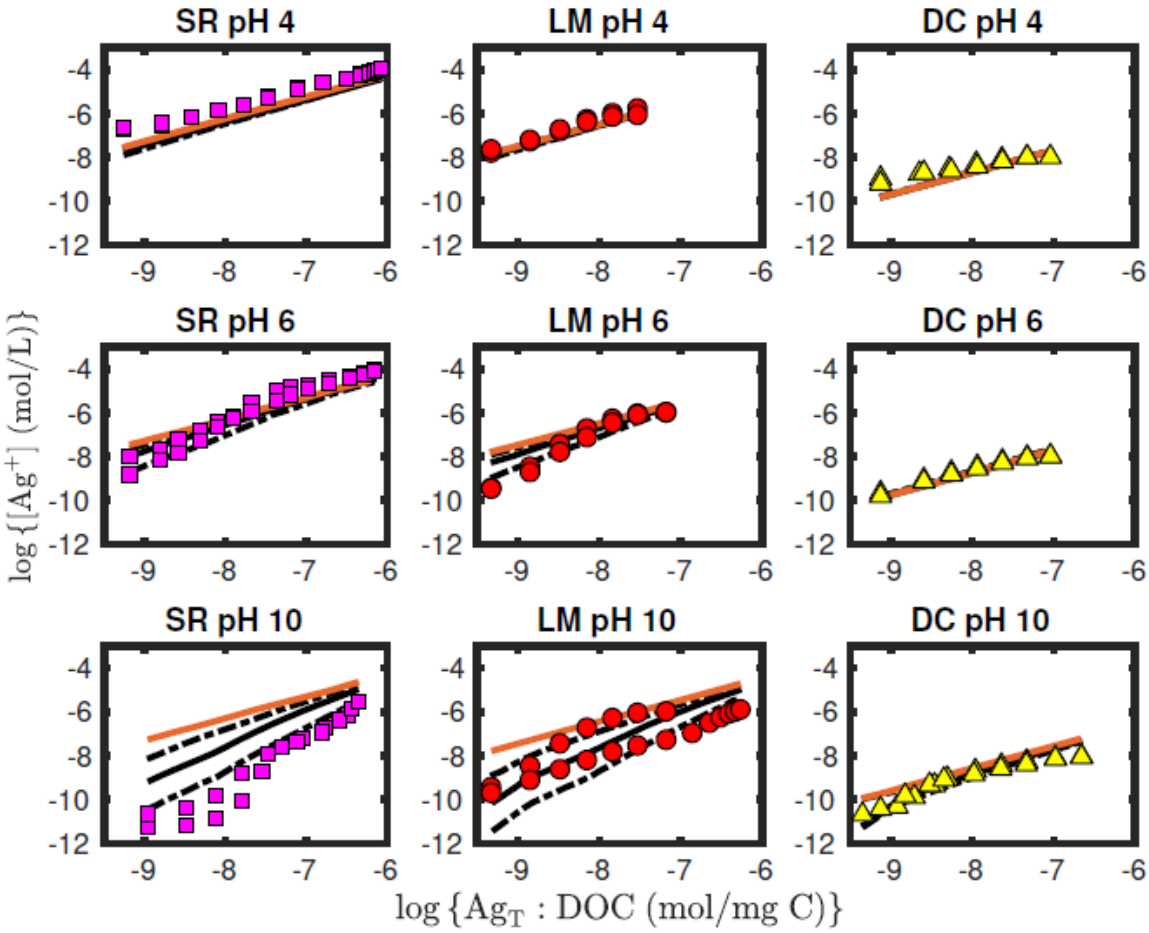

Figure 4: WHAM predictions of silver binding to the SR, LM and DC DOM samples at pH 4.0, 6.0 and 10.0. Points are observations (colours are as in Figure 1). Black lines are WHAM predictions using the default assumption of DOM being 65% chemically active as fulvic acid, dashed lines show predictions with uncertainty of ±0.3 in the binding constant $\log K_{MA}$ for Ag taken into account (Ahmed et al., 2013; Lofts and Tipping, 2011). Orange lines show the predicted free Ag in the absence of DOM, i.e. when complexation is due only to the presence of chloride.

Precipitation of Ag_2S was not included in the modelling because there is no evidence of silver precipitation other than AgCl(s) which forms at the highest added silver concentrations. Precipitation of a silver mineral phase would result in a constant [Ag+] response as is seen when AgCl(s) forms. The amount of cysteine in simulations was determined from the measured chromium-reducible sulphur (CRS) in the DOM samples, assuming all the CRS to have similar binding behaviour to cysteine-type thiol groups (measured thiol, as opposed to CRS, did not correlate with silver binding (Table SI2)). Predictions are shown as the blue line in Figure 3. In all cases the addition of thiol-type groups increases the predicted complexation of Ag, as would be expected. However, the agreement between observation

and prediction is generally poorer, even where observed complexation is higher than that predicted in the absence of thiol-type groups. This suggests that although thiol-type groups may be playing an important role in complexation in some of the DOM samples, the concentration of thiol-type groups that is actively taking part in binding is lower than the measured CRS. In samples NR and BB the silver binding predictions are clearly superior when thiol-type groups are not considered. Thus, for typical surface water samples assuming that the active silver binding ligands are predominantly fulvic acid-based, WHAM is an appropriate approach for assessing silver speciation; there is no improvement when reduced sulphide ligands are considered explicitly using measured CRS concentrations.

Table 3: Formation constants for silver and proton complexation to cysteine (Cys) and histidine (His) using reactions and logK values from Voronkov et al. (2002) and Alekseev et al. (2012) for Cys and from Smith and Martell (2004) for His.

Cys Reaction	logK value	His Reaction	logK value
$Cys^{2-} + H^+ \leftrightharpoons HCys^-$	10.37	$His^- + H^+ \leftrightharpoons HHis^0$	9.28
$Cys^{2-} + 2H^+ \leftrightharpoons H_2Cys$	18.60	$His^- + 2H^+ \leftrightharpoons H_2His^+$	15.25
$Cys^{2-} + 3H^+ \leftrightharpoons H_3Cys^+$	20.58	$His^- + 3H^+ \leftrightharpoons H_3His^{2+}$	16.85
$Cys^{2-} + Ag^+ \leftrightharpoons AgCys^-$	11.14	$His^- + H^+ + Ag^+ + \leftrightharpoons AgHHis^+$	12.41
$Cys^{2-} + H^+ + Ag^+ \leftrightharpoons AgHCys$	20.77	$2His^- + 2H^+ + Ag^+ + \leftrightharpoons Ag(HHis)_2^+$	25.41
$Cys^{2-} + 2Ag^+ \leftrightharpoons Ag_2Cys$	20.32	$2His^- + 2H^+ + 2Ag^+ + \leftrightharpoons Ag_2(HHis)_2^{2+}$	34.29
$Cys^{2-} + H^+ + 2Ag^+ \leftrightharpoons Ag_2HCys$	27.28		

For wastewater though, WHAM significantly underpredicts binding. This is not at all surprising given that WHAM was calibrated for freshwater organic matter with low nitrogen and sulphur content. Figure 5(a) shows WHAM predictions for the UW raw sewage sample. The complexation of silver by the sample is clearly greater than can be predicted from the chemistry of humic substances. For the UW sample a calculation of the amount of silver bound per g of organic matter reveals that the bound Ag exceeds 0.001 mol g DOM^{-1}. This demonstrates that the UW DOM has an unusually high binding capacity compared to humic material; for example, SR at pH 8.0 has a capacity of approximately 10^{-5} mol g DOM^{-1}. This suggests strongly that the DOM in the UW sample is dominated not by humic-type material but by material with a high Ag binding strength and capacity, such as reduced sulphur and/or proteinaceous materials. These materials could include inorganic colloidal materials (i.e., metal sulphides) associated with the DOM.

Predicted Ag complexation in the presence of groups with thiol-type binding strength is shown in Figure 5(b). Assuming the presence of reduced sulphur groups gives a somewhat improved prediction, with excellent agreement at low Ag loading (up to log (AgT:DOC) ~5.8). However, at higher Ag loadings binding remains under predicted by a considerable margin. This suggests that while binding to thiol-type groups is sufficient to explain binding at the low end of the Ag loading range, additional binding sites are required to provide a full description of the overall complexation behaviour. Given the high overall binding capacity, which cannot be explained by binding to humic-type material, the most likely explanation is the presence of additional nitrogenous groups on proteinaceous material.

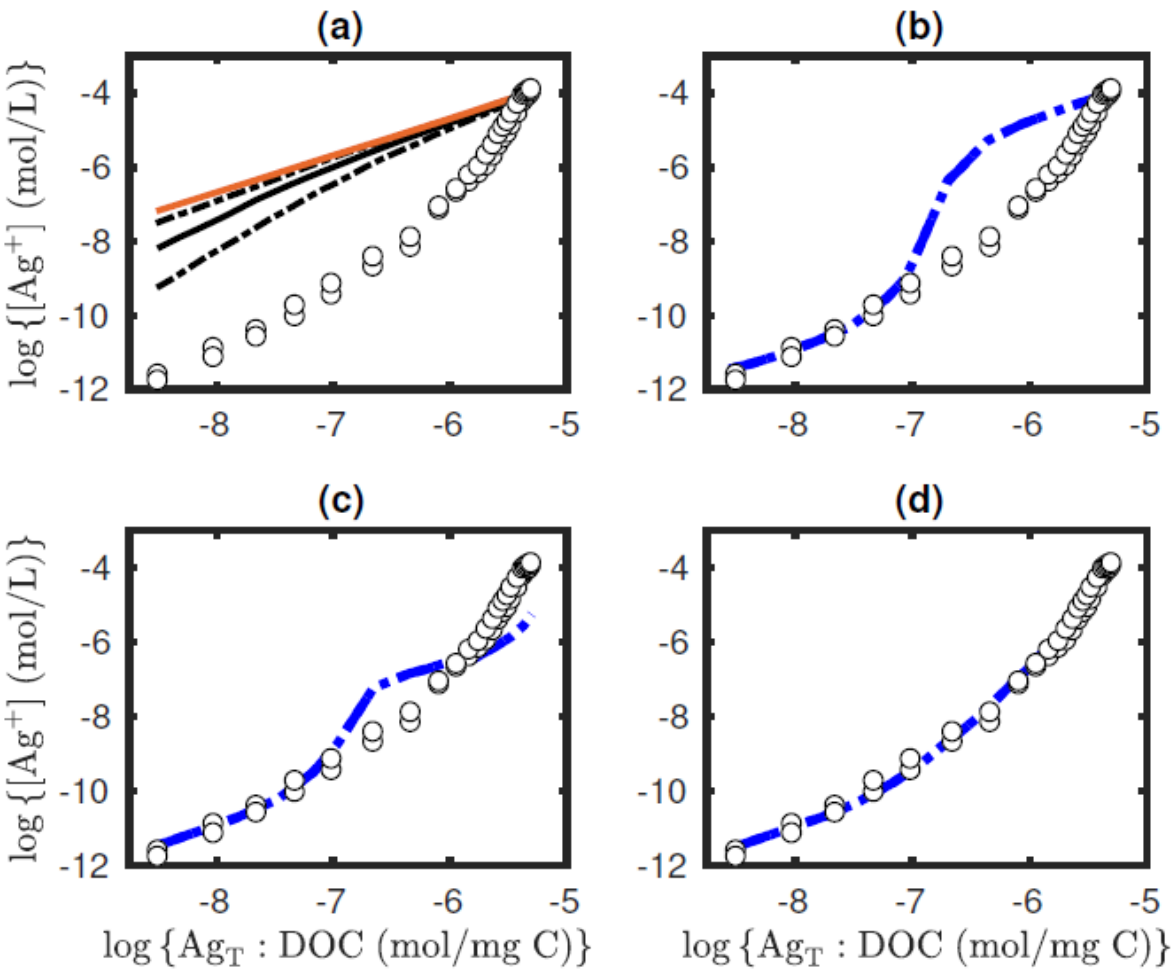

Figure 5: (a) WHAM predictions of silver binding to the UW sample at pH 8. Black lines are WHAM predictions using the default assumption of DOM being 65% chemically active as fulvic acid, dashed lines show predictions with uncertainty of ±0.3 in the binding constant $\log K_{MA}$ for Ag taken into account (Ahmed et al., 2013; Lofts and Tipping, 2011). Orange lines show the predicted free Ag in the absence of DOM, i.e. when complexation is due only to the presence of chloride. Open circles are the measured UW titration data. (b) Observed (open symbols) and predicted (blue line) Ag complexation in the UW sample, assuming the

presence of thiol-type groups (Table 3). (c) Observed (open symbols) and predicted (blue line) Ag complexation in the UW sample, assuming the presence of thiol-type groups as well as amine groups. (d) Observed (open symbols) and predicted (blue line) Ag complexation in the UW sample, using a best-fit empirical model (Table 3).

To test the possible contribution from nitrogenous binding sites, the C:N ratio of the DOM in raw sewage was first estimated. There is a wide literature on the composition of raw sewage, presenting a range of possible C:N ratios. For example, Gray (2004) gives a C:N ratio of raw sewage of 100:17 (5.9), giving an estimated N content for the UW sample of 1350 nmol N/mg C. To probe the possible influence of nitrogenous groups, we assumed this N to be present as histidine. Equilibrium constants (see Table 3) for proton and silver binding to histidine were added to WHAM and the model results shown in Fig 5(c). All binding data were taken from Smith and Martell (2004). Figure 5(c) shows the outcome of a prediction using 100 nmol S/mg C (cf. Figure 5(b)) and 1350 nmol N/mg C. The addition of histidine to represent amino N groups improves the prediction compared to that when thiol groups only are represented. However, the combination of ligands cannot represent the observed shape of the titration curve, since they do not provide enough binding heterogeneity.

An alternative approach to representing binding sites by a collection of small ligands of known chemistry is to represent silver binding to a collection of ligands, assuming a simple 1:1 reaction stoichiometry (as shown in Equation 3), and to fit binding strengths and site densities directly to the data.

$$Ag^{+} + L = AgL+ \qquad (3)$$

This approach has the advantage of allowing an optimal description of the binding behaviour, at the expense of a level of realism in terms of ligand identity. To permit the fitting of a multiligand model with a reasonable number of parameters, we used a formulation whereby the binding strengths (logK values) and site densities (S, mmol/g) were related to each other. We postulated a collection of n ligands denoted L_1, L_2, ... L_n. Defining the silver binding strength and site density of L1 as logK1 and [S1], we then define the binding strengths and site densities of the remaining ligands in relation to the values for L1:

$$\log K_x = \log K_1 + (x - 1)\cdot\Delta\log K \qquad (4)$$

$$[S_x] = [S_1]\cdot\Delta[S]^{x-1} \qquad (5)$$

where x is the ligand number (x = 2,...,n) and logK and [S] are fitting parameters. This scheme allows fitting of any number of ligands greater than one using four binding parameters. The number of ligands required must be set *a priori* by fixing n at different values and optimizing the four adjustable parameters ($\log K_1$, $[S_1]$, $\Delta\log K$ and $\Delta[S]$). The

optimal number of sites is that above which adding another site does not produce a significant improvement in the fit. For the WS data fitting trials suggested that the optimal number of sites was five. Fitting produced the optimal parameter values given in Table 4 and the optimal fit shown in Figure 5(d). The five-site model gives an excellent fit to the data, emphasizing that such a degree of heterogeneity is needed to describe the trend in silver binding. The binding strength of site number 1 (the strongest) is reasonably close to that for 1:1 binding of silver to cysteine ($Ag^+ + HCys^- = AgHCys^0$; logK = 11.37 when I = 0.01 mol/L). However, the binding strengths of the remaining sites are stronger than those for silver binding to amino groups, e.g. on histidine ($Ag^+ + HHis^- = AgHHis^0$; logK = 3.13 when I = 0.01 mol/L). This suggests that other types of sites, such as multidentate sites comprising two or three amino N groups, might be important for silver binding. Such sites would be expected to have logK values for 1:1 binding in the range ~6-9, i.e. approximately double to triple the 1:1 binding affinity for silver to histidine.

Table 4: Optimised parameters for the multiligand model, fitted to the WS sewage titration. The logK values are conditional for the ionic strength of the WS titration (I = 0.01 mol/L).

Parameter	**Optimized value**
$logK_1$	10.24
ΔlogK	-1.15
[S1] nmol/mg C	66.7
Δ[S]	2.24
$logK_2$	9.09
$logK_3$	7.94
$logK_4$	6.79
$logK_5$	5.64
[S2] nmol/mg C	149
[S3] nmol/mg C	334
[S4] nmol/mg C	749
[S5] nmol/mg C	1679

3.5. Silver toxicity to N oxidizing bacteria in sewage, range finder experiment

In wastewater treatment, organic ligands (DOM) that have the potential to complex with silver, may originate in the raw wastewater or may be generated as soluble microbial products (SMP). The chelating properties of SMP have been attributed to various functional groups including carboxylates, hydroxyls, thiols, phenols and amines (Kuo and Parkin, 1996). Holakoo et al. (2006) found that SMP were moderate chelators of copper as indicated

by logK values of 7.6-8.8 and 6.3-6.8 for moderate and weaker ligands, respectively. Weaker ligands contributed more than half of the total ligand concentration. SMP with molecular weights of 1-10 kD were found to have the highest complexation capacity among all the SMP fractions. SMPs are expected to complex silver and reduce silver toxicty to microorganisms in the wastewater matrix.

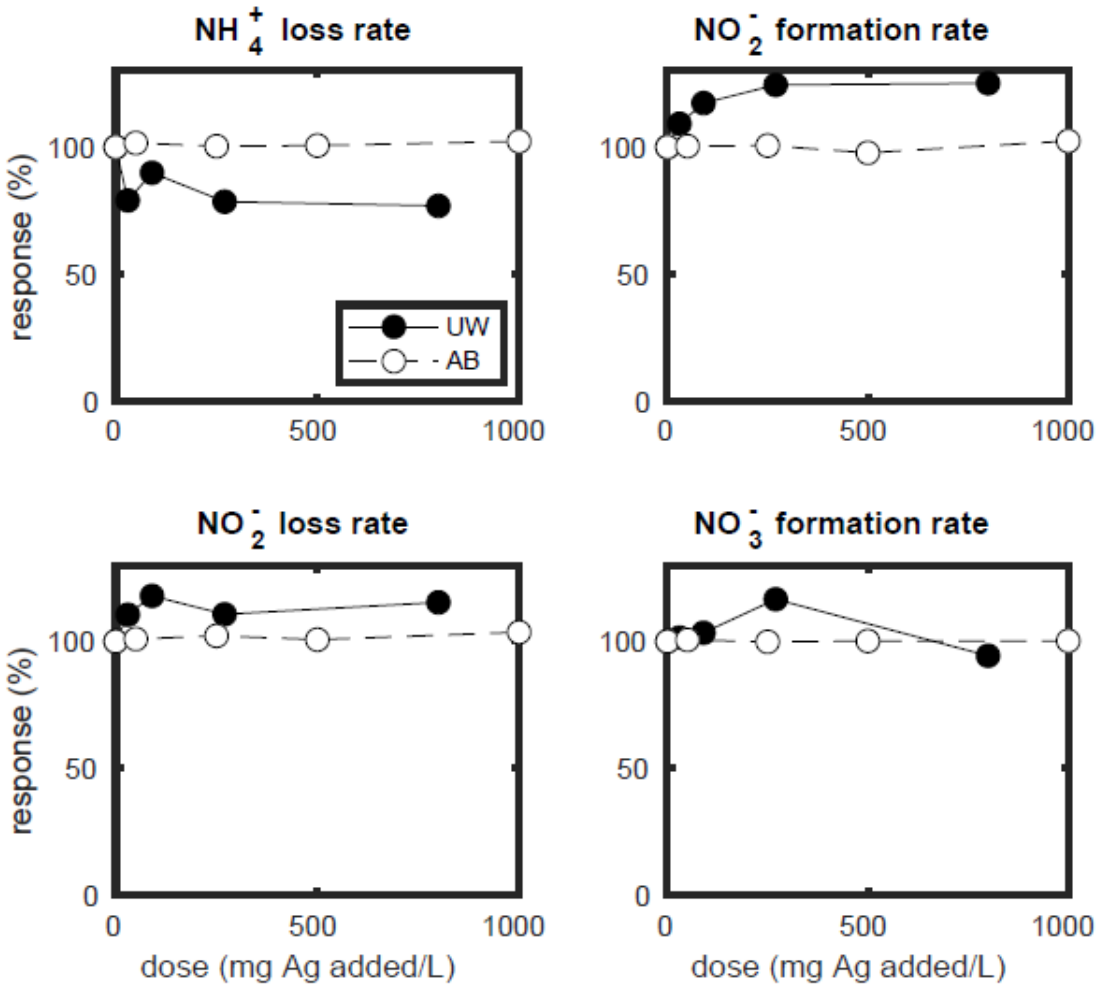

Figure 6: Dose response curves for nitrogen redox transformations in aerated raw sewage from two sources. The silver dose is recorded as the nominal amount of silver added.

SMP are released by microorganisms during substrate metabolism, biomass growth, and biomass decay, and constitute a major part of the residual organic matter in the e uent from biological wastewater treatment plants (Kunacheva and Stuckey, 2014; Xie et al., 2016). SMP comprise a wide range of high and low molecular weight compounds including proteins, polysaccharides, humic and fulvic acids, nucleic acids, enzymes and structural compounds (Rittmann et al., 1987; Parkin and McCarty, 1981). Xie et al. (2016) identified solids retention time, influent chemical oxygen demand (COD) concentration and hydraulic retention time as factors influencing SMP formation in wastewater treatment plants. While,

Hu et al. (2019) demonstrated that the effect of temperature on the chemical characterization of soluble dissolved nitrogen was different from that of soluble dissolved carbon, and hence the composition of SMPs changed with temperature. Thus, the release of ligands that could bind metals in receiving waters will likely vary with location and season; thus, two sources of sewage were studied (see below).

A simple range finder experiment was designed to test the impacts of silver on ammonia oxidation to nitrate with nitrite as an intermediate species; Figures SI2 and SI3 show the experimental setup. Measurements of the concentration of each nitrogen species concentration versus time show the expected trend of ammonia transformation to nitrite and subsequently nitrite transformation to nitrate (Figure SI5 for the UW sample and SI6 for the Ash AB sample). From the species data alone it is hard to discern if there is any e ect of the added silver; the data look very similar. To probe the data further the rates of transformations of each species were calculated from the slopes of each species concentration versus time. In this way rate of NH_4^+ loss, NO_2^- formation and loss and NO_3^- formation were calculated (Figure 6).

There were replicate flasks during the oxidation tests but there were analytical issues and some of the replicate analyses were lost. Figures SI5 and SI6 show what replicate data was available as duplicate data points; for the majority of treatments and times only one data point was measured. This lack of available replicate data makes it impossible to report actual effect concentrations. Still, inspection of Figure 6 reveals that the effects are no greater than 20% even up to 1000 µg Ag/L. The Ashbridges Bay sewage sample (AB) actually shows very minimal variations (less than 5%) with respect to added silver. The UW sample suggests a slowing of about 20% on ammonia loss rates and nitrite formation rates. Similarly, nitrite loss and nitrate formation rates increase by less than 20%.

A previous sediment incubation study on nitrogen oxidation kinetics showed that silver nanoparticles only impacted N_2O production at high silver doses. Zheng et al. (2017) determined that N_2O production was stimulated by silver nanoparticles in estuarine sediments at doses of <534, 1476, 2473 µg/L for 10, 30 and 100 nm sized silver nanoparticles respectively. A study on wastewater biofilms by Sheng et al. (2015) demonstrated that at 200 mg Ag/L silver doses, as silver nanoparticles, silver decreased community diversity but did not significantly affect microbial community function. Sheng et al. (2015) did not investigate any other silver concentrations, and such a high dose is much larger than could reasonably be achieved by discharge of personal care products; Ag concentrations in personal care products has been reported to be between 17 to 30 mg kg□1 (Khaksar et al., 2019). The research presented in this current paper, although at much

lower total silver doses did not investigate impacts on community diversity though, and this should be addressed in follow-up studies. There would be the potential for negative impacts if bacterial community diversity were to be modified in a receiving water (Zeglin, 2015).

Dissolved organic carbon also showed no trends with respect to nominal silver dose (Figure SI7). The DOC did decrease with time from 35 to approximately 10 over 30 days consistent with degradation of organic matter in the raw sewage, but there was no influence of silver dose on the DOC degradation kinetics. Dissolved silver decreased rapidly in all exposures (Figure SI8); by day 18 there was no detectable dissolved silver remaining in solution for any of the treatments. The removal of silver was likely due to sulphide precipitation and this removal would be protective with respect to the bacterial community. The complexation in solution would also render the remaining soluble silver less bioavailable.

The sewage incubation experiments also allowed testing of the hypothesis that sewage organic matter would become more “WHAM-like” as it oxidized; i.e, the silver binding properties of the aged material became more similar to WHAM predictions of humic substance silver complexation. From the Ash AB control incubations subsamples were taken for silver titration at day 0, 2, 15, 18 and 30 (Figure SI9). The youngest two samples showed the strongest binding; i.e., the lowest free silver concentrations. The three later samples were an order of magnitude higher in initial free silver concentrations. The measured values are still 3 orders of magnitude lower than WHAM predictions, but the tendency is towards binding curves more closely resembling allochthonous organic matter, suggesting that the very strong silver binding ligands are effectively reduced by wastewater treatment.

4. Conclusions

WHAM predicts silver speciation very well within the model confidence envelope using DOC as the input variable across pH values from 4 to 10. There are almost two orders of magnitude variation in free silver between sources but, within model uncertainty, WHAM captures this variability. Thus for risk assessment WHAM is an appropriate tool for predicting silver speciation in surface waters.

The raw sewage sample tested was not modelled correctly by WHAM though. Investigation of the data suggested that the binding strength and capacity was much greater than that of humic-type material. We suggest that this is due to ligands derived from components of raw sewage, such as a proteinaceous material, that we would expect to be at low concentrations in natural waters unimpacted by raw sewage. WHAM simulations with thiol-type binding sites could explain the binding of silver at low loadings but not at higher loadings. This suggests the presence of an additional class or classes of strongly binding ligands, possibly nitrogen-based groups.

This project involved testing the performance of existing software (Windermere Humic AqueousModel (WHAM)) for predicting the forms of silver in receiving waters. It was found that this software performs very well for most surface freshwater environments. The only time the model overpredicts the amount of toxic free silver ion is in heavily sewage impacted environments. This additional binding not captured by the modelling means that any management decisions performed using WHAM would be conservative though. Thus, silver stakeholders can proceed with WHAM-based risk assessment. An early prototype sewage-specific silver prediction model has been developed as part of this project. Such a model can serve as a useful starting point for a more comprehensive model taking other variables, such as pH, into account, and specifically a revised modelling approach would relate independent measures of DOM quality to ligand concentrations, rather than fitting these values for a specific “batch” of sewage, as was done here. Ultimately, such a model would be of great use to stakeholders, as a means to perform silver risk assessment for sewage-impacted waters.

In addition, after it was found that WHAM did not predict silver binding in sewage, wastewater was explored further in incubation experiments to test if sewage would become more like humic substances with age and if silver would impact the natural treatment of sewage by bacteria. It was found that sewage does become more WHAM like with age but even after a month binds silver more strongly than organic matter from natural aquatic environments. Silver did not impact the natural attenuation of sewage though and it seems that silver-containing products do not cause unanticipated consequences in regions of low wastewater treatment technology.

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