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Preservation of co-occurring As, Sb and Se species in water samples with EDTA and acidification

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Abstract: The simultaneous preservation of the following redox couples was studied: As(III, V), Sb(III, V) and Se(IV, VI). Over a time period of 11 weeks the stability of these three redox couples was assessed in water samples with different dissolved oxygen content, i.e., groundwater, lake water and river water. High concentrations of Fe (25.0 mg L^{-1}) and Mn (25.0 mg L^{-1}) were added to each of the different matrices to simulate a Fe and Mn rich environment. In addition to their natural concentration, each sample was spiked with 5.0 µg L^{-1} As(III and V) and Sb(III and V) and 15.0 µg L^{-1} Se(IV and VI). As potential preservation strategies, EDTA alone and EDTA combined with HCl, HNO_3, formic acid or acetic acid were investigated and compared to unpreserved samples. In addition, preserved samples were stored at 4°C in the dark, while unpreserved samples were stored at room temperature in the presence of light. The results showed that the addition of EDTA combined with acidification to a pH of 3 successfully preserved all three redox couples for at least 11 weeks stored at 4°C in the dark. EDTA alone ($pH = 6$) failed to preserve the As and Sb species, although it successfully preserved the Se species. Primarily based on observations made for the unpreserved samples, it was concluded that Sb(III) could be oxidized easier than As(III) and Se(IV) at neutral pH, and that the Se species in general were most stable. The formation of Fe-(oxy) hydroxide and possibly Mn-(oxy) hydroxide in the unpreserved samples also allowed an estimation of the relative adsorption behaviour. Arsenic(III), Sb(III), Se(IV)) and As(V) showed a strong adsorption affinity for Fe-(oxy) hydroxide and Mn-(oxy) hydroxide probably due to the fact that they all form inner sphere complexes, whereas Sb(V) and Se(VI) rarely adsorbed because they form outer sphere complexes and thus bond via weak electrostatic adsorption. Antimony(III) could chelate with EDTA and formed several complexes according to pH. The most stable species of $Sb(III)Y^-$ ($Y = EDTA$) existed at a pH range of 1.8 to 3.0. Apparently Sb(V), on the other hand, did not chelate with EDTA and thus should exist mainly in the form of $Sb(OH)_6^-$ and minor $Sb(OH)_5$ in this pH range.

Keywords: preservation; species; stability; As; Sb; Se

Redox sensitive, multi species elements, such as arsenic (As(III, V)), antimony (Sb(III, V)) and selenium (Se(IV, VI)) are intensively studied, because they play an important role in both environmental and health issues. To date, great effort was made to investigate the stability and preservation strategies for individual element species (Kumar & Riyazuddin 2010), however the next step will be to study the behaviour of various element species in different environments simultaneously (Lindemann *et al.* 2000). For example, redox behaviour of these elements in different matrices (Leuz 2006; Wallis *et al.* 2011; Breuer & Pichler 2013; Lazareva *et al.* 2015), toxicity and bioavailability could vary as a function of redox state (Price *et al.* 2007; Mailloux *et al.* 2009; Jamier *et al.* 2010; Price *et al.* 2013) and adsorption onto hydrous ferric oxide (HFO) surfaces (Qi & Pichler 2014). The diverse properties of these species are still not fully understood. The main reason might be the lack of methods allowing simultaneous speciation analysis of these redox couples and the difficulty in preserving the distribution of these species from sampling to measurement. The stability of these species depends on redox condition, pH, microbial activity, photochemical oxidation, organic matter, presence of oxidizing ions like Fe(III), co-precipitation with, and adsorption by, HFO, and adsorption on container walls. Consequently, published preservation methods for the three elements of interest are contradictory. For example, HCl is commonly used for preservation of As speciation, but HCl is known to catalyze oxidation of Sb and Se.

Gómez-Ariza *et al.* (1998) carried out a systematic investigation of the variables that can affect the stability of inorganic Se species, including species concentration, pH, container material, temperature and matrix (seawater and fresh water). The results showed that when the samples were acidified to p $H = 2$ with HCl and stored at −20°C in Teflon containers, Se(VI) remained stable for a whole year in all studied matrices. As for Se(IV), clear losses were observed in fresh water (river and tap water) after 6 months. However, in seawater samples, Se(IV) was stable for a year, indicating a higher stability at high ionic strengths. They also found that Se(VI) was more stable than Se(IV) and higher concentrations were more stable than lower. To the contrary, Cobo *et al.* (1994) suggested that acidification was not necessary for preserving inorganic Se species at −20°C. Selenium(IV) was more stable at pH 6 than at pH 4, with two months being the maximum storage time without Se(IV) loss. Moreover, Héninger *et al.* (1997) pointed out that HCl acidification may catalyze the oxidation of Se(IV) to Se (VI). Wiedmeyer & May (1993) studied the influence of ionic strength, container material and temperature on the stability of Se species. Compared to Se(VI), significant changes of Se(IV) were observed over 120 days, and loss of Se(VI) was observed in a low ionic strength matrix. The least change during storage was observed at 4°C in a glass container.

Preservation of inorganic Sb species seems more complicated than As and Se, especially for time spans in excess of one month, because Sb forms strong complexes and its species are easier affected by adsorption and oxidation. Acidification with HCl proved to be ineffective. Andreae (1983) observed a rapid oxidation of Sb (III) in estuarine water samples preserved with HCl acidification, but total Sb remained stable for several months. Oxidation of Sb(III) was also observed in seawater acidified with HCl by Cabon & Louis

Madec (2004) and Ellwood & Maher (2002). Freezing is somewhat controversial to preserve Sb species. Andreae (1983) observed fast oxidation in the brine formed during partial freezing of estuarine water samples. Cutter & Cutter (1995) found that quickly frozen samples could not be stored longer than one month before the inorganic speciation was compromised. Stabilizing agents such as tartaric acid and EDTA were also investigated and found to prevent oxidation of Sb(III) for six days (Han-wen 1982; Gregori *et al.* 2005).

Arsenic(III, V) preservation has been the most widely studied among the three elements of interest. Although reviews have summarized and evaluated published methods (Mccleskey *et al.* 2004; Kumar & Riyazuddin 2010), there are still discrepancies regarding preservation of As species (Mccleskey *et al.* 2004). Acidification is one of the most common procedures for preserving As species, however, Bednar *et al.* (2002) observed an immediate oxidation of As(III) in synthetic samples preserved with HCl in both dark and light conditions. Hall *et al.* (1999) studied the stability of inorganic As(III) and As(V) in spiked de-ionized water and river water which were acidified with HCl or HNO_3, and the result showed that both HCl and HNO_3 caused oxidation of As(III) to As (V), but HNO_3 led to a higher degree of oxidation. EDTA combined with acetic acid were also investigated as potential preservation for As species (Gallagher *et al.* 2004; Samanta & Clifford 2006; Wang & Liu 2012).

According to the conditions leading to the change of distribution of different species, the preservation of these redox species should address the following aspects.

(1) Filtration. It is a common practice to filter with a 0.20 or 0.45 μm membrane for sample preservation, as it excludes suspended particles and microorganisms. Bacteria are capable of catalyzing As and Sb oxidation (Asta *et al.* 2012).

(2) Acidification. Most researchers prefer acidification, because low pH ($pH < 3$) impedes the hydrolysis of Fe (III), which is one of the major factors that can affect the distribution of the As, Sb and Se redox couples. While acidification, particularly with HCl, works well for the preservation of As species (Mccleskey *et al.* 2004) it is not suited for the preservation of Sb and Se species because HCl is known to catalyze the oxidation of Se(IV) to Se(VI) and Sb(III) to Sb(V) (Ellwood & Maher 2002; Andreae 1983; Cabon & Louis Madec 2004). Sulphuric acid and H_3PO_4 worked well in some cases (Bednar *et al.* 2002; Daus *et al.* 2002, 2006), but were discarded either because they were difficult to purify and might cause precipitation by forming metal phosphate (or sulphate), e.g. strengite ($FePO_4{\cdot}2H_2O$). The use of HNO_3 is somewhat controversial, because it has the potential to be an oxidant, although there are no reliable accounts that it can oxidize, for example As, without the presence of light. The use of HNO_3 is beneficial, however, during analytical detection because it does not cause mass interferences in the plasma such as HCl, i.e., $^{40}Ar^{35}Cl$, which has the same mass as ^{75}As.

(3) Temperature. Temperature influences microbial activity as well as chemical reactions (endothermic/exothermic). Cooling the water samples to different temperatures, e.g. 3°C, 6°C and −20°C was widely investigated for preservation of As, Sb and Se species (Lindemann *et al.* 2000; Daus *et al.* 2006). However, long term storage at very low temperature is not practical for sampling in the field and transportation to the lab. Therefore, freezing is a controversial technique leading to precipitation and repartitioning (Héninger *et al.* 1997).

(4) Light. To our knowledge, no preservative tested could maintain the As(III/V) speciation when exposed to light. When a water sample containing Fe(III) is exposed to light, hydroxyl radicals are produced at pH >2, and dichloro radicals are produced when the sample is acidified with HCl to $pH < 2$. These radicals react with As(III) to produce intermediate As(IV) species reacting with Fe(III) to produce Fe(II) and As(V) (Mccleskey *et al.* 2004). An instant switch from As(III) to As(V) was observed by Bednar *et al.* (2002) with light exposure when hydrochloric acid was used for preservation. In another study Samanta & Clifford (2005) showed that in the presence of strong UV light and Fe(II), neither EDTA-HAc, H_2SO_4 nor H_3PO_4 could prevent the rapid oxidation of As(III). It has been reported that nitrate could also undergo photochemical reaction and produce hydroxyl radicals (Fanning 2000). Therefore, samples should be preserved in opaque bottles to eliminate/minimize the influence of UV light.

(5) (Oxy) hydroxide adsorption and precipitation. Iron-(oxy) hydroxide and/or Mn-(oxy) hydroxide can bind various trace elements, including As, Sb and Se due to their large surfaces, high adsorptive capacities and large abundance in the environment. Thus, the formation of Fe-(oxy) hydroxide and/or Mn-(oxy) hydroxide should be avoided in any case during storage.

To our knowledge, a preservation strategy for inorganic species of As(III, V), Sb(III, V) and Se(IV, VI) has not yet been reported. With the development of a method for the simultaneous speciation of As (III, V), Sb(III, V) and Se(IV, VI) using SF-ICP-MS coupled to HPLC (Wu & Pichler 2014) a preservation strategy for As(III, V), Sb(III, V) and Se(IV, VI) is necessary.

Materials and method

Instrumentation

Detection

A Thermo Scientific ELEMENT 2 sector field ICP-MS (SF-ICP-MS) in high-resolution mode was used for the detection of As(III, V), Sb(III, V), and Se(IV, VI) (Wu & Pichler 2014). The instrumental conditions and tuning information are given in Table 1. For As and Sb, isotopes of ^{75}As and ^{121}Sb were monitored. For Se, the less abundant isotope of ^{78}Se was used due to the $^{40}Ar^{40}Ar$ interference on ^{80}Se.

The sample matrix was analyzed for major cations and anions to calculate ionic strength. Oxygen, pH and temperature were measured in the field by probe. The cations Na^+, Ca^{2+}, K^+ and Mg^{2+} were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES) using a Perkin-Elmer Optima 3300DV instrument. The anions Cl^-, NO_3^- and SO_4^{2-} were measured by ion chromatography using a Metrohm 883 IC plus instrument. The HCO_3^- concentration was determined by assuming electroneutrality and missing ion calculation.

All reagents were analytical grade or better. Certified reference materials (NIST, IAPSO), blind duplicates and repeat analyses were used for quality assurance and control. The analytical uncertainty was estimated to be better than 2%.

Separation

The chromatographic separations were carried out using a Thermo Scientific Accela 1250 Pump and an Hamilton PRP-X100 (Hamilton, Reno, USA) anion exchange column (250 × 4.1 mm, 10 μm) at a constant flow rate of 1.5 mL min^{-1}. The chromatography conditions are listed in Table 1. The outlet of the HPLC column was connected via PEEK capillary tubing to the nebulizer of ELEMENT 2. The pH of all solutions was determined using a pH-meter (pH 340, WTW).

Table 1. *Instrumental conditions for the simultaneous detection and separation of multiple redox couples*

Nebulizer	Conikal nebulizer
Spray chamber	Scott type spray chamber
Detection mode	High resolution (HR)
Monitored isotopes	^{75}As, ^{78}Se, ^{121}Sb
Column	PRP-X100 (250 × 4.1 mm, 10 µm) (Hamilton, Reno, USA)
Mobile phase	0–4.5 min: 5 mM EDTA (97%) + methanol (3%) 4.5–5.5 min: linear ramp to 30 mM EDTA (97%) + methanol (3%) 5.5–11 min: 30 mM EDTA (97%) + methanol (3%)
pH	4.7 (adjusted with formic acid)
Flow rate	1.5 mL min^{-1}
Injection volume	50 µL
Species	As (III, V), Sb (III, V), and Se (IV, VI)

Reagents and solutions

All solutions were prepared with double de-ionized water obtained from a Millipore water purification system (MilliQ Advantage A10, 18 MΩ cm).

Stock solutions (1000 mg L^{-1} for each species) were prepared as follows: As(III) from As(III) oxide (As_2O_3, Sigma-Aldrich) dissolved in 4 g L^{-1} NaOH (Merck). Arsenic(V) from sodium arsenate dibasic heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$, Sigma-Aldrich) dissolved in water. Antimony(III) from potassium antimonyl tartrate trihydrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$, Sigma-Aldrich) dissolved in water. Antimony(V) from potassium hexahydroxoantimonate (H_6KO_6Sb, Fluka) dissolved in water. Selenium(IV) from sodium selenite (Na_2O_3Se, Sigma) dissolved in water. Selenium(VI) from sodium selenate (Na_2O_4Se, Sigma-Aldrich) dissolved in water. Iron(II) (10,000 mg L^{-1}) from iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, Merck) dissolved in water. Manganese(II) (10,000 mg L^{-1}) from manganese(II) chloride dihydrate ($MnCl_2 \cdot 2H_2O$, Merck) dissolved in water. 0.2 M EDTA from EDTA (p.a. AppliChem) dissolved in water. All stock solutions were kept at 4°C in the dark and the standards and mix standards of lower concentration were prepared daily by appropriate dilution with water.

The mobile phase was prepared using EDTA (p.a. AppliChem), the pH of which was adjusted with ammonium (Suprapur, Merck) and formic acid (98–100%, Merck). The mobile phase was filtered through a 0.45 µM membrane (Whatman) before use. Methanol (for HPLC, ≥99.9%, Sigma-Aldrich) was used in combination with EDTA solution as mobile phase.

Sampling and storage condition

Three aqueous matrices: groundwater (G), river water (R) and lake water (L) containing all six desired species were studied. Immediately after collection, temperature (T), dissolved oxygen (DO), Eh and pH were measured (Table 2). The samples were then filtered with a 0.45 µm membrane, and subsequently divided into 6 subsamples of 50 mL. Then 125 µL of a 10,000 mg L^{-1} Fe^{2+} and Mn^{2+} stock solution was added to all subsamples to generate a Fe^{2+} and Mn^{2+} rich matrix (25.0 mg L^{-1} for each). In addition, 500 µL of 0.2 M EDTA was added to five of the six subsamples. To ensure that all six desired species were present, the subsamples were spiked by adding 5.0 µg L^{-1} As(III and V), Sb(III and V) and 15.0 µg L^{-1} Se(IV and VI). It is important that the Fe^{2+} stock solution was a mix of Fe^{2+} and Fe^{3+}, which can be seen from the brown color rather than pale green of its solution. All subsamples were stored in 50 mL PE bottles. The pH for all above prepared samples was measured prior to storage; about 6.2 for the samples without preservative and with EDTA only. For those preserved with EDTA and acid, the pH was adjusted to around 3 with HCl(Cl), HNO_3(N), formic acid(Fo) and acetic acid(Ac) respectively. The subsamples without any preservation were labeled as Gw/o (Rw/o, Lw/o) and stored under normal conditions (room temperature, with the presence of light). The subsamples preserved with EDTA only were labeled as GE (RE, LE). The subsamples preserved with EDTA combined with acid (HCl, HNO_3, formic acid and acetic acid) were labeled as GClE (RClE, LClE), GNE (RNE, LNE), GFoE (RFoE, LFoE) and GAcE (RAcE, LAcE). Those preserved with EDTA or EDTA combined with acid were stored at 4°C in the dark (see Fig. 1). The samples were measured within hours after collection. Those samples without any preservation changed color to a yellow/reddish brown, indicating that Fe-(oxy) hydroxide and possibly Mn-(oxy) hydroxide precipitated. To verify this assumption, Fe and Mn were measured at the end of the experiment in the unpreserved samples. The measurements showed that Fe was completely removed from solution and that between 60% and 80% of the Mn was removed from solution. Those samples preserved with EDTA remained colorless and transparent, indicating that Fe and Mn were chelated by EDTA. The samples without any preservation were filtered with 0.45 µm membrane each time before measurement, due to the dark brown suspension it formed. All the samples were measured periodically; 1 week, 2 weeks, 3 weeks, 7 weeks and 11 weeks. Every time 500 µL of each sample was taken for measurement.

Results and discussion

The matrix effect

The matrix plays an important role in the stability of redox sensitive species, such as As, Sb, Se, Mn and Fe. Numerous experiments reveal that the presence of microbial organisms, dissolved organic carbon and ionic strength all affect the stability of redox species (Gómez-Ariza *et al*. 1998). Therefore it is necessary to analyze the matrix as first step (Table 2). Groundwater had lower dissolved oxygen (2.88 mg L^{-1}) and a higher Fe concentration (2.3 mg L^{-1}) than river water and lake water whose Fe concentrations were almost not detectable (Table 2). As has been indicated, Fe may undergo photochemical reactions and further lead to oxidation of those species. Dissolved oxygen, although it cannot cause remarkable changes to these species alone, may catalyze oxidation of those redox species in combination with Fe. Lake water had higher concentrations of Ca, Mg and sulphate than groundwater and river water. These ions are known to form minerals once

Table 2. *Physicochemical character and constituents of the three matrices*

Sample	pH	*I* mol L^{-1}	*I** mol L^{-1}	T °C	O_2 mg L^{-1}	Ca mg L^{-1}	Fe mg L^{-1}	K mg L^{-1}	Mg mg L^{-1}	Mn mg L^{-1}	Na mg L^{-1}	Cl^- mg L^{-1}	NO_3^- mg L^{-1}	SO_4^{2-} mg L^{-1}	HCO_3^- mg L^{-1}
Groundwater	7.26	0.010	0.012	4.3	2.88	60.2	2.3	10.3	3.5	n.d	91.4	147.9	3.4	47.5	143.9
River water	7.05	0.006	0.009	8.8	9.04	47.8	0.3	4.5	4.4	0.2	34.1	48.0	6.9	38.9	133.6
Lake water	7.35	0.016	0.019	8.3	6.13	98.5	n.d	3.1	14.1	n.d	129.7	265.0	n.d	149.9	74.4

I, ionic strength; ***I****, ionic strength (after addition of Fe and Mn); n.d., not detected

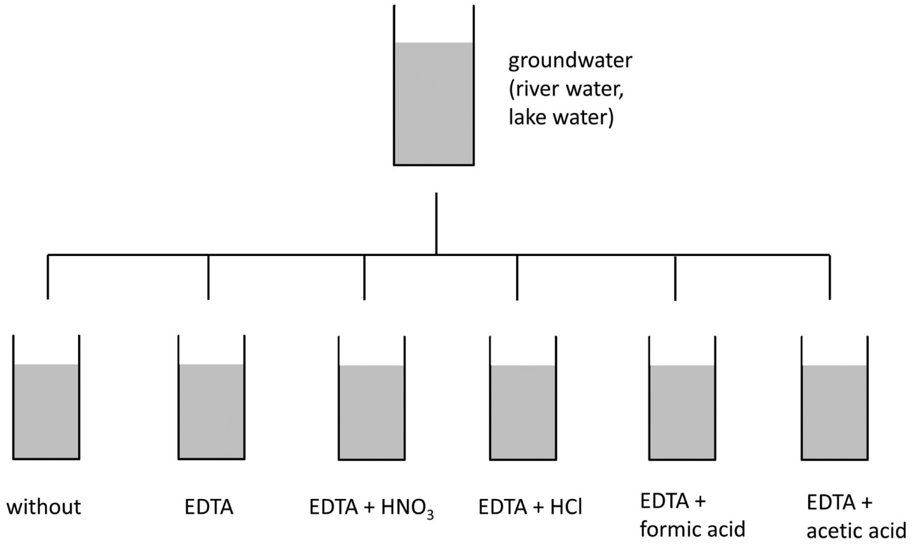

Fig. 1. Scheme of sampling and preservation processes.

physicochemical conditions change and thus they may cause co-precipitation of As, Sb and Se species. The three studied matrices showed similar pH, but varying ionic strength, oxygen content and temperature (Table 2).

Stability of As(III) and As(V)

The initial concentrations of As(III) and As(V) were 18.0 µg L^{-1} of As(III) and 11.0 µg L^{-1} of As(V) in groundwater, around 5.0 µg L^{-1} of both As(III) and As(V) in lake water and river water. The concentration of As(III) and As(V) in original groundwater was around 13.0 and 6.0 µg L^{-1}. In lake and river water, however, hardly any As was detected. In general, As(III) and As(V) were both preserved successfully in those samples prepared with EDTA combined with acid (HCl, HNO_3, formic acid or acetic acid) over a period of 11 weeks (Fig. 2). For the samples without any preservation (Gw/o, Lw/o and Rw/o), both As(III) and As(V) were lost. In particular As(V) immediately disappeared, while As (III) dropped significantly at first but only disappeared completely in week three. This result indicated that both As(III) and As(V) were strongly adsorbed by Fe-(oxy) hydroxide and Mn-(oxy) hydroxide, and the adsorption occurred within an hour. However the adsorption of As(V) seemed even faster than As(III). In contrast, the samples preserved with EDTA alone (GE, LE and RE) showed remarkable behaviour. In lake water (LE) and river water (RE) almost no changes of As(III) and As(V) were observed. But in groundwater, (GE) As(III) was partially oxidized to As(V), which was indicated by the slight decrease of As(III) and the corresponding increase of As(V) up to the third week of the experiment (Fig. 2).

Apparently, EDTA alone does not preserve the distribution of As (III) and As(V). Samanta & Clifford (2006) reported similar results, noting that EDTA alone failed to preserve As species in synthetic water samples without light. Numerous studies show that in the presence of Fe, the preservation of As species became more complicated, because oxidation and adsorption would normally occur. In groundwater with neutral pH, Fe(II) can be oxidized by oxygen and thus facilitate As(III) oxidation (Hug *et al.* 2001; Zhao *et al.* 2011). Oxidation of Fe(II) also leads to the precipitation of Fe (III)(hydr)oxides. However, once Fe(III)(hydr)oxides are formed, the oxidation rate of As(III) becomes slow (Hug *et al.* 2001), and As (V) and to a lesser extent As(III) are adsorbed onto Fe(III)(hydr) oxides. This was in agreement with our results that in EDTA alone in preserved samples where Fe and Mn were chelated by EDTA, oxidation of As(III) was observed in groundwater. On the other hand, in non-preserved samples where Fe-(oxy) hydroxide and/or Mn-(oxy) hydroxide formed, adsorption of As(III) and As(V) was observed. Previous studies showed that As(V) mainly formed inner-sphere complexes on Fe-(oxy) hydroxides and As(III) in some cases existed as a combination of inner-and outer-sphere surface complexes (Goldberg & Johnston 2001; Ona-Nguema *et al.* 2005). This may explain why both As(III) and As(V) showed strong adsorption in our study, and As(V) an even stronger adsorption than As(III) (Fig. 2).

At pH 3, HCl, HNO_3, formic acid and acetic acid had more or less the same influence on preserving As, Sb and Se species (Figs 3–5). Nevertheless, for longer periods of storage, the combination of an organic acid (formic acid and acetic acid) with EDTA may stabilize the pH of samples better than a combination with a strong inorganic acid (HCl and HNO_3) due to the formation of a buffer system. In our study time scale up to 11 weeks, however, no remarkable differences were observed.

Stability of Sb(III) and Sb(V)

About 5.0 µg L^{-1} of Sb(III) and Sb(V) were added to all subsamples. Again, the samples preserved with EDTA combined with acid (HCl, HNO_3, formic acid or acetic acid) successfully preserved Sb(III) and Sb (V) over 11 weeks. In those samples without preservation (Gw/o, Lw/o and Rw/o; with neutral pHs) Sb(III) was missing, indicating adsorption by newly formed Fe-(oxy) hydroxide and/or Mn-(oxy) hydroxide. On the other hand Sb(V) kept constant except in lake water where Sb(V) decreased gradually. Remarkably, EDTA alone failed to preserve Sb (III) and Sb(V) distribution in all matrices. The Sb(III) decreased gradually and disappeared at week three, meanwhile Sb(V) increased accordingly and reached its equilibrium in the third week, indicating that Sb(III) was now completely converted to Sb(V). Those samples preserved with EDTA alone (GE, LE and RE), Sb(III) had a lower concnetration of Sb(III) than spiked (5.0 µg L^{-1}), while Sb(V) was higher than spiked. In addition, in non-preserved samples, especially lake and river samples, Sb(V) was also higher than those preserved with an EDTA acid combination. The results indicate that oxidation of Sb(III) to Sb(V) occurred before measurement. The large decrease of Sb(III) indicates strong adsorption by Fe-(oxy) hydroxide and Mn-(oxy) hydroxide. In all samples, Sb(III) was near 2.0 µg L^{-1} higher than spike, about 7.0 µg L^{-1} in general. However, the increase in Sb(III) was unlikely from reduction of Sb(V), because Sb(V) remained constant during storage. The chromatogram of preserved samples (with EDTA, 5.0 µg L^{-1} Sb(III)) showed a larger and higher peak for Sb(III)

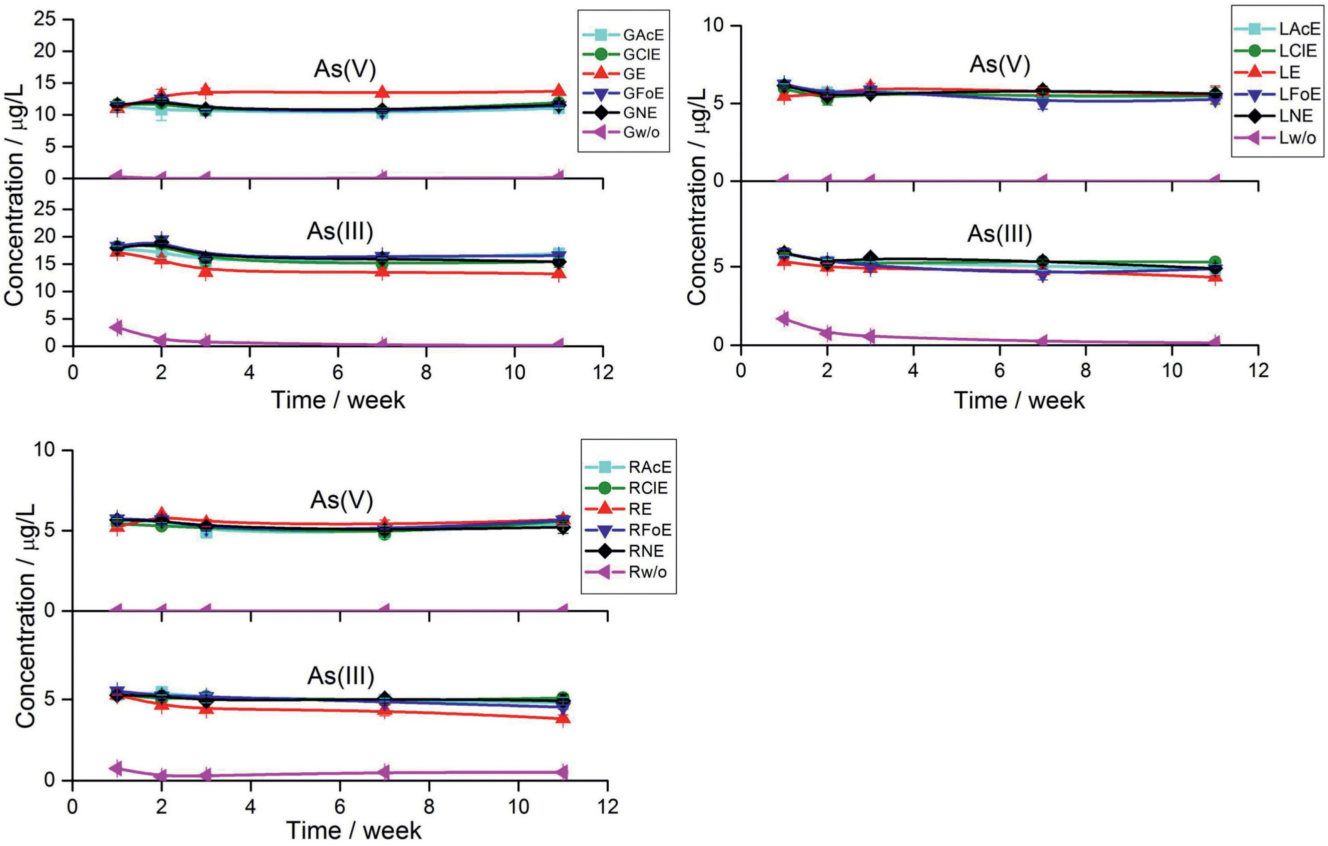

Fig. 2. The stability of As(III) and As(V) in groundwater(G), lake water (L) and river water (R). Preserved samples were stored at 4°C in the dark. Non preserved samples (Gw/o, Lw/o and Rw/o) were stored at room temperature in the presence of light. E-EDTA, Cl-HCl, N-HNO_3, Fo-Formic acid, Ac-Acetic acid, e.g. GAcE is groundwater preserved with EDTA and acetic acid; GE is groundwater preserved with EDTA alone; Gw/o is groundwater without preservation.

than the standard with de-ionized water and 5.0 µg L^{-1} Sb(III). This difference indicates that chelation of Sb(III) with EDTA from the mobile phase in standards was incomplete, thus Sb(III) was partially retained in the column, leading to a smaller and broader peak. As a result, the slope of the calibraton curve was lower than expected, a phenomenon observed previously (Kolbe *et al*. 2012), and suggesting that the addition of EDTA to both the samples and standards is required before measurement.

Since all samples were filtered through a 0.45 µm membrane after sampling and the preserved samples were stored at 4°C in the dark, microbial activity and photochemical reactions were minimized. Dissolved O_2 related oxidation is generally sluggish, although oxygen is a strong oxidant from a thermodynamic point of view (Leuz 2006). Thus the oxidation of Sb(III) in non-preserved samples and EDTA alone preserved samples (Fig. 3) was most likely caused by Fe(III). Belzile *et al.* (2001) observed a fast and complete oxidation of Sb(III) to Sb(V) by Fe-(oxy) hydroxide after a few days following pseudo-first order rate laws. The potential mechanisms involved are: (i) adsorption of Sb(III) and formation of surface complex with Fe(III)-(oxy) hydroxide (one Sb(III) on two Fe(III) sites); (ii) transfer of two electrons from Sb to two Fe atoms; (iii) release of oxidized Sb(V) and reduced Fe(II). The reason why EDTA alone failed to preserve Sb and As species, while EDTA at lower pH showed successful preservation for up to 11 weeks might be due to the pH increase caused by EDTA addition. Higher pH accelerates the oxidation of Fe^{2+} and results in a higher concentration of dissolved Fe(III) that is capable of oxidizing Sb (III) or As(III) (Gault *et al*. 2005). At lower pH (pH = 3), the oxidation of Sb(III) or As(III) was slowed due to the complete chelation of Fe^{3+} with EDTA. In comparison, As(III) was oxidized much less than Sb(III).

Antimony species have more complexing propertes than As and Se. First of all, Sb(V) existed in various forms in solution due to its inability to chelate with EDTA. A previous study (Wu & Pichler 2014) showed that at a pH above 4.5, Sb(V) existed as $Sb(OH)_6{}^-$ and lesser Sb-polymer. In the present work, the behaviour of Sb(V) at pH below 3 indicates Sb(V) is dominantly present as $Sb(OH)_6{}^-$ and trace amounts of $Sb(OH)_5$ due to hydrolysis (Tella & Pokrovski 2012), which is shown by the chromatograms of the three samples (Fig. 5).

Bhat & Iyer (1965) found that the normal complex species of SbY^- (Y = EDTA) exists in the pH range from 1.8 to 3, with the stability constants of log *KSbO SbY* = 24.80. The complex converted to free ions of $Sb(OH)_2^+$ (SbO^+) at pH < 1.5. Hydroxy complexes are formed above pH of 4 ($Sb(OH)Y^{2-}$ in the pH range from 4 to 5.5 and $SbY(OH)_2{}^{3-}$ in the pH range from 5.7 to 7, with stability constants of 8.69, and 7.8 respectively). Therefore, pH was adjusted to 3 when using EDTA as preservative for various water samples due to the high stabiliy constant it forms. In addition, Fe (III) starts forming Fe-(oxy) hydroxide due to hydrolysis when pH is higher than 3. Previous studies reported that Sb(III) was adsorbed on the surface of Fe(III)hydroxides by inner-sphere surface complexation at pH 7.7, and that it has a higher affinity for the solid phase than Sb(V) (Mitsunobu *et al*. 2010). In addition, the solubility of Sb (III) species is much lower than that of Sb(V) species because Sb (III) forms neutral (or no anionic) hydroxide species of $Sb(OH)_3^0$ in this pH range. In agreement in our study, Sb(III) was lacking in non-preserved samples (Gw/o, Lw/o and Rw/o), while Sb(V) remained stable except in lake water (Fig. 3).

Compared to As(V), Sb(V) occupies a wider redox range from Eh = 360 to −140 mV (pH 8), suggesting that Sb(III) could be oxidized at more negative Eh conditions than As (Mitsunobu *et al.* 2006). The strong adsorption by most solid phases and the low

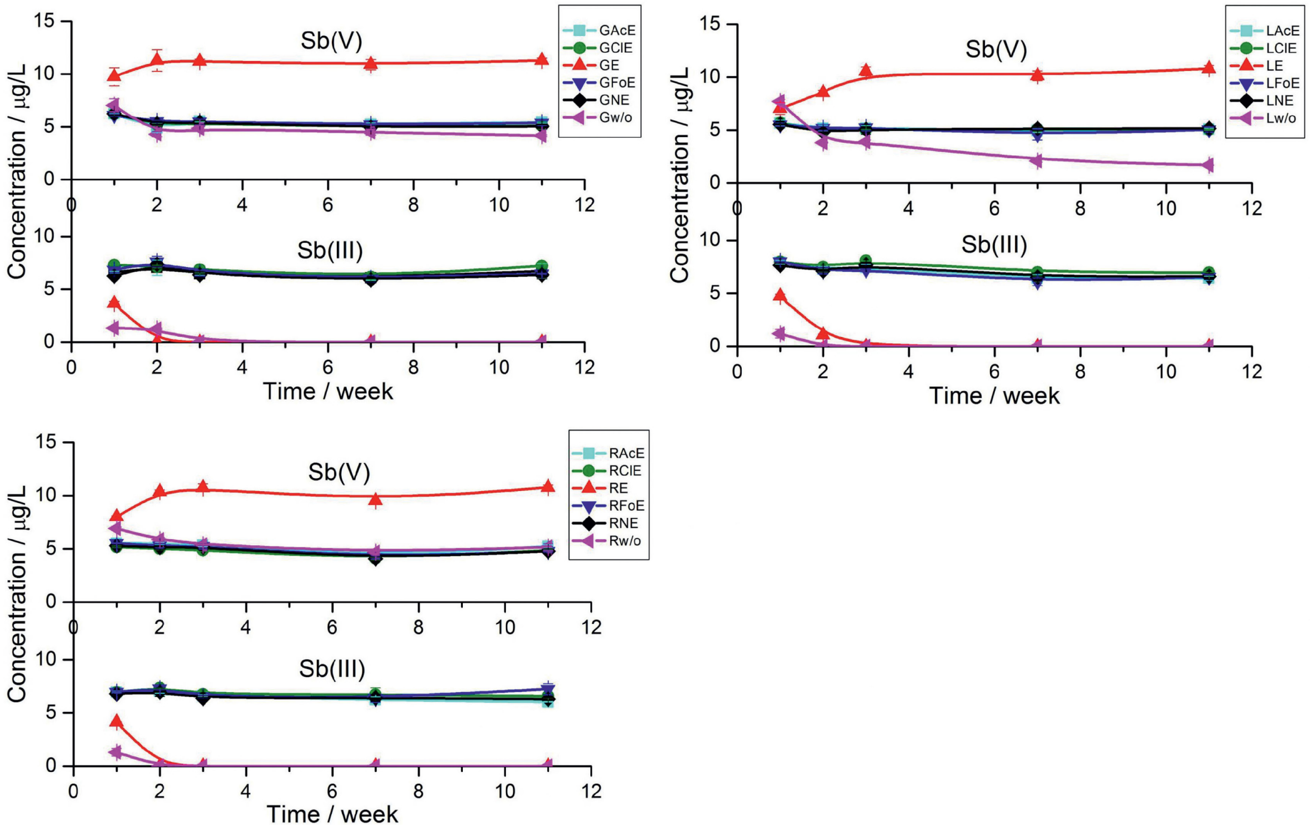

Fig. 3. The stability of Sb(III) and Sb(V) in groundwater (G), lake water (L) and river water (R). Preserved samples were stored at 4°C in the dark. Non preserved samples (Gw/o, Lw/o and Rw/o) were stored at room temperature in the presence of light. E, EDTA; Cl, HCl; N, HNO_3; Fo, Formic acid; Ac, Acetic acid, e.g. GAcE is groundwater preserved with EDTA and acetic acid; GE, groundwater preserved with EDTA alone; Gw/o, groundwater without preservation.

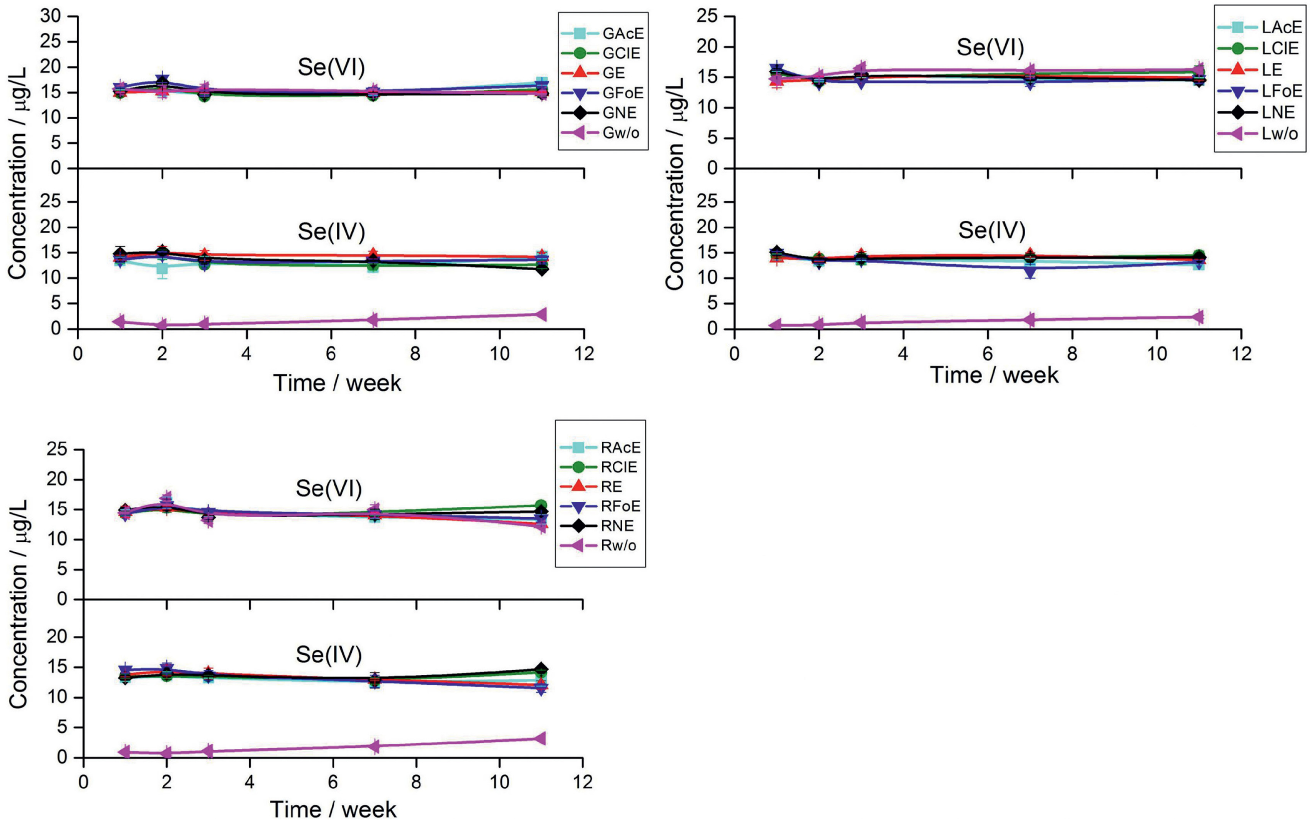

Fig. 4. The stability of Se(IV) and Se(VI) in groundwater (G), lake water (L) and river water (R). Preserved samples were stored at 4°C in the dark. Non preserved samples (Gw/o, Lw/o and Rw/o) were stored at room temperature in the presence of light. E, EDTA; Cl, HCl; N, HNO_3; Fo, Formic acid; Ac, acetic acid, e.g. GAcE is groundwater preserved with EDTA and acetic acid; GE, groundwater preserved with EDTA alone; Gw/o, groundwater without preservation.

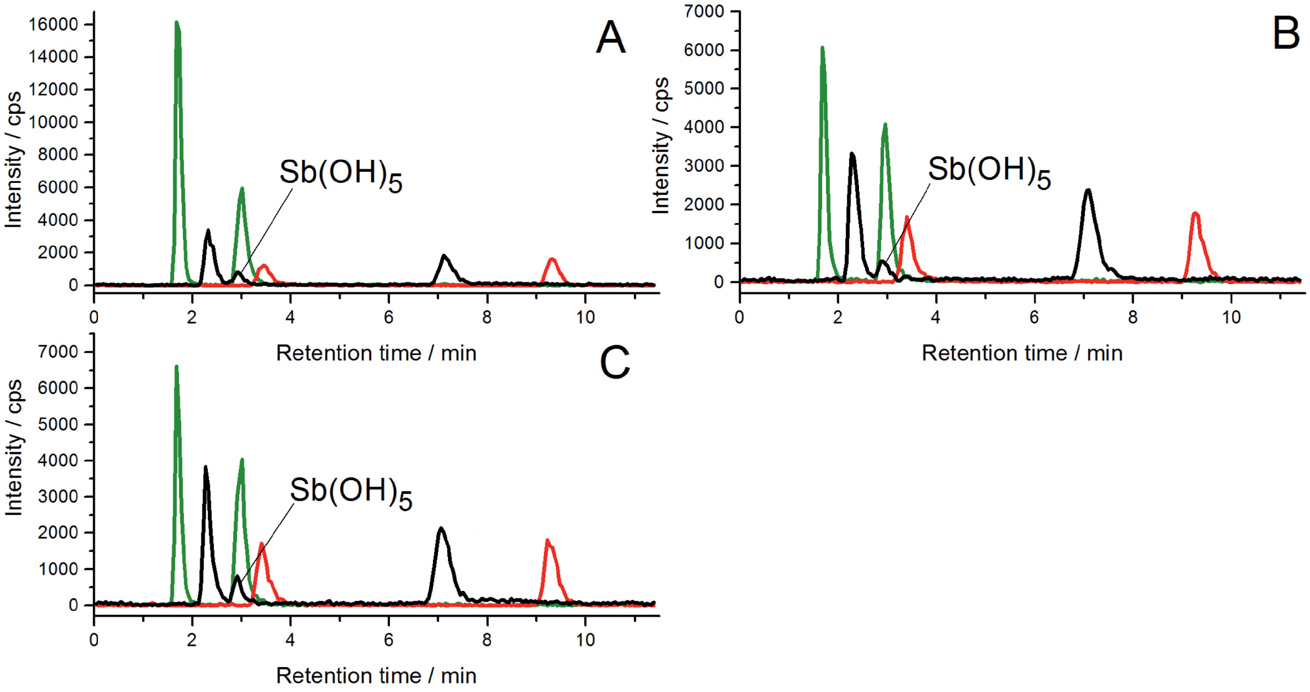

Fig. 5. Chromatogram of As, Sb and Se separation during HPLC-ICP-MS analysis. The samples were preserved at 4°C in the dark acidified to a pH of 3. The peak of Sb(OH)$_5$ is indicated, and the remaining peaks from left to right are As(III), Sb(V), As(V), Se(IV), Sb(III) and Se(VI). A, groundwater; B, lake water; C, river water.

solubility of Sb(III) at neutral pH is a viable explanation of why Sb (V) is the dominant species in most natural environments. Both Sb (III) and As(III) form inner-sphere surface complexes on the goethite surface, thus having similar adsorption behaviour. But Sb (III) is a stronger Lewis base than As(III) and the surface sites can be considered as Lewis acids, which explains the stronger binding of Sb(III) than As(III). The adsorption of Sb(V) is more complicated, based on factors related to pH, ionic strength and initial Sb(V) concentrations (Leuz *et al.* 2006).

Stability of Se(IV) and Se(VI)

Subsamples of groundwater, river water and lake water were spiked with 15.0 μg L^{-1} Se(IV) and Se(VI). In those samples without preservation (Gw/o, Rw/o and Lw/o), Se(VI) concentration remained unchanged during the duration of the 11 week experiment, while Se(IV) was immediately reduced. This indicates that Se(IV) was adsorbed onto Fe-(oxy) hydroxide and Mn-(oxy) hydroxide within hours and that no oxidation of Se(IV) to Se(VI) occurred during this time. In contrast to observations for As and Sb, EDTA alone was able to preserve the distribution of Se(IV) and Se(VI) in the groundwater, river water and lake water matrices. The EDTA combination with acid (HCl, HNO$_3$, formic acid or acetic acid) showed the same results.

Reddy *et al.* (1995) investigated the effects of redox potential on the stability of Se(IV) and Se(VI) in groundwater and considered the more stable species to be Se(VI). They did not observe reduction of Se(VI) to Se(IV) over the Eh range from 444 to −280 mV. Since oxidation of Se(IV) to Se(VI) was not observed, the mobility of Se (IV) seemed more susceptible to adsorption. In our experiment, Se (IV) was lost immediately in non-preserved samples due to adsorption (Fig. 5), however, from the third week a gradual increase of Se(IV) was observed. Considering that the concentration of Se(VI) remained constant, this increase must have been caused by desorption of Se(IV), probably due to the competitive adsorption with the increased OH$^-$ (Mandal *et al.* 2009). as the pH increased from around 6.2 to around 7.1. EXAFS studies of Se(IV) and Se(VI) adsorption on Fe-(oxy) hydroxides suggested that Se(IV) was adsorbed as an inner sphere surface complex, while Se(VI) was adsorbed mainly as an outer sphere hydrated complex (Duc *et al.* 2003; Rovira *et al.* 2008) or a mixture of outer and inner sphere surface complexes on hydrous ferric oxide (HFO) (Peak & Sparks 2002). Thus, the adsorption of Se (VI) is strongly affected by pH and ionic strength, while Se(IV) is not influenced by ionic strength (Duc *et al.* 2003).

Summary and Conclusion

In the present work, preservation strategies for As, Sb and Se redox couples in Fe- and Mn-rich water samples were tested in matrices of groundwater, lake water and river water. When the pH was adjusted to 3 with either HCl, HNO$_3$, formic acid or acetic acid, and in the absence of light, EDTA successfully preserved the six desired species for up to 11 weeks at 4°C.

(1) The metalloids As, Sb and Se are all redox sensitive elements with relatively high mobility in natural waters, nevertheless they behave differently during preservation and storage with respect to stability, redox behaviour, chromatographic complexing and adsorption on Fe-(oxy) hydroxide or/and Mn-(oxy) hydroxide.
(2) EDTA alone could preserve Se species distribution in all studied matrices, but failed to preserve As and Sb. The combination of EDTA with an acid (HCl, HNO$_3$, acetic acid or formic acid) to adjust sample pH to 3 successfully preserved the studied redox couples in groundwater, river water and lake water.
(3) The chromatography of Sb revealed that the abundance of Sb(V) varied with pH. Antimony(V) was present mainly as Sb(OH)$_6^-$ and minor Sb(OH)$_5$ at a pH of 3.
(4) Oxidation affected the redox couples to different degrees. Under the same conditions Sb(III) was completely oxidized to Sb(V), while As(III) was partially oxidized and Se(IV) was not oxidized at all.

(5) Lower-valence state species As(III), Sb(III) and Se(IV) were easily adsorbed on Fe-(oxy) hydroxide and/or Mn-(oxy) hydroxide. In addition, As(V) also showed strong adsorption by Fe-(oxy) hydroxide and Mn-(oxy) hydroxide. The strong adsorption of As(III), Sb(III), Se (IV) and As(V) may be explained by the fact that they mainly form inner sphere complexes. Higher-valence state species such as Sb(V) and Se(VI), however, were rarely adsorbed, because they form outer sphere complexes and thus bond by weak electrostatic adsorption.

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References

Andreae, M.O. 1983. Antimony (by hydride generation/AAS). *In*: Grasshoff, K., Ehrhardt, M. & Kremling, K. (eds) *Methods of Seawater Analysis*. Verlag Chemie, Weinheim, Germany, 225–229.

Asta, M.P., Kirk Nordstrom, D. & Blaine Mccleskey, R. 2012. Simultaneous oxidation of arsenic and antimony at low and circumneutral pH, with and without microbial catalysis. *Applied Geochemistry*, **27**, 281–291.

Bednar, A.J., Garbarino, J.R., Ranville, J.F. & Wildeman, T.R. 2002. Preserving the distribution of inorganic arsenic species in groundwater and acid mine drainage samples. *Environmental Science & Technology*, **36**, 2213–2218.

Belzile, N., Chen, Y.-W. & Wang, Z. 2001. Oxidation of antimony (III) by amorphous iron and manganese oxyhydroxides. *Chemical Geology*, **174**, 379–387, http://dx.doi.org/10.1016/S0009-2541(00)00287-4

Bhat, T.R. & Iyer, R.K. 1965. Studies on EDTA Complexes. V. Antimony(III) and Bismuth(III) EDTA System. *Zeitschrift für Anorganische und Allgemeine Chemie*, **335**, 331–336.

Breuer, C. & Pichler, T. 2013. Arsenic in marine hydrothermal fluids. *Chemical Geology*, **348**, 2–14.

Cabon, J.Y. & Louis Madec, C. 2004. Determination of major antimony species in seawater by continuous flow injection hydride generation atomic absorption spectrometry. *Analytica Chimica Acta*, **504**, 209–215, http://dx.doi.org/10.1016/j.aca.2003.10.048

Cobo, M.G., Palacios, M.A. & Cámara, C. 1994. Effect of physicochemical parameters on trace inorganic selenium stability. *Analytica Chimica Acta*, **286**, 371–379.

Cutter, G.A. & Cutter, L.S. 1995. Behavior of dissolved antimony, arsenic, and selenium in the Atlantic Ocean. *Marine Chemistry*, **49**, 295–306, http://dx.doi.org/10.1016/0304-4203(95)00019-N

Daus, B., Mattusch, J., Wennrich, R. & Weiss, H. 2002. Investigation on stability and preservation of arsenic species in iron rich water samples. *Talanta*, **58**, 57–65.

Daus, B., Weiss, H., Mattusch, J. & Wennrich, R. 2006. Preservation of arsenic species in water samples using phosphoric acid–limitations and long-term stability. *Talanta*, **69**, 430–434, http://dx.doi.org/10.1016/j.talanta.2005.10.012

Duc, M., Lefevre, G., Fedoroff, M. *et al.* 2003. Sorption of selenium anionic species on apatites and iron oxides from aqueous solutions. *Journal of Environmental Radioactivity*, **70**, 61–72.

Ellwood, M.J. & Maher, W.A. 2002. Arsenic and antimony species in surface transects and depth profiles across a frontal zone: The Chatham Rise, New Zealand. *Deep Sea Research Part I: Oceanographic Research Papers*, **49**, 1971–1981, http://dx.doi.org/10.1016/S0967-0637(02)00115-2

Fanning, J.C. 2000. The chemical reduction of nitrate in aqueous solution. *Coordination Chemistry Reviews*, **199**, 159–179.

Gallagher, P.A., Schwegel, C.A., Parks, A., Gamble, B.M., Wymer, L. & Creed, J.T. 2004. Preservation of As(III) and As(V) in drinking water supply samples from across the United States using EDTA and acetic acid as a means of minimizing iron-arsenic coprecipitation. *Environmental Science & Technology*, **38**, 2919–2927.

Gault, A.G., Jana, J., Chakraborty, S. *et al.* 2005. Preservation strategies for inorganic arsenic species in high iron, low-Eh groundwater from West Bengal, India. *Analytical and Bioanalytical Chemistry*, **381**, 347–353, http://dx.doi.org/10.1007/s00216-004-2861-1

Goldberg, S. & Johnston, C.T. 2001. Mechanisms of arsenic adsorption on amorphous oxides evaluated using macroscopic measurements, vibrational spectroscopy, and surface complexation modeling. *Journal of Colloid and Interface Science*, **234**, 204–216, http://dx.doi.org/10.1006/jcis.2000.7295

Gómez-Ariza, J.L., Pozas, J.A., Giráldez, I. & Morales, E. 1998. Stability and storage problems in selenium speciation from environmental samples. *International Journal of Environmental Analytical Chemistry*, **74**, 215–231.

Gregori, I., De, Quiroz, W., Pinochet, H., Pannier, F. & Potin-Gautier, M. 2005. Simultaneous speciation analysis of Sb(III), Sb(V) and (CH3)3SbCl2 by high performance liquid chromatography-hydride generation-atomic fluorescence spectrometry detection (HPLC-HG-AFS): Application to antimony speciation in sea water. *Journal of Chromatography A*, **1091**, 94–101.

Hall, G.E.M., Pelchat, J.C. & Gauthier, G. 1999. Stability of Inorganic Arsenic (III) and Arsenic (V) in Water Samples. *Journal of Analytical Atomic Spectrometry*, **14**, 205–213.

Han-Wen, S. 1982. Selective separation and differential determination of antimony(III) and antimony(V) by solvent extraction with N-benzoyl-N-phenylhydroxylamine and graphite-furnace atomic-absorption spectrometry using a matrix-modification technique. *Talanta*, **29**, 589–593.

Héninger, I., Potin-Gautier, M., Gregori, I. de & Pinochet, H. 1997. Storage of aqueous solutions of selenium for speciation at trace level.pdf. *Fresenius Journal of Analytical Chemistry*, **357**, 600–610.

Hug, S.J., Canonica, L., Wegelin, M., Gechter, D. & von Gunten, U. 2001. Solar oxidation and removal of arsenic at circumneutral pH in iron containing waters. *Environmental Science & Technology*, **35**, 2114–2121.

Jamier, V., Ba, L.A. & Jacob, C. 2010. Selenium- and tellurium-containing multifunctional redox agents as biochemical redox modulators with selective cytotoxicity. *Chemistry - A European Journal*, **16**, 10920–10928, http://dx.doi.org/10.1002/chem.201000884

Kolbe, F., Mattusch, J., Wennrich, R., Weiss, H., Sorkau, E., Lorenz, W. & Daus, B. 2012. Analytical investigations of antimony-EDTA complexes and their use in speciation analysis. *Fresenius Environmental Bulletin*, **21**, 3453–3458.

Kumar, A.R. & Riyazuddin, P. 2010. Preservation of inorganic arsenic species in environmental water samples for reliable speciation analysis. *TrAC Trends in Analytical Chemistry*, **29**, 1212–1223.

Lazareva, O., Druschel, G. & Pichler, T. 2015. Understanding arsenic behavior in carbonate aquifers: Implications for aquifer storage and recovery (ASR). *Applied Geochemistry*, **52**, 57–66, http://dx.doi.org/10.1016/j.apgeochem.2014.11.006

Leuz, A.-K. 2006. Redox reactions of antimony in the aquatic and terrestrial environment (dissertation). *Swiss Federal Institute of Technology Zurich*, Switzerland.

Leuz, A.-K., Mönch, H. & Johnson, C.A. 2006. Sorption of Sb(III) and Sb(V) to Goethite: Influence on Sb(III) Oxidation and Mobilization. *Environmental Science & Technology*, **40**, 7277–7282.

Lindemann, T., Prange, A., Dannecker, W. & Neidhart, B. 2000. Stability studies of arsenic, selenium, antimony and tellurium species in water, urine, fish and soil extracts using HPLC/ICP-MS. *Fresenius Journal of Analytical Chemistry*, **368**, 214–220.

Mailloux, B.J., Alexandrova, E., Keimowitz, A.R. *et al.* 2009. Microbial mineral weathering for nutrient acquisition releases arsenic. *Applied and Environmental Microbiology*, **75**, 2558–2565, http://dx.doi.org/10.1128/AEM.02440-07

Mandal, S., Mayadevi, S. & Kulkarni, B.D. 2009. Adsorption of aqueous selenite [Se(IV)] species on synthetic layered double hydroxide materials. *Industrial & Engineering Chemistry Research*, **48**, 7893–7898.

McCleskey, R.B., Nordstrom, D.K. & Maest, A.S. 2004. Preservation of water samples for arsenic (III/V) determinations : An evaluation of the literature and new analytical results. *Applied Geochemistry*, **19**, 995–1009, http://dx.doi.org/10.1016/j.apgeochem.2004.01.003

Mitsunobu, S., Harada, T. & Takahashi, Y. 2006. Comparison of antimony behavior with that of arsenic under various soil redox conditions †. *Environmental Science & Technology*, **40**, 7270–7276.

Mitsunobu, S., Takahashi, Y. & Terada, Y. 2010. micro-XANES evidence for the reduction of Sb(V) to Sb(III) in soil from Sb mine tailing. *Environmental Science & Technology*, **44**, 1281–1287, http://dx.doi.org/10.1021/es902942z

Ona-Nguema, G., Morin, G., Juillot, F., Calas, G. & Brown, G.E. 2005. EXAFS analysis of arsenite adsorption onto two-line ferrihydrite, hematite, goethite, and lepidocrocite. *Environmental Science & Technology*, **39**, 9147–9155, http://dx.doi.org/10.1021/es050889p

Peak, D. & Sparks, D.L. 2002. Mechanisms of selenate adsorption on iron oxides and hydroxides. *Environmental Science & Technology*, **36**, 1460–1466.

Price, R.E., Amend, J.P. & Pichler, T. 2007. Enhanced geochemical gradients in a marine shallow-water hydrothermal system: Unusual arsenic speciation in horizontal and vertical pore water profiles. *Applied Geochemistry*, **22**, 2595–2605, http://dx.doi.org/10.1016/j.apgeochem.2007.06.010

Price, R.E., London, J., Wallschläger, D., Ruiz-Chancho, M.J. & Pichler, T. 2013. Enhanced bioaccumulation and biotransformation of As in coral reef organisms surrounding a marine shallow-water hydrothermal vent system. *Chemical Geology*, **348**, 48–55, http://dx.doi.org/10.1016/j.chemgeo.2012.02.023

Qi, P. & Pichler, T. 2014. Closer look at As(III) and As(V) adsorption onto ferrihydrite under competitive conditions. *Langmuir: The ACS Journal of Surfaces and Colloids*, **30**, 11110–11116, http://dx.doi.org/10.1021/la502740w

Reddy, K.J., Blaylock, M.J., Vance, G.F. & See, R.B. 1995. *Effects of redox potential on the speciation of selenium in groundwater and coal mine backfill materials*. Proceedings of the 12th Annual National Meeting of the American Society for Surface Mining and Reclamation, Gillette, Wyoming, 3–8 June, 230–236.

Rovira, M., Giménez, J., Martínez, M., Martínez-Lladó, X., de Pablo, J., Martí, V. & Duro, L. 2008. Sorption of selenium(IV) and selenium(VI) onto natural iron oxides: Goethite and hematite. *Journal of Hazardous Materials*, **150**, 279–284.

Samanta, G. & Clifford, D.a 2005. Preservation of inorganic arsenic species in groundwater. *Environmental Science & Technology*, **39**, 8877–8882.

Samanta, G. & Clifford, D.A. 2006. Preservation and field speciation of inorganic arsenic species in groundwater. *Water Quality Research Journal*, **41**, 107–116.

Tella, M. & Pokrovski, G.S. 2012. Stability and structure of pentavalent antimony complexes with aqueous organic ligands. *Chemical Geology*, **292-293**, 57–68.

Wallis, I., Prommer, H., Pichler, T., Post, V., Norton, S.B., Annable, M.D. & Simmons, C.T. 2011. Process-based reactive transport model to quantify arsenic mobility during aquifer storage and recovery of potable water. *Environmental Science & Technology*, **45**, 6924–6931, http://dx.doi.org/10.1021/es201286c

Wang, S. & Liu, C. 2012. *Influence of Iron on the Distribution of Inorganic Arsenic Species in Groundwater Samples*. International Conference on Chemical Processes and Environmental Issues (ICCEEI 2012), Singapore, 15–16 July, 187–191.

Wiedmeyer, R.H. & May, T.W. 1993. Storage characteristics of three selenium species in water. *Archives of Environmental Contamination and Toxicology*, **25**, 67–71.

Wu, D. & Pichler, T. 2014. Simultaneous speciation analysis of As, Sb and Se redox couples by SF-ICP-MS coupled to HPLC. *Analytical Methods*, **6**, 5112–5119, http://dx.doi.org/10.1039/c4ay01013b

Zhao, Z., Jia, Y., Xu, L. & Zhao, S. 2011. Adsorption and heterogeneous oxidation of As(III) on ferrihydrite. *Water Research*, **45**, 6496–6504.