Preservation and analysis of sulfur isotopes in thioarsenates: New methods for the investigation of abiotic and biotic transformation processes

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ABSTRACT

Sulfur is known to exhibit a high affinity towards metal(loid)s. The consequent formation of soluble thiometalloid species is increasingly being recognized as an important process within the biogeochemical sulfur cycle. Taking into account their environmental, but also toxicological impact, thioarsenates ($[H_xAs^{V}S^{-II}_nO_{4-n}]^{x-3}$, x = 1 - 3, n = 1 - 4) have gained special attention. However, exact pathways of thioarsenate formation and transformation are not fully resolved yet. In particular, speciation observed during thioarsenate oxidation still raises some fundamental questions regarding both abiotic and biotic transformation pathways.

Sulfur isotope analysis has been demonstrated to be a helpful tool for investigating the transformation of other sulfur species, such as thiosulfate or elemental sulfur. Yet, a suitable method for the isotopic analysis of thioarsenates does currently not exist. Therefore, the goal of the present study was to develop a method that would allow isotope analysis of thioarsenates, and apply this method to address open questions on thioarsenate transformation in order to elucidate their role within the sulfur cycle.

As the fundamental prerequisite to successful isotope analysis, methods of thioarsenate preservation were investigated first, focusing primarily on the challenges arising from natural, iron-rich waters. Established methods, namely the addition of mineral acids or flash-freezing, are unsuitable in this case because they induce arsenic sulfide or iron oxyhydroxide precipitation, inevitably changing the original thioarsenic speciation. In the present study, a new approach based on separating the anionic thioarsenates from cationic iron by solid phase extraction was investigated. In addition to thioarsenate preservation, this approach could also be used for enrichment to facilitate later isotope analysis. Anion-exchange resin AG2-X8 was found to fully retain monothioarsenate and trithioarsenate, as well as sulfate, thiosulfate, and arsenate. Iron passed the resin without interaction, and was thus separated from the target species as hypothesized. Elution of the retained species was performed in flow-through mode with alkaline salicylate, which proved to conserve speciation and remove all investigated species quantitatively from the resin. However, complete recovery of the strongly bound trithioarsenate required repeated cycles of soaking the resin in salicylate prior to elution. Full recovery of the target species was also obtained by performing batch elution, which allowed lowering the elution volume, and consequently improved species enrichment in the eluates. Finally, the method was applied to iron-rich waters from a mineral spring in the Czech Republic and was found to preserve sulfur and arsenic speciation for up to 6 days.

For the subsequent determination of sulfur isotopes in thioarsenates, routine analytical methods were considered. Isotope analysis is commonly performed by isotope ratio mass spectrometry (IRMS), following precipitation of sulfur species from solution. However, this approach is not feasible

since the high chemical similarity among thioarsenates prevents selective precipitation. Moreover, during standard precipitation of sulfide for IRMS analysis, monothioarsenate was found to co-precipitate, and thus impede correct $\delta^{34}S$ determination of sulfide. To circumvent these issues, a new method was developed based on ion chromatographic species separation and online detection of sulfur isotopes by multi-collector inductively coupled plasma mass spectrometry (IC-MC-ICP-MS). This allowed simultaneous isotopic analysis of sulfide, sulfate, thiosulfate, and for the first time of all four thioarsenates.

The new IC-MC-ICP-MS method was applied to investigate oxidative thioarsenate transformation. Abiotic oxidation of monothioarsenate produced ^{34}S-depleted sulfate, yielding a normal isotope effect of -6.1 ‰. Oxidation of tetrathioarsenate revealed that no isotopic fractionation is associated with the stepwise transformation to tri-, di-, and monothioarsenate. However, monothioarsenate was found enriched in ^{34}S, which was proposed to result from further oxidation of monothioarsenate, but also intermolecular exchange with heavy sulfide. All these findings prove that oxidative monothioarsenate transformation causes a redox change in arsenic-bound sulfur, while all other thioarsenates release sulfidic sulfur in its original form. Furthermore, the current results support a previous hypothesis of thioarsenites occurring briefly as intermediates during formation, as well as decomposition of thioarsenates.

IC-MC-ICP-MS was further employed to investigate the question, if the reduced sulfur in thioarsenates can be utilized as an electron donor by chemolithotrophic bacteria. Previously obtained speciation data seemed to indicate direct microbial oxidation of monothioarsenate, yet abiotic transformation could not be excluded. Incubation experiments with the hyperthermophile *Thermocrinis ruber* performed in this study revealed a pronounced inverse isotope effect. The combination of $\delta^{34}S$ values observed during abiotic and biotic oxidation indicate that arsenic-bound sulfur can in fact not be used directly as a substrate for microbial metabolism. An alternative model was proposed, in which monothioarsenate is first disproportionated abiotically to elemental sulfur and arsenite, followed by microbial oxidation of these intermediates to sulfate and arsenate.

Overall, the methods developed over the course of this study allow for the investigation of both abiotic and biotic pathways of thioarsenate transformation. Where speciation analysis alone did not yield conclusive results until now, isotope analysis was found to provide new, valuable insights. Thus, the findings of the present study assist in elucidating the processes that control the occurrence, stability, and utilization of thioarsenates within the biogeochemical sulfur cycle.

ZUSAMMENFASSUNG

Die ausgeprägte Affinität von Schwefel zu Metallen und Metalloiden führt zur Bildung verschiedener löslicher Thiometall(oid)-Verbindungen, deren Bedeutung für den biogeochemischen Schwefelkreislauf zunehmend an Aufmerksamkeit gewinnt. Ein besonderes Augenmerk liegt dabei auf Thioarsenaten ($[H_xAs^{V}S^{-II}_nO_{4-n}]^{x-3}$, x = 1 - 3, n = 1 - 4), die vor allem wegen ihrer ökologischen und toxikologischen Auswirkungen Gegenstand bisheriger Forschung waren. Allerdings sind die genauen Bildungs- und Umwandlungsprozesse noch nicht vollständig aufgeklärt. Insbesondere Speziesveränderungen, die während der Oxidation von Thioarsenaten beobachtet wurden, werfen nach wie vor grundlegende Fragen zum Verlauf abiotischer und biotischer Umwandlungsprozesse auf.

Für die Untersuchung der Umwandlung von Schwefelspezies wie Thiosulfat oder Elementarschwefel hat sich die Analyse von Schwefelisotopen als äußerst hilfreich erwiesen. Zurzeit fehlt jedoch eine Methode für die Isotopenanalyse von Thioarsenaten. Dementsprechend war das Ziel der vorliegenden Studie, eine Methode zu entwickeln, mit der Schwefelisotope in Thioarsenaten untersucht werden können. Im Anschluss wurde die entwickelte Methode angewendet, um offene Fragen im Hinblick auf die Umwandlung von Thioarsenaten zu untersuchen und somit ihre Rolle innerhalb des Schwefelkreislaufs zu beleuchten.

Die Entwicklung einer Methode zur Probenstabilisierung stellte dabei den ersten, grundlegenden Schritt für die spätere Isotopenanalyse dar. Hierbei wurde ein besonderer Schwerpunkt auf die Probleme der Stabilisierung von Thioarsenaten in natürlichen, eisenreichen Wässern gelegt. Gegenwärtig angewandte Methoden, wie das Ansäuern oder Schockgefrieren von Proben, sind für Thioarsenate ungeeignet, da sie zu Arsensulfid- und Eisenhydroxid-Ausfällungen führen und so die ursprüngliche Speziesverteilung verändern. Im Rahmen der vorliegenden Studie wurde eine neue Herangehensweise getestet, die auf der Trennung der anionischen Thioarsenate von kationischem Eisen mittels Festphasenextraktion (SPE) basiert. Zusätzlich zur Probenstabilisierung kann diese Methode auch zur Anreicherung von Thioarsenaten für die spätere Isotopenanalyse verwendet werden. Das Anionenaustauschmaterial AG2-X8 zeigte vollständige Retention von Mono- und Trithioarsenat sowie Sulfat, Thiosulfat und Arsenat. Eisen hingegen passierte den Anionenaustauscher ohne zu sorbieren und wurde somit erfolgreich von den Thioarsenaten getrennt. Für die anschließende Elution wurden Lösungen von alkalischem Salicylat kontrolliert durch die SPE-Kartusche geleitet und dabei die sorbierten Spezies komplett vom Anionenaustauscher desorbiert. Die starke Adsorption von Trithioarsenat erforderte dabei vor der Elution eine wiederholte Einwirkzeit in Salicylat. Als alternative Elutionsmethode wurde das AG2-X8-Material komplett aus der Kartusche gelöst. Unter Rühren in alkalischem Salicylat wurden so die sorbierten

Spezies ebenfalls vollständig zurückgewonnen, gleichzeitig das Elutionsvolumen wesentlich verringert und so die Anreicherung der Spezies im Eluat verbessert. An natürlichen, eisenreichen Wässern einer tschechischen Mineralquelle konnte abschließend gezeigt werden, dass die neue Methode Schwefel- und Arsenspezies für mindestens 6 Tage stabilisiert.

Für die anschließende Isotopenanalyse der Thioarsenate wurden zunächst etablierte Routinemethoden untersucht. Standardmäßig werden gelöste Schwefelspezies gefällt und das Präzipitat mittels Isotopenverhältnis-Massenspektrometrie (IRMS) analysiert. Die ausgeprägte chemische Ähnlichkeit der Thioarsenate erlaubt jedoch keine selektive Ausfällung. Darüber hinaus zeigten Tests dieser Studie, dass während der standardisierten Fällung von Sulfid für die IRMS-Analyse Monothioarsenat mitausfällt und somit δ^{34}S-Werte von Sulfid verfälschen kann. Um diese Probleme zu umgehen, wurde eine neue Methode entwickelt, die auf ionenchromatografischer Trennung und direkt anschließender Analyse der Schwefelisotope mittels Multikollektor-Massenspektrometrie mit induktiv gekoppeltem Plasma beruht (IC-MC-ICP-MS). Diese Methode erlaubt die gleichzeitige Isotopenanalyse von Sulfid, Sulfat, Thiosulfat und erstmals aller vier Thioarsenate.

Die neue IC-MC-ICP-MS-Methode wurde angewendet, um oxidative Umwandlungen von Thioarsenaten zu untersuchen. Während der abiotischen Oxidation von Monothioarsenat bildete sich ^{34}S-abgereichertes Sulfat, was zu einem normalen Isotopeneffekt von -6.1 ‰ führte. Keine Isotopenfraktionierung wurde hingegen für die stufenweise Oxidation von Tetrathioarsenat über Tri- und Di- zu Monothioarsenat gefunden. Monothioarsenat zeigte jedoch eine deutliche Anreicherung von ^{34}S, die auf weitere Oxidation, aber auch auf intermolekularen Austausch mit schwerem Sulfid zurückzuführen ist. Diese Ergebnisse legen nahe, dass die oxidative Zersetzung von Monothioarsenat mit einer Oxidation des Arsen-gebundenen Schwefels einhergeht, während Tetra-, Tri- und Dithioarsenat sulfidischen Schwefel in seiner ursprünglichen Oxidationsstufe freisetzen. Des Weiteren unterstützen die hier gewonnenen Erkenntnisse die Hypothese früherer Studien, nach denen dreiwertige Thioarsenite kurzzeitig als intermediäre Spezies sowohl während der Bildung als auch der Umwandlung von Thioarsenaten auftreten.

Die neu entwickelte IC-MC-ICP-MS-Methode wurde außerdem eingesetzt, um der Frage nachzugehen, ob Mikroorganismen den in Thioarsenaten gebundenen, reduzierten Schwefel als Elektronendonor verwenden können. In früheren Studien beobachtete Speziesumwandlungen deuteten darauf hin, dass chemolithotrophe Bakterien Monothioarsenat direkt oxidieren. Umwandlungen durch abiotische Prozesse konnten jedoch nicht ausgeschlossen werden. Inkubationsexperimente, die im Rahmen der vorliegenden Studie mit dem hyperthermophilen *Thermocrinis ruber* durchgeführt wurden, offenbarten einen ausgeprägten inversen Isotopeneffekt.

In Verbindung mit Ergebnissen abiotischer Oxidationsexperimente weist dies darauf hin, dass Schwefel in Monothioarsenat nicht wie angenommen als Substrat für mikrobiellen Umsatz dient. Dementsprechend wurde ein alternatives Modell entworfen, in dem Monothioarsenat zunächst abiotisch zu Elementarschwefel und Arsenit disproportioniert, bevor Mikroorganismen diese Zwischenprodukte weiter zu Sulfat und Arsenat oxidieren.

Insgesamt ermöglichten die im Zuge dieser Studie entwickelten Methoden eine eingehendere Untersuchung von abiotischen sowie biotischen Thioarsenatumwandlungen. Die Aussagekraft von Speziesanalysen für die Aufklärung von Transformationsprozessen ist oftmals begrenzt. Diese Studie konnte zeigen, dass die zusätzliche Analyse von Schwefelisotopen wertvolle Informationen liefert. Damit tragen die hier gewonnenen Erkenntnisse zu einem besseren Verständnis von Vorkommen, Stabilität und mikrobieller Umsetzung von Thioarsenaten innerhalb des natürlichen Schwefelkreislaufs bei.

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LIST OF ABBREVIATIONS

EDTA	ethylenediaminetetraacetic acid
IC	ion chromatography
ICP	inductively coupled plasma
MC	multi-collector
MQ	deionized water (resistivity 18 Ω · cm)
MS	mass spectrometry
SAX	strong anion-exchange
SPE	solid phase extraction
V-CDT	Vienna Canyon Diablo Troilite
XAS	X-ray absorption spectroscopy

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EXTENDED SUMMARY

1 Introduction

1.1 Fractionation of sulfur isotopes

1.1.1 Isotopic distribution of sulfur

Sulfur, a classic representative of the non-metallic chalcogens with the atomic number 16, is a ubiquitous element in our environment. Even though it represents only 0.07 % of the earth's crust (Steudel, 1998), it occurs in various forms in all environmental spheres: as gaseous compounds or sulfur-bearing particles in the atmosphere, as minerals most commonly found in the form of evaporites, ore deposits or precipitates in sediments, as dissolved species in the oceans and in freshwater systems, and most importantly for livings organisms in the form of organic compounds such as the amino acids cysteine and methionine. Currently, 24 isotopes of sulfur are known. Considering their natural abundances and half-lifes, only the four stable isotopes ^{32}S, ^{33}S, ^{34}S, and ^{36}S presented in table 1 are of environmental relevance.

Table 1. Natural standard abundances of the four stable sulfur isotopes according to de Laeter et al. (2003).

sulfur isotope	abundance [%]
^{32}S	94.99
^{33}S	0.75
^{34}S	4.25
^{36}S	0.01

However, in the 1930s isotopic investigations of light elements revealed that the standard values of isotopic abundances are variable (Urey et al., 1932; Dole, 1935; Nier and Gulbransen, 1939). Studying the isotopic composition of sulfate ($S^{+VI}O_4^{2-}$) and sulfide ($[HS^{-II}]^-$) from well waters, Thode et al. (1949) were the first to observe these fractionation effects in sulfur isotopes. They hypothesized that the variation in isotopic compositions for different sulfur compounds is directly connected to their chemical properties. Based on these early studies, Bigeleisen (1965) concluded that isotopes of the same element react differently due to their difference in mass, and that any deviation from the standard isotopic distribution observed in nature is the result of physical and chemical processes. On the quantum-mechanical level, these effects are believed to arise from the preferential distribution of energy into vibrational modes for light isotopes, rather than into translational or rotational modes (Hoefs, 2015a). Consequently, less energy is required to break a bond involving a light isotope. In general, this effect will lead to the accumulation of heavy isotopes in the compound providing the strongest bond, which in the case of sulfur is often represented by dissolved sulfate or a solid sulfur compound.

The preferential enrichment or depletion of sulfur isotopes due to these effects is commonly expressed as a deviation from a standard reference. In order to ensure comparability of isotope data, the universal use of Vienna Canyon Diablo Troilite (V-CDT) as a reference material was agreed upon, which is considered to represent the original sulfur isotope composition of the early Earth's crust and mantle (Thode, 1991). Due to the natural sulfur isotopic abundances, most studies focus on deviations in the ratio of ^{34}S to ^{32}S. Using V-CDT as a reference, isotopic fractionation reported for sulfur typically lies between -40 ‰ and +50 ‰ (Fig. 1). However, exceptional $\delta^{34}S$ values of up to -53 ‰ for marcasite associated with uranium deposits, and +87 ‰ for marine barite concretions have been described as well (Austin, 1970; Sakai, 1971; Thode, 1991).

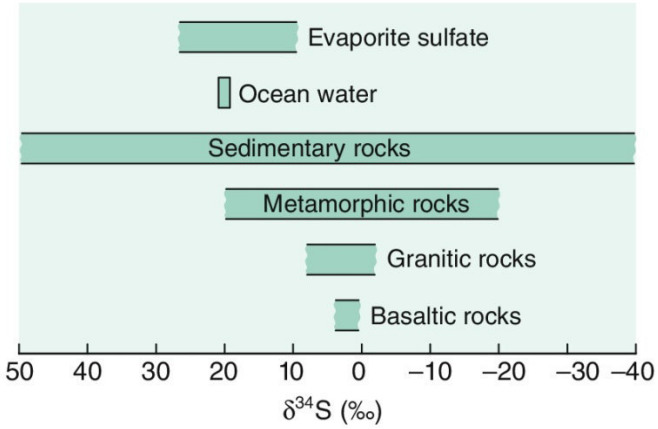

Fig. 1. Extent of sulfur isotope fractionation typically observed in different sulfur-bearing reservoirs (Hoefs, 2015b).

Isotopic fractionation of sulfur has been documented to result from two processes: kinetic fractionation and equilibrium fractionation. While kinetic fractionation is caused by different reaction rates of compounds containing ^{32}S and ^{34}S, equilibrium fractionation originates from isotope exchange reactions between sulfur-bearing compounds. Hence, the isotopic composition serves as a record of all processes that have affected a specific compound. This consideration led to the widespread application of sulfur isotope analysis to identify the origin, as well as transformation, of sulfur in the environment. Research fields to which sulfur isotope analysis has contributed crucial information are numerous, and include paleoenvironmental reconstructions, evolution of early life, formation of mineral deposits, source identification of soluble and gaseous compounds, and elucidation of metabolic pathways.

One field of research that has derived particular benefit from sulfur isotope analysis over the last 50 years is the investigation of the redox chemistry of dissolved sulfur species. The large fractionation associated with redox reactions within the biogeochemical sulfur cycle made analysis of the end-members sulfide and sulfate an early focus of sulfur isotope studies. However, intermediate sulfur species soon became apparent to play a decisive role in sulfur cycling as well. Mainly thiosulfate

($S^{-I}S^{+V}O_3^{2-}$), elemental sulfur (S^0), polysulfides ($[S^0_{n-1}S^{-II}]^{2-}$), and sulfite ($S^{+IV}O_3^{2-}$) were found largely responsible for mediating the overall 8 electron transfer between sulfide and sulfate (Zhang and Millero, 1993). Considering the complex pathways of sulfur transformation, isotope analysis became a much valued tool to elucidate these redox processes within the environment.

1.1.2 Fractionation during abiotic sulfur transformation

Assuming little fractionation would be associated with abiotic sulfur transformations, research into the effects of abiological sulfide oxidation and sulfate reduction has been limited. Indeed, a study by Fry et al. (1988b) reported a maximum fractionation of -5.1 ‰ for sulfate produced from sulfide oxidation. It is noteworthy that this study presents the only detailed investigation of sulfur being fractionated during abiotic oxidation of dissolved sulfide with oxygen. Hence, it currently serves as the sole basis for differentiating between oxidative abiotic and biotic processes when interpreting sulfur isotopic records. In contrast, abiotic sulfate reduction can produce much greater fractionation. However, this process is believed to be of minor environmental importance because of the slow reaction kinetics at temperatures < 150 °C caused by the high symmetry of the sulfate ion (Rickard and Luther, 2007). Nevertheless, Kiyosu and Krouse (1990) argued that in the presence of organic acids, thermochemical sulfate reduction at 50 - 100 °C can yield isotopic fractionation of -20 to -28 ‰.

1.1.3 Fractionation during biotic sulfur transformation

In natural environments, sulfate reduction and sulfide oxidation have been found to be significantly accelerated by microorganisms. More than 100 species are known to use sulfate as a substrate for microbial growth (Canfield, 2001), which produces ^{34}S-depleted sulfide, while the residual sulfate is enriched in ^{34}S. Based on laboratory studies with sulfate-reducing bacteria it was suggested that the extent of isotopic fractionation during sulfate reduction is limited from between -4 to -47 ‰ (Kaplan and Rittenberg, 1964; Thode, 1991; Hoefs, 2015b). Surprisingly, analysis of sulfide from natural environments repeatedly revealed much greater fractionation of more than -70 ‰ (Rudnicki et al., 2001; Wortmann et al., 2001). This clear discrepancy has been explained by repeated redox cycling of intermediate sulfur species, such as elemental sulfur, thiosulfate, and sulfite, introducing additional fractionation for sulfide (Jorgensen, 1990; Canfield and Thamdrup, 1994; Habicht and Canfield, 2001). Yet recently it was suggested that fractionation associated with the final step of sulfate reduction, namely sulfide formation from sulfite, has been previously underestimated, and may in fact be responsible for sulfide depletion beyond -47 ‰ (Brunner and Bernasconi, 2005).

In comparison, microbial sulfide oxidation is generally considered to induce much smaller fractionation (Habicht et al., 1998). Although it is well documented that sulfide oxidation by phototrophic and chemolithotrophic bacteria can exceed abiotic oxidation by more than 3 orders of

magnitude, exact metabolic pathways are not fully understood (Luther et al., 2011). Similar to sulfate reduction, the formation of intermediate sulfur species has been identified as a central aspect for the interpretation of $\delta^{34}S$ values of sulfide and sulfate. Anaerobic sulfide oxidation by the phototrophic bacterium *Chromatium vinosum* was found to produce elemental sulfur enriched in ^{34}S by +1 to +3 ‰ (Fry et al., 1984). This result presents one of the few cases, in which the heavy isotope ^{34}S is preferentially accumulated in the reaction product instead of the substrate. Having made similar observations for nitrogen isotopes, Casciotti (2009) suggested that these inverse isotope effects are most likely the result of two processes: (1) pre-equilibration between the actual substrate and a larger supply of substrate not directly involved in the reaction (for example HS^- versus H_2S as presented by Fry et al. (1986)) or (2) formation of a transition state species along the enzymatic pathway providing a stronger bond, and thus, preferentially attracting the heavier isotopes.

Further oxidation of elemental sulfur by the anaerobe *Chlorobium vibrioforme* was found to yield sulfate depleted in ^{34}S by -1.7 ‰, in accordance with a normal isotope effect (Fry et al., 1988a). Apart from oxidation, disproportionation of intermediate sulfur species also represents a favorable mode of metabolism along the oxidative branch of the sulfur cycle. Using elemental sulfur both as electron donor and acceptor, various bacteria were found to produce sulfate enriched in ^{34}S by +3.6 to +18.4 ‰ and sulfide depleted by -0.9 to -13 ‰ (Canfield and Thamdrup, 1994; Cypionka et al., 1998; Böttcher et al., 2001; Poser et al., 2016). Bacterial disproportionation of sulfite or thiosulfate in pure cultures, as well as natural populations similarly yields heavy sulfate and light sulfide (Habicht et al., 1998).

In large part, these findings on isotopic fractionation during both abiotic and biotic processes constitute the foundation of our current knowledge on the biogeochemical sulfur cycle. Two general concepts have been derived from all of these studies. First and foremost, isotopic analysis provides key information that helps identify abiotic redox processes, as well as pathways of microbial metabolism. Furthermore, the combination of these characteristic fractionation patterns allows for the reconstruction of sulfur transformation processes from both modern and geological $\delta^{34}S$ records.

1.2 Thioarsenates

Much attention was directed over the past century to defining the role that intermediate sulfur species play within the biogeochemical sulfur cycle. At the same time, sulfur speciation analysis, particularly of suboxic to anoxic waters, continuously revealed another group of important soluble species. The presence of metal(loid)s such as arsenic, gold, cadmium, copper, zinc, molybdenum, or antimony, is known to induce formation of so-called thiometalloid species (Seward, 1973; Tossell and Vaughan, 1993; Tossell, 2000a; Ullrich et al., 2013; Lohmayer et al., 2015). Among these species, the

combination of the trace metalloid arsenic (As) with the ubiquitous sulfur has gained special attention.

1.2.1 Identification, formation, and occurrence

Since as early as the 19th century, the strong affinity between arsenic and sulfur and the consequent formation of soluble sulfur-arsenic compounds have been a subject of comprehensive study (Brauner and Tomicek, 1887; McCay, 1888; McCay and Foster, 1904; Höltje, 1929; Babko and Lisetskaya, 1956; Weissberg et al., 1966; Thilo et al., 1970; Spycher and Reed, 1989; Krupp, 1990; Webster, 1990; Eary, 1992). Considering the potential health risks and environmental impact of thioarsenic species, the development of suitable analytical tools became a focus of sulfur chemistry research in the late 20th century. Using capillary zone electrophoresis, Schwedt and Rieckhoff (1996) presented the first analytical proof of dissolved thioarsenic species.

In the following years, substantial advancements in the field of analytical chemistry enabled a more detailed investigation of these species. Wilkin et al. (2003) successfully demonstrated the use of ion chromatography coupled to inductively coupled plasma mass spectrometry (IC-ICP-MS) for the analysis of soluble sulfur-arsenic compounds. In fact, this study prompted a debate on the true nature of the detected species regarding the oxidation state of arsenic. Investigating the formation of thioarsenic species from arsenite and sulfide, Wilkin et al. (2003) reported the detected species as trivalent thioarsenites ($[H_xAs^{III}S^{-II}_nO_{3-n}]^{x-3}$, x = 1 - 3, n = 1 - 3). This was concluded on the grounds that completely anoxic conditions prevailed during the experiments. However, further investigations, including the application of electrospray mass spectrometry, disproved their conclusions, and identified the detected thioarsenic species as pentavalent thioarsenates ($[H_xAs^{V}S^{-II}_nO_{4-n}]^{x-3}$, x = 1 - 3, n = 1 - 4) (Stauder et al., 2005; Wallschläger and Stadey, 2007).

Nevertheless, results from further studies using X-ray absorption spectroscopy analysis (XAS) indicated the occurrence of trivalent thioarsenites (Beak et al., 2008; Suess et al., 2009). The seemingly contradictory results of detecting both thioarsenites and thioarsenates in sulfide-arsenite solutions were revealed by Planer-Friedrich et al. (2010) to be an analytical issue. This study showed that XAS analysis, which requires concentrations in the mmol $\cdot$ L^{-1} range, yields thioarsenites as a result of high SH^- to OH^- ratios, while sample dilution to µmol $\cdot$ L^{-1} or even nmol $\cdot$ L^{-1} and alkaline gradient elution in the course of IC-ICP-MS analysis leads to detection of thioarsenates. Furthermore, contact with minor amounts of atmospheric oxygen during IC-ICP-MS analysis was found to be sufficient to rapidly oxidize thioarsenites to thioarsenates (Planer-Friedrich et al., 2010). Based on these results, it can be concluded that IC-ICP-MS analysis of samples from completely anoxic environments will inevitably assign thioarsenites wrongfully as thioarsenates. However, trace

amounts of oxygen present in most natural waters will lead to the prevailing occurrence, and thus correct identification via IC-ICP-MS, of thioarsenates.

Despite their limited importance as naturally occurring species, thioarsenites have been hypothesized to be key intermediates during the formation of thioarsenates (Planer-Friedrich et al., 2010; Planer-Friedrich et al., 2015). The early study by Schwedt and Rieckhoff (1996) demonstrated the synthesis of thioarsenates under alkaline conditions from arsenite and elemental sulfur. In addition, monothioarsenate was found to form during reaction of sulfide with arsenate at pH < 4 (Rochette et al., 2000). A study by Helz and Tossell (2008) also discussed thioarsenate formation by consecutive ligand exchange of OH^- with SH^- on arsenate, but was later disproved based on the identification of thioarsenites as precursors of thioarsenates (Planer-Friedrich et al., 2010). Even though exact thioarsenate formation pathways are still subject to debate, the combination of all currently available data suggests a model with two complementary processes: (1) spontaneous reaction of (thio)arsenite with zerovalent sulfur, either in the form of elemental sulfur or polysulfides, or (2) oxidation of thioarsenites to the respective thioarsenates by trace amounts of oxygen (Planer-Friedrich et al., 2015).

In the environment, thioarsenates are typically found in sulfidic, suboxic to anoxic settings, such as geothermal waters (Planer-Friedrich et al., 2007; Ullrich et al., 2013; Guo et al., 2017), anoxic, haloalkaline lake water (Hollibaugh et al., 2005; Wallschläger and Stadey, 2007), landfill environments (Zhang et al., 2014), marina sediments (Mamindy-Pajany et al., 2013), and anoxic ground waters (Stauder et al., 2005; Wallschläger and Stadey, 2007; Pi et al., 2016). Along with other commonly found species, such as elemental sulfur or thiosulfate, thioarsenates can represent a significant share of the reduced sulfur of up to 40 % in these environments (Planer-Friedrich et al., 2009).

Interestingly, thioarsenates have also been reported in sulfidic, iron-rich environments irrespective of the potential of iron sulfide formation (Suess et al., 2011; Stucker et al., 2014). Moreover, thioarsenates have been shown to occur in the presence of nanohematite, goethite, ferryhydrite, mackinawite, and pyrite (Suess and Planer-Friedrich, 2012; Burton et al., 2013; Couture et al., 2013; Stucker et al., 2014). All of these findings seem to indicate that thioarsenate formation surpasses iron sulfide precipitation, and thus plays a significant role in both iron-free and iron-rich sulfidic environments.

Although thioarsenates have been studied intensively over the past decade, exact pathways of their transformation are still not fully resolved. So far, knowledge on thioarsenate transformation processes, particularly on their oxidation, has been derived exclusively from speciation analysis. For many unresolved transformation pathways of other species, such as thiosulfate or elemental sulfur,

analysis of isotopic fractionation was found to provide pivotal information. However, up to now the potential of elucidating thioarsenate transformations by isotope analysis has not been pursued. Consequently, there is currently no knowledge on isotopic fractionation of thioarsenates.

Furthermore, it is unknown how fractionation of thioarsenates may contribute to $\delta^{34}S$ values observed for the end-members sulfide and sulfate. Inconclusive fractionation patterns were for example documented in sulfidic, high arsenic groundwater (Xie et al., 2009). A subsequent study by Wang et al. (2014) hypothesized that the unusually high $\delta^{34}S$ values for sulfate resulted from increased sulfate reduction, although it was noted that thioarsenate formation may be a contributing factor. Investigating these aspects could not just further our understanding of sulfur-arsenic redox chemistry, but also allow a more refined interpretation of sulfur isotope data from both modern and ancient environments.

1.2.2 Stability and abiotic transformation of thioarsenates

Abiotic transformation of thioarsenates has been studied with respect to the effects of temperature, pH, and oxidation. For the individual thioarsenates, work by Planer-Friedrich et al. (2009) found surprisingly different results of stability towards elevated temperatures. Tri- and tetrathioarsenate became unstable starting at 50 °C, whereas monothioarsenate showed only minor signs of decomposition after 2 h at 80 °C. Thioarsenate decomposition as a result of contact with atmospheric oxygen and changing pH was reported by Stauder et al. (2005). While monothioarsenate is stable over a pH range of 1 to 13, the higher thiolated species di-, tri-, and tetrathioarsenate are only stable at pH values > 4, > 7, and > 13, respectively (Planer-Friedrich and Wallschläger, 2009). In addition to decomposition of the dissolved species, a decrease in pH will ultimately lead to precipitation of arsenic sulfides (Samanta and Clifford, 2006). Oxidation due to contact with atmospheric oxygen was found to cause partial trithioarsenate transformation to arsenite within 1 h, yet monothioarsenate showed no decomposition despite continuous purging with air for 24 h (Planer-Friedrich et al., 2009). All of these findings suggest a general sequence of decreasing abiotic stability of monothioarsenate >> dithioarsenate > trithioarsenate > tetrathioarsenate.

These previous studies demonstrate the high instability of thioarsenates and the potentially fast reaction kinetics, which pose a serious challenge for the investigation of specific transformation pathways. Additionally, the discussion of possible transformation products based merely on speciation analysis is similarly difficult due to their fast abiotic decomposition. For the oxidative transformation, thioarsenites as well as sulfide and elemental sulfur have been hypothesized to be of importance (Planer-Friedrich et al., 2015), yet their specific role remains to be fully resolved. Here, sulfur isotope analysis could provide new insight in order to address these issues. Therefore, sulfur

isotope fractionation during oxidation of tetra- and monothioarsenate was investigated in study 2 in order to elucidate abiotic thioarsenate transformation pathways.

1.2.3 Biotic transformation of thioarsenates

Considering thioarsenates can represent a substantial share of reduced sulfur in natural waters, possible biotic transformation pathways were investigated over the past decade. The fact that thioarsenates comprise both an electron donor, in the form of sulfidic sulfur, as well as an electron acceptor, in the form of arsenate, suggests that their microbial transformation might be a favorable process. Thioarsenate transformation was found in the presence of the anaerobic, chemoautotrophic bacterium MLMS-1 isolated from Mono Lake (California, USA), but was attributed to abiotic processes (Hoeft et al., 2004). Direct involvement of thioarsenates in microbial metabolism was discussed by Fisher et al. (2008), as results obtained from enrichment cultures indicated biotic oxidation of arsenic-bound sulfur in thioarsenates. Furthermore, monitoring thioarsenates along geothermal drainage channels in Yellowstone National Park (Wyoming, USA), Planer-Friedrich et al. (2009) concluded that the observed transformations are most likely microbially catalyzed. Especially in the case of monothioarsenate, which is comparatively stable towards abiotic decomposition, potential microbial utilization could accelerate transformation substantially.

Based on these previous findings, a number of studies investigated the question of whether the arsenic-bound sulfur in thioarsenates could serve as a substrate for microbial growth in more detail (Haertig and Planer-Friedrich, 2012; Edwardson et al., 2014; Haertig et al., 2014; Planer-Friedrich et al., 2015). Indeed, studying monothioarsenate transformation by the hyperthermophile *Thermocrinis ruber*, Haertig et al. (2014) found indications that arsenic-bound sulfur is directly oxidized in the course of chemolithotrophic metabolism. However, this conclusion was based exclusively on observed speciation changes and equilibrium considerations. Consequently, Haertig et al. (2014) acknowledged that the detected speciation changes may have been, in part, the result of abiotic transformation processes.

In order to resolve the question, if thioarsenates can be utilized as electron donors by chemolithotrophic bacteria, sulfur isotope analysis was applied in study 3. The main goal was to differentiate between abiotic and biotic transformation processes, and thus elucidate the role of thioarsenates for microbial growth.

1.3 Sample preservation

1.3.1 Standard preservation methods and challenges associated with the preservation of thioarsenates

The application of a species-conserving preservation method is unquestionably the most important prerequisite for the successful analysis of sulfur isotope fractionation in thioarsenates. While preservation of the highly redox-sensitive thioarsenates in synthetic solutions already presents a challenge, the task becomes even more complicated for natural samples. The difficulty of preserving thioarsenates in natural solutions is mainly arising from the almost ubiquitous occurrence of iron (Fe). In unpreserved samples, iron can readily cause changes of sulfur-arsenic speciation, as well as provide abundant adsorption sites upon oxidation and precipitation in the form of iron oxyhydroxides, leading to substantial loss of dissolved species from solution (Bednar et al., 2002; Dixit and Hering, 2003).

In order to prevent iron oxidation and precipitation, mineral acids such as HNO_3, HCl, H_2SO_4, or H_3PO_4 are commonly added to iron-rich samples (Cherry et al., 1979; Cheam and Agemian, 1980; Aggett and Kriegman, 1987; Edwards et al., 1998; Hall et al., 1999; Daus et al., 2002). This approach aims at decreasing the sample pH to < 2 in order to keep iron in solution, which has been shown to preserve the oxy-anions arsenite and arsenate for up to 6 weeks in the presence of 300 µM Fe(II) (Aggett and Kriegman, 1987). However, sample preservation using HNO_3 or HCl was also found to promote arsenite oxidation due to photochemically induced nitrate reduction or dichloro radical formation, respectively (Hall et al., 1999; Emett and Khoe, 2001; Bednar et al., 2002). As an alternative to mineral acids, ethylenediaminetetraacetic acid (EDTA) has been investigated as a potential preservation agent (Gallagher et al., 2001; Bednar et al., 2002). Chelation of iron by EDTA combined with sample storage in opaque polyethylene bottles was found to ensure preservation of arsenite and arsenate for up to 3 months (Bednar et al., 2002).

Even though these methods are well established for the preservation of the oxy-anions arsenite and arsenate, their application is not suitable for thioarsenic species. Acidification of a sample containing thioarsenates will lead to instant precipitation of arsenic sulfides, and thus loss of dissolved sulfur and arsenic species (Smieja and Wilkin, 2003). Addition of EDTA was found to successfully chelate iron, as proposed by Bednar et al. (2002), but also to promote considerable artifact thioarsenate formation within less than a day of sample storage (Suess et al., 2015). Generally, samples containing thioarsenates are preserved by flash-freezing, which has been shown to preserve speciation for up to 67 days in iron-free waters (Planer-Friedrich et al., 2007). However, application of this method to iron-rich solutions will lead to oxidation of Fe(II) and iron oxyhydroxide precipitation upon thawing the sample prior to analysis, consequently inducing co-precipitation and adsorption of thioarsenates.

So far, only one study addressed the difficulties associated with thioarsenate preservation in iron-rich samples, and proposed the use of anoxic septum bottles amended with 1 % ethanol as the best method currently available (Suess et al., 2015). Nevertheless, this technique also suffers from some considerable limitations as trithioarsenate was not fully preserved and decreased by 34 % in the presence of 90 µM iron within 3 days of storage. Hence, the first step towards the successful analysis of sulfur isotopes in thioarsenates is to address the current lack of a suitable preservation method, and develop a technique that will preserve thioarsenates, even in the presence of iron.

1.3.2 Potential of solid phase extraction for sample preservation

Solid phase extraction (SPE) has been studied intensively as a tool for separating dissolved compounds of a solution, and thus achieving species preservation. This method presents a promising approach for thioarsenate preservation as well, yet has never been investigated before. In 1983, Ficklin proposed a now widely-used SPE method for on-site separation of arsenite and arsenate, thus ensuring preservation of the original arsenic speciation (Kim, 2001; Yalcin and Le, 2001; Bednar et al., 2002; Watts et al., 2010; Bennett et al., 2011; Sugar et al., 2013). According to the Ficklin method, a sample is passed through a cartridge filled with anion-exchange resin, which retains anionic arsenate and allows uncharged arsenite to pass without interaction. A similar approach was presented for preservation of sulfur species in geothermal solutions by Druschel et al. (2003), using anion-exchange SPE to retain sulfate and thiosulfate.

Typically, these SPE procedures entail 4 steps: (1) conditioning the cartridge filled with anion-exchange resin, (2) passing a sample through the SPE cartridge, (3) washing the resin to remove weakly-bound matrix components, and (4) eluting the target species from the resin. Anion-exchange resins generally consist of a styrene divinylbenzene copolymer lattice equipped with functional groups, such as quaternary ammonium groups. In its original form, the positively charged quaternary ammonium group binds a counter ion, e. g. chloride or acetate, which is then replaced by the anionic target species during sample application. Once retained on the anion-exchange resin, the target species can be recovered later by passing an eluting agent through the SPE cartridge.

The concept of separating anionic from cationic or uncharged species by SPE was employed for development of a preservation method for thioarsenates in iron-rich waters. In contrast to the existing methods of adding mineral acids or EDTA to a sample in order to keep iron in solution, this approach aims to separate iron completely from the target thioarsenic species. All four thioarsenates can be expected to bind to anion-exchange resin as they occur as anionic species under environmentally relevant conditions (Thilo et al., 1970). At the same time, cationic iron is expected to pass through the resin without interaction. Based on these considerations, the hypothesis was

investigated as to whether SPE can be used to separate thioarsenates from iron, and thus preserve the sulfur-arsenic speciation.

For subsequent elution of the retained thioarsenates, both total recovery, as well as species-conservation within the eluate needed to be considered. For elution of arsenate from anion-exchange resin, mineral acids such as HCl or HNO_3 are commonly used (Le et al., 2000; Bednar et al., 2002; Jay et al., 2004; Watts et al., 2010), however these are unsuitable for thioarsenates due to the potential of arsenic sulfide precipitation (Smieja and Wilkin, 2003). The use of KCl was proposed for elution of thiosulfate and sulfate (Druschel et al., 2003), yet the applicability for thioarsenates is still unknown.

Furthermore, for complete method assessment the stability of thioarsenates retained on the anion-exchange resin over time was examined. Aside from sample preservation, SPE can also be used for enrichment of target species by choosing an elution volume smaller than the volume of the applied sample (Hanousek et al., 2016). This can also be of potential interest for the sometimes low concentrations of thioarsenates. All of these aspects were investigated during the development of a preservation method using SPE for thioarsenates in the absence and presence of iron as presented in study 1.

1.4 Sulfur isotope analysis

1.4.1 Standard precipitation procedures and IRMS analysis

Determination of $\delta^{34}S$ is routinely performed by combustion of a solid sample to generate SO_2 or SF_6, which are subsequently analyzed by isotope ratio mass spectrometry (IRMS) (Rees, 1978). While originally SO_2 and SF_6 had to be prepared off-line in difficult and laborious procedures (Robinson and Kusakabe, 1975), development of online sample combustion using an elemental analyzer enabled significantly faster and more sensitive sulfur isotope analysis by EA-IRMS (Giesemann et al., 1994; Grassineau, 2006). Despite these methodological advancements, standard sulfur isotope analysis still requires a comparatively large amount of sulfur, and most importantly that it be present in a solid form. This poses an additional challenge for the investigation of aqueous samples, as it necessitates precipitation of dissolved species prior to analysis.

Development of species-selective precipitation methods has mainly focused on sulfide and sulfate, as the end-members typically occur in concentrations sufficiently high for successful precipitation. Sulfide is routinely precipitated by addition of zinc acetate ($ZnAc_2$), followed by filtering off the produced zinc sulfide (ZnS) and converting it to more stable silver sulfide (Ag_2S). The remaining solution is treated with barium chloride ($BaCl_2$) to precipitate dissolved sulfate as barium sulfate ($BaSO_4$) (Carmody et al., 1998). Both Ag_2S and $BaSO_4$ are then directly analyzed by EA-IRMS.

Apart from the end-members sulfide and sulfate, intermediate sulfur species have been acknowledged as crucial for interpretation of sulfur isotope fractionation. As a result, a number of different procedures have been proposed for the precipitation of elemental sulfur (Fry et al., 1984; Zerkle et al., 2009; Knoeller and Schubert, 2010; Kamyshny et al., 2011; Einsiedl et al., 2015; Knossow et al., 2015), thiosulfate (Agarwala et al., 1965; Fry et al., 1985; Uyama et al., 1985; Habicht et al., 1998), and sulfite (Fry et al., 1985; Habicht et al., 1998). The most comprehensive procedure so far has been suggested by Kusakabe et al. (2000), with the aim of precipitation of sulfide, elemental sulfur, thiosulfate, sulfite, polythionates, and sulfate from a single sample (Fig. 2).

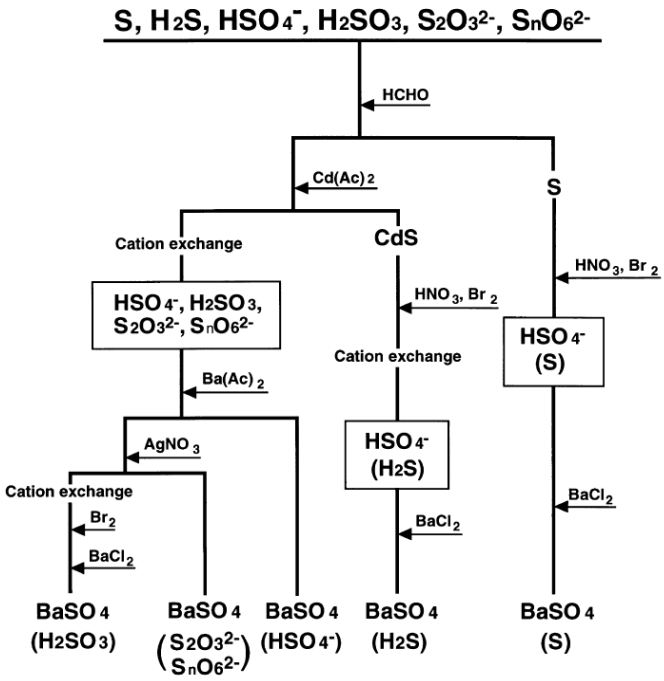

Fig. 2. Sample preparation procedure presented by Kusakabe et al. (2000) for the species-selective precipitation of dissolved sulfide, elemental sulfur, thiosulfate, sulfite, polythionates, and sulfate.

However, all of these methods entail various disadvantages, most notably the substantial preparation effort as evident from figure 2. Artifact species transformation during sample preparation, incomplete recovery, and photochemical instability of the precipitated ZnS can also be of concern (Xu et al., 1998). Furthermore, the species-selective precipitation method typically requires large sample volumes, as high as 9.5 L, to obtain sufficient precipitate for IRMS analysis (Knossow et al., 2015).

Even if one were to accept all the limitations associated with this approach, there is currently no method available for the precipitation of thioarsenates for IRMS analysis. The strong chemical similarity between mono-, di-, tri-, and tetrathioarsenate prevents a clean separation of the four thioarsenates via precipitation. Moreover, thioarsenates may in fact even impede correct $\delta^{34}S$ determination of sulfide. Previous studies have discussed the potential of partial thioarsenate co-

determination together with sulfide when applying the methylene blue method, which is also based on precipitation of sulfide with $ZnAc_2$ (Planer-Friedrich et al., 2007; Suess et al., 2011; Planer-Friedrich et al., 2015). The questions of whether thioarsenates potentially co-precipitate during routine preparation of sulfide for IRMS analysis, and to what extent this may affect $\delta^{34}S$ values are addressed in study 2.

1.4.2 Potential of IC-MC-ICP-MS for species-selective isotope analysis

Over the years, the limitations associated with standard precipitation procedures and IRMS analysis led to investigation of alternative methods for sulfur isotope determination. Prohaska et al. (1999) demonstrated the use of double-focusing sector-field ICP-MS for $\delta^{34}S$ analysis, and achieved precision of < 1 ‰ for sulfur amounts as low as 4 nmol. However, as a result of the instrumental setup of the single collector ICP-MS, data acquisition for ^{32}S and ^{34}S had to be performed separately. This problem was resolved by development of multi-collector ICP-MS (MC-ICP-MS), which has been established as a competitive alternative to IRMS for sulfur isotope analysis over the last decade. In contrast to IRMS, MC-ICP-MS is compatible with various modes of sample introduction, such as solution or laser ablation, requires no combustion of the sample and allows high sample throughput. Typical long-term reproducibility for MC-ICP-MS of 0.08 - 0.2 ‰ is comparable to IRMS, yet the sulfur amount required is several orders of magnitude lower, with as little as 5 - 40 nmol S needed (Grassineau, 2006; Craddock et al., 2008; Paris et al., 2013; Albalat et al., 2016).

Aside from these advantages, MC-ICP-MS most importantly provides the opportunity for simultaneous determination of multiple isotopes. Consequently, this potential was soon employed for analysis of transient signals as typically produced during chromatographic separation. Numerous studies have demonstrated online coupling of gas chromatography to MC-ICP-MS (Rodriguez-Gonzalez et al., 2012). The use of liquid ion chromatography coupled to MC-ICP-MS is less common, but has also been reported (Clough et al., 2006; Santamaria-Fernandez et al., 2008; Zakon et al., 2014; San Blas et al., 2015).

Employing this new approach for species-selective sulfur isotope analysis of thioarsenates could provide two major advances over the standard precipitation and IRMS method: (1) separation of all four thioarsenates, as well as additional sulfur species, could be achieved in one analytical run without time-consuming sample preparation, and (2) online detection by MC-ICP-MS would require significantly lower amounts of sulfur, improving the overall detection capabilities for sulfur isotopes. Thus, the possibility of analyzing sulfur isotopes in thioarsenates by IC-MC-ICP-MS was investigated, and the developed method was evaluated in comparison to standard IRMS analysis. These results are presented in study 2.

1.5 Objectives

The main aim of this study was to gain new insight on the processes governing speciation in sulfur-arsenic systems. From previous work on sulfur redox chemistry the overall hypothesis was derived that sulfur isotope analysis can assist in resolving open questions of thioarsenate formation and decomposition. However, there are currently no suitable analytical methods facilitating such investigations. Thus, the first essential step was to develop a method for the preservation and subsequent analysis of sulfur isotopes in thioarsenates. Following this, isotopic fractionation of thioarsenates was examined in order to elucidate both abiotic and biotic transformation pathways.

In short, the specific objectives of the 3 studies presented in this work were the following:

(1) develop a method for the preservation and enrichment of thioarsenates using solid phase extraction, including a particular focus on iron-rich solutions (study 1)

(2) examine the effect of applying standard analytical procedures of sulfur isotope determination to thioarsenates, develop an alternative IC-MC-ICP-MS method for analysis of isotopic fractionation in thioarsenates, and compare this new method to routine IRMS analysis (study 2)

(3) determine sulfur isotope fractionation during abiotic thioarsenate oxidation and evaluate this data with respect to previous hypotheses on thioarsenate formation and transformation (study 2)

(4) examine fractionation during chemolithotrophic oxidation of monothioarsenate and evaluate the potential of thioarsenates serving as a substrate for microbial growth (study 3)

2 Method development

2.1 Sample preservation and enrichment using SPE (study 1)

2.1.1 Criteria for method development and preliminary results

To be able to study the isotopic fractionation of sulfur in thioarsenates, first a sample preservation method needed to be developed that would prevent speciation changes, even in the presence of interfering compounds such as iron. Method development was based on the capability of anion-exchange SPE to retain anionic target species from a sample solution while cationic and uncharged species simply pass through the resin without interaction. Thus, the main focus of this study was to examine the potential of SPE to separate anionic thioarsenates from cationic iron. Considering the entirety of sulfur-arsenic speciation, method development was extended to also include sulfide, sulfate, thiosulfate, arsenite, and arsenate.

In order to develop an efficient and reliable SPE method, two main aspects were examined: (1) selection of a suitable anion-exchange resin, and (2) elution of target species from the resin. Method optimization was performed using both synthetic solutions as well as natural samples from mineral and geothermal spring waters from Yellowstone National Park (USA), Frantiskovy Lazne (Czech Republic), and Wai-o-Tapu (New Zealand). To assess and compare method quality the two parameters of retention and recovery were used. Efficiency of species retention was evaluated based on equation 1, which represents the share of the target species adsorbed during sample application (Eq. 1):

$$\text{retention} = \frac{n_{\text{initial}} - n_{\text{passage}}}{n_{\text{initial}}} \times 100\ \% \quad (1)$$

where $n_{initial}$ is the molar amount of a species in the initial sample solution and $n_{passage}$ is the molar amount of this species found in the solution passing the SPE cartridge. Recovery during elution was assessed based on equation 2, demonstrating to what extent the target species is removed from the resin by the eluting agent:

$$\text{recovery} = \frac{n_{\text{eluate}}}{n_{\text{initial}} - n_{\text{passage}} - n_{\text{wash}}} \times 100\ \% \quad (2)$$

where n_{eluate} is the molar amount of the target species in the eluate compared to the molar amount bound on the resin prior to elution, expressed as $n_{initial} - n_{passage} - n_{wash}$.

As the first step of method development, several strong anion-exchange (SAX) resins were tested including Bond Elut SAX, Discovery DSC-SAX, Empore Anion-SR SPE Disks, and Bio-Rad AG2-X8. Except for AG2-X8 resin, all investigated resins showed insufficient retention. Based on these results, AG2-X8 was selected for further method development. Kaasalainen and Stefánsson (2011) suggested

conditioning AG2-X8 resin prior to sample application with 0.1 M KOH to promote retention of thiosulfate and sulfate. However, within the present study this led to iron hydroxide precipitation on the resin beads when applying iron-rich samples. Hence, SPE cartridges were conditioned using MQ water to prevent high pH conditions between resin beads prior to sample application.

The second and most crucial aspect of SPE method development was to devise an elution procedure that would remove the retained target species from the resin. While complete recovery was the main criteria for selecting a suitable eluting agent, stability of target species in the eluate, as well as compatibility of the eluting agent with subsequent analytical methods also required consideration. Druschel et al. (2003) proposed the use of 0.5 M KCl for elution of thiosulfate and sulfate from AG2-X8 resin, which was additionally found to yield complete recovery of monothioarsenate during the current study. Nevertheless, elution with KCl could not be used to recover trithioarsenate completely, even though concentrations of up to 3 M KCl were applied. Based on established ion chromatographic analysis of thioarsenates (Planer-Friedrich et al., 2007), 0.1 and 1 M NaOH were investigated as alternative eluting agents, but these also yielded insufficient recoveries. Furthermore, 0.5 M sodium citrate was considered as an eluting agent. However, sodium citrate was found to impede subsequent IC-ICP-MS analysis of the eluate by negatively affecting the peak shape of arsenate. Finally, complete recovery of target species was achieved using 0.5 M sodium salicylate, which all further method optimization was based on.

2.1.2 Preservation method using AG2-X8 resin and sodium salicylate as eluting agent

Taking into account all preliminary results, a procedure for the preservation of sulfur and arsenic species using AG2-X8 resin and sodium salicylate was developed and presented in study 1. Retention of sulfate, thiosulfate, arsenate, monothioarsenate, and trithioarsenate was tested individually in the absence of iron, and yielded values of 100.0 ± 0.0 %, 100.0 ± 0.0 %, 96.8 ± 0.2 %, 98.8 ± 0.2 %, and 99.6 ± 0.3 %, respectively. In the presence of iron, monothioarsenate as well as trithioarsenate were completely retained, while iron passed through the resin as hypothesized (retention 0.4 ± 0.2 %), thus achieving the main goal of separating thioarsenates from iron.

In contrast to all other investigated species, arsenite was not retained on the anion-exchange resin as it was fully protonated (pK_a = 9.2), and passed the SPE cartridge without interaction. However, against expectations, uncharged arsenite was found to be partially retained on the anion-exchange resin in mixtures containing sulfide. A recent study discussed that arsenite could bind to organic matter via thiol groups (Hoffmann et al., 2012). An additional test, in which arsenite was applied after the AG2-X8 resin had been conditioned with sulfide, revealed arsenite retention of 47.3 %. These results suggest that sulfide acting as a bridge between arsenite and the organic lattice of the resin is responsible for the unexpected arsenite retention.

This mechanism has two significant implications. Most importantly, anion-exchange SPE is routinely applied for arsenite and arsenate determination assuming that the passage contains the total amount of uncharged arsenite, while only arsenate is retained on the resin (Jay et al., 2004; Samanta and Clifford, 2006; Lord et al., 2012). Considering the current results, this approach will wrongfully assign the retained part of the arsenite as arsenate, and thus lead to severe underestimation of arsenite in sulfidic solutions. Furthermore, elution of AG2-X8 resin with salicylate will release the retained arsenite as well as sulfide. This will produce a high ionic strength solution containing both arsenite and sulfide. In accordance with previous findings by Planer-Friedrich et al. (2008), the high ionic strength of the eluate was found to cause substantial artifact thioarsenate formation from arsenite and sulfide. Within only 4 hours, 86.4 % of the arsenite was found to be transformed to thioarsenates. Since an excess of SH^- over OH^- in the solution is known to be essential for this reaction (Planer-Friedrich et al., 2010), the pH of salicylate was raised to maintain OH^- excess over SH^-. Applying this approach, a share of only 1.7 % thioarsenates was found to form from arsenite and sulfide within 2 hours, and thus artifact thioarsenate formation was successfully prevented.

For quantitative recovery of the retained target species, an elution protocol was developed based on controlled flow-through conditions on a vacuum manifold. Elution with 3 increments, each of 15 mL 0.5 M alkaline salicylate, resulted in the recovery of 101.5 ± 0.9 %, 101.7 ± 2.2 %, 95.5 ± 1.8 %, and 91.9 ± 2.0 %, of sulfate, thiosulfate, arsenate, and monothioarsenate, respectively. While the major share (≥ 85 %) of these species was readily removed by the first 15 mL increment of salicylate, elution of trithioarsenate required repeated soaking of the resin in salicylate, which finally yielded a recovery of 89.7 ± 3.4 %. From these observations a sequence of preferential adsorption on AG2-X8 of trithioarsenate >> monothioarsenate > arsenate > thiosulfate ≈ sulfate was derived, which is in accordance with previous observations during ion chromatography analysis (Planer-Friedrich et al., 2007).

Besides controlled flow-through elution, which is generally recommended for SPE, batch elution of AG2-X8 resin was also investigated. In cases where only small sample volumes can be applied to the resin, the elution volume must be reduced accordingly to maintain detection of the target species. Here, batch elution presents a viable alternative. Starting from the first results of this approach presented in study 1, further investigations demonstrated the potential to reduce the elution volume to a total of 15 mL salicylate (not included in study 1). The AG2-X8 resin was removed from the SPE cartridge, transferred to a beaker, and stirred in 5 mL 0.5 M alkaline salicylate. After 1 hour, the eluate was separated from the resin. This process was performed three times, resulting in complete recovery of the target species and a total enrichment factor of 17 between the initial sample solution and the eluate.

To complete assessment of the SPE procedure, the stability of the species retained on the resin was investigated through time. Water from an iron-rich mineral spring in Frantiskovy Lazne (Czech Republic) was spiked with the target species and applied to AG2-X8 resin, upon which SPE cartridges were stored in the dark at 4 °C. Elution after 1, 3, and 6 days of storage using controlled flow-through conditions revealed good stability of sulfate and thiosulfate over the investigated time period. Arsenite, arsenate, monothioarsenate, and dithioarsenate were equally well-preserved, and only minor variations within analytical uncertainty were found over 6 days. However, trithioarsenate showed a decrease of 25.2 % over 6 days, most likely as a result of dethiolation. So far, only one other study has investigated a method for thioarsenate preservation in iron-rich solutions. Storing samples in anoxic septum bottles was suggested to prevent iron oxidation and precipitation, and thus preserve thioarsenates (Suess et al., 2015). Yet, using this approach Suess et al. (2015) found trithioarsenate stability suffered from an even more pronounced decrease with a loss of 34 % within only 3 days of storage. Hence, the SPE procedure proposed in study 1 currently presents the best method available for preservation of thioarsenates in iron-rich solutions.

To summarize, study 1 demonstrated the successful separation of thioarsenates as well as sulfate, thiosulfate, and arsenate from iron using anion-exchange resin. Thus, the new method prevented both speciation changes and loss of dissolved species typically caused by iron oxidation and precipitation. Elution with 0.5 M alkaline salicylate, in flow-through or batch mode, was demonstrated to recover all investigated species quantitatively, and renders the proposed procedure a valuable method for the preservation of sulfur and arsenic species.

2.1.3 Potential of removing salicylate from eluates

As previously discussed, elution of higher thiolated thioarsenic species presented the main challenge during method development, and ultimately made the use of high ionic strength 0.5 M salicylate as an eluting agent necessary. However, this inevitably led to the need to dilute eluates before IC-ICP-MS analysis to ensure adequate chromatographic separation. As a result, the enrichment achieved for the target species by SPE is partially diminished. Therefore, additional tests, not included in study 1, were performed to investigate the possibility of decreasing the concentration of salicylate in the eluate.

First, the potential of slightly reducing the initial concentration used for elution to 20, 50, or 200 mM salicylate was tested, but yielded insufficient recovery, particularly for trithioarsenate. Furthermore, salicylate was found to precipitate during flash-freezing, which was investigated as a possible mechanism to remove salicylate from solution. However, upon thawing the precipitate re-dissolved and 99 % of initial salicylate was found in solution again. In addition, the potential of removing salicylate by SPE was examined. Considering that salicylate contains a phenyl group, as well as a

charged carboxyl group, and thus exhibits both nonpolar and polar characteristics, retention by SPE is challenging. The tested SPE resins Discovery DSC-Ph and Supelselect HLB yielded insufficient retention of salicylate of 13.4 and 3.8 %, respectively. Complete retention of salicylate was achieved using Chromabond Easy resin (95.9 %) and Chromabond HR-P resin (97.8 %). However, passing the target sulfur and arsenic species through these resins led to retention of sulfide and tetrathioarsenate (up to 100 %). Due to this substantial loss of target species the SPE resins tested here are not suitable for the removal of salicylate from the eluate. Therefore, future work should focus on investigating alternative SPE resins for removal of salicylate in order to further improve applicability of the preservation method presented in study 1.

2.2 Species-selective isotope analysis of thioarsenates (study 2)

2.2.1 Species separation prior to IRMS analysis by preparative chromatography

The second objective of this study was to develop a method that would permit investigation of sulfur isotope fractionation of individual thioarsenates, and thus gain new insight into thioarsenate transformation pathways (study 2). The ability to separate individual species constitutes the key prerequisite to achieve this goal. For the separation and subsequent isotope analysis of polysulfides, Amrani et al. (2006) demonstrated the use of preparative ion chromatography followed by IRMS. It should be noted that in order to obtain sufficient material for IRMS analysis, working at excessively high sulfur concentrations (up to 100 mM) was necessary for this method. Furthermore, individual polysulfides collected at the outlet of the chromatographic column had to be significantly concentrated by extraction in pentane prior to IRMS analysis.

In preparation of study 2, the potential of separating thioarsenates by preparative ion chromatography similar to the work of Amrani et al. (2006) was investigated. This was based on the established method of thioarsenate analysis employing anion-exchange chromatography and alkaline gradient elution with 20 - 100 mM NaOH (Planer-Friedrich et al., 2007). According to their known retention times, thioarsenates were automatically collected in separate vials, oxidized by excess H_2O_2, precipitated as $BaSO_4$, and analyzed by IRMS. Individual thioarsenates could not be extracted and concentrated in organic solvent after IC separation, as proposed for polysulfides (Amrani et al., 2006). Therefore, operational parameters of the preparative chromatography had to be adjusted to yield a sufficient amount of sulfur for IRMS analysis. This included the use of a 30 mL sample loop, a 22 x 250 mm preparative AS16 column, and an ICS 5000 dual-pump system operating at a flow rate of 18 mL · min^{-1} (Thermo Fisher Scientific, Germany).

These high flow rates precluded the use of gradient mixers, which are dimensioned for much smaller flow rates with a maximum of 1 to 2 mL · min^{-1}. Hence, in-situ mixing of NaOH and MQ water for gradient elution was not possible. Instead, elution had to be performed in discrete steps using four

pre-mixed eluents with increasing concentrations. During optimization of elution, a split of eluent flow was installed that directed approximately 1 mL · min^{-1} of the total flow to ICP-MS for online sulfur and arsenic detection. Separation of sulfide, thiosulfate, sulfate, arsenite, arsenate, mono-, di-, tri-, and tetrathioarsenate was achieved by applying the elution steps presented in table 2.

Table 2. Elution steps used for separation of sulfide, thiosulfate, sulfate, arsenite, arsenate, mono-, di-, tri-, and tetrathioarsenate during preparative chromatography on an AS16 column.

time [min]	NaOH [mM]
0 → 23	5
23 → 32	35
32 → 38	75
38 → 48	100
48 → 52	5

Nevertheless, long-term routine use of the preparative chromatography system proved to be difficult. The exceptionally high flow rates and volumes required, and as a consequence the high pressure within the entire system, was found to be the main cause of complications. Loading of a solution into the sample loop required an additional peristaltic pump and positive pressure on the sample vial because of the back-pressure of the sample loop. Furthermore, injection of a sample into the column was accompanied by a significant pressure surge. In combination with the constantly high flow rate of 18 mL · min^{-1}, this led to significant compaction of the column material. As a result, column performance decreased substantially over time. Therefore, preparative chromatography was excluded from further method development and alternative options of species separation for isotope analysis were investigated.

2.2.2 Species separation prior to IRMS analysis by precipitation

Species-selective precipitation constitutes a commonly used method to separate sulfur species prior to IRMS analysis. While sulfide and sulfate are routinely separated by applying different precipitation agents ($ZnAc_2$ and $BaCl_2$), separating mono-, di-, tri-, and tetrathioarsenate in this way is not possible since they exhibit extremely similar chemical characteristics. Moreover, previous studies have reported occasional thioarsenate co-determination when precipitating sulfide for photometric analysis (Planer-Friedrich et al., 2007; Suess et al., 2011; Planer-Friedrich et al., 2015). These observations suggest that thioarsenates may have to be considered as a potential source of interference during routine sulfide precipitation for IRMS analysis. Hence, the first goal of study 2 was to examine if thioarsenates precipitate when treated according to routine sample preparation methods for sulfide isotope analysis.

Different amounts of $ZnAc_2$ were added to solutions of monothioarsenate, yielding $ZnAc_2$/monothioarsenate ratios of 1:1, 10:1, and 100:1, and sampled after 10 min, 2 h, 5 h, and 24 h of reaction. Monothioarsenate precipitation was found to vary substantially (1.2 - 90 %), mainly as a function of $ZnAc_2$/monothioarsenate ratios. While almost no precipitate was found under 100-fold $ZnAc_2$ excess, maximum precipitation was observed under 10-fold $ZnAc_2$ excess, and precipitation generally increased with time. Interestingly, comparison of the results from total and species analysis revealed the presence of an additional compound, which is < 200 nm and not a dissolved, charged species detectable by IC-ICP-MS. Previous work by Tossell (2000b) on metal-thiometalloid species suggests that this compound is most likely a Zn-monothioarsenate complex.

The obtained results have a number of implications regarding analysis of sulfur species in the presence of arsenic. First and foremost, the observed monothioarsenate precipitation with $ZnAc_2$ demonstrates the need to consider possible thioarsenate co-precipitation when preparing sulfide for analysis by IRMS. It should also be noted that there is currently no general consensus on the $ZnAc_2$ excess that should be used for sulfide precipitation. Reported values of actual $ZnAc_2$ excess applied vary widely, ranging from 10-fold to 80-fold excess over sulfide (Habicht et al., 1998; Kamyshny et al., 2011; Brabec et al., 2012; Knossow et al., 2015). The current results of monothioarsenate precipitating only at specific $ZnAc_2$/monothioarsenate ratios clearly demonstrate that $ZnAc_2$ excess should be chosen carefully, also considering other acid volatile species. Finally, these findings are also of importance beyond sulfur isotope analysis, taking into account the potential to overestimate sulfide due to co-precipitation of thioarsenates when applying the methylene blue method.

2.2.3 Species-selective isotope analysis by ion chromatography coupled to MC-ICP-MS

Thus far, both preparative chromatography and species-selective precipitation were found to be unsuitable methods to separate thioarsenates for subsequent isotope analysis. Essentially, the main problems stem from the need to produce a solid sample with sufficiently high concentration for IRMS analysis. In order to circumvent these issues, a new method was developed in the course of study 2 that combines the advantages of chromatographic species separation with the superior detection capabilities of multi-collector ICP-MS.

Chromatographic separation was adapted from the work of Planer-Friedrich et al. (2007) with slight changes to allow for coupling with MC-ICP-MS as proposed by Zakon et al. (2014) (Fig. 3). Samples were injected using a 500 µL sample loop, followed by separation on a 4 x 250 mm AG/AS16 column (Dionex) using gradient elution of 20 - 90 mM KOH. To reduce the salt load of the eluent, and thus improve MC-ICP-MS detection, the sample was passed through a self-regenerating suppressor and nebulized into a desolvation unit. Using online coupling, the sample was directly introduced into MC-ICP-MS and analyzed for ^{32}S and ^{34}S simultaneously.

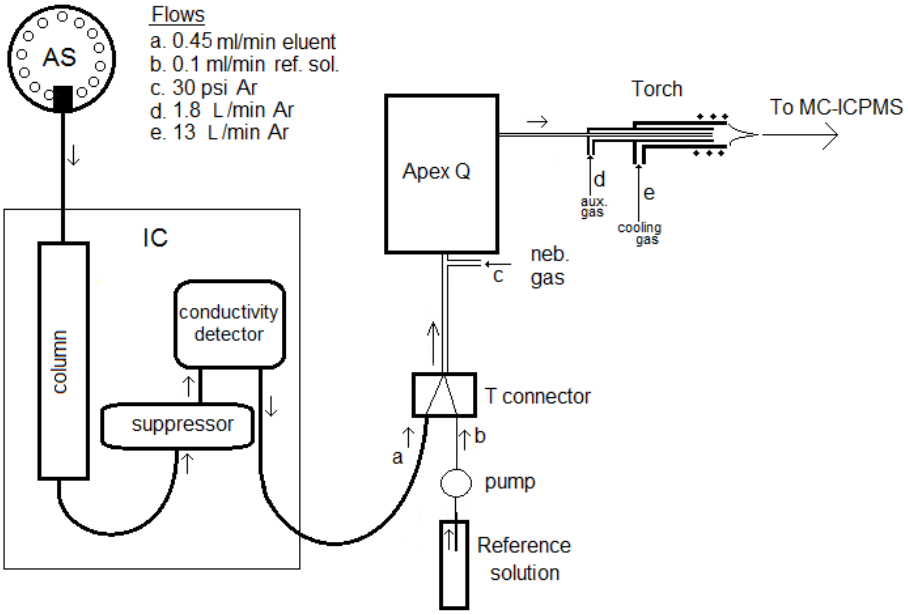

Fig. 3. Instrumental setup of ion chromatography coupled to multi-collector ICP-MS as presented by Zakon et al. (2014).

Using standard-sample bracketing, the obtained results were corrected for instrumental drift and overall method uncertainty was found to be ± 0.3 ‰. Results were presented in the conventional delta notation relative to Vienna Canyon Diablo Troilite according to equation 3.

$$\delta^{34}S\ (‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \cdot 1000 \qquad (3)$$

As part of the quality control process, potential sources of analytical error were assessed by investigating if: (1) chromatographic separation introduces additional fractionation, (2) simultaneous introduction of arsenic into the MC-ICP-MS during thioarsenate elution produces matrix effects, and (3) varying amounts of sulfur (6 - 130 nmol) affect MC-ICP-MS performance. Variations caused by these factors were all found to be smaller than the overall method uncertainty of ± 0.3 ‰, and consequently are negligible.

Thus, the new IC-MC-ICP-MS method developed during the course of study 2 for the first time allowed for the separation of individual thioarsenates and subsequent sulfur isotope analysis. In addition, $\delta^{34}S$ values for sulfide, sulfate, and thiosulfate can be attained. While analytical precision is similar to IRMS analysis, overall detection capabilities of the new IC-MC-ICP-MS method are unquestionably superior. For IRMS analysis of low concentration sulfur species, sample volumes in the range of several liters are often needed to produce sufficient precipitate. Considering the new IC-MC-ICP-MS method requires injection of only 500 µL sample containing approximately 12 µmol · L^{-1} sulfur (depending on daily instrument performance), this clearly presents an important analytical advancement for sulfur isotope analysis.

3 Application of IC-MC-ICP-MS to study isotope fractionation of thioarsenates

3.1 Abiotic oxidation of thioarsenates (study 2)

The developed IC-MC-ICP-MS method significantly expands analytical possibilities and presents a new approach to address open questions regarding sulfur-arsenic redox chemistry. In the current study, the method was applied to elucidate thioarsenate transformation pathways and to investigate if, and to what extent, thioarsenates contribute to the overall isotopic fractionation within the sulfur cycle.

3.1.1 Abiotic monothioarsenate oxidation

First, fractionation during oxidation of monothioarsenate was studied, which is the most stable thioarsenic species (Planer-Friedrich et al., 2009). Monothioarsenate has been argued to occupy a special position among the four thioarsenates, as it only contains elemental sulfur and no additional exchangeable sulfide groups as the higher thiolated thioarsenates (Planer-Friedrich et al., 2015). Stepwise oxidation of monothioarsenate using increasing amounts of H_2O_2 was found to produce minor amounts of thiosulfate and sulfate as the final reaction product. Analysis of sulfur isotopes by IC-MC-ICP-MS showed that monothioarsenate became increasingly enriched in ^{34}S, while sulfate was accordingly depleted in ^{34}S. As a result, a maximum fractionation of -6.1 ‰ between monothioarsenate and sulfate was found, presenting the first report of sulfur isotope fractionation for thioarsenates.

Furthermore, obtained fractionation values were compared to a second experiment, where monothioarsenate samples were similarly oxidized by H_2O_2, but analyzed using the standard precipitation and IRMS method. This allowed quantification of the artifact fractionation that is introduced as a result of monothioarsenate precipitation with $ZnAc_2$. The previously discussed incomplete precipitation and formation of Zn-monothioarsenate complexes was found to cause additional artifact fractionation of up to 3.2 ‰.

3.1.2 Abiotic tetrathioarsenate oxidation

Stepwise oxidation of tetrathioarsenate via tri-, di-, and monothioarsenate yielded sulfide and thiosulfate as intermediate species, and finally sulfate. The final $\delta^{34}S$ value of sulfate (16.2 ‰) was in good agreement with the initial value of tetrathioarsenate (16.8 ‰), as well as with the overall mass balance (16.2 - 17.9 ‰), clearly demonstrating the capacity of the new IC-MC-ICP-MS method. Interestingly, no fractionation was found for oxidation of tetrathioarsenate via tri- and di- to monothioarsenate. However, during further oxidation significant ^{34}S-enrichment was observed for monothioarsenate that exceeded findings from the first monothioarsenate oxidation experiment. This effect was proposed to result from intermolecular isotope exchange between monothioarsenate

and ^{34}S-enriched sulfide, similar to what has been found to explain ^{34}S-enrichment in thiosulfate (Chu et al., 2004). Considering previous findings on thioarsenate transformations, the intermolecular exchange observed in this study most likely proceeds via zerovalent sulfur, either in the form of elemental sulfur or polysulfides (Planer-Friedrich et al., 2015).

Until now, identification of thioarsenate transformation pathways was based exclusively on analysis of speciation changes. The isotopic values obtained in this study added new information to unresolved issues such as the role of pentavalent thioarsenates versus trivalent thioarsenites. The lack of fractionation observed for tetra-, tri-, and dithioarsenate indicates that trivalent thioarsenites do occur only briefly as intermediates as previously hypothesized (Planer-Friedrich et al., 2015), since their rapid oxidation prevents any isotopic enrichment through intermolecular exchange. In comparison, slower oxidation of intermediate arsenite provides sufficient opportunity for intermolecular exchange yielding ^{34}S-enriched monothioarsenate. Moreover, the obtained data adds to previous observations that decomposition of individual thioarsenates can differ fundamentally (Planer-Friedrich et al., 2009). From the lack of fractionation for tetra-, tri-, and dithioarsenate it can be concluded that their decomposition is not associated with any redox change for sulfur, and thus leads to the release of sulfidic sulfur. In contrast, monothioarsenate has been suggested to only decompose via direct oxidation of arsenic-bound sulfur, which is clearly supported by the considerable fractionation found during monothioarsenate oxidation.

Overall, study 2 presents the first report of sulfur isotope fractionation in thioarsenates, impossible until now due to analytical limitations. Application of the new IC-MC-ICP-MS analysis revealed substantial fractionation of monothioarsenate as a result of oxidation as well as intermolecular isotope exchange. While the results assisted in elucidating thioarsenate transformation pathways, study 2 also provided new information on the limits of fractionation associated with abiotic sulfide oxidation in general. Essentially, based on a single study by Fry et al. (1988b), abiotic oxidation processes are customarily assumed to produce only minor fractionation of a maximum of -5.1 ‰ between sulfide and sulfate. This generalization is often used to distinguish oxidation from reduction processes, which are believed to generate higher fractionation. However, study 2 showed that sulfidic sulfur in thioarsenates may become enriched in ^{34}S by up to 15 ‰, and thus demonstrated the importance of including thioarsenates in the interpretation of isotopic records.

3.2 Chemolithotrophic oxidation of monothioarsenate (study 3)

Recently, thioarsenates have gained attention as possible sources of reduced sulfur for microbial growth. Several studies have investigated the potential of arsenic-bound sulfur serving as an electron donor, similar to what has been described for thiosulfate or elemental sulfur (Haertig and Planer-Friedrich, 2012; Edwardson et al., 2014; Planer-Friedrich et al., 2015). In particular, Haertig et al.

(2014) proposed that the chemolithotrophic hyperthermophile *Thermocrinis ruber* can grow by oxidizing arsenic-bound sulfur in monothioarsenate. However, the authors acknowledged that this conclusion was drawn exclusively from observed speciation changes, and that a potential contribution of abiotic monothioarsenate transformation could not be excluded.

Therefore, the aim of study 3 was to investigate if sulfur isotope analysis can assist in differentiating between biotic and abiotic transformation processes, and thus answer the question of whether thioarsenates can serve as a microbial substrate. Incubation experiments with native filamentous mats of *T. ruber* were conducted on-site in the drainage channel of a geothermal spring at Yellowstone National Park (USA). Monothioarsenate solutions were incubated at 80 °C for 4 h in both the presence and absence of *T. ruber*, and repeatedly sampled for speciation and isotopic analysis. In accordance with previous studies, monothioarsenate was quantitatively transformed to sulfate, arsenite, and arsenate during biotic incubation, while only minor transformation was observed for the abiotic treatment (Planer-Friedrich et al., 2009; Haertig and Planer-Friedrich, 2012; Haertig et al., 2014).

Intriguingly, sulfur isotope analysis revealed an inverse isotope effect, indicated by a pronounced decrease in $\delta^{34}S$ values for monothioarsenate, whereas the sulfate produced was accordingly enriched. This resulted in fractionation between monothioarsenate and sulfate of +1.2 and +4.4 ‰ for the biotic and abiotic incubation, respectively. In contrast, oxidation of monothioarsenate with H_2O_2 performed in study 2 produced a normal isotope effect of -6.1 ‰. This finding strongly suggests that a fundamentally different process controlled the transformation of monothioarsenate to sulfate in the incubation experiment conducted in study 3.

Previously, inverse isotope effects of up to +2.5 ‰ have been reported for the biotic oxidation of sulfide to elemental sulfur (Fry et al., 1984; Fry et al., 1988a; Kelly, 2008). Furthermore, Tudge and Thode (1950) calculated ^{34}S-enrichment for elemental sulfur of +3 ‰ as a result of isotopic exchange with sulfide. In view of these results, it was concluded that disproportionation of monothioarsenate, which essentially translates to oxidation of sulfidic arsenic-bound sulfur to elemental sulfur, is the main process responsible for the observed inverse isotope effect. Fossing and Jørgensen (1990) discussed the formation of polysulfides as a crucial step during isotopic exchange between sulfide and elemental sulfur. Considering that polysulfides have been identified as important intermediates during monothioarsenate disproportionation, polysulfide formation would facilitate the proposed ^{34}S-enrichment of elemental sulfur. Furthermore, high pH and high temperature conditions, as applied during the incubation experiment, have been shown to accelerate isotopic exchange between sulfide and elemental sulfur (Voge and Libby, 1937).

All of these aspects support a transformation pathway in which monothioarsenate is subject to disproportionation. This leads to the formation of ^{34}S-enriched elemental sulfur and ultimately generates the detected inverse isotope effect. Further oxidative transformation to sulfate most likely includes biotic elemental sulfur disproportionation, abiotic sulfide oxidation, and biotic sulfite disproportionation, but exact enzymatic pathways remain to be resolved (Cypionka et al., 1998; Frederiksen and Finster, 2003; Zerkle et al., 2009). It is noteworthy that this presents another case in which IC-MC-ICP-MS analysis could deliver new insights. However, this would require method development outside the scope of the present work through the implementation of reverse phase instead of anion-exchange chromatography coupled to multi-collector ICP-MS.

The combination of results from study 2 and 3 yielded some new information to address the question of whether thioarsenates serve directly as electron donors for microbial growth. Study 2 demonstrated a normal isotope effect for the direct oxidation of arsenic-bound sulfur to sulfate, whereas study 3 showed an inverse isotope effect indicative of monothioarsenate disproportionation. This finding suggests that arsenic-bound sulfur in monothioarsenate is in fact not directly oxidized by *T. ruber*. Furthermore, the inverse isotope effect was found during both abiotic and biotic incubation, implying monothioarsenate disproportionation proceeds abiotically. The much smaller extent of fractionation detected in biotic (+1.2 ‰) compared to abiotic incubation (+4.4 ‰) can be attributed to increased transformation rates in the presence of *T. ruber*. This observation is in accordance with similar effects reported for sulfite disproportionation (Habicht et al., 1998) as well as sulfate reduction (Sim et al., 2011).

Overall, the incubation experiments of study 3 revealed that abiotic disproportionation presents the first step of monothioarsenate transformation, producing elemental sulfur and arsenite. Although previously hypothesized, direct utilization of monothioarsenate by chemolithotrophic bacteria could not be confirmed based on the observed isotopic fractionation. Nevertheless, considering their fast abiotic transformation, thioarsenates present an important sink of reduced sulfur that can easily be released in the form of sulfide or elemental sulfur (Planer-Friedrich et al., 2009; Planer-Friedrich et al., 2015). As a consequence, arsenic-bound sulfur in thioarsenates plays a key role for chemolithotrophic growth. Hence, study 3 demonstrated how valuable information can be gained from species-selective isotope analysis, contributing new aspects to the on-going challenge of elucidating biotic and abiotic sulfur transformation processes.

4 Conclusions

The present study addressed the challenges associated with isotope analysis of thioarsenates and demonstrated the value that can be derived from this new approach for the elucidation of unresolved transformation pathways. Thioarsenate chemistry has been studied in detail, but until now analytical capabilities were limited to speciation analysis alone, and thus often delivered ambiguous results. The methods developed over the course of this study significantly broadened the analytical repertoire and were found to shed new light on redox processes in sulfur-arsenic systems.

Preservation of thioarsenates was achieved by employing a new method based on solid phase extraction (study 1). Thioarsenates, sulfate, thiosulfate, and arsenate were successfully separated from iron, which typically causes transformation or even loss of these species from solution. The combination of the anion-exchange resin AG2-X8 and 0.5 M alkaline salicylate as an eluting agent was found to yield complete retention and recovery of the investigated species. Examination of different elution modes suggests that batch elution presents the most favorable option on which further application of the SPE procedure should be based. Overall, the developed method presents an inexpensive technique that can be used even in remote sampling areas and ensures preservation of sulfur-arsenic speciation for at least 6 days. Considering the straightforward principle of retaining anionic species on resin, this method could be easily transferable to the preservation of other thiometalloids, such as thioantimonates.

For the species-selective isotope analysis of thioarsenates, three different approaches were investigated (study 2). First, the possibility of separating species by preparative chromatography followed by precipitation for IRMS analysis was assessed. Even though it was shown that implementation of this approach is possible, high pressure conditions during chromatographic separation rendered it unsuitable for long-term use. Further, separation of species by selective precipitation, as routinely performed for the isotopic analysis of sulfide or sulfate, was considered. However, this approach is not feasible for separation of thioarsenates since the chemical properties they exhibit are too similar. Moreover, thioarsenates were in fact found to be co-determined together with sulfide during routine sample preparation procedures for IRMS analysis. This effect can lead to substantial over- or underestimation of $\delta^{34}S$ values for sulfide, and consequently needs to be taken into account when sulfur isotopes are determined in the presence of arsenic. Furthermore, these findings suggest other acid-volatile sulfur species should be investigated with respect to potential co-determination with sulfide as well. Finally, thioarsenate separation, and subsequent isotope analysis, was achieved by employing a new method of ion chromatography coupled to multi-collector ICP-MS. In comparison to the established IRMS technique, the new method enables a

significant reduction in sample volume, requires minimal sample preparation effort, and permits simultaneous isotope analysis of up to 7 different sulfur species, including all four thioarsenates.

Application of the presented IC-MC-ICP-MS method delivered the first analytical proof of sulfur isotope fractionation in thioarsenates. Abiotic thioarsenate oxidation can proceed quickly, which renders process identification by speciation analyses difficult. Additional isotope analysis performed over the course of study 2 elucidated the dethiolation of thioarsenates and confirmed the brief intermediate occurrence of thioarsenites as previously hypothesized. Strong indications were found that intermolecular exchange with sulfide controls the isotopic signature of monothioarsenate to a large extent. Furthermore, IC-MC-ICP-MS analysis revealed that oxidation of monothioarsenate is accompanied by a change in oxidation state for arsenic-bound sulfur, while di-, tri-, and tetrathioarsenate simply release sulfidic sulfur. These findings highlight the special role that monothioarsenate holds among thioarsenates in terms of abiotic stability, but also interaction with other reduced sulfur species.

In addition to resolving abiotic processes, the potential of the new IC-MC-ICP-MS method to elucidate biotic thioarsenate transformation was demonstrated in study 3. Previously, certain bacteria were hypothesized to oxidize arsenic-bound sulfur during chemolithotrophic metabolism. In contrast to the normal isotope effect found during abiotic monothioarsenate oxidation, incubation experiments with the chemolithotrophic bacterium *Thermocrinis ruber* revealed a pronounced inverse isotope effect. In light of these findings, the previous hypothesis of thioarsenates serving as electron donors for chemolithotrophic growth has to be reconsidered. The results of this study have allowed for differentiating between abiotic and biotic processes, suggesting that the first step of monothioarsenate transformation is in fact not direct biotic oxidation, but abiotic disproportionation to elemental sulfur and arsenite. Ultimately, specific pH, temperature, and redox conditions will control this abiotic release of reduced sulfur from thioarsenates. Considering their generally low abiotic stability, especially of the higher thiolated species, thioarsenates may represent a significant source of reduced sulfur for chemolithotrophic bacteria. A similar role of other thiometalloid species for microbial growth seems likely based on these results, but has yet to be investigated in detail. Furthermore, the enzymatic pathways bacteria use to oxidize elemental sulfur released from thioarsenates are still a subject of debate. Future development of an IC-MC-ICP-MS method employing reverse phase chromatography could yield a better understanding of these particular processes.

Overall, the newly developed methods proved to be powerful tools for the investigation of redox chemistry in sulfur-arsenic systems. IC-MC-ICP-MS analysis of thioarsenates revealed, for the first time, sulfur isotope fractionation in thiometalloid species. The obtained results led to refining, but

also reconsidering previous concepts of abiotic and biotic thioarsenate transformation. Moreover, this study clearly demonstrated that fractionation of thioarsenates will also affect the isotopic composition of other sulfur species, such as sulfide, thiosulfate, or sulfate. All of these aspects need to be considered when isotopic records are used to retrace or predict processes of sulfur transformation in the environment. Thus, a profound understanding of isotopic fractionation for all sulfur species, including thiometalloids, is fundamental for our ability to elucidate the biogeochemical sulfur cycle in its entirety.

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Contribution to studies 1 - 3

STUDY 1: A new method for thioarsenate preservation in iron-rich waters by solid phase extraction

Maria K. Ullrich	85 %	development of research concept, laboratory and field work, analyses and data interpretation, preparation of manuscript
Valentina Misiari	5 %	assistance with laboratory and field work, discussion of results
Britta Planer-Friedrich	10 %	development of research concept, field work, discussion of results, comments on manuscript

STUDY 2: Sulfur isotope analysis by IC-MC-ICP-MS provides insight into fractionation of thioarsenates during abiotic oxidation

Maria K. Ullrich	75 %	development of research concept, laboratory work, analyses and data interpretation, preparation of manuscript
Faina Gelman	10 %	laboratory work, analyses, comments on manuscript
Yevgeni Zakon	2.5 %	comments on manuscript
Ludwik Halicz	2.5 %	comments on manuscript
Kay Knöller	2.5 %	discussion of results, comments on manuscript
Britta Planer-Friedrich	7.5 %	development of research concept, discussion of results, comments on manuscript

STUDY 3: Differentiation of abiotic and biotic monothioarsenate transformation by analysis of sulfur isotopes using IC-MC-ICP-MS

Maria K. Ullrich	80 %	development of research concept, field and laboratory work, analyses and data interpretation, preparation of manuscript
Faina Gelman	5 %	analyses, comments on manuscript
Yevgeni Zakon	2.5 %	comments on manuscript
Ludwik Halicz	2.5 %	comments on manuscript
Kay Knöller	2.5 %	comments on manuscript
Britta Planer-Friedrich	7.5 %	development of research concept, discussion of results, comments on manuscript

APPENDIX: Studies 1 - 3

STUDY 1

Ullrich, M.K., Misiari, V., Planer-Friedrich, B. (2016): **A new method for thioarsenate preservation in iron-rich waters by solid phase extraction.** Water Research, 102: 542-550.

STUDY 2

Ullrich, M.K., Gelman, F., Zakon, Y., Halicz, L., Knöller, K., Planer-Friedrich, B.: **Sulfur isotope analysis by IC-MC-ICP-MS provides insight into fractionation of thioarsenates during abiotic oxidation.** submitted to Chemical Geology.

STUDY 3

Ullrich, M.K., Gelman, F., Zakon, Y., Halicz, L., Knöller, K., Planer-Friedrich, B.: **Differentiation of abiotic and biotic monothioarsenate transformation by analysis of sulfur isotopes using IC-MC-ICP-MS.** submitted to Applied Geochemistry.

STUDY 1

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A new method for thioarsenate preservation in iron-rich waters by solid phase extraction

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A new method for thioarsenate preservation in iron-rich waters by solid phase extraction

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ABSTRACT

In order to preserve iron-rich samples for arsenic speciation analysis, mineral acids or EDTA are typically added to prevent oxidation and precipitation of iron. However, when sulfide is present, and thioarsenates ($[HAs^{V}S^{-II}_{n}O_{4-n}]^{2-}$, n = 1–4) can form, these methods are unsuitable due to arsenic sulfide precipitation or artifact speciation changes. Here, a new method based on separating the anionic arsenic species from cationic iron in the presence of sulfide via solid phase extraction (SPE) has been investigated. Synthetic solutions containing arsenite, arsenate, monothioarsenate, and trithioarsenate were passed through the anion-exchange resin AG2-X8, after which the resin was washed, eluted, and speciation of each step analyzed by IC-ICP-MS. Retention on the resin of 96.8 ± 0.2%, 98.8 ± 0.2%, and 99.6 ± 0.3% was found for arsenate, monothioarsenate, and trithioarsenate, respectively. Cationic iron (90 μM Fe(II)) was not retained (0.4 ± 0.2%). Uncharged arsenite passed through the resin in the absence of sulfide, while 47.3% of arsenite were retained at tenfold sulfide excess via thiol groups binding to the organic resin structure. Elution with 3 × 15 mL of 0.5 M salicylate, including a soak time, resulted in quantitative recovery of all retained species. Stability of the retained species on the resin was tested with iron-rich, natural waters from a Czech mineral spring. Arsenate, monothioarsenate, dithioarsenate, and trithioarsenate were successfully separated from iron and recovered after 6 d. Thus, SPE presents a viable answer to the problem of preserving arsenic in the presence of both iron and sulfide.

1. Introduction

It is well documented that speciation is a key factor for understanding the fate of arsenic in the environment. Over the years, numerous studies have shown how the oxidation state of arsenic controls not just its mobility in natural systems (Ferguson and Gavis, 1972; Campbell and Nordstrom, 2014), but also its toxicity (Styblo et al., 2000; Dopp et al., 2008; Hinrichsen et al., 2014). In order to assess the potential impact of arsenic in the environment, it is therefore imperative to measure the correct arsenic speciation in natural samples. However, maintaining original speciation in a sample during collection and storage can be challenging, particularly in the presence of iron.

Iron has the potential to impair both the speciation and total arsenic concentration of a sample. In less than 1 d, 0.3 μM arsenite can be fully oxidized to arsenate in an unpreserved sample with 18 μM Fe(III) (Bednar et al., 2002). Furthermore, arsenite and arsenate readily co-precipitate with, or adsorb on, iron oxyhydroxides depending on pH (Dixit and Hering, 2003), which leads to loss of total dissolved arsenic. To overcome these problems, different preservation methods have been developed. Most of the published methods are based on the addition of mineral acids, such as HNO_3, HCl, H_2SO_4, or H_3PO_4 to the sample (Cherry et al., 1979; Cheam and Agemian, 1980; Aggett and Kriegman, 1987; Edwards et al., 1998; Hall et al., 1999; Daus et al., 2002). This approach is used to decrease the pH to <2 in order to keep iron in solution, which has been shown to preserve arsenic speciation in the presence of 300 μM Fe(II) for up to 6 weeks (Aggett and Kriegman, 1987). However, the addition of mineral acids has also been reported to change arsenic speciation and total concentrations. Rapid oxidation of arsenite has been found in samples preserved with HNO_3 or HCl (Hall et al., 1999; Bednar et al., 2002), as a result of photochemically-induced nitrate reduction or dichloro radical formation (Emett and Khoe, 2001), respectively. Other studies have evaluated the use of EDTA to chelate iron, and thus avoid oxidation and precipitation of iron oxyhydroxides (Gallagher et al., 2001; Bednar et al., 2002). Samples containing arsenite and arsenate

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were stable for up to 3 months when treated with EDTA and stored in opaque polyethylene bottles (Bednar et al., 2002).

Method development for arsenic preservation in iron-rich waters has focused on arsenite and arsenate so far. In sulfidic environments, however, thioarsenates ($[HAs^{V}S^{-II}_{n}O_{4-n}]^{2-}$, n = 1–4) can also play a significant role for arsenic speciation. Initially, thioarsenates were found in iron-free geothermal systems, representing up to 83% of total arsenic (Planer-Friedrich et al., 2007; Planer-Friedrich and Wilson, 2012; Ullrich et al., 2013). Despite the potential formation of iron sulfides, thioarsenates have also been shown to exist in iron-rich environments (Suess et al., 2011; Stucker et al., 2014). Furthermore, several laboratory studies on the mobility of thioarsenates reported their occurrence in the presence of nanohematite, goethite, ferrihydrite, mackinawite, and pyrite (Suess and Planer-Friedrich, 2012; Burton et al., 2013; Couture et al., 2013; Stucker et al., 2014). The reaction of free sulfide with arsenite to form thioarsenates is kinetically favored over the formation of iron sulfides. Therefore, thioarsenate formation will occur prior to precipitation of any iron sulfides such as mackinawite, greigite, or pyrite.

Preserving thioarsenates in a solution that contains iron and sulfide presents a delicate task. Acidification of such a sample would lead to precipitation of arsenic sulfides (Smieja and Wilkin, 2003). Preservation with EDTA, as recommended by Bednar et al. (2002), was found to accelerate oxidation of arsenite, while also causing artifact thioarsenate formation (Suess et al., 2015). Flash-freezing, which preserved thioarsenates in iron-free solutions for up to 67 d (Planer-Friedrich et al., 2007), leads to oxidation of Fe(II) and precipitation of iron oxyhydroxides upon thawing the sample prior to analysis. This promotes co-precipitation and adsorption of arsenic, which renders it unsuitable for the preservation of iron-rich samples. Overall, none of the established methods can preserve arsenic speciation in the presence of sulfide and iron.

This lack of a suitable preservation method for sulfidic, iron-rich waters can be addressed by applying solid phase extraction (SPE). It has been shown that arsenite and arsenate can be separated using SPE cartridges packed with aluminosilicate (Meng et al., 2001), or more commonly with anion-exchange resin according to Ficklin (1983). When a sample is passed through an anion-exchange resin, anionic arsenate will be adsorbed, while uncharged arsenite will pass without interaction (Kim, 2001; Yalcin and Le, 2001; Bednar et al., 2002; Watts et al., 2010; Bennett et al., 2011; Sugar et al., 2013). Most anion-exchange resins consist of a functional group, such as a quarternary ammonium group, attached to a styrene divinylbenzene copolymer lattice. In its original form the positively charged functional group binds a counter ion such as chloride or acetate. When a sample is applied to the resin, this counter ion is replaced with an anionic compound from the sample. The retained anionic compounds can be removed later from the resin by applying an eluting agent. In order to elute arsenate from an anion-exchange resin, 1 M HCl (Le et al., 2000), 6 M HCl (Jay et al., 2004), 0.16 M HNO_3 (Bednar et al., 2002), 1 M HNO_3 (Watts et al., 2010), or 2 M HNO_3 (Bennett et al., 2011) have been proposed.

The capability of SPE to retain anionic compounds on a resin can be employed for the preservation of thioarsenates in sulfidic, iron-rich waters. Under environmentally relevant conditions, thioarsenates occur as anionic species (Thilo et al., 1970) and can be expected to adsorb to the anion-exchange resin. At the same time, cationic iron should pass through the resin without interaction. Thus, arsenic speciation changes are prevented by separating thioarsenates on-site from iron. However, the main challenge consists of eluting the retained thioarsenates for analysis without changing speciation. Lord et al. (2012) used SPE to investigate arsenic speciation of geothermal waters shown to contain thioarsenates (Planer-Friedrich and Wilson, 2012; Ullrich et al., 2013). They speculated that thioarsenates were retained on the anion-exchange resin, but were unable to elute them for analysis. Elution with HCl or HNO_3, as previously presented for arsenate, will cause species conversion and arsenic sulfide precipitation. Druschel et al. (2003) studied SPE for the preservation of sulfate and thiosulfate in geothermal waters and showed that 0.5 M KCl can elute sulfate and thiosulfate without speciation changes, which might be transferable to thioarsenates.

The aim of this study was to explore the potential of SPE to preserve arsenic speciation in sulfidic, iron-rich waters. Based on the hypothesis that thioarsenates can be separated on-site from iron, this approach will prevent arsenic speciation changes and arsenic adsorption on iron oxyhydroxides. After ensuring full retention of the arsenic species on the anion-exchange resin, the development of a species-conserving elution was a major focus. Furthermore, the stability of the retained species on the resin was investigated over time. The method developed in this study is summarized in a detailed application protocol that demonstrates the preservation of arsenic in the presence of sulfide and iron using SPE.

2. Material & methods

2.1. Preparation of cartridges

Cartridges for SPE were prepared with 1 g of strongly basic anion-exchange resin (AG2-X8, Cl-form, BioRad). A polyethylene frit was inserted into a 6 mL polypropylene tube (Supelco, Sigma-Aldrich), and a slurry of resin and deionized water (MQ, 18 MΩ cm) was poured into the tube. Excess water was removed by applying vacuum at the tube outlet producing a compact resin bed. No top frit was inserted to promote mixing with the applied solutions. Prior to sample application the resin was conditioned with 12 mL of 0.1 M KOH (≥85%, Sigma-Aldrich) or 12 mL of MQ water, ensuring that the resin was fully equilibrated.

2.2. SPE procedure

During the first step of the SPE procedure, 250 mL of initial sample solution was passed through the resin at a flow rate of 1.7–2.0 mL min^{-1} (Fig. 1). A vacuum manifold was used to control constant flow during sample application. While applying the sample at the top of the cartridge, the passage was collected at the outlet of the tube. Hence, the passage contains the compounds not retained by the resin. The second step consisted of quickly washing the resin by applying 4 mL of MQ water to eliminate excess sample solution from between the resin beads. When a natural sample was applied, this step also removed any weakly bound matrix compounds.

The third step of the SPE procedure constitutes the elution, during which the retained compounds were removed from the resin. Elution of the target sulfur and arsenic species was investigated using 0.5 M and 3 M KCl (≥99.8%, VWR), 0.1 M NaOH (50% w/w, Sigma-Aldrich), 0.5 M sodium citrate ($C_6H_5Na_3O_7$, ≥99%, Sigma-Aldrich), and 0.5 M sodium salicylate ($C_7H_5NaO_3$, ≥99.5%, Sigma-Aldrich). Since sodium salicylate yielded the best results it was chosen for all further experiments. For the elution, 15 mL of 0.5 M salicylate were applied to the resin in three increments of 5 mL each, including 20 min soak time before eluting each increment. These three increments were collected as one sample. Eluting with 15 mL salicylate in this way was repeated three times generating eluates A, B, and C.

Synthetic sample solutions containing 31.2 μM ammonium sulfate ($[NH_4]_2SO_4$, ≥99.99%, Sigma-Aldrich), 46.8 μM sodium thiosulfate ($Na_2S_2O_3$, ≥99.99%, Sigma-Aldrich), 2.7 μM sodium

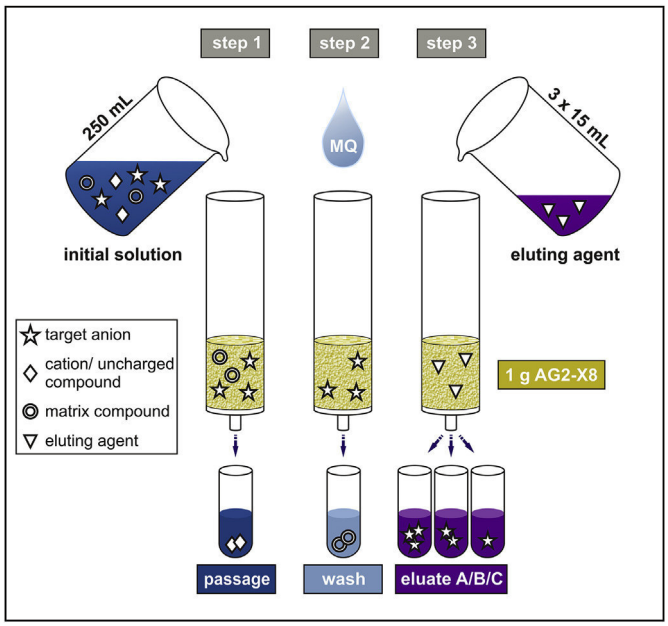

Fig. 1. SPE procedure including the 3 steps of (1) passing 250 mL of the initial solution through AG2-X8 resin, (2) washing with 4 mL of MQ water, and (3) eluting with 3 × 15 mL 0.5 M salicylate.

arsenite ($NaAsO_2$, ≥90%, Sigma-Aldrich), 2.7 μM sodium arsenate (Na_2HAsO_4 × 7 H_2O, ≥99.99%, Sigma-Aldrich), 2.7 μM monothioarsenate (Na_3AsO_3S × 7 H_2O), or 5.7 μM trithioarsenate (Na_3AsOS_3 × 8 H_2O, both synthesized as stated in Suess et al. (2009)) were treated according to the described SPE procedure. To investigate the applicability for iron-rich samples, 90 μM $FeCl_2$ (≥99.0%, Sigma-Aldrich) was added to initial sample solutions of 5 μM monothioarsenate (with and without 4 mM NaCl) and a mixture of arsenite, arsenate, monothioarsenate, trithioarsenate, and sulfide. In the course of method development, a slightly different elution was tested for the monothioarsenate solutions with iron (1 h soak time with 15 mL instead of 3 × 20 min with 3 × 5 mL salicylate). All experiments were performed in triplicate, and under anoxic conditions, when oxidation of thioarsenates or Fe(II) was a concern. Aliquots of each step of the SPE procedure were collected for total and species concentration analysis. Samples for total analysis were stored at 4 °C after adding H_2O_2 (30%, VWR) and 7.25 M HNO_3 (65%, Sigma-Aldrich) to a final concentration of 0.4% and 0.8%, respectively. Only H_2O_2 was added to the aliquots from eluates A, B, and C to avoid precipitation of salicylic acid. Samples for speciation were analyzed immediately as described in section 2.4.

Considering potential interactions between arsenite and sulfide during the sample application, an additional test was performed in which 50 mL of 133.5 μM sodium sulfide (Na_2S × 9 H_2O, >98%, Sigma-Aldrich) was passed through the resin, followed by 50 mL of 13.35 μM arsenite. The resin was washed and only the first elution, step A, was performed with 1 × 15 mL of 0.5 M alkaline salicylate, since the focus of this test was to investigate the sample application process. Samples of each step were analyzed for arsenic speciation and sulfide.

Potential reactions of arsenite with sulfide leading to artifacts in the eluates were examined. Solutions containing 25.8 μM arsenite and 2.8 mM sulfide were monitored over time in different matrices of MQ water, 0.5 M salicylate (dissolved in MQ water, pH 6.5) or 0.5 M alkaline salicylate (dissolved in NaOH, pH 12.3). Samples for arsenic speciation were collected after 0.25 h, 0.5 h, 1 h, 2 h, 4 h, and 1 d.

2.3. Stability test with natural waters

The stability of the target species on the resin during storage was tested with natural iron-rich waters from Frantiskovy Lazne, Czech Republic, which are known to contain thioarsenates and have been characterized in more detail in Suess et al. (2011). Waters from the mineral spring Stepanka (pH 5.6, 186 μM Fe) were spiked with 13.35 μM arsenite and 133.5 μM sulfide to generate a sufficient amount of each sulfur and arsenic species for method assessment. A volume of each 250 mL of this initial sample solution was passed through 9 cartridges. After the wash step the cartridges were dried by applying vacuum and stored at 4 °C under exclusion of light. Three cartridges each were eluted after 1, 2, and 6 d of storage using 3 × 15 mL of 0.5 M salicylate as previously described (Fig. 1).

2.4. Analytical methods

The solutions derived from the SPE procedure (passage, wash, eluate A, B, and C), and the initial sample solutions were analyzed for sulfur and arsenic species, as well as total arsenic and iron. Speciation of sulfur and arsenic was determined immediately following a previously established method (Planer-Friedrich et al., 2007). Species were separated by ion chromatography (IC, IonPac AG/AS16, ICS 3000 SP, Dionex) using gradient elution of 20–100 mM NaOH at 1.2 mL min^{-1}, and detected by inductively coupled plasma mass spectrometry (ICP-MS, XSeries2, Thermo Scientific). Sulfur and arsenic species were determined in oxygen mode at m/z = 48 and 91, respectively. Sulfate, thiosulfate, arsenite, and arsenate were quantified via their respective calibration standards. Due to the lack of commercially available standards for thioarsenates, these species were quantified using the calibration of arsenate. The validity of this approach has been discussed in Planer-Friedrich et al. (2007).

Preliminary tests on the IC-ICP-MS showed that salicylate increases the signal intensity of the investigated sulfur and arsenic species considerably. To account for this sensitivity change, calibration solutions in salicylate were used for all samples. Moreover, the high salt load of salicylate in the eluates was found to impair chromatographic separation, rendering sample dilution necessary. Taking into account both IC performance and detection limits, a minimum tenfold dilution to 50 mM salicylate and a 25 μL injection volume were chosen for the eluates. This means that the proposed SPE procedure will allow detection of arsenic species >54 nM in a sample, assuming an initial sample volume of 250 mL is applied and eluted with 3 × 15 mL salicylate.

Solutions from the SPE procedure were also sampled for total arsenic and iron determination. Samples were measured by ICP-MS in oxygen mode for arsenic at m/z = 91 and in kinetic energy discrimination mode (KED, −3 V) for iron at m/z = 56. Besides external calibration corrections every 10 to 15 samples, rhodium was used to correct for internal instrument drift. Quality of analysis was monitored with a certified reference material (TM-DWS, Environment Canada, National Water Research Institute).

Sulfide was determined photometrically by the methylene-blue method in samples from the experiment considering arsenite-sulfide interactions. Aliquots from the initial sulfide and arsenite solutions, the respective passages, wash, and the one eluate A were analyzed in triplicate using a microplate reader at 650 nm (Infinite 200 Pro, Tecan).

2.5. Retention and recovery calculations

The two parameters retention and recovery were used to assess

the efficiency of the proposed SPE procedure. Concentrations derived from sample analysis were converted to molar amounts n, based on the respective volumes of each step in the SPE procedure. The retention was calculated according to equation (1), and demonstrates how much of a given compound is adsorbed while the initial solution passes the resin:

$$\text{retention} = \frac{n_{initial} - n_{passage}}{n_{initial}} \times 100\ \% \quad (1)$$

where $n_{initial}$ is the molar amount of a compound in the initial sample solution, $n_{passage}$ is the molar amount of this compound found in the passage, and their difference essentially constitutes the amount retained on the resin during sample application.

The recovery indicates to what extent a retained compound is removed from the resin during elution (Eq. (2)):

$$\text{recovery} = \frac{n_{eluate\ A+B+C}}{n_{initial} - n_{passage} - n_{wash}} \times 100\ \% \quad (2)$$

where $n_{eluate\ A+B+C}$ is the sum of the molar amounts in all three eluates compared to the molar amount bound on the resin prior to elution ($n_{initial}-n_{passage}-n_{wash}$). This calculation inevitably combines samples containing two different matrices. The initial solution is in MQ water, while the eluates are in salicylate, which was found to increase the sensitivity of IC-ICP-MS. This would underestimate the target species in the initial solution based on the calibration in salicylate. To account for the sensitivity change, the initial sample was analyzed both in MQ water and salicylate matrix. The initial in MQ water was used for calculating the retention, while the initial in salicylate was used for calculating the recovery. For the small concentrations in the passage and wash samples the sensitivity change is negligible.

3. Results & discussion

3.1. Development of SPE procedure

In order to develop a preservation method for sulfur and arsenic species in iron-rich waters, a number of different SPE procedures were investigated. The results were evaluated with respect to (1) retention of sulfur and arsenic species on the anion-exchange resin, and (2) recovery of these target species through elution, and parameters were adjusted accordingly. Prior to detailed method optimization, a number of preliminary tests were conducted, of which the results are briefly summarized in the following (data not shown).

Conditioning the resin prior to sample application was first conducted with 0.1 M KOH as suggested by Kaasalainen and Stefánsson (2011). However, for samples that also contained iron this led to precipitation of iron oxyhydroxides visible on the resin surface. Thus, the conditioning step was conducted using MQ water instead of KOH to avoid high pH conditions between the resin beads.

Several eluting agents were tested to achieve complete recovery of the retained species. Druschel et al. (2003) reported the use of 0.5 M KCl to elute sulfate and thiosulfate from AG2-X8 resin. In accordance with this, our results showed complete recovery for sulfate, thiosulfate, and monothioarsenate, but not for trithioarsenate. Even higher concentrations of up to 3 M KCl did not elute trithioarsenate completely. Based on the established method for thioarsenate detection by IC-ICP-MS (Planer-Friedrich et al., 2007), 0.1 M NaOH was tested as an alternative eluting agent, but did not yield full recoveries. The possibility of using 0.5 M citrate for the elution was also investigated. However, citrate was found to negatively affect peak shape of arsenate in ion chromatography, and was therefore excluded from further consideration. Finally, 0.5 M salicylate was found to yield complete recoveries, and was used for all further experiments. A detailed description of the optimized SPE procedure can be found in the supporting information (SI-10).

3.2. Synthetic solutions without iron

3.2.1. Retention and recovery of individual sulfur and arsenic species

First, the retention of sulfur and arsenic species on the anion-exchange resin was investigated for each species individually in solutions without iron. When the sample solution passed through the resin, the applied sulfate, thiosulfate, arsenate, monothioarsenate, and trithioarsenate were adsorbed completely, yielding retention values of 100.0 ± 0.0%, 100.0 ± 0.0%, 96.8 ± 0.2%, 98.8 ± 0.2%, and 99.6 ± 0.3%, respectively (Table 1, speciation data in SI-1, 2, 4, 5, 6). However, arsenite was found to pass the resin without adsorbing (retention < 3.0 ± 0.6%, SI-3). Following the sample application, the resin was washed with MQ water, which did not remove any of the target species.

During the three elution steps A, B, and C, the retained sulfur and arsenic species were recovered quantitatively. Recovery of the sulfur species sulfate and thiosulfate was 101.5 ± 0.9% and 101.7 ± 2.2%, respectively. During the first elution step A, 95.5% of the retained sulfate and 98.5% of the retained thiosulfate were removed. Compared to the method of Druschel et al. (2003) using KCl for the elution of sulfate and thiosulfate, salicylate delivered similar recovery values, but provided the additional advantage of simultaneous arsenic elution. The elution of the retained arsenate and monothioarsenate removed the major share of these species during the first elution step A (SI-4, 5, 7). However, for trithioarsenate only 51.1% of the retained amount was recovered during the first elution step A (SI-6 & SI-7). Conducting the second and third elution steps B and C, as well as soaking the resin in salicylate for 20 min prior to elution, led to satisfactory recovery of trithioarsenate as well. Results from total arsenic and speciation analysis were in good agreement (<14% difference), taking into account that two different methods were employed.

Considering the obtained retention and recovery values, the anion-exchange resin AG2-X8 clearly shows a preferential adsorption of trithioarsenate >> monothioarsenate > arsenate > thiosulfate ≈ sulfate >> arsenite. Arsenite does not interact with the resin since it is present as an uncharged species. At the sample pH of 6.6, arsenite was fully protonated ($pK_{a1} = 9.2$), thus passed through the resin and was collected in the passage. This must be considered for most environments with pH values < 9, and the passage analyzed accordingly to acquire complete arsenic speciation.

3.2.2. Partial arsenite retention in mixtures with sulfide

Contrary to passing the resin without any adsorption in the absence of sulfide, arsenite was found to be partially adsorbed in the presence of sulfide. In preliminary tests, up to 60% retention was found for arsenite in combination with sulfide. It has been suggested recently that arsenite can bind to organic matter via thiol groups (Hoffmann et al., 2012; Langner et al., 2012; Catrouillet et al., 2015). Therefore, when a sulfidic solution is applied to the cartridge, sulfide may act as a bridge between the uncharged arsenite and the organic structure of the resin. In order to study this hypothesis, a 133.5 μM sulfide solution was passed through the resin in a first step to provide potential binding sites for arsenite. The sulfide solution presented a tenfold excess over the 13.35 μM arsenite solution, which was applied during a second step. Out of

the 6.0 μmol sulfur in the initially applied sulfide solution, 3.2 μmol remained on the resin (Fig. 2). Passing the arsenite solution through the resin then resulted in 47.3% retention.

The results from this experiment have two major implications. Firstly, SPE is widely used for species determination, assuming that the arsenic in the passage equals the total amount of uncharged arsenite, while the eluate holds only the charged pentavalent arsenic species (Jay et al., 2004; Samanta and Clifford, 2006; Lord et al., 2012). Based on this assumption, the passage and eluate are normally analyzed only for total arsenic, inevitably leading to severe underestimation of arsenite in sulfidic samples. Secondly, eluting with 0.5 M salicylate produces a high ionic strength solution containing both arsenite and sulfide, which is known to promote the formation of thioarsenates (Planer-Friedrich et al., 2008). This effect must be considered in terms of potential artifacts forming in the eluate prior to analysis.

3.2.3. Controlling artifact thioarsenate formation

To investigate the possible formation of artifact thioarsenates in the eluate, a mixture of arsenite and sulfide in different matrices was monitored over time (Fig. 3). In MQ water, the formation of thioarsenates showed an almost linear increase, yielding 78.6% thioarsenates out of all detected arsenic species after 1 d. An even faster thioarsenate formation was seen in 0.5 M salicylate. The maximum of 86.4% thioarsenates was already reached after 4 h. Here the high ionic strength of 0.5 M salicylate led to an accelerated thioarsenate formation from arsenite and sulfide producing mainly trithioarsenate (SI-8).

The observed reaction of arsenite with sulfide in salicylate would pose a serious problem for the elution step of the SPE procedure, because a considerable amount of artifact thioarsenates could form. In order to suppress this reaction, the salicylate was alkalized. A previous study showed that thioarsenates only form from arsenite and sulfide at an excess of SH^- versus OH^- in solution (Planer-Friedrich et al., 2010). Thus, alkalization of the salicylate will ensure excess of OH^- over SH^- and impede thioarsenate formation. Indeed, when the salicylate was alkalized to pH 12.3, the reaction evidently slowed down yielding only 1.7% thioarsenates after 2 h (Fig. 3). The efficiency of this approach can also be seen in Fig. 2, where the alkaline salicylate was used for elution. The eluate containing both arsenite and sulfide was analyzed within 2 h and showed no thioarsenate formation. As evident from these results, artifact thioarsenates can be excluded by immediate analysis of the alkaline eluates. It must be noted that the extremely high pH during elution may lead to a minor conversion of arsenite to arsenate (SI-8). This can be addressed by adjusting the pH according to the sulfide content of the sample solution. To ensure OH^- excess for samples containing less than 100 μM sulfide for example, a pH of 10 is sufficient. At this pH, arsenite will neither convert to arsenate nor react with sulfide to form thioarsenates.

The problem of artifact thioarsenates forming from arsenite and sulfide could also be avoided by including another SPE cartridge with mercapto-modified silica prior to the anion-exchange resin that will retain uncharged arsenite (Howard et al., 1987). However, it would require another elution for the additional cartridge, and the retention of thioarsenates has yet to be tested on this resin. Hence, the much simpler alkalization of salicylate was given preference as it was shown to effectively prevent artifact thioarsenate formation.

Table 1
Retention and recovery values of individual sulfur and arsenic species in the absence of iron (speciation analysis of target species by IC-ICP-MS and total arsenic analysis by ICP-MS of 3 experimental replicates).

	Retention [%]		Recovery [%]	
	Species	Total As	Species	Total As
Sulfate	100.0 ± 0.0		101.5 ± 0.9	
Thiosulfate	100.0 ± 0.0		101.7 ± 2.2	
Arsenite	–	3.0 ± 0.6		
Arsenate	96.8 ± 0.2	97.7 ± 0.3	95.5 ± 1.8	100.2 ± 1.2
Monothioarsenate	98.8 ± 0.2	97.1 ± 0.6	91.9 ± 2.0	105.9 ± 0.6
Trithioarsenate	99.6 ± 0.3	94.2 ± 0.8	89.7 ± 3.4	102.4 ± 1.6

3.3. Synthetic solutions with iron

3.3.1. Monothioarsenate & iron

To test the hypothesis that the anionic thioarsenates will be retained on the anion-exchange-resin, while cationic iron will pass through, a solution containing monothioarsenate and iron was applied to the resin. Monothioarsenate was completely retained (98.6 ± 0.5%) and all of the applied iron passed through, with negligible retention of 0.4 ± 0.2% (Fig. 4). During elution, the retained monothioarsenate was fully recovered with 104.3 ± 2.7%. Thus, the separation of monothioarsenate and iron was achieved as expected.

In an additional experiment, chloride was added to the initial solution of monothioarsenate and iron to investigate the applicability of the method for saline groundwater or even slightly brackish water, where chloride concentrations typically lie in the high μM to low mM range. In the presence of 4 mM chloride the retention of monothioarsenate decreased slightly to 91.9 ± 2.1%, likely due to the high load of competing anions (SI-9). Nevertheless, all of the retained monothioarsenate was recovered (102.4 ± 2.0%). This also demonstrates the possibility of employing the SPE method for sampling waters with increased chloride concentrations, which often poses serious problems for analysis.

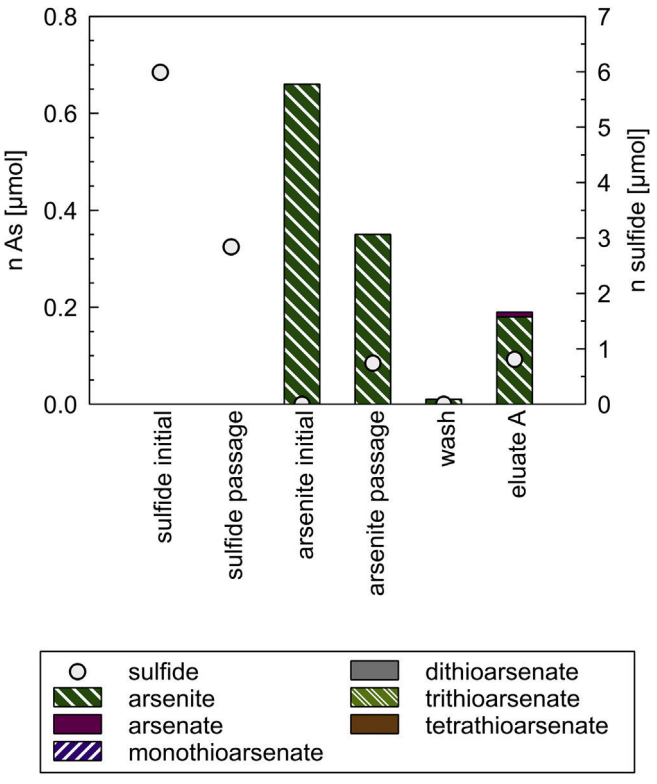

Fig. 2. Molar amounts of sulfide and arsenic species after first passing a 133.5 μM sulfide, then a 13.35 μM arsenite solution through AG2-X8 resin (one elution step with 15 mL alkaline salicylate was performed).

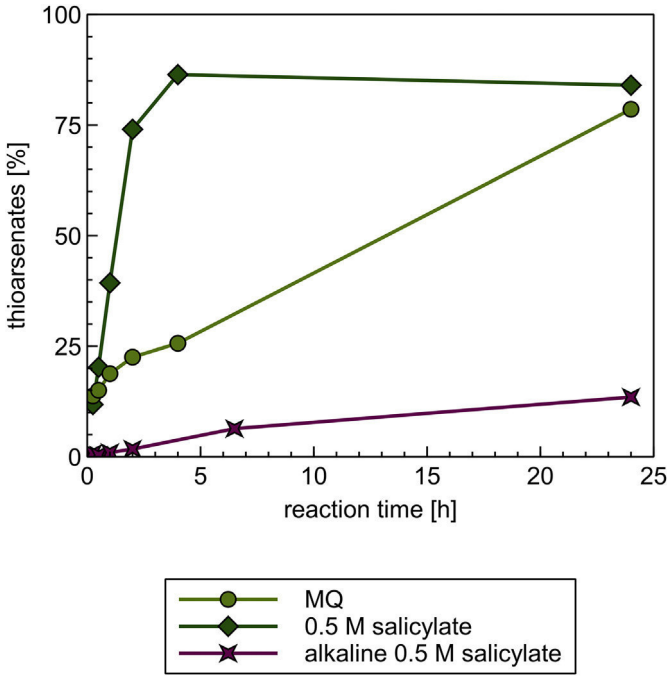

Fig. 3. Formation of thioarsenates in a solution with 25.8 μM arsenite and 2.8 mM sulfide in MQ water, salicylate, and alkaline salicylate over time as percentage of the sum of arsenic species.

By performing the SPE procedure, monothioarsenate was successfully separated from iron. As a result, any potential iron oxyhydroxides cannot change the arsenic speciation once the sample has been subjected to the SPE procedure. However, while passing the sample solution through the resin, Fe(II) could potentially be oxidized and provide sorption sites for arsenic, which is then no longer retained on the resin. In the presence of 180 μM Fe(II), a 10% decrease in arsenate retention was found for a similar resin (Mondal et al., 2007). Bednar et al. (2002) suggested adding EDTA to the initial sample to chelate iron before passing it through the resin. Although this method satisfactorily preserved arsenite and arsenate, it is not suitable for sulfidic solutions containing thioarsenates as it has been shown to not only induce arsenite oxidation but also artifact thioarsenate formation (Suess et al., 2015). Consequently, potential oxidation of Fe(II) during sample passage must be limited by working under anoxic conditions in the laboratory and using gas-tight syringes in the field. Faster sample application could also be considered (Impellitteri, 2004), but complete retention of target compounds should be verified beforehand.

3.3.2. *Arsenic mix & iron - considerations for small sample volumes*

The SPE procedure shown in Fig. 1 was demonstrated to separate thioarsenates from iron as hypothesized. The proposed elution with 3 × 15 mL alkaline salicylate allowed quantitative detection of thioarsenates despite high iron concentrations. In this form, the developed SPE procedure can be used as a preservation method for arsenic species in the presence of iron and sulfide. In addition to that, further potentials of the presented SPE method were considered. The possibility to reduce the sample volume of 250 mL applied to the resin was examined. It needs to be factored in that a decrease in sample volume, and with that a decrease in the amount of target species on the resin, will lead to lower concentrations in the eluates. The ratio of sample versus eluate volume ultimately determines satisfactory IC-ICP-MS detection of the target species. Consequently, the volume and number of elution steps could be reduced to increase enrichment of the target species in the eluate and also expedite analysis.

Considering all these aspects, a modification of the presented SPE procedure was tested. A volume of only 50 mL of initial sample was applied and eluted in four increments of 5 mL alkaline salicylate, each including 20 min soak time. The four increments were collected as one eluate and analyzed immediately. An initial sample containing arsenite, arsenate, monothioarsenate, trithioarsenate, sulfide, and iron was treated according to this modified SPE procedure and all arsenic species were retained except for arsenite (Fig. 5). Consequently, arsenite was collected together with iron in the passage, which requires acidification of such samples to keep iron in solution (Aggett and Kriegman, 1987). Since no arsenic species other than arsenite was found in the passage, total arsenic analysis is sufficient. As previously discussed, a share of the applied arsenite (31.4%) was also bound on the resin via sulfide.

Calculation of recovery values for the modified elution had to be based on the initial solution in MQ water in this case. Analysis of the initial solution in alkaline salicylate was impossible, because mixing the iron-rich initial sample with alkaline salicylate led to iron oxyhydroxide precipitation. Therefore, recovery values of all species are >100% here due to the increased sensitivity for the species in the eluate. For arsenate and monothioarsenate the modified elution with 4 × 5 mL alkaline salicylate was complete and yielded recoveries of 152.1% and 107.5%, respectively. For arsenate, the oxidation of arsenite induced by the high pH salicylate (see section 3.2.3) may have also contributed to the high recovery value. As discussed before, this can be easily avoided by using a slightly less alkaline salicylate.

Trithioarsenate was not completely eluted during the modified elution procedure. In order to recover the trithioarsenate still bound on the resin, a more forceful approach was chosen. The resin material was emptied into a beaker and stirred in 5 mL 0.5 M

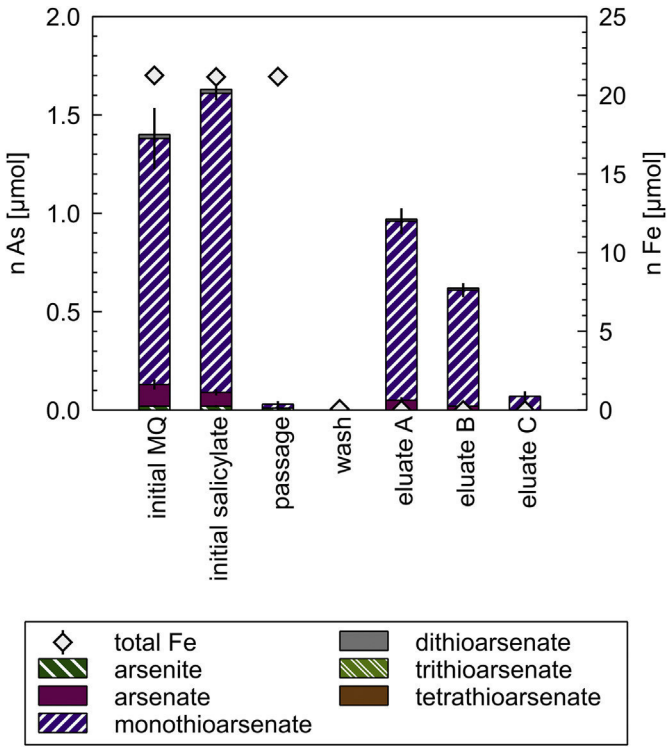

Fig. 4. Molar amounts of arsenic species and total iron for each step of the SPE procedure applied to a 5 μM monothioarsenate solution with 90 μM Fe(II) (average of 3 experimental replicates).

salicylate for 1 h (batch elution). Only total arsenic was measured in the batch eluate since there was no species other than trithioarsenate still bound on the resin. The batch elution completely removed the amount of trithioarsenate remaining on the resin after the first elution (Fig. 5). Combining the results from first elution and batch elution yielded a recovery of 115.4% for trithioarsenate, and a recovery of 112.1% for all arsenic species summed up, demonstrating complete elution. Again, the recovery values >100% are the consequence of the increased IC-ICP-MS sensitivity of the eluted arsenic species in salicylate compared to the initial solution in MQ water.

The results from this test clearly showed that decreasing the volume and number of elution steps must be considered carefully. When only a small sample volume is applied to the resin, the elution must be adjusted accordingly to ensure detection of the eluted species. In general, controlled flow-through elution, as presented in Fig. 1, is recommended for SPE cartridges. However, when only a limited sample volume can be applied to the resin, batch elution could provide an alternative.

3.4. Stability of natural samples

Stability of the target species over time is a key factor for the successful use of SPE on field samples. To investigate this aspect, water from the iron-rich mineral spring Stepanka in Frantiskovy Lazne (Czech Republic), known to contain various sulfur and arsenic species and 186 μM Fe, was passed through the resin. During sample application, arsenite was partially retained (33.2%) on the anion-exchange resin via sulfide as previously discussed. Back in the laboratory, cartridges were stored for 1, 2, and 6 d before elution. In this case, sample volume was not limited and sufficient sample was applied to perform controlled flow-through elution as presented in Fig. 1.

For comparison over time, the amounts of each sulfur and arsenic species in eluates A, B, and C were summed up. The sulfur species showed good stability for storage up to 6 d, with sulfate only slightly decreasing by 7.6% between day 1 and 2 (Fig. 6). Arsenic was also preserved well over time in terms of both total amount and speciation. Arsenite, arsenate, monothioarsenate, and dithioarsenate showed only small variations over the investigated period within analytical uncertainty. The amount of trithioarsenate eluted after 6 d demonstrated a decrease of 25.2% compared to day 1. The equivalent 0.13 μmol trithioarsenate were probably subject to dethiolation, which might be indicated by the slight arsenate and monothioarsenate increase on day 6.

To our knowledge, only one other preservation method has been proposed for thioarsenates in sulfidic, iron-rich waters so far. In the work of Suess et al. (2015), anoxic septum bottles were suggested for sample storage, but showed even bigger limitations with respect to trithioarsenate stability compared to the SPE method. After only 3 d of storage a 34% decrease was found for trithioarsenate in the presence of 90 μM Fe. Thus, SPE currently presents the best available preservation method for arsenic species in the presence of sulfide and iron. Despite the described decrease in trithioarsenate, the SPE method achieved a consistent reproduction of arsenic speciation over 6 d. Furthermore, the total arsenic amount remained stable over the entire investigated period. Separation of thioarsenates from iron by SPE clearly prevented any arsenic loss typically caused by adsorption on iron oxyhydroxides, and allowed thioarsenate detection even after 6 d of storage.

4. Conclusions

This work has shown the successful application of SPE to preserve arsenic species in sulfidic, iron-rich waters. We accomplished the separation of thioarsenates from iron by adsorption on an anion-exchange resin. Speciation changes or loss of total arsenic normally caused by iron were thus excluded. Complete and species-conserving elution of arsenate, monothioarsenate, dithioarsenate, and trithioarsenate was achieved by repeated elution with 0.5 M salicylate. Arsenite, which was not retained in the absence of sulfide, was found to be partially adsorbed on the resin in the presence of sulfide. In this case, thiol groups acted as a bridge between the organic structure of the resin and the uncharged arsenite, and caused partial retention. This effect must be considered when SPE is to be applied under sulfidic conditions. Furthermore, it was shown that the combination of arsenite and sulfide in the high ionic strength eluate can lead to artifact thioarsenate formation. These speciation changes can be prevented by using alkaline salicylate for the elution.

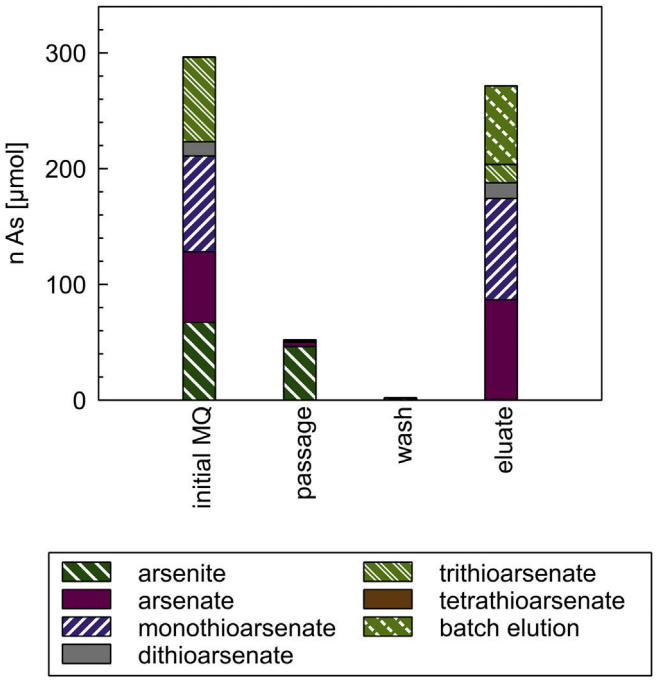

Fig. 5. Molar amounts of arsenic species for each step of the modified SPE procedure applied to a solution with arsenite, arsenate, monothioarsenate, trithioarsenate, and 90 μM Fe(II). Batch elution was performed after incomplete recovery by flow-through elution and analyzed for total arsenic.

The proposed SPE method presents a promising alternative to established methods like the addition of mineral acids or EDTA, which may suffer from limitations such as arsenic sulfide precipitation or arsenite oxidation. The method is inexpensive and the prepared SPE cartridges can be easily transported even to remote sampling points, making it a convenient field technique. Moreover, the retained sulfur and arsenic species were found to be stable on the resin for at least 6 d. To further exploit the potential of SPE, the limitations and possibilities of batch elution should be considered. This might allow a reduction in the required sample volume and application time.

The SPE method presented here can serve as a basis for future studies to investigate arsenic species in the presence of sulfur and iron. In addition, the method has also been shown to work well under increased chloride conditions, rendering application for saline groundwater or even slightly brackish waters possible. Overall, SPE provides the opportunity to investigate the role of thioarsenates in environments where they may have been previously

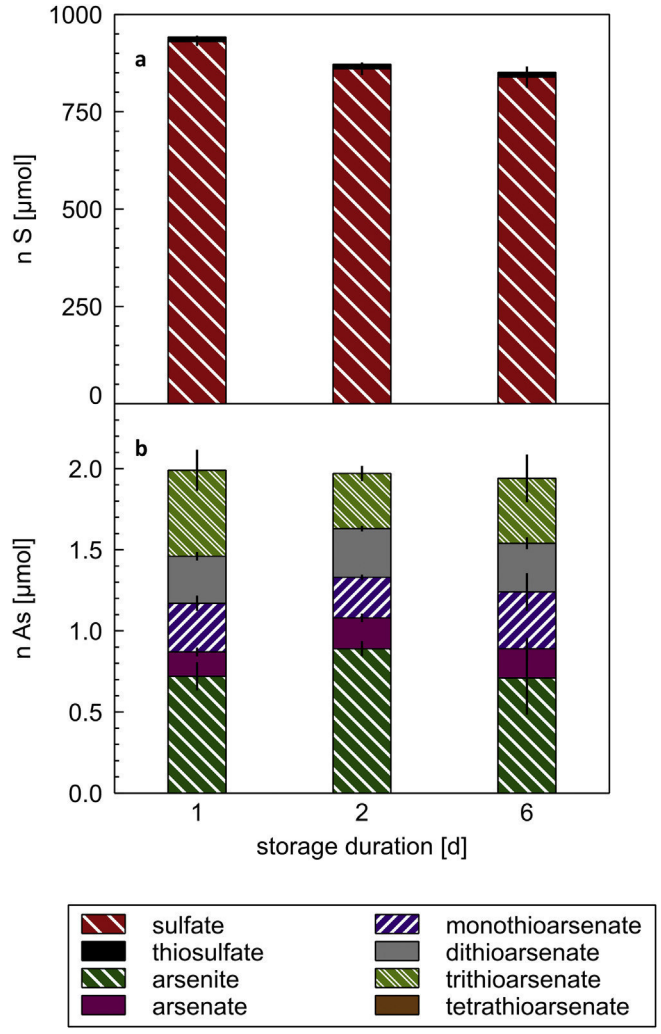

Fig. 6. Molar amounts of (a) sulfate and thiosulfate and (b) arsenic species eluted (sum of eluate A, B, and C) from SPE cartridge 1, 2, and 6 d after applying a sample from Stepanka spring, Frantiskovy Lazne (Czech Republic; pH 5.6, 186 µM Fe) spiked with 13.35 µM arsenite and 133.5 µM sulfide (average of 3 experimental replicates).

overlooked due to the lack of suitable preservation methods.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2016.07.008.

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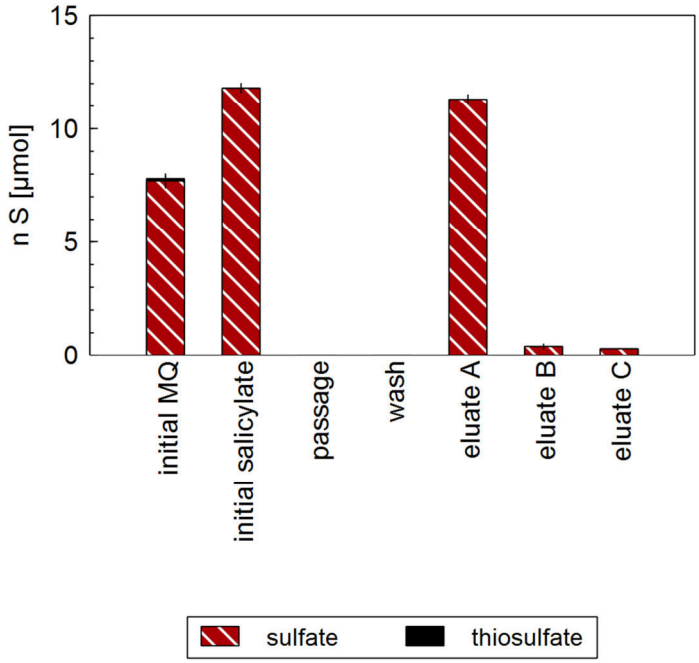
SUPPORTING INFORMATION

A new method for thioarsenate preservation in iron-rich waters by solid phase extraction

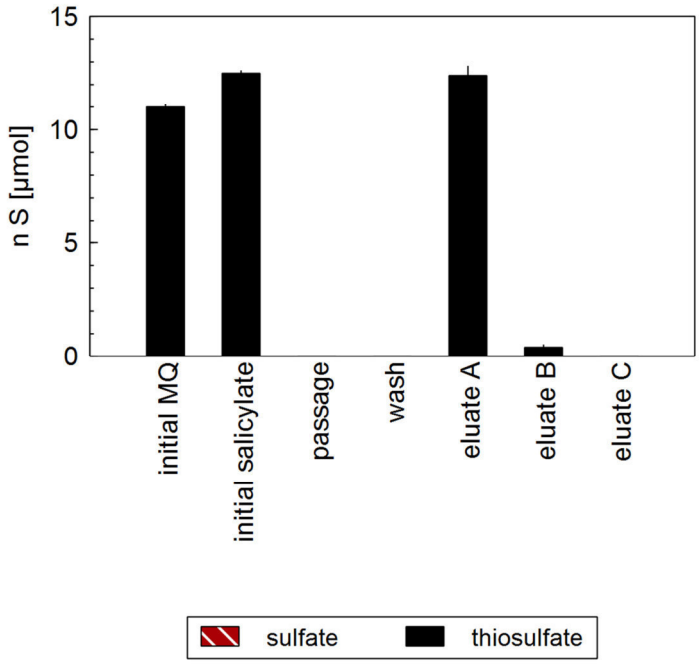
Maria K. Ullrich, Valentina Misiari, Britta Planer-Friedrich

Number of pages: 9

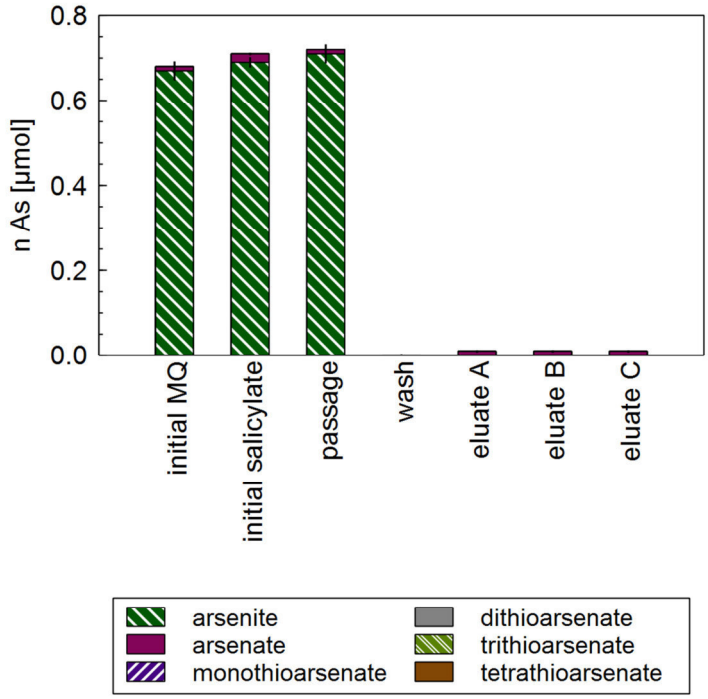
Content: Page



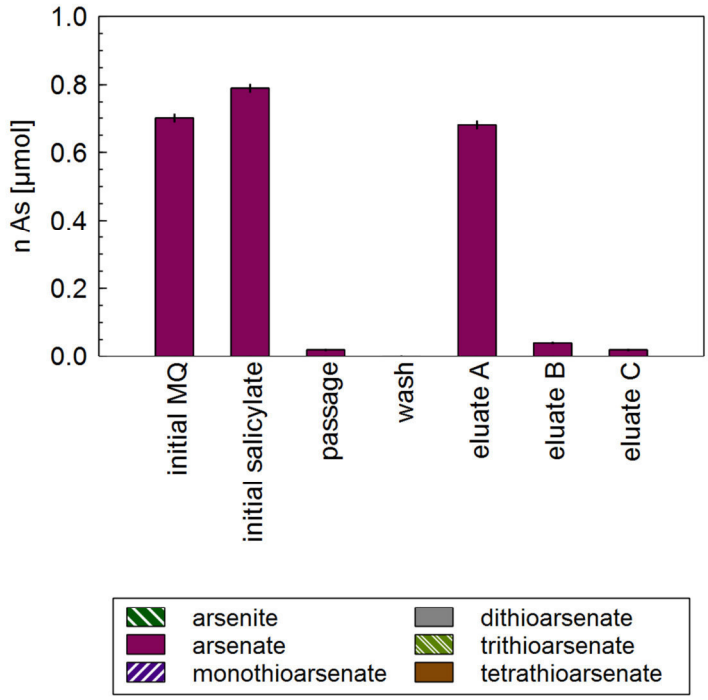
SI-1: Molar amounts of sulfur species for each step of the SPE procedure applied to a solution containing sulfate (average of 3 experimental replicates).



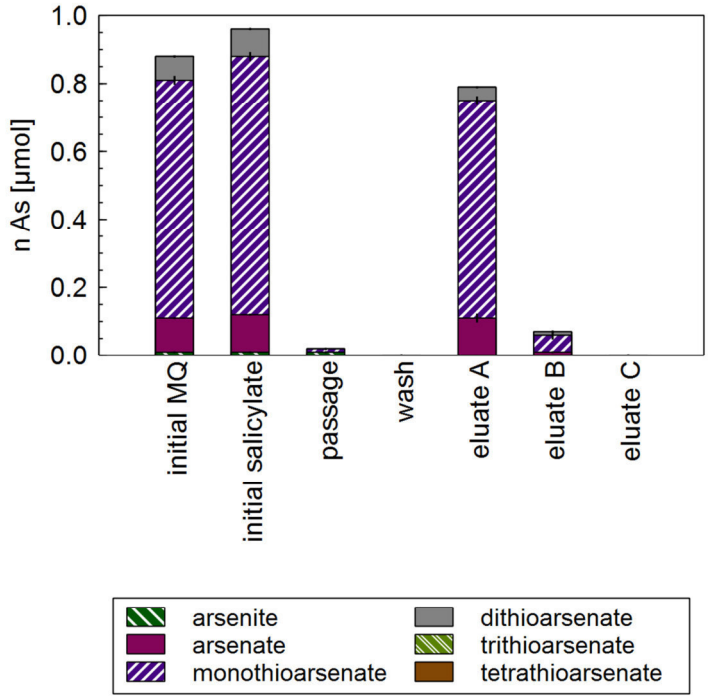
SI-2: Molar amounts of sulfur species for each step of the SPE procedure applied to a solution containing thiosulfate (average of 3 experimental replicates).



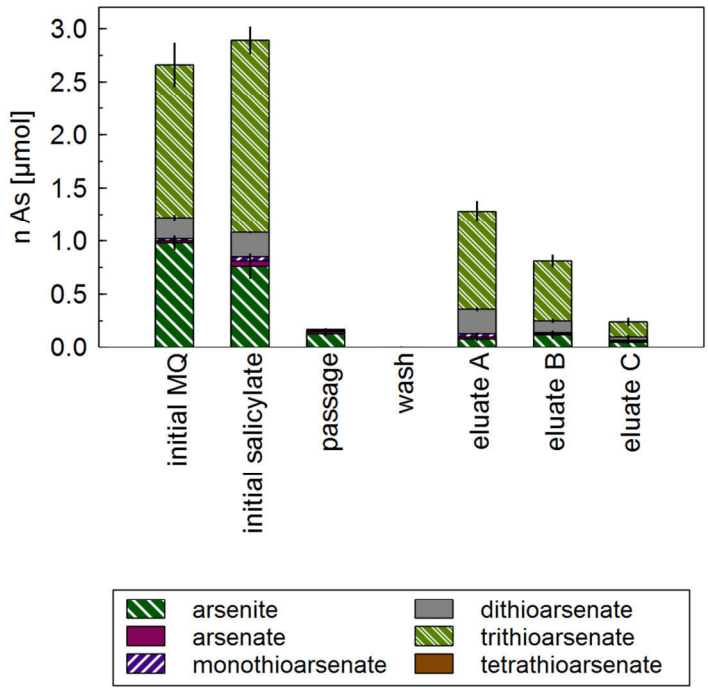
SI-3: Molar amounts of arsenic species for each step of the SPE procedure applied to a solution containing arsenite (average of 3 experimental replicates).



SI-4: Molar amounts of arsenic species for each step of the SPE procedure applied to a solution containing arsenate (average of 3 experimental replicates).



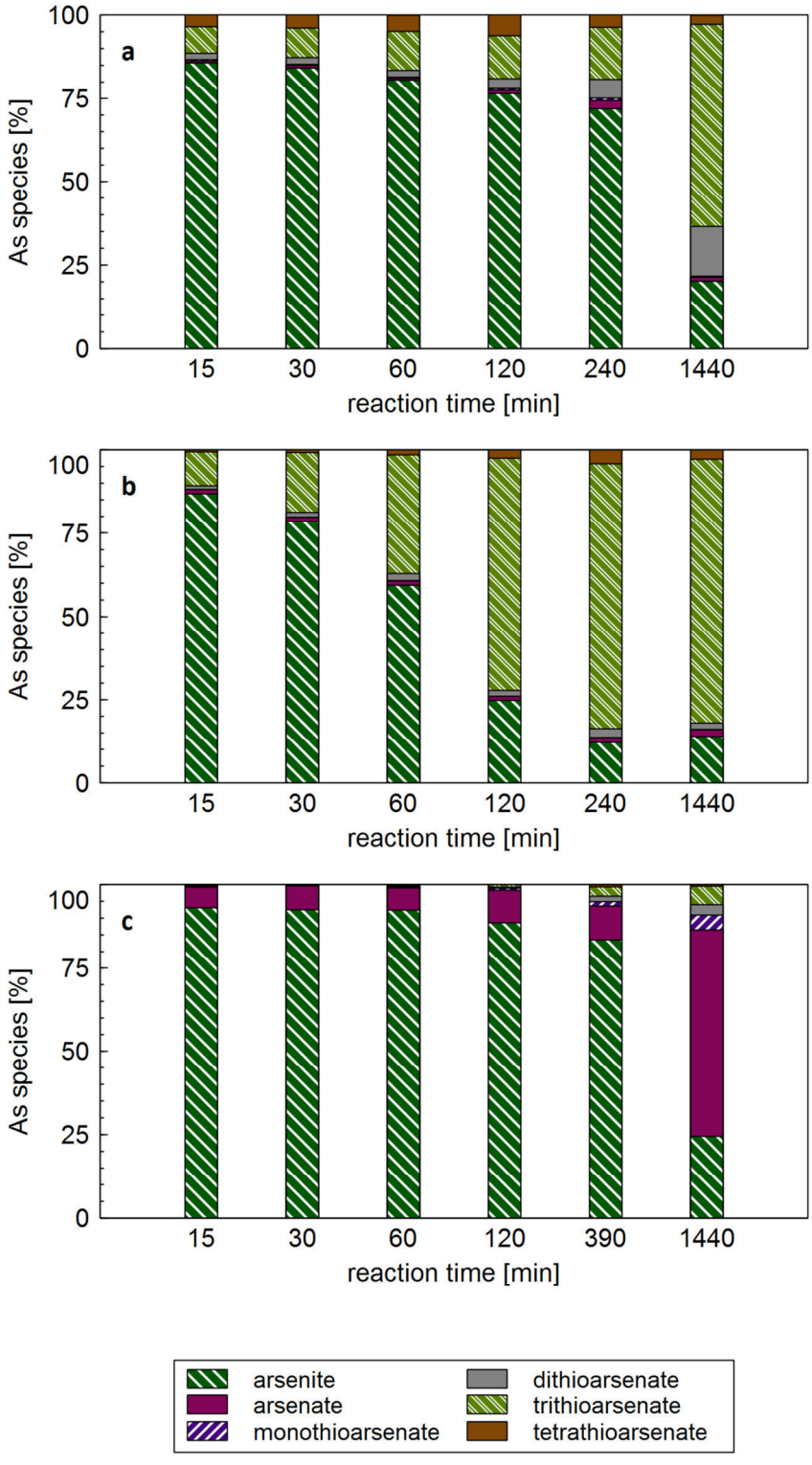
SI-5: Molar amounts of arsenic species for each step of the SPE procedure applied to a solution containing monothioarsenate and minor amounts of arsenate and dithioarsenate due to synthesis (average of 3 experimental replicates).



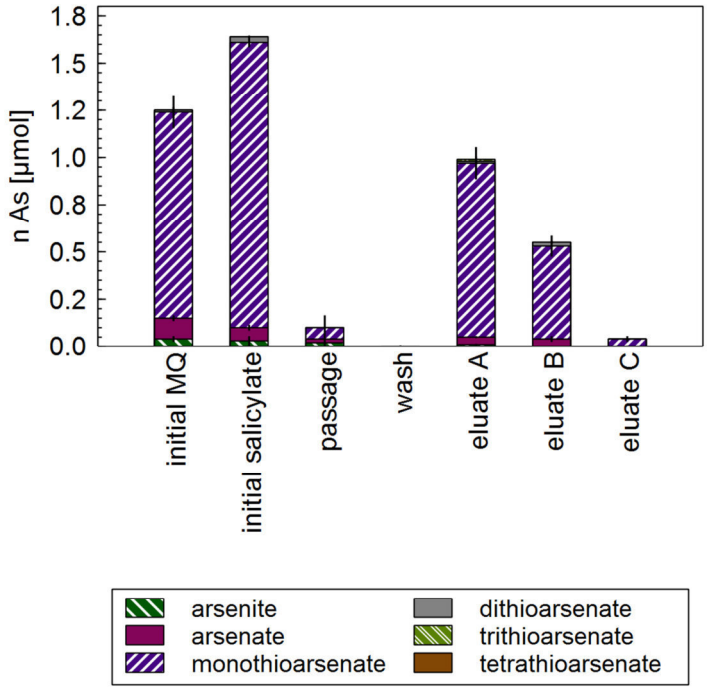
SI-6: Molar amounts of arsenic species for each step of the SPE procedure applied to a solution containing trithioarsenate as well as arsenite and dithioarsenate due to synthesis (average of 3 experimental replicates).

SI-7: Recovery values of arsenic species for the individual eluates A, B, and C in the absence of iron (speciation analysis of target species by IC-ICP-MS and total arsenic analysis by ICP-MS of 3 experimental replicates).

	As species			total As		
	eluate A	eluate B	eluate C	eluate A	eluate B	eluate C
arsenate	88.3 ± 1.9	4.7 ± 0.2	2.6 ± 0.1	96.7 ± 1.3	3.0 ± 0.1	0.5 ± 0.0
monothioarsenate	85.2 ± 0.9	6.5 ± 1.4	0.2 ± 0.1	96.5 ± 0.9	8.5 ± 0.3	0.9 ± 0.0
trithioarsenate	51.1 ± 5.0	31.1 ± 2.4	7.6 ± 1.7	59.6 ± 0.1	34.2 ± 1.7	8.6 ± 0.9



SI-8: Arsenic speciation in a solution with 25.8 µM arsenite and 2.8 mM sulfide in (a) MQ water, (b) 0.5 M salicylate, and (c) alkaline 0.5 M salicylate over time as percentage of the sum of all detected arsenic species.



SI-9: Molar amounts of arsenic species for each step of the SPE procedure applied to a solution containing monothioarsenate, 90 µM Fe(II) and 3.9 mM NaCl as well as minor amounts of arsenate and dithioarsenate due to synthesis (average of 3 experimental replicates).

SI-10: Detailed description of performing the proposed SPE method for preservation of thioarsenates in iron-rich waters including preparation of cartridges, SPE procedure, and analysis.

A) Preparation of cartridges

1) a polyethylene frit is inserted into a 6 mL polypropylene cartridge (Supelco, Sigma-Aldrich)
2) 1 g of AG2-X8 resin (Cl-form, BioRad) is poured as a slurry with MQ water into the cartridge
3) excess MQ water is removed by connecting the cartridge to a vacuum manifold and applying vacuum
4) no top frit is inserted to promote mixing between the resin and the applied solutions (and later facilitate removal of resin for batch elution)
5) the top of the cartridge is wrapped with parafilm and sealed with a rubber stopper for transport

B) SPE procedure

1) the cartridge is connected to a vacuum manifold including a valve at the outlet of the cartridge (alternatively for the field: a gas-tight syringe can be connected via a 0.2 µm cellulose-acetate filter and an adapter to the cartridge to pass the solutions manually through the resin)
2) conditioning: 12 mL of MQ water are passed through the cartridge to condition the resin, applying vacuum will start flow (note: do not let resin run dry at the end of conditioning; if acidic sample solutions are supposed to be applied, conditioning with acetate instead of MQ water should be considered to buffer pH)
3) sample application: 250 mL of initial sample are passed through the cartridge at a flow rate of 1.7 - 2.0 mL · min^{-1} (note: the applied volume can be adjusted, but concentrations in the initial sample and elution volumes must be considered for IC-ICP-MS detection); while the initial solution is being applied, the passage is collected at the outlet of the cartridge (150 µL 30 % H_2O_2 and 250 µL 7.25 M HNO_3 are added to a 10 mL aliquot of the passage for stabilization)
4) wash: 4 mL of MQ water are quickly passed through the cartridge (note: when cartridges are supposed to be stored, the resin needs to be dried after this step by applying vacuum)
5) elution:
    a. controlled flow-through elution: 3 increments of each 5 mL alkaline 0.5 M salicylate (≥ 99.5 %, Sigma-Aldrich) are passed through the cartridge by letting each increment soak for 20 min, then eluting it within 2 min and collecting all three increments in one tube. This step is repeated three times resulting in eluates A, B, and C with 15 mL

volume each (note: the alkaline salicylate is produced by dissolving sodium salicylate in NaOH to ensure OH^- excess over SH^- in the eluates, e.g. for samples containing less than 100 µM sulfide pH 10 is sufficient)

b. batch elution: as an alternative to controlled flow-through elution, the resin material can be removed from the cartridge, transferred to a beaker and stirred in at least 5 mL alkaline 0.5 M salicylate for 1 h (this step should be repeated once to verify full elution by separating the resin from the first salicylate and adding fresh salicylate for another 1 h of stirring)

C) Analysis

1) samples are analyzed for speciation according to Planer-Friedrich et al. (2007) by IC-ICP-MS
2) eluates must be diluted at least tenfold and analyzed within 2 h of elution
3) calibration must be matrix-matched according to final salicylate concentration in the eluates after dilution
4) passages are analyzed for total As by ICP-MS

STUDY 2

submitted to Chemical Geology

Ullrich, M.K., Gelman, F., Zakon, Y., Halicz, L., Knöller, K.,Planer-Friedrich, B.

Sulfur isotope analysis by IC-MC-ICP-MS provides insight into fractionation of thioarsenates during abiotic oxidation

Sulfur isotope analysis by IC-MC-ICP-MS provides insight into fractionation of thioarsenates during abiotic oxidation

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ABSTRACT: Standard sulfur isotope analysis of aqueous samples is aimed at sulfide and sulfate, while often disregarding intermediate species, mainly due to analytical limitations. However, sulfur can form numerous intermediate species in the environment, including thiometal(loid)s such as thioarsenates ($[HAs^{V}S^{-II}_{n}O_{4-n}]^{2-}$, n=1-4), which can play a key role in isotope fractionation. The standard precipitation procedure for separation of sulfide and sulfate was applied to monothioarsenate solutions. Monothioarsenate was found to co-precipitate together with sulfide, thus potentially impeding correct sulfide isotope analysis. To overcome the limitations associated with this standard precipitation procedure, a new method was developed based on separating sulfur species by ion chromatography followed by online isotope detection on multi-collector ICP-MS (IC-MC-ICP-MS). Applying this new method, fractionation between monothioarsenate and sulfate of up to -6.1 ‰ was found during monothioarsenate oxidation. In contrast, oxidation of tetrathioarsenate via tri- and di- to monothioarsenate did not result in fractionation. However, the released sulfide became increasingly enriched in ^{34}S due to oxidation to sulfate. This process was found to introduce additional enrichment of up to 9.1 ‰ to monothioarsenate through intermolecular isotope exchange between arsenic-bound sulfur and sulfide in solution. These results help elucidate pathways of thioarsenate transformation, and thus provide valuable information for the interpretation of isotope fractionation patterns in sulfidic environments.

KEYWORDS: sulfide oxidation; thio-arsenic species; isotope ratio mass spectrometry; thioarsenites

INTRODUCTION

Sulfur isotope analysis can provide key data for understanding abiotic and biotic processes in our environment. Fractionation is mainly a result of sulfide oxidation and sulfate reduction, during which a number of intermediate sulfur species are produced. Elemental sulfur and thiosulfate are the most commonly studied intermediate sulfur species. However, in the presence of metal(loid)s such as Au, Mo, Zn, Cd, Cu, Sb, or As, sulfur can also form thiometal(loid) species (Lohmayer et al., 2015; Planer-Friedrich et al., 2007; Seward, 1973; Tossell, 2000a; Tossell and Vaughan, 1993; Ullrich et al., 2013). Considering their environmental impact, thioarsenates ($[HAs^{V}S^{-II}_{n}O_{4-n}]^{2-}$, n = 1 - 4) are of particular interest. The formation of thioarsenates is promoted in sulfidic environments, e.g. geothermal waters, remediated waste sites, or reduced ground waters (Pi et al., 2016; Planer-Friedrich et al., 2007; Stucker et al., 2014; Ullrich et al., 2013; Wallschlaeger and Stadey, 2007). Concentrations of sulfur bound in thioarsenates typically lie in the order of 1 to 10^2 µmol, presenting a considerable amount of reduced sulfur in these environments in addition to e.g. thiosulfate or elemental sulfur. All of these sulfur species mediate the overall electron transfer between sulfide and sulfate, and thus are crucial for our interpretation of sulfur isotopic data. However, isotopic fractionation of sulfur bound in thioarsenates and how thioarsenate occurrence may affect standard sulfur isotope analysis is unknown.

The isotopic composition of sulfur species is commonly analyzed by isotope ratio mass spectrometry (IRMS). This method requires precipitation of individual aqueous sulfur species prior to analysis. Based on standard methods, sulfide

is precipitated as ZnS by the addition of zinc acetate ($ZnAc_2$), after which sulfate is precipitated as $BaSO_4$ by adding $BaCl_2$ (Knoeller and Schubert, 2010). To precipitate intermediate sulfur species, a number of different species-selective methods have been suggested (Habicht et al., 1998; Knossow et al., 2015; Kusakabe et al., 2000; Uyama et al., 1985). However, the precipitation approach often requires large sample volumes of up to 9.5 L (Knossow et al., 2015), and entails the potential of incomplete species recovery as well as accidental species transformation during sample preparation. Moreover, there is currently no method available for species-selective precipitation of thioarsenates. In fact, several studies have reported partial thioarsenate co-determination during photometric sulfide analysis by the methylene blue method (Planer-Friedrich et al., 2015; Planer-Friedrich et al., 2007; Suess et al., 2011), which is also based on ZnS precipitation using $ZnAc_2$. Consequently, thioarsenate co-precipitation together with sulfide might be of concern for sulfur isotope analysis as well. However, this aspect has yet to be investigated in detail.

Limitations associated with standard species-selective precipitation have led to the investigation of alternative methods of species separation for isotope analysis such as chromatography. Initially, chromatography was used as an offline separation method prior to IRMS analysis (Amrani et al., 2006). However, introduction of multi-collector ICP-MS provided the opportunity for high precision, multiple isotope analysis (Paris et al., 2013), thus enabling online coupling of chromatographic separation with isotope detection by MC-ICP-MS. While numerous studies have focused on gas chromatography coupled to MC-ICP-MS ((Rodriguez-Gonzalez et al., 2012) and references therein), work on employing ion chromatography for species separation has been limited (Clough et al., 2006; Gunther-Leopold et al., 2004; Santamaria-Fernandez et al., 2008; Zakon et al., 2014).

The aim of the present study was to develop a method that would allow investigating fractionation of thioarsenates, and thus elucidate their oxidative transformation pathways, which are not fully understood yet. We examined the potential of the standard precipitation method followed by IRMS analysis, taking into account the possibility of thioarsenate co-determination with sulfide. In addition, we developed an alternative method for species-selective isotope analysis based on IC-MC-ICP-MS, enabling simultaneous detection of up to 7 different species in one analytical run. This new method was applied to investigate the oxidative transformation of monothioarsenate, which is the most stable thioarsenate. Finally, tetrathioarsenate oxidation was studied to gain new insight on how all four thioarsenates and their oxidation products may contribute to sulfur isotope fractionation observed in the environment.

MATERIALS & METHODS

Experimental Setup & Sample Preparation

To investigate the potential of applying the standard precipitation method for samples containing thioarsenates, 3 different $ZnAc_2$/monothioarsenate ratios in combination with 4 different reaction times were tested. Twelve vials were prepared with 5 mM monothioarsenate ($Na_3AsO_3S \cdot 7\ H_2O$, synthesized according to Suess et al. (2009)), and alkaline 3 % $ZnAc_2$ solution (≥ 99.5 %, Grüssing, 10 v/v % NH_3) was added at molar ratios of 1:1, 10:1, and 100:1. After 10 min, 2 h, 5 h, and 24 h reaction times, during which solutions were stored in the dark to avoid photooxidation (Xu et al., 1998), the pH was measured and solutions were filtered (0.2 µm, cellulose acetate, Carl Roth). An aliquot for immediate speciation analysis via IC-ICP-MS was taken from this filtrate. The remaining solution was treated by adding H_2O_2 (30 %, VWR) and HNO_3 (65 %, Sigma-Aldrich) to a final concentration of 1.5 % and 1.6 %, respectively, and analyzed for total arsenic by ICP-MS. The precipitate on the filter was digested in 30 mL of 1.3 % HNO_3 on a heating block at 95 °C for 90 min. Precipitation of monothioarsenate was quantified based on arsenic because of lower detection limits compared to sulfur. However, to verify concurrent sulfur precipitation, sulfur analysis was conducted for 10-fold $ZnAc_2$ excess after a 2 h reaction time.

Sulfur isotope fractionation during monothioarsenate oxidation was studied by applying increasing amounts of 0 - 69 mM H_2O_2 to 100 mL solutions of 5 mM monothioarsenate. As determined during a preliminary test, H_2O_2 had fully reacted with monothioarsenate after 4 d and an aliquot for sulfur and arsenic species analysis by IC-ICP-MS was taken. Initial solutions with H_2O_2 > 15 mM showed a slight milky coloring indicating elemental sulfur formation. The sample containing 69 mM H_2O_2 was prepared again and analyzed for elemental sulfur by HPLC-UV (ThomasArrigo et al., 2016). Monothioarsenate in the oxidized solutions was precipitated with 10-fold $ZnAc_2$ excess. After 24 h, the solutions were filtered and the precipitates converted to Ag_2S by addition of $AgNO_3$ (≥ 99.0 %, Sigma-Aldrich) according to routine procedures (Knoeller and Schubert, 2010). The filtrate was sampled for total and species analysis of sulfur and arsenic, followed by acidification of the remaining solution to pH 3.0 - 3.5 and subsequent precipitation of $BaSO_4$ with $BaCl_2$ (> 99.0 %, Fluka). Both Ag_2S and $BaSO_4$ were analyzed by IRMS as described in the section "Analytical Procedures".

To compare standard isotope analysis by IRMS with the new IC-MC-ICP-MS method presented here, the monothioarsenate oxidation experiment was repeated. The superior sensitivity of the IC-MC-ICP-MS method allowed conducting the experiment with an initial 0.25 mM monothioarsenate as opposed to 5 mM required for IRMS analysis. Monothioarsenate solutions were again oxidized with H_2O_2 and analyzed after 4 d reaction time. No species transformation, and accordingly no fractionation, was observed during this reaction time for the initial monothioarsenate solution without H_2O_2.

In addition, the IC-MC-ICP-MS method was used to study isotope fractionation during tetrathioarsenate oxidation. Solutions containing 0.15 mM tetrathioarsenate ($Na_3AsS_4 \cdot 8\ H_2O$, synthesized according to Suess et al. (2009)) were

oxidized with 0 - 2.5 mM H_2O_2. During the reaction time of 4 d, solutions were stored under an oxygen-free atmosphere to avoid additional oxidation by O_2, which is known to affect all thioarsenates except monothioarsenate (Planer-Friedrich et al., 2009).

Analytical Procedures

Sulfur and arsenic speciation were determined according to a previously established method by IC-ICP-MS (Planer-Friedrich et al., 2007). In short, species were separated by anion exchange chromatography (IonPac AG/AS16, ICS 3000, Dionex) using gradient elution with 20 - 100 mM NaOH, and subsequently detected by quadrupole ICP-MS (XSeries2, Thermo Scientific). Total sulfur and arsenic concentrations in solutions treated with H_2O_2 and HNO_3 were determined by ICP-MS.

Standard sulfur isotope analysis of Ag_2S and $BaSO_4$ followed routine procedures (Knoeller and Schubert, 2010), using an elemental analyzer connected to IRMS (delta S, Thermo Finnigan). Results were normalized using International Atomic Energy Agency reference materials, by assigning IAEA-S1 = -0.3 ‰ (Ag_2S) and IAEA-SO-5 = 0.5 ‰ ($BaSO_4$). Analytical error was better than ±0.5 ‰ and results are presented in the conventional delta notation relative to Vienna Canyon Diablo Troilite:

$$\delta^{34}S\,(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \cdot 1000 \qquad (1)$$

where R is the ratio of $^{34}S/\,^{32}S$. Sulfur isotopic mass balance was assessed based on:

$$\sum \delta^{34}S = f_i \cdot \delta^{34}S_i \qquad (2)$$

where f_i is the molar fraction of species i relative to total sulfur and $\delta^{34}S_i$ is the corresponding isotopic composition.

As an alternative to standard IRMS analysis, a new species-selective method was developed based on a combination of work by Zakon et al. (2014) and Planer-Friedrich et al. (2007). Species separation was achieved using ion chromatography followed by online sulfur isotope detection using MC-ICP-MS. A schematic of the instrumental setup is presented in Zakon et al. (2014). The system consists of an ion chromatography pump (ICS 2100, Dionex) fitted with an AS40 autosampler (Dionex) and an eluent generator equipped with a KOH eluent cartridge (EGC III, Dionex). Samples were injected via a 500 µL sample loop, and species separated using an anion exchange column (IonPac AG/AS16, Dionex) and gradient elution (20 - 90 mM KOH). Following separation, the sample was passed through a self-regenerating suppressor and a conductivity detector, nebulized into an Apex Q desolvation unit and introduced into the MC-ICP-MS (Nu Plasma II, UK). For compatibility with nebulizer flow and MC-ICP-MS sample introduction the eluent flow was adjusted to 0.45 mL · min^{-1}. Isotopic composition was analyzed using the MC-ICP-MS parameters listed in the Supporting Information (SI-1).

Online chromatographic separation produced transient signals as each species eluted from the column. Thus, the whole area of each peak was integrated to calculate isotopic values. An average of 40 - 50 points before each peak was assigned as background and subtracted from the integrated peak on a point-by-point basis to account for baseline contribution. Standard-sample bracketing was used to correct for instrumental drift by averaging two analyses of the standard (Na_2SO_4, $\delta^{34}S$ = 5.9 ‰, Merck) conducted before and after each sample.

Since part of the analyzed sulfur species contain arsenic as well, it was important to evaluate possible matrix effects of arsenic on $\delta^{34}S$ values. The laboratory standard (Na_2SO_4) was analyzed with and without introducing arsenic acid (H_3AsO_4 in HNO_3, Merck) via a T-connector (see instrumental setup in Zakon et al. (2014)). The observed $\delta^{34}S$ values were identical within method uncertainty (±0.3 ‰). Furthermore, it was investigated whether chromatographic separation introduces additional fractionation. Solutions of sulfide, monothioarsenate, and tetrathioarsenate were analyzed directly by MC-ICP-MS and after IC separation, and showed identical $\delta^{34}S$ values within method uncertainty. Additionally, the influence of variations of the sulfur concentration on instrument performance was examined. Values of $\delta^{34}S$ remained within the range of method uncertainty for peaks with magnitudes of 1 - 20 V, corresponding to a total sulfur amount of 6 - 130 nmol.

RESULTS & DISCUSSION

Interferences of Monothioarsenate during Standard IRMS Analysis

Sample Preparation: Precipitation with $ZnAc_2$

Precipitation of monothioarsenate with $ZnAc_2$ was investigated to assess the potential of thioarsenate co-determination with sulfide during standard precipitation for IRMS analysis. Monothioarsenate precipitation varied extensively with 1.2 - 90 % of the initial monothioarsenate found as a solid after $ZnAc_2$ addition (Fig. 1). Surprisingly, quantification of the solid precipitate and speciation analysis of the remaining solution left a large part of the initial monothioarsenate unaccounted for, especially for samples with 100-fold $ZnAc_2$ excess. Therefore, the filtered solution remaining after precipitation was treated with H_2O_2/HNO_3 and analyzed for total arsenic. This revealed an additional compound < 200 nm, which was not detected as a dissolved, charged species by IC-ICP-MS, thus termed colloid during further discussions.

At a $ZnAc_2$/monothioarsenate ratio of 1:1 only 12 % precipitation was found after 10 min, leaving the predominant share of monothioarsenate as a dissolved species in solution. In contrast, at 10-fold $ZnAc_2$ excess the precipitation efficiency increased substantially to 48 %, and even further to 82 % after 2 h, while the colloidal share decreased over time. Concurrent sulfur analysis of precipitate and solution verified the predominance of monothioarsenate precipitation at 10-fold $ZnAc_2$ excess (71 % at 2h). However, at 100-fold excess the majority of monothioarsenate did not precipitate and the colloidal share reached a maximum of 84

% after 24 h. These colloids are most likely Zn-As-S complexes as previously discussed by Tossell (2000b). According to that study, such metal-thiometalloid complexes can form without breaking any of the As-S bonds, which supports the hypothesis of the formation of a Zn-monothioarsenate complex.

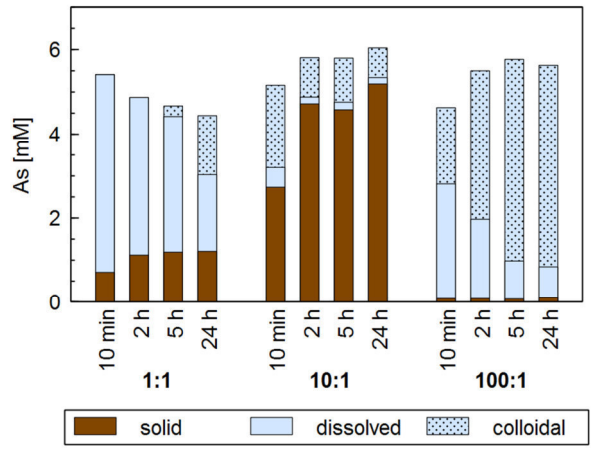

Figure 1. Monothioarsenate (monoTA) precipitation at $ZnAc_2$/monoTA ratios of 1:1 (pH 10.3), 10:1 (pH 10.0), and 100:1 (pH 10.3); colloidal arsenic constitutes the difference between the total arsenic determined by ICP-MS in the filtered, H_2O_2/HNO_3-treated solution and the sum of dissolved, charged species determined by IC-ICP-MS.

The results from this precipitation experiment have two major implications regarding analysis of sulfidic waters that contain arsenic. To fix sulfide for isotope analysis an excess of $ZnAc_2$ is routinely applied, but specific values can vary substantially from 10-fold to even 80-fold $ZnAc_2$ excess (Brabec et al., 2012; Habicht et al., 1998; Kamyshny et al., 2011; Knossow et al., 2015). Depending on particular concentrations of thioarsenates and other acid volatile species, the addition of $ZnAc_2$ will lead to partial co-determination of thioarsenates with sulfide during IRMS analysis. Furthermore, potential co-determination of thioarsenates should be equally considered for photometric sulfide analysis. Applying the methylene blue method, which is also based on sulfide precipitation with $ZnAc_2$, several studies have reported observations indicative of thioarsenate co-determination with sulfide (Planer-Friedrich et al., 2015; Planer-Friedrich et al., 2007; Suess et al., 2011). Taking into account the current findings of partial monothioarsenate precipitation upon $ZnAc_2$ addition, thioarsenates need to be considered as a source of potential over-estimation of sulfide concentrations.

IRMS Analysis of Monothioarsenate Oxidation

These results raise the question to which extent monothioarsenate co-determination with sulfide may affect isotopic values determined for sulfide during oxidation. Using increasing concentrations of H_2O_2, monothioarsenate was oxidized to sulfate and arsenate via thiosulfate and minor amounts of arsenite (Fig. 2a, SI-2). To investigate the maximum effect of monothioarsenate co-precipitation with sulfide, $ZnAc_2$ was added at 10-fold excess. Similar to figure 1, this led to partial precipitation of monothioarsenate (71 - 86 %), as well as formation of the previously discussed colloid (Fig. 2b). Thiosulfate and sulfate did not react with $ZnAc_2$. Hence, sulfur only precipitated in samples containing monothioarsenate.

In contrast, arsenic analysis showed continuous precipitation even after all monothioarsenate had already been oxidized to arsenate (SI-2). It is well documented that Zn and arsenate form insoluble precipitates (Nordstrom et al., 2014). In fact, co-precipitation of arsenate with $ZnAc_2$ also supported monothioarsenate precipitation. The ongoing oxidation caused the $ZnAc_2$/monothioarsenate ratio to increase from initially 10 to 26. This could have led to an extensive loss of precipitation efficiency, considering that monothioarsenate precipitation was found to decrease substantially between 10-fold and 100-fold $ZnAc_2$ excess (Fig. 1). However, increasing formation of arsenate, consequent precipitation with Zn and thus removal of Zn from solution restrained the $ZnAc_2$ excess at a value of 8.8 - 10.4 ensuring continuous monothioarsenate precipitation.

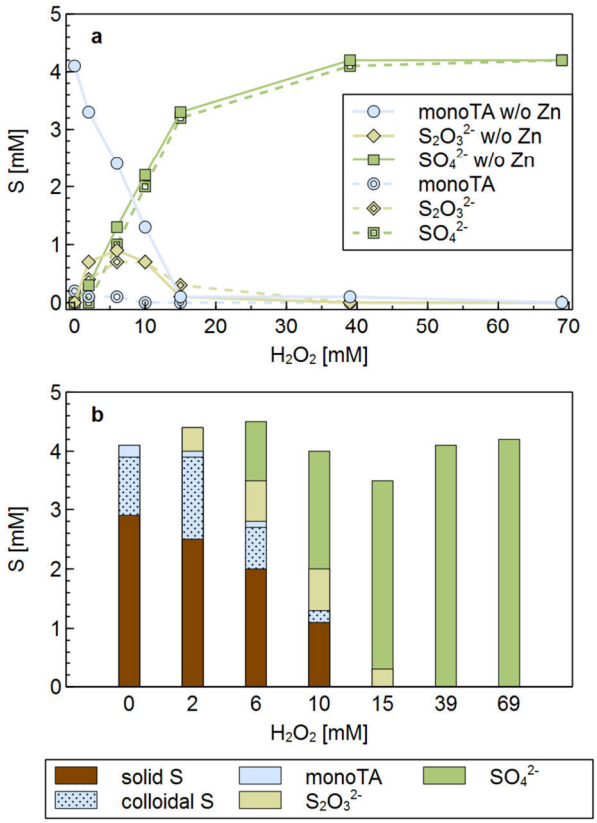

Figure 2. Oxidation of monothioarsenate (monoTA) with H_2O_2, followed by 10-fold excess $ZnAc_2$ addition: (a) sulfur speciation before and after $ZnAc_2$ addition; (b) distribution of precipitated and colloidal sulfur (difference between the total sulfur determined by ICP-MS in the filtered, H_2O_2/HNO_3-treated solution and the sum of dissolved, charged species determined by IC-ICP-MS) in comparison with dissolved sulfur species after $ZnAc_2$ addition.

IRMS analysis of the precipitates showed a clear increase in $\delta^{34}S$-enrichment for monothioarsenate from 4.2 to 8.0 ‰

as oxidation progressed (Fig. 3a). Based on the standard precipitation procedure, these values would be wrongfully assigned to sulfide. At the same time, $\delta^{34}S$ values of produced sulfate decreased from initially 2.1 ‰ to a minimum of -1.9 ‰ at 15 mM H_2O_2, after which $\delta^{34}S$ increased again to a final value of -0.1 ‰. Direct analysis of the solid monothioarsenate standard delivered a $\delta^{34}S$ value of 0.7 ‰. Firstly, this value is slightly higher than the final $\delta^{34}S$ of sulfate, but more importantly it stands in strong contradiction to the dissolved monothioarsenate standard at 0 mM H_2O_2, which is much heavier. In addition, assessment of the sulfur mass balance for this experiment revealed major discrepancies indicated by values ranging between 4.2 ‰ and -1.6 ‰ (Fig. 3b).

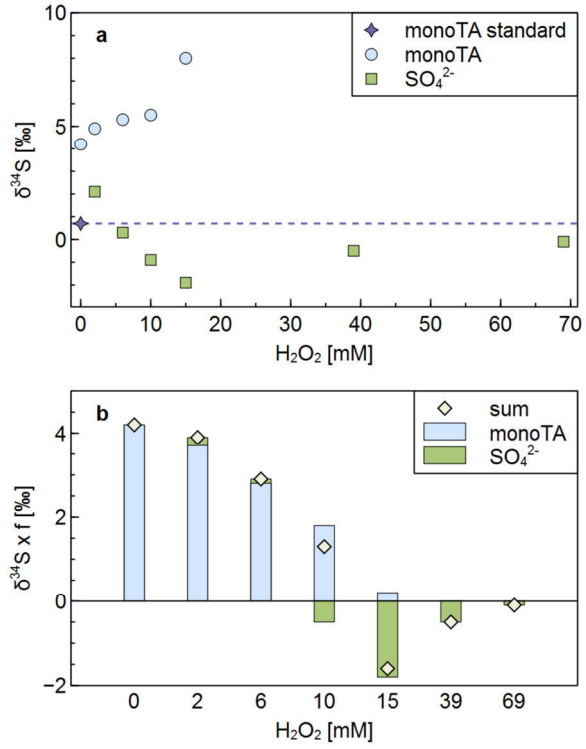

Figure 3. Standard IRMS analysis of sulfur isotope fractionation during oxidation of monothioarsenate (monoTA) with H_2O_2: (a) isotopic composition determined from precipitates (dashed line indicates $\delta^{34}S$ value of the solid monoTA standard); (b) sulfur isotope mass balance calculated from equation (2).

Most likely these findings are the result of incomplete precipitation of monothioarsenate and formation of Zn-monothioarsenate complexes. While it can be expected that fractionation occurs during these processes, it is unclear how the dissolved monothioarsenate remaining in solution, as well as the Zn-monothioarsenate complex, are affected by further sample treatment for IRMS analysis. The first $\delta^{34}S$ value of sulfate surpassing the solid monothioarsenate standard seems to indicate at least partial co-determination of heavy monothioarsenate in the sulfate pool. The standard acidification prior to $BaCl_2$ addition is likely to destroy the Zn-monothioarsenate complex as seen in solutions treated with H_2O_2/HNO_3. Acidification of the complex may release sulfide, elemental sulfur, or even intact monothioarsenate. Depending on the released species this could lead to degassing of sulfide, oxidation and co-determination with sulfate, or loss of monothioarsenate during further sample preparation, consequently impairing precise $\delta^{34}S$ analysis.

To complete the assessment of the mass balance, formation of elemental sulfur from monothioarsenate oxidation also needs to be considered. Initial sample solutions with H_2O_2 > 15 mM were slightly milky, suggesting formation of elemental sulfur (Chen and Morris, 1972). HPLC analysis verified the occurrence of 78 µM elemental sulfur at 69 mM H_2O_2. Even though this corresponds to only 1.6 % of initial monothioarsenate, elemental sulfur might be important for the mass balance, yet goes undetected with this precipitation procedure.

As evident from all these results, the standard method for species-selective isotope analysis based on precipitation of sulfide and sulfate suffers from a number of limitations. The potential of thioarsenate co-determination with sulfide or even sulfate needs to be considered for sulfur isotope studies in arsenic-rich environments. The results presented here could help interpret anomalies observed in isotope fractionation patterns as e.g. reported for arsenic-rich ground waters (Xie et al., 2009). Apart from thioarsenates, the co-determination of other reduced sulfur species, like polysulfides, together with sulfide can also be of major concern when using the standard precipitation procedure. Furthermore, important species like thiosulfate or elemental sulfur might be completely disregarded. Kusakabe et al. (2000) suggested a more elaborate precipitation procedure that is capable of separating sulfide, elemental sulfur, thiosulfate, polythionates, sulfite, and sulfate. However, their proposed procedure is accordingly complex, time-consuming and requires large sample volumes or concentrations to gain sufficient precipitate for IRMS analysis of each species. Moreover, in the case of mono-, di-, tri-, and tetrathioarsenate, species-selective precipitation is not possible due to their chemical similarities, rendering the precipitation method unsuitable for investigating sulfur isotopes in thioarsenates.

Application of IC-MC-ICP-MS to study fractionation of thioarsenates

Fractionation during Monothioarsenate Oxidation

In order to overcome the aforementioned limitations of the standard precipitation, a new method based on separation of sulfur species by ion chromatography and online isotope analysis by MC-ICP-MS was employed. For comparison with results from standard IRMS analysis (Fig. 3), the monothioarsenate oxidation experiment was repeated (speciation data in SI-3). Using IC-MC-ICP-MS, monothioarsenate was found to become increasingly enriched in ^{34}S from 1.0 to 4.9 ‰ as oxidation progressed (Fig. 4a). The corresponding sulfate showed a $\delta^{34}S$-depletion up to -1.5 ‰ at 1 mM H_2O_2, followed by an increase to a final $\delta^{34}S$ value of 0.2 ‰, which is in agreement with the solid monothioarsenate standard (0.7 ‰) considering analytical

uncertainty. Minor amounts of thiosulfate were detected at 0.2 and 0.3 mM H_2O_2, but were too low for reliable sulfur isotope analysis and thus excluded. Overall, the oxidation resulted in a maximum fractionation between monothioarsenate and sulfate of -6.1 ‰.

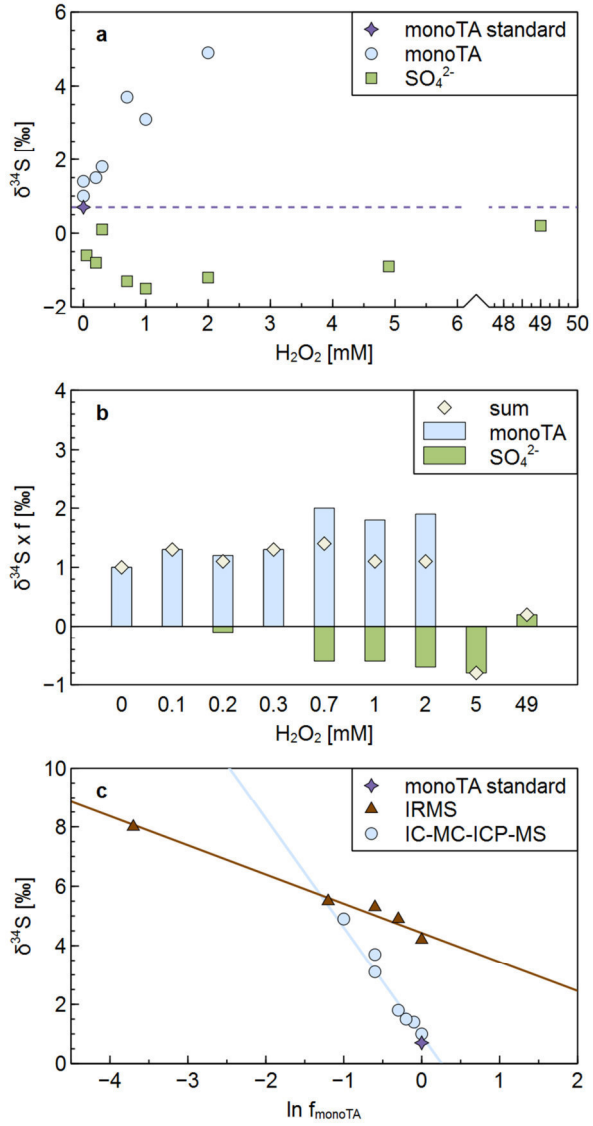

Figure 4. IC-MC-ICP-MS analysis of sulfur isotope fractionation during oxidation of monothioarsenate (monoTA) with H_2O_2: (a) isotopic composition determined by IC-MC-ICP-MS (note broken axis at 6 mM H_2O_2; dashed line indicates $\delta^{34}S$ value of the solid monoTA standard); (b) sulfur isotope mass balance calculated from equation (2); (c) comparison of isotopic composition of monoTA determined by IRMS and IC-MC-ICP-MS as a function of the molar amount f of monoTA remaining in solution (monoTA standard marks the $\delta^{34}S$ value of the solid monothioarsenate standard).

Mass balance calculations show generally consistent values of 1.0 - 1.4 ‰ (Fig. 4b). Only the two samples with the highest H_2O_2 concentrations presented a discernible exception, which is most likely a result of elemental sulfur formation as previously discussed. While the chromatographic method presented here allows detection of the anionic sulfur species sulfide, thiosulfate, sulfate, mono-, di-, tri-, and tetrathioarsenate based on alkaline elution from an AS16 column, it is not possible to determine elemental sulfur or polysulfides with this IC-MC-ICP-MS method. This would require completely different method development outside the focus of this work, considering the need for reverse phase chromatography as addressed by Amrani et al. (2006).

The results obtained from IC-MC-ICP-MS analysis show some significant differences compared to results from standard IRMS analysis (Fig 4c). Initial enrichment of monothioarsenate was much higher in samples analyzed with IRMS (4.2 ‰) than in samples analyzed with IC-MC-ICP-MS (1.0 ‰). Moreover, the $\delta^{34}S$ value of the solid monothioarsenate standard plots well along the line of the IC-MC-ICP-MS results, whereas IRMS results are clearly too high. Overall, this seems to indicate additional artifact fractionation within samples analyzed by the standard method. Combining all findings, it can be assumed that sample preparation, including incomplete monothioarsenate precipitation and colloid formation, caused this over-estimation of ^{34}S-enrichment.

Fractionation during Tetrathioarsenate Oxidation

Development of the IC-MC-ICP-MS method permitted to study for the first time the isotope fractionation of the reduced sulfur bound in thioarsenates. Sulfur isotope fractionation was monitored during stepwise oxidation of tetrathioarsenate to tri-, di-, and monothioarsenate, producing sulfide, thiosulfate, and finally sulfate (Fig. 5a). Negligible to no fractionation was found for the transformation from tetra- via tri- to dithioarsenate, all yielding similar $\delta^{34}S$ values of 16.8 - 18.4 ‰ (Fig. 5b). Monothioarsenate on the other hand only showed a comparable $\delta^{34}S$ value of 17.0 ‰ at 0.05 mM H_2O_2 where it was first observed. Starting at 0.1 mM H_2O_2 however, ^{34}S-enrichment of monothioarsenate rapidly increased to 25.9 ‰. This marked also the point after which $\delta^{34}S$ of monothioarsenate was almost identical to that of sulfide. As oxidation progressed, monothioarsenate became heavier up to a final $\delta^{34}S$ value of 29.8 ‰, while enrichment of sulfate increased from initially 7.2 to 14.7 ‰ at 0.5 mM H_2O_2.

By adding an excess 2.5 mM H_2O_2 complete oxidation to sulfate was achieved, yielding a final value of 16.2 ‰, which corresponds well with the starting value of 16.8 ‰ for tetrathioarsenate (data not shown). Results from mass balance calculations, ranging from 16.2 to 17.9 ‰, were also in good agreement with the starting value, considering that $\delta^{34}S$ values of up to 7 different sulfur species were combined (Fig. 5c). Furthermore, the mass balance did not show any major deficit as observed during the monothioarsenate experiment due to elemental sulfur formation (Fig. 4b). Elemental sulfur is likely to form during oxidation of tetrathioarsenate as well. However, in combination with 7 other sulfur species covering a broad range of $\delta^{34}S$ values it might not affect the mass balance as severely as for monothioarsenate.

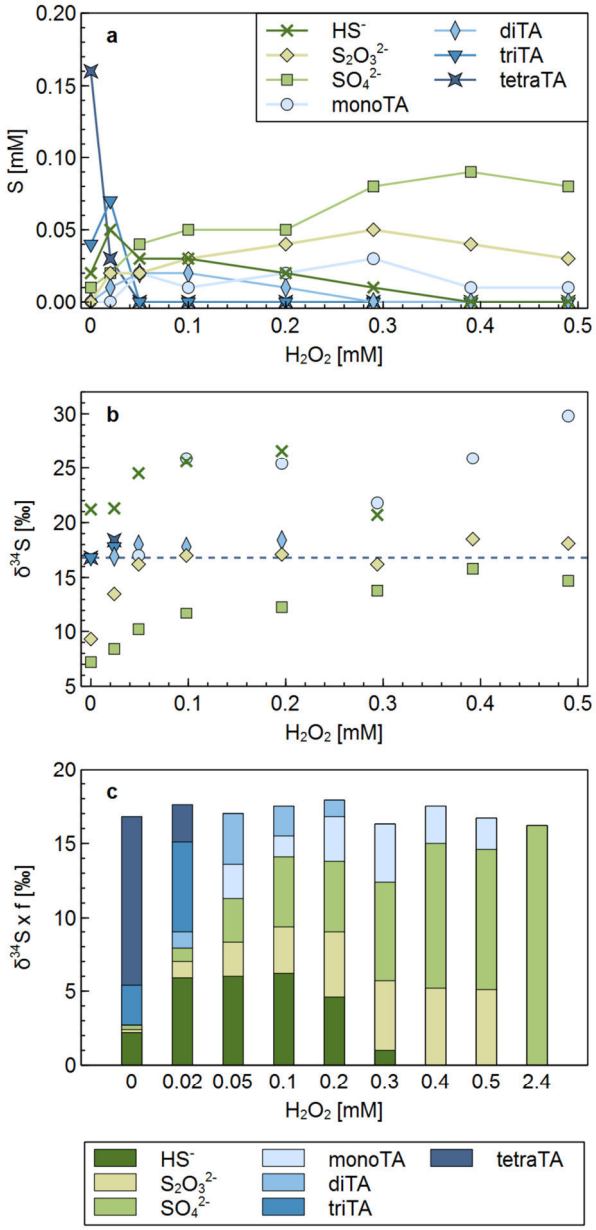

Figure 5. IC-MC-ICP-MS analysis of sulfur isotope fractionation during oxidation of tetrathioarsenate (tetraTA) with H_2O_2 (trithioarsenate: triTA, dithioarsenate: diTA, monothioarsenate: monoTA): (a) sulfur speciation (b) isotopic composition determined by IC-MC-ICP-MS (dashed line indicates starting $\delta^{34}S$ value of tetraTA); (c) sulfur isotope mass balance calculated from equation (2).

These results seem to indicate a lack of sulfur isotope fractionation during oxidation of tetrathioarsenate to the less thiolated tri- and dithioarsenate. However, as sulfide is being oxidized it becomes up to 8.2 ‰ heavier than the tetra-, tri-, and dithioarsenate from which it originated. This enriched sulfide could represent the source of the high $\delta^{34}S$ values of monothioarsenate if the potential of intermolecular isotope exchange between free sulfide and arsenic-bound sulfur is taken into account. For the intermolecular exchange between sulfide and sulfane sulfur of thiosulfate, the rapid formation of polysulfides has been shown to play a key role (Chu et al., 2004). In combination with previous findings on monothioarsenate formation and transformation (Planer-Friedrich et al., 2015), we thus propose the following reactions to explain isotope exchange between free sulfide in solution and arsenic-bound sulfur in monothioarsenate:

$$[HAs^{V}S^{-II}O_3]^{2-} + H_2O \rightarrow [H_2As^{III}O_3]^{-} + S^0 + [OH]^{-} \quad (3)$$

$$S^0 + [H{*}S^{-II}]^{-} \rightarrow {*}S^0 + [HS^{-II}]^{-} \quad (4)$$

$$[H_2As^{III}O_3]^{-} + {*}S^0 + [OH]^{-} \rightarrow [HAs^{V}{*}S^{-II}O_3]^{2-} + H_2O \quad (5)$$

where the *S denotes the heavy isotope ^{34}S. The exchange (Eq. 4) may proceed via formation of zerovalent sulfur either in the form of elemental sulfur or more likely polysulfides. The onset of intermolecular exchange starting at 0.1 mM H_2O_2 indicates that a certain excess of H_2O_2 is required for the formation of polysulfides facilitating the exchange according to equation (4). As a result, the intermolecular exchange led to almost identical $\delta^{34}S$ values for sulfide and monothioarsenate. The pronounced decrease in enrichment of both sulfide and monothioarsenate observed at 0.3 mM H_2O_2 lends further support to this hypothesis. While monothioarsenate formation reached the maximum, sulfide concentrations decreased (Fig. 5a), leading to a relatively smaller pool of heavy sulfide available for intermolecular exchange. This caused $\delta^{34}S$ of monothioarsenate to shift to a value more similar to the initial tetra-, tri-, and dithioarsenate.

Hence, both intermolecular exchange at low H_2O_2 and fractionation associated with oxidation at high H_2O_2 concentrations contributed to the overall fractionation of 15.0 ‰ between monothioarsenate and sulfate. Using the initial $\delta^{34}S$ value of 16.8 ‰ as a reference point, enrichment for monothioarsenate of 4.9 - 9.1 ‰ can be assigned to intermolecular exchange in samples containing ≤ 0.3 mM H_2O_2, assuming negligible monothioarsenate oxidation. Calculation of enrichment caused directly by monothioarsenate oxidation then yields 5.9 - 10.1 ‰, which is comparable to the extent of fractionation found in the monothioarsenate experiment (-6.1 ‰, Fig. 4).

In contrast, no signs of similar isotope fractionation for di-, tri-, or tetrathioarsenate were observed, which adds some valuable information to the ongoing challenge of identifying thioarsenate transformation pathways. The obtained isotopic data lends support to a previous hypothesis of the brief occurrence of trivalent thioarsenites as intermediate species and their rapid oxidation to pentavalent thioarsenates (Planer-Friedrich et al., 2015). The fast oxidation of the intermediate thioarsenites exceeds any potential intermolecular exchange with heavy sulfide and thus prevents formation of ^{34}S-enriched tetra-, tri-, and dithioarsenate. In the case of monothioarsenate however, oxidation of the intermediate arsenite is sufficiently slow, allowing intermolecular exchange with sulfide according to equations (3) - (5). Moreover, the lack of fractionation found during the transformation from tetra- to monothioarsenate further elucidates the dethiolation process of thioarsenates. The obtained isotopic data supports the hypothesis that decomposition of tetra-, tri-, and dithioarsenate is merely a

result of decreasing SH/OH ratios in solution and the consequent release of sulfidic sulfur is not associated with any oxidation change (Planer-Friedrich et al., 2015). In comparison, dethiolation of the much more stable monothioarsenate only proceeds via direct oxidation of the arsenic-bound sulfur to thiosulfate and sulfate. This unique characteristic of monothioarsenate is reflected in the considerable fractionation observed in contrast to the higher thiolated species.

Finally, the obtained data delivered new aspects for defining the limits of fractionation associated with abiotic sulfide oxidation. Almost exclusively based on a single study, it is generally assumed that abiotic sulfide oxidation generates small fractionation between sulfide and sulfate of maximum -5.1 ‰ (Fry et al., 1988). In agreement with that study, abiotic oxidation of sulfidic sulfur in monothioarsenate was found to produce a similar fractionation of -6.1 ‰. However, in the presence of excess sulfide, in this case originating from higher thiolated species, monothioarsenate can be additionally enriched in ^{34}S due to the discussed intermolecular exchange. Considering that fractionation associated with abiotic thioarsenate oxidation may even exceed fractionation during sulfide oxidation, it is crucial that thioarsenates are included in the analysis and interpretation of sulfur isotopes from sulfidic environments.

CONCLUSIONS

This work demonstrates the limitations associated with standard sulfur isotope analysis by IRMS based on standard species-selective precipitation procedures. In addition to general disadvantages like the requirement for large sample volumes and time-consuming sample preparation, the method was found specifically unsuitable for investigating sulfur isotopes in solutions containing thioarsenates. Monothioarsenate was found to partially precipitate when applying the standard procedure for sulfide isotope analysis by $ZnAc_2$ addition. Moreover, depending on $ZnAc_2$ excess, the formation of a Zn-monothioarsenate complex was hypothesized, further impeding precise $\delta^{34}S$ analysis. These limitations were successfully avoided by employing a new method based on species separation via ion chromatography followed by online MC-ICP-MS analysis. This approach is much faster, requires less sample volume, and allows direct isotope analysis of up to 7 different sulfur species without any sample preparation.

We successfully applied the IC-MC-ICP-MS method to investigate fractionation during thioarsenate oxidation, and present here, to our knowledge, the first study of the sulfur isotope chemistry of thio-metalloid species. Oxidation of tetra-, tri-, and dithioarsenate generated no isotope fractionation. However, monothioarsenate was enriched by up to 6.1 ‰ in comparison to the produced sulfate. An additional enrichment of up to 9.1 ‰ was found for monothioarsenate as the result of intermolecular isotope exchange with sulfide.

The IC-MC-ICP-MS results provide a first constraint on the extent to which thioarsenates can contribute to sulfur isotope fractionation in sulfidic environments. Furthermore, the obtained data delivered new aspects for the identification of previously hypothesized thioarsenate transformation processes, including abiotic dethiolation and oxidation. Species-selective IC-MC-ICP-MS analysis thus proved to be a powerful tool for the investigation of sulfur redox chemistry and should be considered for other open research questions, where speciation analysis alone does not deliver conclusive results. A future field of application is e.g. the investigation of microbially catalyzed transformations of thioarsenates. In combination with the obtained results on abiotic oxidation, IC-MC-ICP-MS analysis could assist in elucidating the potential of thiometalloids serving as substrate for microbial growth, and thus further our understanding of both abiotic and biotic processes in the sulfur cycle.

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SUPPORTING INFORMATION

Sulfur isotope analysis by IC-MC-ICP-MS provides insight into fractionation of thioarsenates during abiotic oxidation

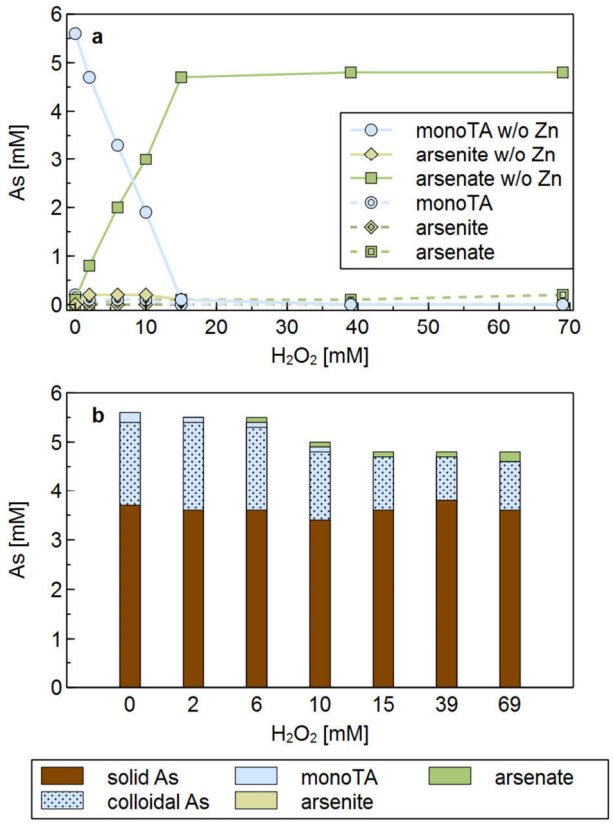
Maria K. Ullrich, Faina Gelman, Yevgeni Zakon, Ludwik Halicz, Kay Knoeller, Britta Planer-Friedrich

Number of pages: 4

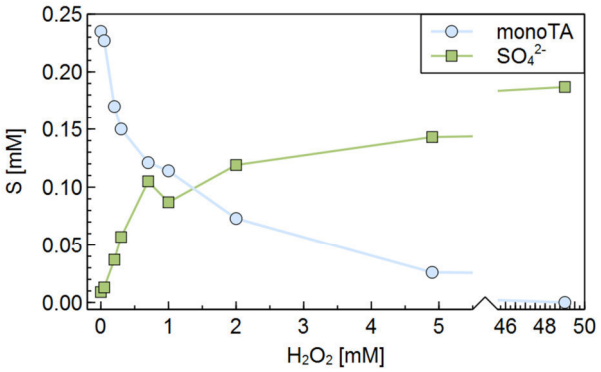
Content: Page

MC-ICP-MS parameters	
RF power	1300 W
coolant flow	13 L · min^{-1}
auxiliary flow	1.8 L · min^{-1}
nebulizer gas flow	35 psi
interface cones	Ni
Measurement parameters	
resolution mode (ΔM/M)	~ 6000
cup configuration	L3: m/z 32 H6: m/z 34
integration time	0.5 s
Apex-Q desolvator parameters	
heater	140 °C
chiller	2 °C

SI-1. Instrumental parameters used for isotope analysis of sulfur species by IC-MC-ICP-MS.



SI-2. Oxidation of monothioarsenate (monoTA) with H_2O_2, followed by 10-fold excess $ZnAc_2$ addition: (a) arsenic speciation before and after $ZnAc_2$ addition; (b) distribution of precipitated and colloidal arsenic (difference between total arsenic determined by ICP-MS in digest of filtered solution and sum of species detected by IC-ICP-MS) in comparison with dissolved arsenic species after $ZnAc_2$ addition.



SI-3. Oxidation of monothioarsenate (monoTA) with H_2O_2 for IC-MC-ICP-MS analysis: sulfur speciation (note broken axis at 5.5 mM H_2O_2).

STUDY 3

submitted to Applied Geochemistry

Ullrich, M.K., Gelman, F., Zakon, Y., Halicz, L., Knöller, K.,Planer-Friedrich, B.

Differentiation of abiotic and biotic monothioarsenate transformation by analysis of sulfur isotopes using IC-MC-ICP-MS

Differentiation of abiotic and biotic monothioarsenate transformation by analysis of sulfur isotopes using IC-MC-ICP-MS

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ABSTRACT: Recent analytical advancements have allowed identifying thiometalloid species, such as thioarsenates ($[HAs^{V}S^{-II}_{n}O_{4-n}]^{2-}$, n = 1 - 4), as important sink of reduced sulfur in our environment. It has been proposed that arsenic-bound sulfur in thioarsenates can serve as electron donor for chemolithotrophic bacteria. Here, we employ species-selective isotope analysis to pursue this hypothesis and differentiate abiotic and biotic processes. Incubation experiments with monothioarsenate as sole sulfur source and native filamentous mats of *Thermocrinis ruber* were conducted at pH 9.1 and 80 °C in the drainage channel of a geothermal spring in Yellowstone National Park. As monothioarsenate was successively transformed to sulfate, it became depleted in ^{34}S by up to 1.5 ‰ compared to the initial $\delta^{34}S$ value, indicating an inverse isotope effect. Maximum fractionation between monothioarsenate and sulfate of +1.2 ‰ and +4.4 ‰ was found for biotic and abiotic incubation, respectively. We propose that monothioarsenate is first disproportionated abiotically to elemental sulfur and arsenite. In a second step, elemental sulfur is then utilized by *T. ruber*, eventually yielding ^{34}S-enriched sulfate. Thus, thioarsenates cannot serve as microbial substrate directly. However, given their fast abiotic transformation to elemental sulfur or sulfide, thioarsenates present a substantial source of reduced sulfur for chemolithotrophic bacteria.

KEYWORDS: microbial sulfide oxidation; zerovalent sulfur; thio-arsenic species; normal isotope effect; sulfur cycling

1. INTRODUCTION

Analysis of isotope fractionation has become an indispensable tool for the investigation of the sulfur cycle in our environment. Numerous studies have demonstrated how isotopic analysis can assist in elucidating transformation pathways of sulfur in both ancient (Canfield and Teske, 1996; Farquhar et al., 2000; Johnston et al., 2005; Wacey et al., 2010) and modern environments (Jorgensen and Bak, 1991; Kamyshny et al., 2011; Knossow et al., 2015; Pellerin et al., 2015). Observed fractionation of sulfur isotopes mainly arises from redox processes between the end-members sulfide and sulfate. Reduction of sulfate, primarily driven by microorganisms, is known to generate extensive fractionation of > -70 ‰ for sulfide (Rudnicki et al., 2001; Wortmann et al., 2001). In contrast, both abiotic and biotic sulfide oxidation is typically accompanied by much smaller fractionation of 0 - 5 ‰ (Fry et al., 1984; Fry et al., 1988b; Habicht et al., 1998). However, various studies have demonstrated that repeated redox cycling involving intermediate sulfur species can produce fractionation higher than customarily associated with the oxidative branch of the sulfur cycle (Canfield and Thamdrup, 1994; Jorgensen, 1990).

Consequently, knowledge on fractionation of intermediate species has gained attention as a crucial element for the identification of sulfur transformation processes. Several intermediate species are known to form along the oxidative pathway, with elemental sulfur, thiosulfate, and sulfite presenting the most commonly found species (Chen and Morris, 1972; Zhang and Millero, 1993). Their formation from sulfide and further transformation to sulfate is catalyzed considerably by microorganisms. Both phototrophic and chemolithotrophic bacteria use reduced sulfur species as metabolic substrate, and thus exert significant control on

isotope fractionation of intermediate sulfur species. For the anaerobic oxidation of sulfide to elemental sulfur, Fry et al. (1984) reported a ^{34}S-enrichment factor ε of +2.5 ‰, indicative of an inverse isotope effect. Further anaerobic oxidation of elemental sulfur to sulfate was found to produce a small normal isotope effect of -1.7 ‰ (Fry et al., 1988a). In addition, disproportionation of elemental sulfur to sulfide and sulfate has also been identified as a favorable metabolic process, considering that no other electron acceptor is required (Poser et al., 2013; Thamdrup et al., 1993). Sulfide produced from elemental sulfur disproportionation is generally depleted in ^{34}S by -0.9 to -13 ‰, whereas sulfate is enriched by +3.6 to +18.4 ‰ (Böttcher et al., 2001; Canfield and Thamdrup, 1994; Cypionka et al., 1998; Poser et al., 2016). Similar effects have been observed for the disproportionation of sulfite and thiosulfate, where the simultaneous reduction and oxidation yield sulfide depleted and sulfate enriched in ^{34}S (Habicht et al., 1998).

All of these intermediate sulfur species have been studied intensively over the past decades using routine methods. However, recent analytical advances revealed that another group of previously undetected species contributes to the overall sulfur budget. Particularly in suboxic to anoxic environments, sulfur readily combines with metal(loid)s like Cd, Zn, Sb, Mo, Au, or As to form thiometalloid species (Lohmayer et al., 2015; Planer-Friedrich et al., 2007; Seward, 1973; Tossell and Vaughan, 1993; Ullrich et al., 2013). Due to the environmental impact, the formation of thioarsenates ($[HAs^{V}S^{-II}_{n}O_{4-n}]^{2-}$, n = 1 - 4) has gained particular attention lately. These arsenic-sulfur complexes were first detected in geothermal waters (Planer-Friedrich et al., 2007), but have since been found in a variety of environments, including haloalkaline, anoxic lake water (Wallschläger and Stadey, 2007), landfill environments (Zhang et al., 2014), marina sediments (Mamindy-Pajany et al., 2013), and naturally anoxic and contaminated ground waters (Pi et al., 2016; Stucker et al., 2014; Wallschläger and Stadey, 2007).

Thioarsenates contain sulfur in oxidation state -II, and thus can act as potential electron donors that need to be considered when investigating the oxidative branch of the sulfur cycle. Next to other electron donors such as sulfide or thiosulfate, sulfur in thioarsenates can contribute up to 40 % to the sum of all reduced sulfur species (Planer-Friedrich et al., 2009). Based on the widespread occurrence of thioarsenates in anoxic environments, several studies examined whether the arsenic-bound sulfur can be utilized for microbial growth (Edwardson et al., 2014; Haertig et al., 2014; Haertig and Planer-Friedrich, 2012; Planer-Friedrich et al., 2015). Investigating the oxidation of monothioarsenate by the hyperthermophile *Thermocrinis ruber*, Haertig et al. (2014) proposed that arsenic-bound sulfur can be directly used as a substrate for chemolithtrophy similarly to thiosulfate or elemental sulfur. However, it was also discussed that abiotic processes may have contributed to the observed speciation changes as well. This presents a typical case, in which the application of species-selective isotope analysis can provide additional information for differentiating biotic from potential abiotic processes.

To further pursue the hypothesis of direct microbial thioarsenate utilization, we monitored sulfur isotope fractionation during monothioarsenate transformation in the presence of *T. ruber*. This chemolithotrophic bacterium was first isolated from the drainage channel of a geothermal feature called Octopus Spring at Yellowstone National Park, where it grows in the form of filamentous microbial mats (Huber et al., 1998). Neutral to alkaline, low salinity conditions and temperatures of 82 - 88 °C facilitate optimum growth of *T. ruber*. Similar to what has been shown for other members of *Aquificales* (Caldwell et al., 2010; Eder and Huber, 2002), *T. ruber* can grow on reduced sulfur species such as elemental sulfur and thiosulfate (Huber et al., 1998).

Using native filamentous mats of *T. ruber* from a geothermal feature in Yellowstone National Park, we investigated the transformation of arsenic-bound sulfur in monothioarsenate within the natural environment of the drainage channel. The aim of the current study was to (1) monitor speciation changes associated with monothioarsenate transformation in the presence and absence of *T. ruber*, (2) use the additional information provided by isotope analysis to differentiate abiotic and biotic processes, and (3) combine these results with previous findings to assess the potential of direct microbial utilization of arsenic-bound sulfur in thioarsenates.

2. MATERIALS & METHODS

2.1. Incubation experiment

To investigate isotope fractionation accompanying abiotic and chemolithotrophic monothioarsenate transformation, incubation experiments were performed on-site at Yellowstone National Park. A geothermal feature called Conch Spring, located on the east bank of the Firehole River in the Lower Geyser Basin, was selected based on known occurrence of *T. ruber* (Haertig and Planer-Friedrich, 2012). The feature comprises the alkaline, sulfidic spring itself as well as an approximately 35 m long drainage channel, in which *T. ruber* grows in the form of brownish-pink filamentous mats starting at approximately 7 m. A detailed description of *T. ruber* regarding phylogeny, morphology, and metabolism can be found in Huber et al. (1998). To determine sulfur species occurring in the geothermal fluid of Conch Spring, 2 mL of filtered sample (0.2 µm, cellulose-acetate, Membrex) were taken from the drainage channel at 0, 4, 7, 11, 16, 22, 29, and 35 m and immediately flash-frozen on dry ice. Results for sulfur speciation changes along the drainage channel are presented in SI-1.

Abiotic and biotic incubation was carried out in LDPE bottles containing 150 mL of 200 µM monothioarsenate solution ($Na_3AsO_3S \cdot 7\ H_2O$, synthesized according to Suess et al. (2009)). Monothioarsenate was chosen over di-, tri-, and tetrathioarsenate since it is much less susceptible to oxidation by atmospheric oxygen, and thus biotic transformation can be expected to clearly exceed any abiotic

oxidation. In order to avoid potential interferences from other naturally occurring sulfur species in the geothermal fluid, synthetic Octopus Spring (OS) water described by Huber et al. (1998) was used as medium instead of Conch Spring water. The composition of the OS medium was slightly modified by excluding salts that contain sulfate in order to produce a matrix free of any additional sources of sulfur (SI-2).

For biotic incubation, brownish-pink filamentous mats of *T. ruber* were collected aseptically from the drainage channel of Conch Spring at approximately 9 m. Bottles for both abiotic and biotic treatment were incubated in the drainage channel at 8 m and approximately 80 °C for 4 h. A sampling port was created by inserting a needle through the lid of the bottle, allowing limited exchange between the headspace and ambient air. Incubation bottles were sampled after 0, 5, 10, 15, 30, 60, and 240 min for sulfur and arsenic speciation as well as sulfur isotope analysis. All samples were filtered (0.2 µm, cellulose-acetate, Membrex) and immediately flash-frozen on dry ice.

In addition to the monothioarsenate incubation at Conch Spring, a laboratory test was conducted to investigate the potential of abiotic arsenite oxidation under the experimental conditions. Reconstructing the experimental field setup, arsenite was incubated in OS medium (SI-2) at 80 °C for 240 min. Different concentrations of 1.3, 13, and 130 µM arsenite were tested and samples for arsenic speciation analysis taken after 0, 5, and 240 min. Additionally, arsenite was also spiked into OS medium at room temperature to examine potential oxidation independent of elevated temperature. Results from this test are presented in the Supporting Information SI-3.

2.2. Speciation and isotope analysis

Immediately before analysis, frozen samples for species and isotope determination were thawed under an anoxic atmosphere. Analysis of sulfur and arsenic species was performed using IC-ICP-MS based on a previously established method (Planer-Friedrich et al., 2007). Briefly, anion-exchange chromatography (IonPac AG/AS16, ICS 3000, Dionex) together with gradient elution of 20 - 100 mM NaOH was used to separate sulfur and arsenic species, followed by online detection via ICP-MS (XSeries2, Thermo Scientific). Sulfide concentrations were determined photometrically in triplicate on a microplate reader employing the methylene-blue method (Infinite 200 Pro, Tecan).

Species-selective sulfur isotope analysis of monothioarsenate and sulfate was performed using IC-MC-ICP-MS (Ullrich et al., 2017, submitted). Based on speciation results obtained from IC-ICP-MS analysis, specific samples were selected for IC-MC-ICP-MS analysis, namely samples from 0, 5, 10, 30, 60, and 240 min of biotic incubation and 0, 60, and 240 min of abiotic incubation. Sulfur species were separated prior to isotope analysis by anion-exchange chromatography (IonPac AG/AS16, ICS 2100, Dionex) with an adjusted gradient elution of 20 - 90 mM KOH and detected via online coupling to MC-ICP-MS (Nu Plasma II, UK). A schematic of the analytical setup can be found in Zakon et al. (2014). To account for instrumental drift during analysis, a standard solution of Na_2SO_4 ($\delta^{34}S$ = 5.9 ‰, Merck) was analyzed before and after every sample. Considering the transient nature of the signal introduced into MC-ICP-MS, isotopic values were calculated based on integration of the entire area of each peak. Baseline contribution was corrected for by assigning an average of 40 - 50 points before each peak as background and point-by-point subtraction from the integrated peak. It has to be noted that small amounts of sulfide were also detected during biotic incubation, but were too low to yield reliable isotope values.

Isotopic values obtained from IC-MC-ICP-MS analysis are presented using the conventional delta notation relative to Vienna Canyon Diablo Troilite (VCDT):

$$\delta^{34}S = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \text{ x } 1000 \qquad (1)$$

where R is the ratio of $^{34}S/^{32}S$. Analytical precision of sulfur isotope determination was better than ±0.3 ‰. Sulfur isotopic mass balance was calculated according to:

$$\sum \delta^{34}S = f_i \text{ x } \delta^{34}S_i \qquad (2)$$

where f_i is the molar fraction of species i of the sum of all detected sulfur species and $\delta^{34}S_i$ is the corresponding isotopic composition.

3. RESULTS

3.1. Sulfur & arsenic speciation during monothioarsenate incubation

Speciation analysis of samples obtained during monothioarsenate incubation showed marked differences between biotic (Fig. 1 a&b) and abiotic treatment (Fig. 1 c&d). Within 240 min, monothioarsenate was almost completely (91 %) transformed to sulfate in the presence of *T. ruber* (Fig. 1a). The only other sulfur species detected was sulfide in concentrations of up to 20 µM during the first 60 min of biotic incubation. Analysis of arsenic speciation showed both arsenite and arsenate formation as monothioarsenate decreased, reaching a final arsenite to arsenate ratio of 2:1 after 240 min. Furthermore, two phases could be identified during biotic incubation with respect to the rate of monothioarsenate transformation. While monothioarsenate decreased by 163.2 µM · min^{-1} within the first 15 min of the experiment, transformation significantly slowed down to 33.3 µM · min^{-1} during the second phase of the incubation (15 - 240 min).

In contrast to the nearly complete conversion of monothioarsenate to sulfate during biotic incubation, abiotic incubation of monothioarsenate generated only minor transformations (Fig 1 c&d). Monothioarsenate was stable for the first 60 min, after which the concentration decreased slightly. This was accompanied by the formation of small amounts of sulfate and arsenate at 240 min.

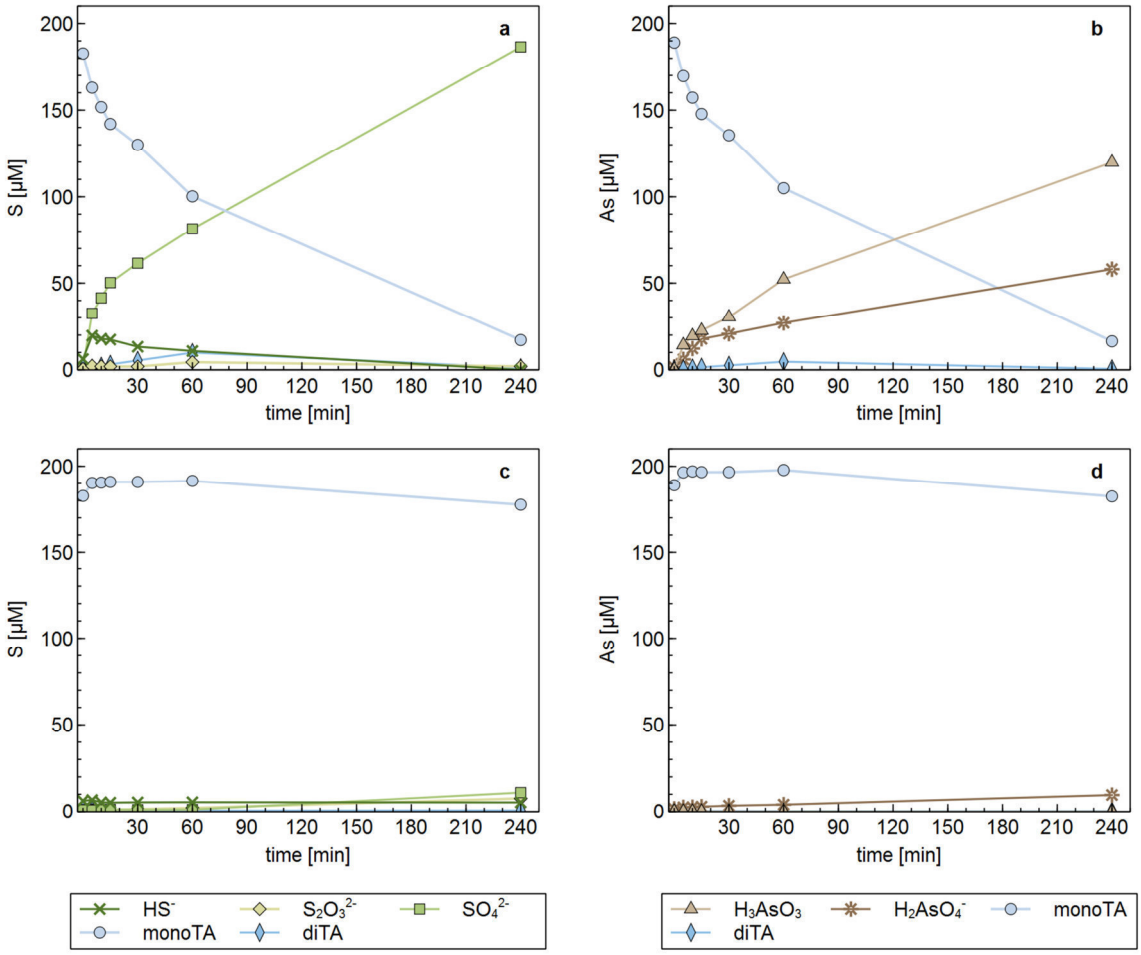

Fig. 1: Sulfur and arsenic speciation during incubation of monothioarsenate (200 µM monoTA, 80 °C, pH 9.1, synthetic Octopus Spring medium): (a) & (b) biotic incubation with *T. ruber* from Conch Spring, Yellowstone National Park; (c) & (d) abiotic incubation.

3.2. Isotope fractionation during monothioarsenate incubation

To investigate the redox processes controlling the observed speciation changes in more detail, sulfur isotopic composition of monothioarsenate and sulfate were determined by IC-MC-ICP-MS (Fig 2). In general, both biotic and abiotic incubation led to formation of sulfate that was isotopically heavier than the remaining monothioarsenate. During the first 10 min of biotic incubation, monothioarsenate became lighter by up to -1.3 ‰ compared to the starting $\delta^{34}S$ value of 1.7 ‰ (Fig 2a). However, as transformation progressed, monothioarsenate was not further depleted and showed comparatively stable $\delta^{34}S$ values of 0.2 - 0.4 ‰ for the second phase of the experiment. At the same time, $\delta^{34}S$ values of the produced sulfate decreased continuously to 0.9 ‰ at 60 min, after which $\delta^{34}S$ increased again to a value of 1.6 ‰ at 240 min. This final value is in good agreement with the initial $\delta^{34}S$ value for monothioarsenate of 1.7 ‰ and demonstrates the nearly complete conversion of monothioarsenate to sulfate in the presence of *T. ruber*.

During abiotic incubation, $\delta^{34}S$ values were also found to decrease for monothioarsenate, but sulfate was much more enriched compared to biotic incubation with a $\delta^{34}S$ value of 5.3 ‰ at 240 min (Fig. 2c). This resulted in a higher fractionation between monothioarsenate and sulfate of +4.4 ‰ for abiotic incubation, clearly exceeding the maximum of +1.2 ‰ observed for the biotic treatment.

In addition, mass balance calculations were performed to assess the pool of detected sulfur isotopes. A clear discrepancy between the initial $\delta^{34}S$ value and the balance calculated for samples from 5 to 60 min was found for the biotic treatment (Fig. 2b). This result strongly indicates the presence of another undetected sulfur species. Based on isotopic mass balance considerations, this sulfur species must be enriched in ^{34}S. Furthermore, it occurred only as an intermediate during incubation since the final mass balance at 240 min is in accordance with the initial value at 0 min. Finally, considering the IC-MC-ICP-MS method is capable of detecting all anionic sulfur species, the undetected species is most likely elemental sulfur, which will be discussed in further detail in section 4.1.

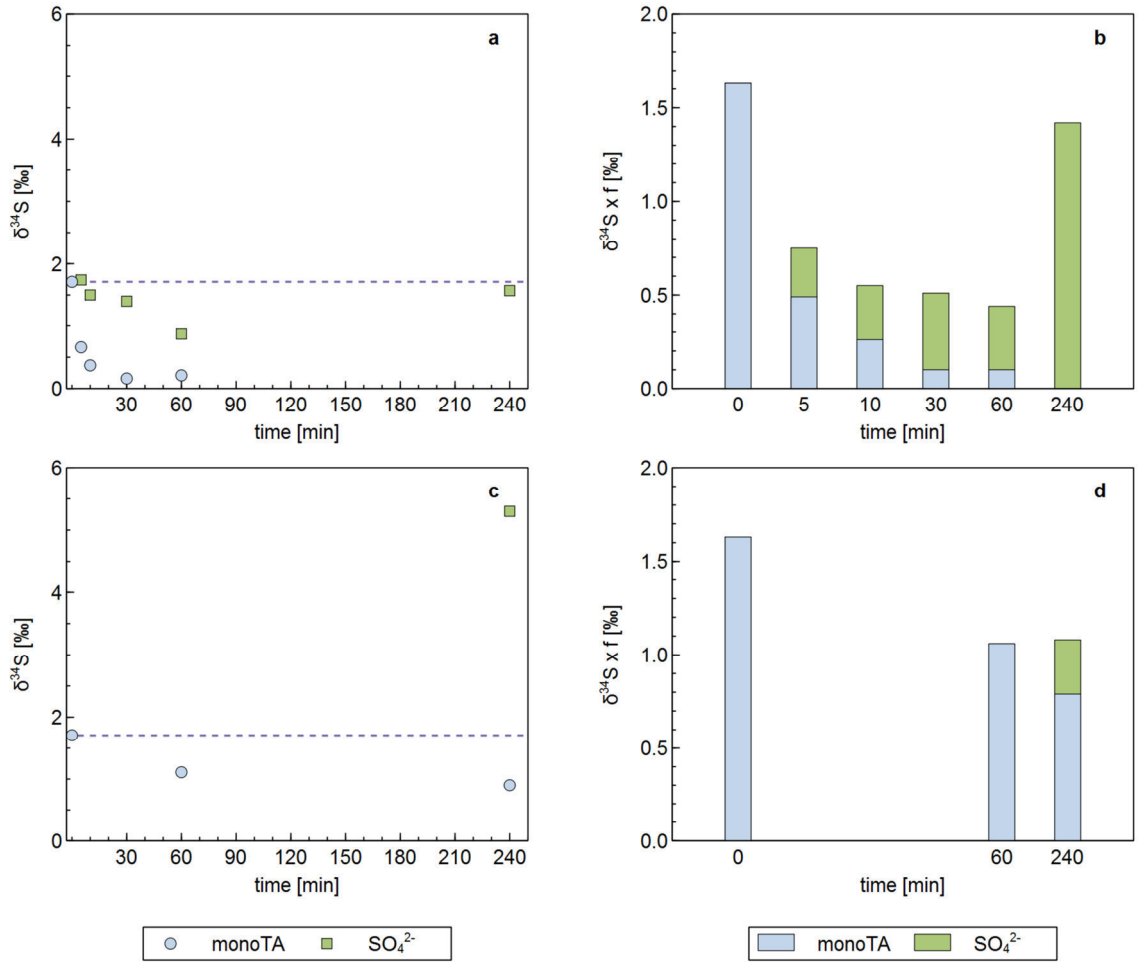

Fig. 2: Isotopic composition determined by IC-MC-ICP-MS during incubation of monothioarsenate (dashed line indicates starting $\delta^{34}S$ value of monoTA) and sulfur isotope mass balance calculated from equation (1): (a) & (b) biotic incubation with *T. ruber* from Conch Spring, Yellowstone National Park; (c) & (d) abiotic incubation (note that for abiotic incubation, samples from 0, 60, and 240 min were selected for isotope analysis based on the absence of speciation changes between 5 and 60 min shown in figure 1 c&d).

4. DISCUSSION

4.1. Monothioarsenate disproportionation

At present, knowledge on isotope fractionation of thioarsenates is limited. In a previous work, fractionation during monothioarsenate oxidation was investigated by application of H_2O_2 (Ullrich et al., 2017, submitted), yielding thiosulfate and sulfate in accordance with products of sulfide oxidation (Chen and Morris, 1972; O'Brien and Birkner, 1977; Zhang and Millero, 1993). Isotope analysis revealed ^{34}S-enrichment of monothioarsenate and ^{34}S-depletion of the produced sulfate, indicative of a normal isotope effect (Ullrich et al. 2017, submitted). However, in the current study we found an inverse isotope effect. Monothioarsenate becoming lighter over the course of the incubation suggests that in this case an entirely different process may control the transformation, and thus the isotopic fractionation.

Some conclusions for interpretation of the current data set can be drawn from the much broader knowledge available on the fractionation of sulfide, considering the strong similarity between the arsenic-bound sulfur of monothioarsenate and sulfide. Similar to our observations, several studies on the oxidation of sulfide have reported an inverse isotope effect. This is most commonly attributed to an equilibrium isotope effect (Fry et al., 1984, 1988a; Kelly, 2008), although kinetic isotope effects have been discussed as well (Zerkle et al., 2009). Investigating the anaerobic oxidation of sulfide to elemental sulfur by the purple sulfur bacterium *Chromatium vinosum*, Fry et al. (1984) found a fractionation factor ε of +2.5 ‰. Similarly, sulfide oxidation to elemental sulfur by the green sulfur bacterium *Chlorobaculum parvum* produced a fractionation factor ε of +2.4 ‰ (Kelly, 2008). Furthermore, Tudge and Thode (1950) calculated an inverse isotope effect for the isotopic exchange between sulfide and elemental sulfur. According to this study, isotopic exchange yields elemental sulfur enriched by +3 ‰ and most likely proceeds via the formation of polysulfides.

Based on all of these previous findings, we propose that monothioarsenate disproportionation and associated ^{34}S-depletion cause the observed inverse isotope effect (Fig. 3). The disproportionation of monothioarsenate to elemental sulfur and arsenite essentially equals an oxidation of the arsenic-bound sulfur:

$$[HAs^{V}S^{-II}O_3]^{2-} + H_2O \leftrightarrow [H_2As^{III}O_3]^{-} + S^0 + [OH]^{-} \quad (3)$$

At room temperature and neutral pH values, monothioarsenate is stable and does not disproportionate (Planer-Friedrich et al., 2009). However, figure 1c clearly shows that the high temperature conditions of 80 °C and pH of 9.1 in combination with an incubation time > 60 min are sufficient to start the monothioarsenate transformation even in the absence of *T. ruber*. At the same time, the remaining monothioarsenate is increasingly depleted in ^{34}S, yielding the observed inverse isotope effect. Taking into account the results from mass balance calculations (Fig. 2 b&d), we thus propose that elemental sulfur constitutes the increasingly enriched product of this reaction.

As previously discussed, elemental sulfur formed from monothioarsenate could not be analyzed by IC-MC-ICP-MS directly since it is not present as an anionic species. Nevertheless, there are several observations that support the hypothesis of the formation of ^{34}S-enriched elemental sulfur during monothioarsenate disproportionation. Firstly, the reverse reaction of equation 3 presents the equilibrium for the synthesis of monothioarsenate from arsenite and elemental sulfur, which needs to be performed at a temperature of 65 °C at high pH for 2 h (Suess et al., 2009). Conducting the incubation experiments at 80 °C and pH 9.1 for 4 h thus likely led to monothioarsenate transformation to the original substances arsenite and elemental sulfur. Furthermore, Voge and Libby (1937) showed that isotopic exchange between sulfide and elemental sulfur requires a temperature of 100 °C, high pH and a reaction time of at least 1 h. The reaction between sulfide and elemental sulfur proceeds via the rapid formation of polysulfides (Fossing and Jørgensen, 1990), which also appear as intermediate species during monothioarsenate disproportionation (Planer-Friedrich et al., 2015). Additionally, polysulfides have been found to be preferentially enriched in ^{34}S in the middle of the chain (Amrani et al., 2006), which constitutes the part of the chain eventually becoming elemental sulfur during further reaction. All of these aspects support the hypothesis that disproportionation of monothioarsenate produces elemental sulfur enriched in ^{34}S, thus causing the observed inverse isotope effect.

Apart from elemental sulfur, monothioarsenate disproportionation also produces arsenite according to equation 3. However, during abiotic incubation only arsenate was found as a transformation product of monothioarsenate (Fig. 1c). Additional incubation tests revealed the potential of rapid abiotic arsenite oxidation, particularly when the initial concentration of arsenite was low (SI-3). Within 5 min, 74 % of 1.3 µM arsenite were oxidized to arsenate, while no oxidation was observed over 240 min for the highest arsenite concentration of 130 µM. Hence, arsenite most likely formed as the original product of monothioarsenate disproportionation, but was not detected during abiotic incubation because of immediate oxidation of the small amount of arsenite. In contrast, the large amount of arsenite produced during biotic incubation was much more stable towards abiotic oxidation.

The combination of all these observations suggests that disproportionation to elemental sulfur and arsenite is the first step of monothioarsenate transformation. Due to the strong similarities in terms of experimental conditions, observed species, and isotopic fractionation, previous findings for the system sulfide-elemental sulfur are likely transferable to the oxidation of the arsenic-bound sulfidic sulfur in monothioarsenate. The consequent formation of ^{34}S-enriched elemental sulfur plays a crucial role as an intermediate step during further transformation to sulfate.

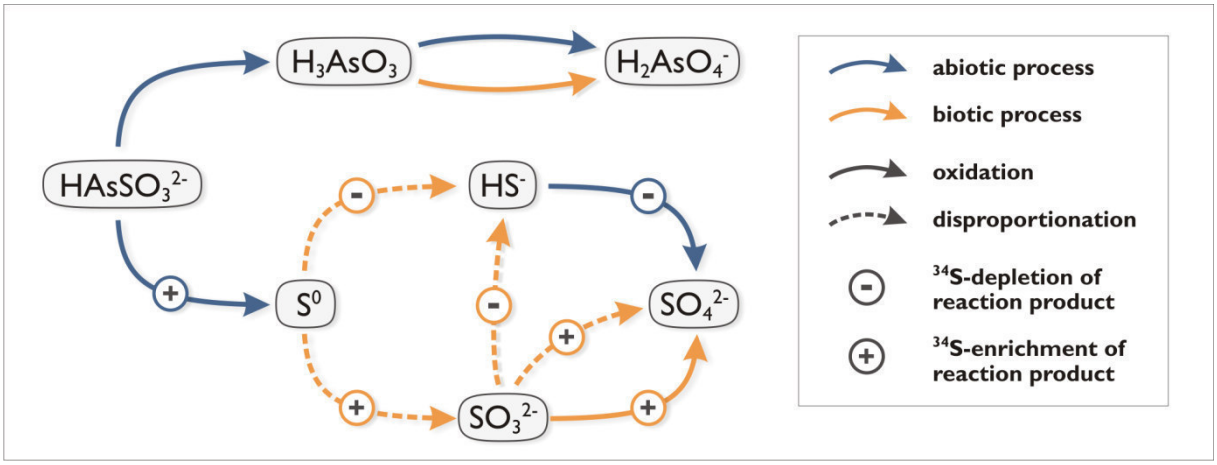

Fig. 3: Model of monothioarsenate transformation to sulfate and arsenate by a combination of abiotic and biotic disproportionation (dashed lines) and oxidation processes (solid lines), resulting in ^{34}S-enrichment (+) or ^{34}S-depletion (-) of the respective reaction products. Note that zerovalent sulfur S^0 can occur in the form of elemental sulfur and/or polysulfides.

4.2. Transformation processes of elemental sulfur

Considering that speciation analysis identified sulfate as the final product of biotic incubation (Fig. 1a), elemental sulfur produced from monothioarsenate is most likely subject to either (1) oxidation or (2) disproportionation. A previous study reported the capability of *T. ruber* to grow on elemental sulfur, thiosulfate, and hydrogen, with elemental sulfur presenting the best electron donor (Huber et al., 1998). However, the exact path of elemental sulfur utilization remains unclear. The early phase of the incubation experiment gives some indication towards direct oxidation of elemental sulfur to sulfate by *T. ruber*. After 5 min of incubation, the isotopic composition of the produced sulfate is identical to the initial $\delta^{34}S$ value of monothioarsenate (Fig. 2a). This result suggests that the ^{34}S-enrichment of the intermediate elemental sulfur is compensated by a ^{34}S-depletion as elemental sulfur is being further oxidized to sulfate. Fry et al. (1988a) reported a similar result for the consecutive steps of sulfide oxidation to elemental sulfur and elemental sulfur oxidation to sulfate, which yielded first an inverse isotope effect of +1.7 ‰ followed by a normal isotope effect of -1.7 ‰, respectively.

Direct oxidation of elemental sulfur to sulfate by *T. ruber* presents a plausible pathway, yet it cannot account for the formation of sulfide detected during the first 60 min of biotic incubation. Consequently, the alternative path of sulfur disproportionation depicted in figure 3 should be considered, which is known to produce sulfate and sulfide according to Thamdrup et al. (1993):

$$4\ S^0 + 4\ H_2O \rightarrow SO_4^{2-} + 3\ HS^- + 5\ H^+ \qquad (4)$$

Sulfate formation during elemental sulfur disproportionation has been proposed to proceed via sulfite, although the specific enzymatic pathway is not fully resolved (Cypionka et al., 1998; Frederiksen and Finster, 2003; Zerkle et al., 2009). The formation of sulfide at a 3-fold molar excess over sulfate according to equation 4 may seem to be in conflict with the obtained speciation data (Fig. 1a). However, sulfide is subject to rapid abiotic oxidation under the experimental conditions leaving only small amounts of sulfide to be detectable (Eq. 5). The removal of sulfide by this process is crucial for reaction 4 to stay thermodynamically favorable (Thamdrup et al., 1993). It should be noted that *T. ruber* may also be able to utilize sulfide, but abiotic oxidation will exceed any potential biotic oxidation as previously shown (Haertig et al., 2014).

$$HS^- + 2\ O_2 \rightarrow SO_4^{2-} + H^+ \qquad (5)$$

Sulfur isotope fractionation accompanying the bacterial disproportionation of elemental sulfur has been studied intensively and generates ^{34}S-depletion for sulfide and ^{34}S-enrichment for sulfate (Böttcher et al., 2001; Canfield and Thamdrup, 1994; Cypionka et al., 1998; Poser et al., 2016). Furthermore, the immediate abiotic oxidation of sulfide to sulfate (Eq. 5) is known to cause a ^{34}S-depletion of -5.1 ‰ for sulfate (Fry et al., 1988b). Hence, sulfide is first depleted in ^{34}S as the product of elemental sulfur disproportionation and then enriched during abiotic oxidation to sulfate (Fig. 3). Sulfite produced from elemental sulfur disproportionation undergoes further disproportionation according to equation 6a (Bak and Cypionka, 1987; Cypionka et al., 1998; Habicht et al., 1998), although direct oxidation of sulfite to sulfate (Eq. 6b) has also been discussed (Fry et al., 1985; Zerkle et al., 2009).

$$4\ SO_3^{2-} + H^+ \rightarrow 3\ SO_4^{2-} + HS^- \qquad (6a)$$

$$SO_3^{2-} + ½\ O_2 \rightarrow SO_4^{2-} \qquad (6b)$$

In the case of sulfite disproportionation, a fractionation of up to -37 ‰ for sulfide and +12 ‰ for sulfate relative to the source sulfite can be expected (Habicht et al., 1998). Less ^{34}S-enrichment for sulfate of +5 ‰, or potentially even ^{34}S-depletion of -5 ‰, is associated with sulfite oxidation (Fry et al., 1985). Even though the exact process of sulfite transformation in *T. ruber* is not known, the overall ^{34}S-enrichment of sulfate compared to monothioarsenate points towards sulfite disproportionation, which generates higher ^{34}S-enrichment for sulfate than sulfite oxidation. Nevertheless, the precise pathways of elemental sulfur transformation to sulfate by chemolithotrophic bacteria have yet to be fully resolved.

Finally, for comprehensive interpretation of observed $\delta^{34}S$ values, the potential of abiotic monothioarsenate formation from elemental sulfur and arsenite also needs to be considered. The high temperature conditions during incubation can also favor the reverse reaction shown in equation 3, essentially yielding an equilibrium between monothioarsenate disproportionation and recombination. The pronounced decrease in the monothioarsenate transformation rate starting at 15 min implies that monothioarsenate recombination may be a contributing process during the second phase of the incubation experiment (Fig. 1 a&b). Indeed, the corresponding $\delta^{34}S$ values of monothioarsenate became comparatively stable (Fig. 2a), suggesting a balance between ^{34}S-depletion during monothioarsenate disproportionation and ^{34}S-enrichment during the reverse reaction.

Nonetheless, the disproportionation of monothioarsenate according to equation 3 is favored due to the continuous removal of the reaction products. In this context, it should be noted that not only elemental sulfur produced from monothioarsenate but also arsenite is subject to further transformation. As previously shown, *T. ruber* can also use arsenite directly as electron donor, which leads to the formation of arsenate observed during biotic incubation (Haertig et al., 2014; Haertig and Planer-Friedrich, 2012). Thus, rapid monothioarsenate disproportionation during biotic incubation can essentially be ascribed to microbial utilization of both arsenite and elemental sulfur by *T. ruber*.

Furthermore, the much faster transformation of monothioarsenate in the presence of *T. ruber* also controlled

the extent of fractionation. Small fractionation of maximum +1.2 ‰ between monothioarsenate and sulfate was found as a result of faster reactions during biotic incubation. In comparison, the much slower transformation in the abiotic treatment produced a fractionation of +4.4 ‰. Similar effects have been described for different rates of sulfite disproportionation (Habicht et al., 1998) and sulfate reduction (Sim et al., 2011).

In general, observed fractionation is much smaller than values typically reported for microbial disproportionation of reduced sulfur species. Interestingly, the only other work conducted under comparably high pH conditions also found only small fractionation (Poser et al., 2016). Studying elemental sulfur disproportionation at pH 10, Poser et al. (2016) observed fractionation of -1 ‰ and +4.7 ‰ for sulfide and sulfate, respectively. It was proposed that under high pH conditions, increased bioavailability of zerovalent sulfur in the form of polysulfides accelerated disproportionation, and thus decreased fractionation. Considering polysulfides are known intermediates of monothioarsenate transformation (Planer-Friedrich et al., 2015), this effect may have similarly contributed to the small fractionation observed during monothioarsenate incubation. Nevertheless, further investigations are needed to confirm that the different extent of fractionation is not merely a result of different enzymatic pathways under neutral and alkaline conditions.

4.3. Abiotic vs. biotic oxidation of monothioarsenate

All of the obtained results clearly show how isotope analysis can assist in elucidating intermediate steps of sulfur metabolism in chemolithotrophic bacteria. Identifying electron donors for bacterial growth based exclusively on observed speciation changes can be rendered difficult by simultaneous abiotic transformations. The isotopic data presented here allows to revisit the hypothesis of arsenic-bound sulfur in thioarsenates serving as electron donor for chemolithotrophic bacteria like *T. ruber*. Certainly, microbial oxidation of thioarsenates appears favorable in the light of their abundant availability in geothermal systems (Planer-Friedrich et al., 2007; Ullrich et al., 2013). At the site from where *T. ruber* was collected for the incubation experiment, arsenic-bound sulfur in thioarsenates was found to represent up to 54 % of the sum of reduced sulfur species (SI-1).

Indications for direct microbial oxidation of monothioarsenate to sulfate were presented by Haertig et al. (2014) based on observed speciation changes and equilibrium considerations. Previous investigations of the fractionation that accompanies direct oxidation of monothioarsenate to sulfate showed a distinct normal isotope effect of -6.1 ‰ (Ullrich et al., 2017 submitted). Hence, if the formation of sulfate during incubation was a result of direct monothioarsenate oxidation as hypothesized by Haertig et al. (2014), a similar normal isotope effect should have been detected.

However, the inverse isotope effect found during incubation in the current study demonstrates that monothioarsenate was disproportionated rather than directly oxidized. The corresponding ^{34}S-depletion of monothioarsenate was detected both in abiotic and biotic incubation, suggesting that monothioarsenate disproportionation is an abiotic process and arsenic-bound sulfur is not directly used by *T. ruber* as previously assumed. The abiotic disproportionation of monothioarsenate is most likely initiated by the elevated temperature in the drainage channel. As a result, the high temperature environment, in which *T. ruber* typically grows, facilitates the abiotic transformation of monothioarsenate to elemental sulfur and makes the arsenic-bound sulfur available for microbial utilization. Considering that the higher thiolated species di-, tri-, and tetrathioarsenate exhibit even less stability under high temperatures than monothioarsenate (Planer-Friedrich et al., 2009), it can be assumed that they present an easily accessible pool of reduced sulfur as well. Thus, once abiotic transformation is initiated, thioarsenates can act as an important source of electron donors for chemolithotrophic growth.

5. CONCLUSIONS

The current study presents, for the first time, values of isotope fractionation associated with both biotic and abiotic transformation of arsenic-bound sulfur in thioarsenates. Results from incubation experiments in a geothermal drainage channel revealed a pronounced inverse isotope effect for monothioarsenate, as it became increasingly depleted compared to the initial δ^{34}S value. This effect was observed in both abiotic and biotic treatments and stands in strong contrast to the normal isotope effect previously found during oxidation of monothioarsenate to thiosulfate and sulfate. Combining these findings, we propose that the observed inverse isotope effect is a result of abiotic monothioarsenate disproportionation rather than oxidation, leading to the formation of ^{34}S-enriched elemental sulfur and arsenite.

In the presence of the chemolithotroph *T. ruber*, elemental sulfur from monothioarsenate disproportionation was further transformed. Even though exact enzymatic pathways are not resolved, the observed sulfur speciation and fractionation are best supported by a model comprising biotic elemental sulfur disproportionation, followed by abiotic sulfide oxidation and biotic sulfite disproportionation. Based on these results, we conclude that monothioarsenate cannot serve directly as electron donor for chemolithotrophic bacteria as previously assumed. Nevertheless, thioarsenates present a substantial source of reduced sulfur. The specific environmental setting, in particular temperature, pH, and redox conditions, will essentially control to what extent thioarsenates are converted to a more bioavailable form of sulfur, such as elemental sulfur or sulfide.

The current study clearly demonstrated the additional value of sulfur isotope analysis for the differentiation of abiotic and biotic processes. The results presented here can serve as a first step towards a better understanding of how thiometalloid occurrence and transformation contributes to

isotope fractionation. Overall, this will improve our ability to interpret sulfur isotope data from both modern and ancient environments, and thus help define processes within the sulfur cycle.

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SUPPORTING INFORMATION

Differentiation of abiotic and biotic monothioarsenate transformation by analysis of sulfur isotopes using IC-MC-ICP-MS

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SI-1. Sulfur speciation for the drainage channel of Conch Spring (Lower Geyser Basin, Yellowstone National Park)

distance	sulfide	sulfate	thio-sulfate	monoTA	diTA	triTA	tetraTA	thioarsenates/ reduced S[a]
[m]				S [µmol · L^{-1}]				[%]
0	62.3	203.6	7.1	2.7	2.5	19.3	31.9	44.9
4	47.4	205.2	8.3	2.4	3.2	37.1	1.1	44.1
7	45.7	208.4	9.0	4.9	2.3	16.2	28.8	48.9
11	28.9	276.3	9.1	7.6	2.0	11.1	24.6	54.4
16	34.0	240.9	12.3	6.6	2.0	13.1	13.9	43.4
22	29.7	254.2	14.2	8.1	1.9	8.5	11.7	40.8
29	15.4	272.7	15.3	8.1	1.5	5.1	4.1	38.0
35	14.8	256.8	16.6	8.4	1.1	2.4	2.0	30.7

[a] reduced S refers to the pool of detected S species presenting possible electron donors (i.e. sulfide, thiosulfate, monoTA, diTA, triTA, and tetraTA)

SI-2. Composition of sulfur-free, low-mineral Octopus Spring (OS) medium used for abiotic and biotic incubation of monothioarsenate (modified from Huber et al., 1998).

substance	concentration [mg · L^{-1}]
NaCl	256.0
KH_2PO_4	1.7
$CaCl_2 \cdot 2\ H_2O$	0.8
$NaNO_3$	0.3
KCl	15.0
$FeCl_3 \cdot 6\ H_2O$	0.1
$NaHCO_3$	1000.0

SI-3. Arsenic speciation after abiotic incubation of solutions containing 1.3, 13, or 130 µM arsenite at 80 °C for 240 min in synthetic, low-mineral Octopus Spring medium (room temperature as reference for arsenic speciation in OS medium without heating).

	incubation time	temperature		arsenite	arsenate
	[min]	room temperature	80 °C	[%]	
1.3 µM arsenite	0	x		78.4	21.6
	0		x	33.1	66.9
	5		x	26.3	73.7
	240		x	42.4	57.6
13 µM arsenite	0	x		90.3	9.7
	0		x	87.2	12.8
	5		x	84.7	15.3
	240		x	80.9	19.1
130 µM arsenite	0	x		98.7	1.3
	0		x	97.7	2.3
	5		x	98.1	1.9
	240		x	98.9	1.1

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