

2017-01-01

A New Method For Ultra-Low Sulfate Extraction And A Pilot Study In Arid Soils

Marisela Montelongo

University of Texas at El Paso, mariselamontelongo4@gmail.com

Follow this and additional works at: https://digitalcommons.utep.edu/open_etd



Part of the [Environmental Sciences Commons](#), and the [Geochemistry Commons](#)

Recommended Citation

Montelongo, Marisela, "A New Method For Ultra-Low Sulfate Extraction And A Pilot Study In Arid Soils" (2017). *Open Access Theses & Dissertations*. 505.

https://digitalcommons.utep.edu/open_etd/505

This is brought to you for free and open access by DigitalCommons@UTEP. It has been accepted for inclusion in Open Access Theses & Dissertations by an authorized administrator of DigitalCommons@UTEP. For more information, please contact lweber@utep.edu.

A NEW METHOD FOR ULTRA-LOW SULFATE EXTRACTION
AND A PILOT STUDY IN ARID SOILS

MARISELA MONTELONGO

Master's Program in Environmental Science

APPROVED:

Benjamin Brunner, Ph.D., Chair

Gail Lee Arnold, Ph.D., Co-Chair

Lixin Jin, Ph.D.

Vanessa Lougheed, Ph.D.

Charles Ambler, Ph.D.
Dean of the Graduate School

Copyright ©

by

Marisela Montelongo

2017

DEDICATION

This thesis is dedicated to my husband, Jorge, my parents, Aurelio, and María and my siblings Blanca and Osvaldo. The reason I am where I am right now, is because of you. You have and continue to motivate me in continuing on this educational path. Even though sometimes it seems too much, I know you will always be there for me. Thank you for being you!

A NEW METHOD FOR ULTRA-LOW SULFATE EXTRACTION
AND A PILOT STUDY IN ARID SOILS

by

MARISELA MONTELONGO, B.S.

THESIS

Presented to the Faculty of the Graduate School of
The University of Texas at El Paso
in Partial Fulfillment
of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Geological Sciences
THE UNIVERSITY OF TEXAS AT EL PASO

May 2017

ACKNOWLEDGEMENTS

In the Fall of 2015, Dr. Benjamin Brunner and Dr. Gail L. Arnold decided to give me the opportunity to be part of their research group. Up until that moment I have heard rumors that they were really good professors and decided to continue, I mean, what could go wrong, right? These two and a half years with them, I do not regret not even for a second that we chose each other for this adventure. With them, I learned the importance of being punctual, not that I was not before, but that ten minutes before is being on time, 5 minutes before is being late and “being on time” will get you fired. Also, that it is OK to take baby steps in learning so that at the end you understand the whole “picture” and really learn and know what you are doing. All I have learned with them has made me a better person, not only in a professional way, but in my personal life. I deeply appreciate your patience and support; I know this accomplishment would never have been possible without you. Dr. Brunner and Dr. Arnold, you rock!

Secondly, I would like to thank Michael Mathuri, my partner in crime, for all your help throughout my Master’s Thesis. I would never have made it this far if it were not for your help in the lab and your always positive attitude. Also, I would like to acknowledge Anna Ortiz and Andre Llanos for providing me with soil samples used in my research.

Finally, Dr. Thomas Gill. Thank you very much for your all your support and believing in me since the beginning.

ABSTRACT

Sulfur in soils is a crucial nutrient for all organisms including plants. Sulfate (SO_4^{2-}), a water soluble anion, is a good source of this important element. Because of the importance of sulfur for agriculture, the use of sulfate has been well studied in temperate climate zones, and it is generally accepted that microbes play a critical role in controlling the availability of sulfate. Organically bound sulfur, such as sulfate esters and carbon-bonded sulfur, compounds that are generated and decomposed by microbes, are essential sulfur sources for plants under these conditions. For arid environments, knowledge of sulfate turnover in soils by microbes and plants is much more limited. It is not known whether sulfate turnover by microbes in desert soils is more or less critical than in temperate climates.

El Paso, Texas is located in the Chihuahuan Desert, an arid environment. It is also surrounded by agricultural areas, which receive sulfate through application of fertilizers and irrigation waters; is located in proximity of White Sands National Park, a source of gypsum dust; and receives water from two aquifers, Mesilla and Hueco Bolson that have relatively high sulfate concentrations. These contrasts make El Paso a unique site for sulfur cycling studies.

Theoretically, it is possible to trace the sulfate turnover by measuring changes in the sulfur and oxygen isotope of sulfate. The challenge for this approach is that sulfate in arid soils exists only in low concentration, which renders accurate determination of isotope compositions difficult. The objectives of this study were 1) to develop a new method to extract low sulfate from soils for isotope analysis and 2) to carry out a pilot study on microbial turnover of sulfate in soils from nearby White Sands National Park, El Paso Texas and the Indio Mountains.

So far, methods for the extraction of sulfate from solutions with low sulfate concentrations relied on techniques that employed ion exchange resins. This approach requires large volumes of the

solution from which sulfate is to be extracted, and only works for solutions with low ion strength. Thus, sulfate extraction from high-chloride, low-sulfate solutions was not possible. My now proven technique uses the classical approach of collecting sulfate as barium sulfate, followed by a chelator-assisted dissolution-precipitation step which returns a pure barium sulfate sample. The method can be used on solutions with high chloride levels, up to the equivalent of chloride encountered in seawater (>500 mM), and enables extraction of sulfate from samples with sulfate concentrations as low as 0.03 μM and volumes below of 50 ml.

The application of the developed technique to soils from a site on the ‘Lost Dog Trail’ (El Paso), and a location in the Indio Mountains revealed that arid soils have indeed extremely low sulfate content. The observed values for the Indio Mountains are $\sim 0.5 \mu\text{mol}$ of sulfate per gram of soil. No sulfate was recovered from the Lost Dog Trail samples, suggesting a sulfate content of less than $0.1 \mu\text{mol}$ of sulfate per gram of soil for this site. The sulfur isotope composition of the sulfate obtained from the Indio Mountains is almost identical to the isotope composition of sulfate found near the White Sands National Park, indicating that transport of dust from this site could be a major sulfur source to this region. This input could be in form of dry deposition, or happen during rain events. In order to obtain insight if biological sulfur cycling takes place in the investigated arid soils, a strong rainfall event was mimicked. The soils were covered with water that was amended with sulfate ($\sim 210 \mu\text{M}$, approximately 3 times the typical value for rainwater over North America) and labeled with ^{18}O -enriched water. Decreasing sulfate concentrations and an enrichment in ^{18}O of sulfate revealed that microbial sulfur cycling takes place after rainfall events, and that oxygen isotopes of sulfate can be successfully applied to trace soil sulfur cycling in arid soils.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	V
ABSTRACT.....	VI
TABLE OF CONTENTS	VIII
LIST OF TABLES.....	X
LIST OF FIGURES	XI
 1. BACKGROUND AND MOTIVATION.....	 1
1.1 SULFUR AS BIO-ESSENTIAL NUTRIENT	1
1.2 MOBILITY OF SULFATE-SULFUR, A CRITICAL NUTRIENT FOR PLANTS	1
1.3 DYNAMICS BETWEEN ORGANIC SULFUR AND INORGANIC SULFUR	2
1.4 SULFUR CYCLING IN SOILS FROM ARID ENVIRONMENTS	4
 2. THE CHALLENGE: TRACING MICROBIAL SULFUR CYCLING IN SOILS FROM ARID AND SEMI-ARID ENVIRONMENTS	 6
2.1 SULFUR ISOTOPES AS TRACERS FOR SULFUR CYCLING.....	7
2.2 OXYGEN ISOTOPES AS TRACERS FOR SULFUR CYCLING	8
2.3 EXTRACTION OF SULFATE FROM ARID SOILS FOR ISOTOPE ANALYSIS	9
 3. GOALS OF THIS STUDY	 11
 4. A NEW METHOD FOR ULTRA-LOW SULFATE EXTRACTION	 12
4.1 BACKGROUND.....	12
4.2 PREPARATION FOR EXPERIMENTS.....	13
4.2.1 Diethylenetriaminepentaacetic acid (DTPA) stock solution.....	13
4.2.2 Cleaning glassware	14
4.2.3 Sulfate-free artificial seawater (SF-ASW).....	14
4.2.4 Sodium sulfate stock solution	14
4.2.5 BaCl ₂ stock solution.....	15
4.3 NEW EXTRACTION METHOD	15
4.4 PREPARATION FOR SULFUR AND OXYGEN ISOTOPE ANALYSIS	18
4.4.1 Preparation for sulfur and oxygen isotope analysis	18
4.4.2 Using acetone for extremely low barium sulfate	20

4.4.3	Additional preparation for oxygen isotope analysis of ultra-low concentration samples.....	20
4.5	RESULTS AND DISCUSSION.....	21
4.5.1	Preliminary Experiments	21
4.5.2	Artificial seawater (ASW) Experiments	22
4.5.3	Sources of contamination in the ASW experiments	23
4.5.4	18MΩ water experiments.....	24
4.6	CONCLUSIONS – ROBUSTNESS OF NEW ULTRA-LOW SULFATE EXTRACTION METHOD	25
4.6.1	Caveats of the method.....	26
5.	PILOT STUDY –	28
	APPLICATION OF NEW ULTRA-LOW SULFATE EXTRACTION METHOD	28
5.1	SOIL COLLECTION AND PREPARATION	29
5.2	EXPERIMENT SET UP.....	29
5.3	METHODS – SEQUENTIAL LEACHING	30
5.3.1	Readily Available Sulfate (RAS).....	30
5.3.2	Easily Soluble Sulfate (ESS)	31
5.4	INITIAL RESULTS	31
5.5	RAIN EVENT EXPERIMENTS	33
5.5.1	Experimental design.....	34
5.5.2	Rainwater experiment results.....	34
5.6	CONCLUSIONS OF THE RAINWATER EXPERIMENTS.....	35
6.	CONCLUSIONS AND FUTURE WORK.....	37
7.	TABLES.....	38
8.	FIGURES	46
	REFERENCES	60
	APPENDIX.....	64
	VITA.....	78

LIST OF TABLES

Table 1. Example experimental design for ~45ml test solutions for ultra-low sulfate extraction.	38
Table 2. Preliminary experiments testing the reproducibility of low sulfate extraction method using an ASW media.	39
Table 3. Isotope composition of sodium sulfate and sulfate stock solution.	40
Table 4. Test of variable concentrations with the low sulfate extraction method with artificial seawater.....	41
Table 5. Sources of sulfur contamination.	42
Table 6. Results of low sulfate extraction method with 18MΩ water.	43
Table 7. Results of pilot study.	44
Table 8. Results of rainwater experiments. 'Rainwater' added 4/13/2017.	45

LIST OF FIGURES

Figure 1. Importance of sulfur for organisms and agriculture.	46
Figure 2. Sulfur deposition in Denmark through time (Eriksen, 2009).	47
Figure 3. Sulfate ion concentration of precipitation-weighted mean from 1985 to 2014 in mg/L (http://nadp.isws.illinois.edu).	48
Figure 4. Concept design for testing new extraction method.	49
Figure 5. Sulfate extraction protocol for low sulfate concentrations.	50
Figure 6. Flow chart for ultra-low sulfate extraction method testing.	51
Figure 7. Important steps during filtration process.	52
Figure 8. Preliminary extraction method results.	53
Figure 9. Results from variable concentration sulfate in ASW experiments for the sulfur isotope composition (A.) and oxygen isotope composition (B.) of sulfate.	54
Figure 10. Results from variable concentration sulfate in ultra-pure water experiments for the sulfur isotope composition (A.) and oxygen isotope composition (B.) of sulfate.	55
Figure 11. Locations for sample collection in Pilot Study.	56
Figure 12. Measured $\delta^{34}\text{S}$ composition from sulfate extraction in Hwy54-I, Indio Mtn I, Indio Mtn II and Positive Control in sequential leaching process.	57
Figure 13. RAS leaching results. Three time points (Day 1, 5 and 13) vs. Oxygen Isotope Composition for Lost Dog Trail soil sample.	58
Figure 14. RAS leaching results. Three time points (Day 1, 5 and 13) vs. Oxygen Isotope Composition for Indio Mountain soil sample.	59

1. BACKGROUND AND MOTIVATION

1.1 SULFUR AS BIO-ESSENTIAL NUTRIENT

Sulfur (S) is an essential nutrient for all living organisms. For plants, sulfur is one of the four most important macro-nutrients, including nitrogen, phosphorus, and potassium (Eriksen et al., 1998). Sulfur is instrumental for nitrogen fixation and the synthesis of amino acids such as cysteine and methionine, coenzymes (A, biotin, thiamine, glutathione), chlorophyll and other secondary sulfur compounds (Figure 1; Eriksen et al., 1998).

Over the last two decades an increase in sulfur deficiency has been recorded in many regions of the world (Eriksen, 2009). This sulfur deficiency is mainly caused by the decrease of acid rain due to environmental policies to reduce sulfur dioxide (SO₂) emissions, the increased use of low-sulfur fertilizers and a decrease in the use of pesticides and fungicides that contain sulfur (Eriksen, 2009). For example, as a side effect of the industrial revolution, sulfur deposition in Denmark rose since 1800, reaching a peak in 1970 followed by a sharp decrease due to environmental policies to reduce sulfur dioxide emissions (Figure 2; Eriksen, 2009). A similar pattern is observed in the United States (Figure 3). The decrease in sulfate input has led to a renewed interest in soil sulfur cycling, as sulfate deficiency could negatively impact crop yields.

1.2 MOBILITY OF SULFATE-SULFUR, A CRITICAL NUTRIENT FOR PLANTS

In soils, sulfur is commonly found as organically bound sulfur accounting for approximately 95% of the total sulfur (Eriksen et al., 1998). Sulfate (SO₄²⁻) or inorganically bound sulfur accounts for approximately 5% of the total sulfur (Scherer, 2009). Sulfate is a crucial sulfur source for plants, because organically bound sulfur must first be liberated from the organic compounds and converted into sulfate before it can be taken up by the root system (Eriksen et al., 1998; Scherer,

2009). In soils, inorganic sulfate can be found in three different forms; sulfate in soil solution (i.e. aqueous sulfate), adsorbed sulfate, and mineral sulfate (Scherer, 2001). The content of sulfate in soils usually depends on plant uptake, contribution from external sulfate sources such as fertilizers, sulfate on dust particles or sulfate in rainwater (acid rain), sulfate immobilization through processes such as adsorption or precipitation, re-mobilization of sulfate by the same processes operating in the opposite direction, formation of organically bound sulfur as well as the mineralization of these compounds, and removal of sulfate by aqueous or wind transport (Scherer, 2009). The ability of soils to retain sulfate by adsorption is controlled by the concentration of anions and cations including the pH-dependent abundance of protons and hydroxide ions that compete with sulfate for adsorption sites on minerals (e.g. clays, iron oxides), soil organic matter, and colloids (Scherer, 2009). Presence of organic compounds in solution (dissolved organic carbon, DOC) also can strongly affect sulfate adsorption via occupation of charged mineral surfaces or as chelators that decrease the activity of cations in solution (e.g. calcium). The soil pH also strongly affects the sequestration or release of sulfate by controlling the precipitation or dissolution of minerals that can trap sulfate, either as coprecipitates (e.g. in carbonates) or as minerals like gypsum or anhydrite (Mayer et al., 1995b). Moreover, the pH plays a critical role in the mineralization of organically bound sulfur, as the activity of the enzymes that catalyze these processes are pH dependent (Ganeshamurthy and Nielsen, 1990; Klose et al., 1999; Kertesz, 2000).

1.3 DYNAMICS BETWEEN ORGANIC SULFUR AND INORGANIC SULFUR

Organically bound sulfur is likely to play a key role in the sulfur cycle of soils, because it constitutes 95% to 98% of the total soil sulfur (Ghani et al., 1993; Kertesz and Mirleau, 2004; Scherer, 2009). The dominance of organically bound sulfur in soils is probably owed to two facts. First, in soils, organically bound sulfur is typically less mobile than sulfate (Scherer, 2001; Scherer,

2009), and second, microbial conversion of inorganic sulfate to organically bound sulfur appears to be very efficient (Eriksen, 2009). As organic sulfur is unavailable for plants the transformation of abundant organically bound sulfur to inorganic sulfur becomes a critical process for plant access and uptake (Castellano and Dick, 1991; Scherer, 2001).

Organically bound sulfur is a heterogeneous mixture of compounds derived from organisms in the soil, including microbes, plants, and animals (Eriksen, 2009). There are two major groups of sulfur compounds that can be identified within this complexity, namely ester sulfates (C-O-S) and carbon-bonded sulfur (C-S) (Scherer, 2009). Microbes play a key role both in the conversion of sulfate into ester sulfates and carbon-bonded sulfur and in the mineralization of these compounds (Ghani et al., 1993). Microbial mineralization of carbon-bound sulfur compounds is probably driven by energetic needs. This means that the reduced carbon moiety of the compound is oxidized to carbon dioxide in order to gain energy, with sulfate being released as a metabolic by-product (Ghani et al., 1993). In contrast, mineralization of ester sulfates is driven by the need for inorganic sulfate. When the supply with inorganic sulfate does not cover the microbial sulfate demand in the soil, enzymes called sulfatases are produced or activated (Scherer, 2009). These sulfatases act as catalysts for the hydrolysis of ester sulfates (C-O-S). One group of sulfatases, the S-O cleaving sulfatases (aryl sulfate monoesters) have been found to be among the most powerful biological catalysts known (Edwards et al., 2012). The two reasons for mineralization of organically bound sulfur are similar to the reasons for mineralization of organically bound phosphorus, where phosphate is either released to the environment because of phosphorous limitation or during the breakdown of organic compounds for dissimilatory processes.

1.4 SULFUR CYCLING IN SOILS FROM ARID ENVIRONMENTS

Approximately, 41% of the world's land surface is composed of arid and semi-arid ecosystems; regions that are vulnerable to climate change (Reynolds et al., 2007; Luo et al., 2015). Global changes in precipitation and temperature may lead to an increase of arid and semi-arid areas and consequently affect plant nutrition and the ecosystem's function (Reynolds et al., 2007; Knapp et al., 2008; Schimel, 2010; Dai, 2011; Luo et al., 2015). Studies suggest that the increase in aridity can negatively affect the biogeochemical cycle of not just sulfur, but also other nutrients such as nitrogen, phosphorus, and carbon (Luo et al., 2015). Only a few studies have investigated the impact of changing climate on the biogeochemical sulfur cycle in arid and semi-arid region (Luo et al., 2015).

The interest in sulfur cycling in arid and semi-arid soils is driven by the insight that such soils could be sulfur-deficient, an observation that was made as early as 1958 (Ensminger, 1958 *vide* Dracy et al., 2013), and has been corroborated by findings that show that sulfur availability in such systems is tightly coupled to other nutrient cycles (i.e. P and N), and also strongly dependent on water addition. Moreover, there appears to be a threshold in aridity at which the availability of sulfur in soils changes from decreasing availability to increasing availability (Luo et al., 2015). Arid soils from the Southwestern United States appear to harbor microbial communities that rely on the availability of thiosulfate for dissimilatory processes, which raises the question if those communities are important for sulfur and carbon cycling in arid soils (Dracy et al., 2013). The dependence of sulfur cycling in arid and semi-arid soils on the multitude of variables that could drastically change due to climate change, such as different rain patterns in terms of duration, intensity, and seasonality and concomitant change of land use (agriculture, cattle, intensity of irrigation) could heavily impact sulfur availability for plants. For temperate environments, it is

commonly accepted that microbial communities exert a strong control on sulfur availability for the plants, as the microbes play a dominant role in the interconversion of soil sulfur fluxes between inorganic and organically bound sulfur pools. For arid environments, this may not be the case, or, vice versa, may be especially true. Obviously, there is an urgent need to elucidate the role of microbial sulfur cycling in soils from arid and semi-arid environments.

2. THE CHALLENGE: TRACING MICROBIAL SULFUR CYCLING IN SOILS FROM ARID AND SEMI-ARID ENVIRONMENTS

Based on what is known about arid environments and biogeochemical sulfur cycling in soils, I make the following predictions about soil sulfur cycling in arid environments (Text Box 1).

Text Box 1: Predictions about soil sulfur cycling in arid environments

- Arid soils contain diverse inorganic (e.g. mineral-bound sulfate) and organic sulfur pools, with sulfur inventories that can be very large.
- The spatial distribution of these sulfur pools is highly variable.
- The bio-availability of sulfur stored in these pools varies greatly, and the largest sulfur pools may be the most inaccessible sulfur reservoirs (e.g. sulfate trapped in carbonates such as caliche). As such, there may be vast sulfur pools that can never be tapped into.
- Soil sulfur cycling in arid environments is highly episodic, and tied to precipitation. This means that there are long periods of inactivity, which are interrupted by bursts of activity.

If these presumptions are correct, tracing of sulfur cycling in arid environments faces a suite of challenges. First, bulk analyses of inorganic and organic sulfur and other nutrient (e.g. C, N) inventories are likely not adequate to assess if there is a sulfur deficiency, as presence of sulfur does not indicate availability. And even if plant-available sulfur, such as sulfate, is detected, it is not known what process made the compound available – e.g. mineralization of organically bound sulfur, deposition of sulfate via dust transport, or sulfate in acid rain. Finally, general sulfur availability may not be representative for the sulfur that is bio-available during the burst of activity after precipitation or flooding events. These circumstances highlight the need for tracers of sulfur

cycling that allow to distinguish processes and sulfur sources. Isotopes of sulfur and oxygen (Text Box 2) have the potential to address this need.

Text Box 2: Sulfur and oxygen isotopes as tracers for sulfur cycling

- Sulfur has four stable isotopes (^{32}S , ^{33}S , ^{34}S , ^{36}S) and a radioactive isotope, ^{35}S with a half-life of 87 days, all of which can be used as tracers.
- Oxygen has three stable isotopes (^{16}O , ^{17}O , ^{18}O).
- The ratio of heavy to light sulfur and oxygen isotopes varies between different sulfur and oxygen pools because biogeochemical processes fractionate isotopes. Sulfur and oxygen isotope signatures can therefore be used to detect different sulfur and oxygen sources, and also to identify individual biogeochemical processes with the help of the isotope signature that is left by the isotope fractionation.
- Natural abundance isotope compositions are reported in the standard delta notation, where the ratio of heavy to light isotope of a sample is compared to the ratio of heavy to light isotope of a standard, e.g.

$$\delta = \left(\frac{R_{\text{sample}}}{R_{\text{std}}} - 1 \right) \cdot 1000\text{‰}$$

- For sulfur isotopes, the reporting standard is Vienna Canyon Diablo Troilite (VCDT). For oxygen isotopes, the reporting standard is Vienna Standard Mean Ocean Water (VSMOW).

2.1 SULFUR ISOTOPES AS TRACERS FOR SULFUR CYCLING

Stable sulfur isotopes are extremely powerful tracers for dissimilatory processes, especially sulfate reduction, and have been successfully applied to elucidate marine sulfur cycling. This success is owed to the fact that dissimilatory processes in the sulfur cycle tend to impose strong sulfur isotope fractionation (e.g. Canfield, 2001). Unfortunately, this is not the case for assimilatory processes, where sulfur isotope fractionation is small (Thode, 1991; Trust and Fry, 1992 and references therein). Consequently, stable sulfur isotopes may not be the ultimate tracer to decipher soil sulfur

cycling. This setback can be partially overcome by the use of radioactive (^{35}S) or stable isotope tracers (e.g. ^{33}S , ^{34}S) in experiments, which allow to track sulfur transformations (e.g. O'donnell et al., 1994; Mayer et al., 1995a; Mayer et al., 1995b; Kang et al., 2014). Such approaches have been extensively used to investigate sulfur cycling in temperate soils and grasslands. These studies focused on sulfur mobility, transformation of soil sulfur, and identification of the source of dissolved organic sulfur by employing sulfur isotope analyses of sulfur bearing compounds obtained by different sulfur extraction methods. The caveat with such methods is that the isotope label must be used in compounds that are relevant for the study object. While this can be achieved for sulfate, it is difficult for organically bound sulfur – as the exact compound and its geochemical context (e.g. bound to clay matrix) may not be known, or the labeled compound not be available. In this respect, isotopically labeled organic sulfur compounds are of limited use, and the use of isotopically labeled sulfate can only reveal the transfer rate of label to the organically bound sulfur pool, but not the mineralization of such compounds. For elucidating the role of microbes in making organically bound sulfur available for plants, this is rather inconvenient.

2.2 OXYGEN ISOTOPES AS TRACERS FOR SULFUR CYCLING

In principle, the restrictions mentioned for sulfur isotope studies also apply to oxygen isotopes. However, the great advantage of oxygen studies is the fact that during dissimilatory and assimilatory sulfur cycling sulfur-oxygen bonds are repeatedly made and broken. During these processes, the oxygen in newly formed sulfur-oxy compounds is derived from other compounds. In most cases, this compound is water, and to a much lesser extent phosphorous compounds, or dioxygen (O_2). These compounds are not only well known, they are also available as isotope labels which makes then the ideal tool to monitor the processes that yield plant-available sulfate, i.e. the conversion of a sulfate-ester to inorganic sulfate.

Moreover, the incorporation of new oxygen into sulfate by these processes is likely to yield distinct natural abundance isotope fractionations. The enzymatically catalyzed processes that yield sulfate from organically bound sulfur compounds are similar to the processes that yield phosphate from organically bound phosphorous compounds (Edwards et al. 2011). For the latter, it was shown that different enzyme systems leave distinct oxygen isotope patterns, that have the potential to be traced with the help of the oxygen isotope signature of phosphate (Tamburini et al. 2014, von Sperber et al. 2014, von Sperber et al. 2015). The expected natural variability in the stable oxygen isotope composition of sulfate opens the possibility to monitor soil sulfur transformations in the environment, without the need for the application of isotope labels.

In situ experiments with ^{18}O -labeled sulfate in acid forest soils demonstrated that the oxygen isotope composition of sulfate can be rapidly altered, presumably by microbial activity in the top 30 cm of the soil profile (Mayer et al., 1995b). These observations have been corroborated by the finding that also the natural abundance oxygen isotope composition of sulfate in forest soils changes with increasing depth (Mayer et al., 1995a). Combinations of stable isotope systems including stable sulfur and oxygen isotopes ($\delta^{34}\text{S}$, $\delta^{18}\text{O}$; Shanley et al., 2005) and radioactive ^{35}S as well as approaches using $\delta^{17}\text{O}$ (Bao et al., 2001; Johnson et al., 2001; Lee et al., 2001) have proven to be powerful at tracing sulfur turnover in the environment. To my knowledge, incorporation of oxygen from water into newly formed sulfate with the help of ^{18}O labeling has not been tried so far.

2.3 EXTRACTION OF SULFATE FROM ARID SOILS FOR ISOTOPE ANALYSIS

From the discussion above, it is evident that the sulfur and oxygen isotope analysis of sulfate from soils has a great potential to elucidate microbial sulfur cycling in soils from arid and semi-arid environments. Whereas the standing pools of organically bound sulfur and inorganic sulfur may

be large, this is likely not the case for or targeted inorganic sulfur pool, namely plant-available sulfate that may intermittently be released by microbial activity after rainfall events. For this pool, I expect low sulfate concentrations, in limited amounts of water. The technical challenge is to extract this sulfate without altering its isotope composition for subsequent isotope analysis. This is by no means a trivial endeavor, sulfate can be extracted from freshwater for isotope analysis (Carmody et al., 1998), but there is no extraction protocol that works for waters with a substantial anion (e.g. chloride or nitrate) content, which is exactly the type of soil water we expect to encounter in arid and semi-arid soil material. The reasons for the discrepancy between freshwater and anion-rich waters is as follows: for freshwater samples resin extraction techniques can be used (Carmody et al., 1998; Révész and Coplen, 2007; Kang et al., 2012; Turchyn et al., 2013). This technique fails with anion-rich solutions because sulfate is no longer quantitatively trapped since other anions compete with sulfate anions for the positively charged sites on the resin (Arnold et al., 2014).

3. GOALS OF THIS STUDY

With this study, I pursued two goals (Text Box 3).

Text Box 3:

Goals

- Establish a protocol for the extraction of sulfate from low-sulfate, high salinity solutions for sulfur and oxygen isotope analysis.
- Carry out a pilot study to quantify sulfate release by mineralization of organically bound sulfur in arid soils.

To achieve my goals, I defined the following objectives (Text Box 4 and 5):

Text Box 4:

Objective 1: Sulfate extraction protocol

- Production of sulfate-free artificial seawater and sulfate stock solution so that a suite of low-sulfate, high salinity samples can be tested.
- Precipitation of sulfate as barium sulfate from the test solutions by established procedures.
- Recovery of barium sulfate precipitate with the help of a chelator.
- Re-precipitation and collection of sulfate in a small-volume centrifuge tube.
- Testing of protocol for different sulfate concentrations, with test criteria being sulfate recovery, and comparison of sulfur and oxygen isotope composition of sample to original sulfate stock.

Text Box 5:

Objective 2: Pilot Study

- Selection of field sites with a variety of sulfate sources.
- Quantification and isotope analysis of different sulfur pools from selected sites to establish inventory of sulfur compounds.
- Experiments to establish sulfur turnover after rainfall events by monitoring change in sulfur and oxygen isotope composition of sulfate extracted from soil water and determine changes in the isotope composition of sulfate for experiments with natural abundance and also ^{18}O labeled water.

4. A NEW METHOD FOR ULTRA-LOW SULFATE EXTRACTION

4.1 BACKGROUND

A method to collect dissolved sulfate and sulfide was published by the USGS (U.S. Geological Survey) in 1998 (Carmody et al., 1998), where an anion exchange resin method is used to collect sulfate from water samples. In Carmody et al. (1998), sulfate collection method was performed in samples which contain high concentrations of sulfate equal or greater than 1 mM with approximately 200 to 1000 mM of chloride present, low concentrations samples which contain less than 0.2 mM sulfate with chloride present and samples with low sulfate concentration (0.1 to 0.01 mM) that were chloride free, and low sulfate samples (0.1 to 0.01 mM) which contained high chloride (50 to 300 mM).

Three types of samples (high-sulfate + high Cl^- , low-sulfate + less Cl^- , and low-sulfate + no Cl^-) perform well under the anion exchange resin procedure. However, samples with low sulfate concentration and high chloride did not perform well with the use of the anion exchange resin method compared to the other set of samples. Low sulfate recovery is complicated in presence of dissolved chloride as sulfate co-elutes with chloride through the anion exchange resin and yielded highly variable sulfur isotope values (Carmody et al., 1998). At low concentrations of sulfate in seawater (0.5 mM), the usual method of barium sulfate precipitation method is also not successful (Arnold et al., 2014).

In this study, I developed an alternative approach to this challenge – I used a chelator to re-dissolve and concentrate barium sulfate that would normally be unrecoverable in large volume / low sulfate concentration sulfate samples with both high (e.g. seawater) and low (e.g. rainwater) chloride concentrations.

Why test with seawater matrix? High salinity samples represent the most difficult separation chemistry we can face in low concentration sulfate isotope analysis. My goal was to develop a method that can be used across all sample types, from freshwater to brine and a method that is capable of working with high ionic strength solutions, such as hydrochloric acid, which is used to dissolve carbonates for collection of carbonate associated sulfate.

4.2 PREPARATION FOR EXPERIMENTS

To begin development of a new ultra-low sulfate extraction, it was fundamental to prepare a set of reagents that are indispensable to obtain satisfactory results. Due to the ultra-low sulfate concentrations, all experimental steps are susceptible to contamination, either from the reagents directly or from residue on the glassware. To cope with the potential of contamination from reagents, one should purchase reagent chemicals that contain nominally less than 0.002% sulfate. To prevent contamination from residues on the glassware, all glassware used must be pre-cleaned with a diethylenetriaminepentaacetic acid (DTPA) solution. Here I briefly describe the preparation of glassware and reagents. Full details for recipes and chemicals used are provided in the appendix (Table A1).

4.2.1 Diethylenetriaminepentaacetic acid (DTPA) stock solution

Diethylenetriaminepentaacetic acid (DTPA) is a strong chelator, which can be used to dissolve barium sulfate (Bao, 2006). In my new method, DTPA had two uses, as a glassware cleaner and to dissolve barium sulfate in new extraction method. It is crucial to keep DTPA stock solution out of light and well-sealed, an amber bottle is preferred, but in the absence of an amber bottle, a normal clear glass bottle can be wrapped in aluminum foil. The average shelf life is about 6 months if stored and handled properly.

To produce a 0.05 M DTPA in 1 M NaOH stock solution, dissolve ~20 grams of DTPA in 1 L of 1 M NaOH solution (Appendix R1). Store in an amber or foil wrapped bottle out of direct light.

4.2.2 Cleaning glassware

To use of DTPA stock solution to clean glassware, add enough DTPA solution to assure an even coating on all surfaces of glassware (Appendix R2). Glassware should be closed with DTPA solution inside. Gently swirl glassware and cover walls with solution. Glassware can now be rinsed three times with ultrapure water.

4.2.3 Sulfate-free artificial seawater (SF-ASW)

To prepare sulfate-free artificial seawater (SF-ASW), I used (in descending order of quantity), sodium chloride, magnesium chloride, calcium chloride, potassium chloride, potassium bromide, sodium bicarbonate, boric acid, and strontium chloride (Appendix R3). It is important to make sure that the sodium chloride and magnesium chloride are as low in sulfate content as possible. The solution should be left on a magnetic stirrer overnight to ensure all the salts are completely dissolved. The recipe I followed does not control for precise concentrations, however, this preparation is appropriate enough for the preparation of the base stock of SF-AWS as the concentrations of salts in solution only needs to approximate the chloride concentration and ionic strength of seawater and not match exactly.

4.2.4 Sodium sulfate stock solution

In order to prepare the experimental samples, a pre-determined amount of sodium sulfate stock solution was added to a known amount of SF-ASW. To prepare the sodium sulfate stock solution (~10 mM), I dissolved ~ 0.7 g of sodium sulfate in 500 ml of ultra-pure (18 M Ω) water (Appendix R4).

4.2.5 BaCl₂ stock solution

Barium chloride stock solution (~1M BaCl₂ in 0.05M HCl) was added to each replicate of the experiment to precipitate sulfate in solution as barium sulfate. To prepare this solution, ~123 g barium chloride dihydrate, 2.5 ml of hydrochloric acid and ultra-pure water are needed (Appendix R5). The acidification of the solution with hydrochloric acid prevents the precipitation of barium carbonate, which would take place because carbon dioxide from the atmosphere is taken up into the solution.

4.3 NEW EXTRACTION METHOD

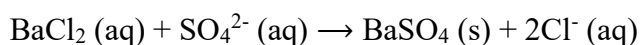
The basic principle to test the new extraction method is as follows; sulfate with a known sulfur and oxygen isotope composition is added in varying amounts to either SF-ASW or ultra-pure (18M Ω) water to obtain a range of sulfate concentration. This sulfate is collected using the new extraction method and analyzed for its sulfur and oxygen isotope composition. The goal is to obtain full sample recovery and a match in isotope composition to the known composition of the originally added sulfate (Figure 4, Appendix R6). The new extraction method can be summarized in six stages that take the sample from initial barium sulfate precipitation, through collection, dissolution and final re-precipitation (Figures 5 and 6).

Stage 1 – Preparation of solutions with a range of sulfate concentrations

The ultra-low sulfate extraction experiments were constructed using a combination of sulfate stock solution and either SF-ASW or ultra-pure (18M Ω) water. Sample preparation was implemented on a series of varying sulfate concentration samples, spanning a range of 0.5 to 0.01 mM sulfate (for example see Table A2). Samples of SF-ASW and ultra-pure (18M Ω) water alone were also prepared. All experimental samples were prepared in replicates of three. Using the sodium sulfate stock solution (Appendix R4), I prepared each replicate and added the correct amount for each

target concentration (Table 1). Preparation was started by weighing empty 50 ml centrifuge tubes, adding the SF-ASW or ultra-pure water, followed by the addition of the appropriate amount sulfate stock solution. At each step the weight of the solutions was recorded so that the concentration and the total amount of sulfate could be gravimetrically determined.

To precipitate the dissolved sulfate as solid barium sulfate, I added 1 ml of ~1 M barium chloride stock solution to each replicate to form barium sulfate precipitate as described in the reaction below. The samples were left to sit overnight.



Before proceeding to stage 2 in the extraction protocol, one needs to prepare the micro centrifuge tubes that will be needed mid-protocol. Two 1.5 ml centrifuge tubes will be needed for each replicate. One of these two 1.5 ml centrifuge tubes will be weighed using a microbalance. Remaining centrifuge tube will be used to store filter in **Step 5**. Record replicate description and weight of each centrifuge tube and label tubes. Samples are filtered using a 0.2 μm PES filter and filtration device (Figure 7).

Stage2. Collection of suspended barium sulfate – the centrifuge tube (50 ml) with barium sulfate precipitates was mixed using a vortex mixer for at least five seconds to ensure solution is well mixed and as much barium sulfate was in suspension as possible. Next, the solution from centrifuge tube is poured into filtration device trying to aim the center not the walls of the device. I used a handpump to pull the vacuum for the filtration device. Pump until entire solution is filtered. Using ultra-pure water in a small squeeze bottle, the filtration device is rinsed down three times to assure coverage of filtration device. It is important to thoroughly rinse the bottom of container of filtration device to ensure that all barium sulfate gets stuck to the filter paper and is not clinging to the walls of the filtration device (Figure 7).

After filtering, the filter must be recovered and placed into a 1.5 ml centrifuge tube (tube with pre-recorded weight), by removing it carefully with tweezers and rolling filter to fit into the centrifuge tube without touching or displacing the barium sulfate in the filter.

Stage 3. Collection of barium sulfate from centrifuge tube – Barium sulfate precipitates formed from adding barium chloride will adhere to the walls of the centrifuge tube used in the initial sample preparation. Since the main goal of this new method is to recover all sulfate and avoid any losses. To accomplish this, DTPA is used to dissolve this barium sulfate from the walls of the centrifuge tube. To proceed, add 1 ml of DTPA stock solution to 50 ml centrifuge tube that initially contained barium sulfate solution and artificial seawater. Swirl DTPA through 50 ml centrifuge tube in order to dissolve barium sulfate from centrifuge tube walls.

Stage 4. Dissolution of barium sulfate from filter – Once barium sulfate from the 50 ml centrifuge tube is dissolved by the DTPA, pipet the DTPA into 1.5 ml centrifuge tube with filter. Ensure that the volume of DTPA solution added to the 1.5 ml tube completely covers the filter. This will dissolve the barium sulfate from the filter paper. Let samples dissolve overnight.

Stage 5. Re-precipitate barium sulfate – Carefully remove the filter from 1.5 ml centrifuge filter and place filter in remaining 1.5 ml centrifuge tube for storage. To the 1.5 ml centrifuge tube with the dissolved barium sulfate solution, add 120 μ l of hydrochloric acid. The addition of the hydrochloric acid ‘breaks’ the chelating abilities of the DTPA solution and frees the barium and sulfate to reprecipitate. Next add 50 μ l of barium chloride stock to ensure excess barium in solution. Let solution re-precipitate barium sulfate overnight.

Step 6. Wash and dry barium sulfate in preparation for isotope analysis – Vortex 1.5 ml centrifuge sample for 5 seconds to assure solution is in suspension and ready to centrifuge. Next, the sample was centrifuged for 10 minutes. Carefully pipet off supernatant from tube, add 1.5 ml of ultrapure

water, vortex and centrifuge for 10 minutes. Repeat previous step one more time. After pipetting supernatant from centrifuge tube, add 1.5 ml of acetone, vortex and centrifuge one more time for 10 minutes. Carefully pipet the supernatant and place 1.5 ml centrifuge tube in a rack with lid open and cover with a large sheet of aluminum foil without touching tubes, then let samples dry overnight.

4.4 PREPARATION FOR SULFUR AND OXYGEN ISOTOPE ANALYSIS

4.4.1 Preparation for sulfur and oxygen isotope analysis

The sulfur and oxygen isotope composition of my sulfate samples was measured in-house in the EaSI Lab (**E**lemental **a**nd **S**table **I**sotope **L**aboratory) of Dr. Brunner in the Department of Geological Sciences at UTEP using a continuous-flow isotope ratio mass spectrometer (CF-IRMS). For sulfur isotope analysis, approximately 0.48 mg to 0.55 mg of sample was weighed into a tin cup. An equal amount of vanadium pentoxide, a catalyst that aids in the conversion of sulfate into sulfur dioxide, was added. Using tweezers, the tin cup was then carefully crimped at the top, and gently shaken to mix the catalyst with the sample. The tin cup was then folded into a small ball by sealing and folding edges of tin capsule and/or boat, making sure that sample and catalyst do not leak out. Samples were then combusted for conversion of sample sulfur into sulfur dioxide using an Elementar Pyrocube, followed by isotope analysis of the evolved gas with an IsoPrime GeoVisION CF-IRMS. Isotope compositions are reported in delta notation relative to the Vienna Canyon Diablo Troilite (VCDT) standard, as follows:

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

For oxygen isotope analysis, the preparation of samples is similar to preparation for sulfur isotope analysis, but the use of tin capsules/boats is replaced by silver capsules/boats. After the sample

(~0.45 mg) is added to the silver capsule, approximately 0.2 mg to 0.4 mg of graphite (C) is added to each silver boat and silver capsule. Carbon from graphite binds to oxygen from barium sulfate creating carbon monoxide (CO). Next, as a catalyst, about 0.2 mg to 0.4 mg of silver chloride (AgCl) is added to each sample. Silver from silver chloride will bind to sulfur from barium sulfate creating silver sulfide (Ag₂S). Chloride from silver chloride will bind to barium from barium sulfate forming barium chloride (BaCl₂). Once sample (of recorded weight) and additives are added to the silver capsules, the capsules are carefully crimped and rounded into a ball-shaped form. Samples were then pyrolyzed for conversion of sample oxygen into carbon monoxide using an Elementar Pyrocube, followed by isotope analysis of the evolved gas with an IsoPrime GeoVisION CF-IRMS. Isotope compositions are reported in delta notation relative to the Vienna Standard Mean Ocean Water (VSMOW) standard, as follows:

$$\delta^{18}\text{O} = \left(\frac{R_{\text{sample}}}{R_{\text{VSMOW}}} - 1 \right) \cdot 1000 \text{ ‰}$$

For all isotope analyses, international standards, NBS-127, IAEA SO-5, IAEA SO-6 as well as in-house standards were used for calibration. Based on repeated analysis of standards, the reproducibility of the sulfur isotope composition measurements is +/- 0.3‰ and the reproducibility of the oxygen isotope composition measurements is +/- 0.5‰.

For easily visible (after centrifugation and drying procedure) barium sulfate samples (e.g. experimental samples 0.5 mM, 0.25 mM and 0.1 mM) the barium sulfate can be transferred to sample capsules, as described above. Unfortunately, for extremely low sulfate concentrations (0.05 mM, 0.03 mM and 0.01 mM) it was not possible to precipitate an amount of barium sulfate that could be manually transferred in dry powder form. For such sample samples (< 0.1 mg), I employed an acetone transfer procedure.

4.4.2 Using acetone for extremely low barium sulfate

Samples with barely visible barium sulfate were transferred from the 1.5 centrifuge tube with acetone. For visible samples, I used 3.5 x 5 mm tin or 3.2 x 4 mm silver capsules. For barely visible samples it was necessary to use larger (4 x 4 x 11 mm) tin or silver sample boats since the volume of acetone used for the transfer needs to be accommodated. For extremely small samples, it is crucial to weigh the sample boats; the weight was recorded along with the sample name description in my laboratory notebook. It is important to weigh the sample boats since the weight of the transferred barium sulfate sample is unknown and post-analysis yield calculations require that the weight of the sample is known prior to combustion/pyrolysis. In total, about ~400 μl of acetone was added to the sample boats into which the small barium sulfate samples were transferred. First, ~200 μl of acetone was pipetted into 1.5 centrifuge tube with the dry barium sulfate sample. Using the same pipette, acetone was distributed through the centrifuge tube by pipetting in and out, assuring most of barium sulfate found on the tube walls and bottom was collected. A small amount of this 200 μl acetone plus barium sulfate was meticulously transferred to its corresponding sample boat. Adding more acetone than the sample boat can hold will cause an overflow and loss of sample. After the first round of acetone has been let to dry in the boat, the rest of the acetone was added. Sample drying was accelerated by placing the tin boats on a hot plate set to the lowest heat setting. After all sample was transferred and dried into the sample boat, the final weight of the boat plus sample was recorded.

4.4.3 Additional preparation for oxygen isotope analysis of ultra-low concentration samples

The use of acetone in sample preparation for oxygen isotope analysis may contribute to sample contamination due to the interaction of acetone with the plastic sample tubes, contribution excess

mass and carbon and/or oxygen content to the sample. Following the acetone procedure, to eradicate any organic residue from the acetone transfer process in the samples, these ultra-low samples were combusted using a muffle oven after the final acetone dry-down. To do this, a muffle oven was pre-heated to 450 °C. Open silver boats with ultra-low samples were placed on a Pyrex glass petri dish. When muffle oven's temperature reached 450 °C, the petri dish was carefully placed inside the muffle oven using a long crucible tong. The muffle oven was closed and the samples 'baked' in the oven for 1 hour. After 1 hour, the hot petri dish was carefully removed and placed on a high temperature safe surface and the samples were left to cool for approximately 30 minutes. When silver boats were cool enough, the samples were weighed on a microbalance.

4.5 RESULTS AND DISCUSSION

4.5.1 Preliminary Experiments

Preliminary experiments were performed in order to test the new ultra-low extraction method's reproducibility using artificial seawater (Figure 8, Table 2). The first three preliminary experiments with five replicate samples each were carried out using 0.5 mM sulfate concentration samples. The first set of samples (A_MMo, B_MMo, C_MMo, D_MMo, and E_MMo) have an average $\delta^{34}\text{S} = 1.4 \pm 0.3\text{‰}$. This first sample set was likely contaminated with sulfate from tap water. The second set of samples (1A_MMo, 1B_MMo, 1C_MMo, 1D_MMo, and 1E_MMo) have an average $\delta^{34}\text{S} = 2.1 \pm 0.1\text{‰}$. The third set of samples (2A_MMo, 2B_MMo, 2C_MMo, 2D_MMo, 2E_MMo) have an average $\delta^{34}\text{S} = 2.1 \pm 0.1\text{‰}$. Results from the second and third sets of samples in the preliminary experiments show a constant robust recovery of sulfate, validating the reproducibility of this new method. When comparing only the second and third experiments the average sulfur isotope composition of the recovered sulfate is $2.1 \pm 0.1\text{‰}$. I observe an offset

of $\sim 0.7\text{‰}$ between the samples and the sulfur isotope composition of the sodium sulfate used to make the stock sulfate and as well as the sulfur isotope composition of the barium sulfate precipitated directly for the stock sulfate solution (Table 3).

To explore the extraction method at lower sulfate concentrations, I employed two sets of experiments with three replicates each (A_0.5_MMo, B_0.5_MMo, C_0.5_MMo, A_0.25_MMo, B_0.25_MMo and C_0.25_MMo). Samples for 0.5 mM concentration (A,B,C) resulted in a $\delta^{34}\text{S}$ of $2.3 \pm 0.1\text{‰}$ and 0.25 mM samples (A,B,C) in a $\delta^{34}\text{S}$ of $2.9 \pm 0.1\text{‰}$.

Although it appears that the sulfur isotope composition of the 0.5 mM concentration samples are slightly heavier than the previous experiments, it should be noted that these sulfur isotope samples were among the first ‘real’ analysis runs after the establishment of the EaSI Lab and during this time moderate drift in the stability of the reporting standards was observed. None the less, the reproducibility of each replicate set is good. The difference of $\sim 0.6\text{‰}$ between the 0.5 mM and 0.25 mM samples, as well as the offset between the 0.5 mM and the sodium sulfate are significant and were the first hints of the presence of a sulfur contaminant in my experiments with variable sulfate concentrations. With these first variable sulfate concentration samples I also measured the oxygen isotope composition of the recovered sulfate. The oxygen isotope composition of the recovered sulfate was $8.9 \pm 0.4\text{‰}$ (Table 2), a result in strong agreement with the oxygen isotope composition of barium sulfate precipitated from the stock sulfate solution ($9.1 \pm 0.4\text{‰}$, Table 3).

4.5.2 Artificial seawater (ASW) Experiments

My next set of experiments was performed using artificial seawater and six different concentrations were measured with three replicates each (0.01 mM, 0.03 mM, 0.05 mM, 0.1 mM, 0.25 mM and 0.5 mM). The ‘high’ concentrations of 0.5 mM, 0.25 mM and 0.1 mM, respectively,

resulted in a yield of $\delta^{34}\text{S} = 1.9 \pm 0.0\text{‰}$, $\delta^{34}\text{S} = 2.5 \pm 0.1\text{‰}$, and $\delta^{34}\text{S} = 4.4 \pm 0.1\text{‰}$ (Table 4). Low concentrations of 0.05 mM, 0.03 mM and 0.01 mM, respectively, yielded results of $\delta^{34}\text{S} = 6.6 \pm 0.3\text{‰}$, $\delta^{34}\text{S} = 8.6 \pm 0.0\text{‰}$ and $\delta^{34}\text{S} = 6.1 \pm 3.0\text{‰}$ (range of 4.0 to 11.6‰). Following the observation of the first variable concentration experiments, sulfur isotope composition tends to get heavier as sulfate concentration decreases (Figure 9A). Considering that as concentration decreases, the amount of barium sulfate decreases, and sulfur isotope composition increases, this raises the question if a sulfate contaminant is present and where it is coming from.

The oxygen isotope composition of sulfate recovered in these experiments displays no such trend (Figure 9B). The oxygen isotope compositions of the variable concentration sample sets (from high to low concentration, excluding the 0.01 mM sample set) are $9.7 \pm 0.5\text{‰}$, $8.9 \pm 0.2\text{‰}$, $9.2 \pm 0.4\text{‰}$, $9.5 \pm 0.1\text{‰}$, $9.3 \pm 1.3\text{‰}$, with an overall average of $9.2 \pm 0.6\text{‰}$ (Table 4), is in strong agreement with the oxygen isotope composition of barium sulfate precipitated from the stock sulfate solution ($9.1 \pm 0.1\text{‰}$, Table 3). The fact that the oxygen isotope composition is in strong agreement with the known value of the sulfate, while the sulfur isotope composition gets progressively heavier with decreasing experimental sulfate concentrations should not necessarily be interpreted as a sulfur contaminant absent of an additional sulfate source. Instead, it may reflect the presence of a sulfate contaminant with an oxygen isotope composition that is similar to the sulfate used in the experiment.

4.5.3 Sources of contamination in the ASW experiments

To clarify the source of the contaminant, I prepared a series of experiments where both the 18M Ω H₂O used to prepare the SF-ASW and the SF-ASW itself were tested using the ultra-low sulfate extraction method where no additional of sulfate was added to the samples.

These experiments begin with the assessment of a potential contaminant in the 18M Ω water. This experiment also serves to verify that any sulfur contaminant was not a procedural blank, present in 18M Ω H₂O, glassware, or filter. Although there is an apparent calculated weight of sample recovered at the end of these experiments (Table 5), the residue failed to produce any sulfur signal during isotopic analysis, indicating that whatever the residue in the sample vials is, it is not sulfate. These results confirm that the contaminant is not present in the 18M Ω H₂O, filter, and/or glassware, nor is it a procedural error.

Similar experiments with the SF-ASW yielded a different result all together. The extraction method produced larger samples and these samples produced measurable sulfur isotope compositions with a sulfur isotope composition of $\sim 34\%$. This value agrees well with the value predicted from the trend of the curve plotted through the experimental data (Figure 9A). This indicates that in all likelihood, the source of the contaminant sulfate in the SF-ASW are the salts used to make the solution and not the 18M Ω water.

After finding that the contamination source was not the extraction method procedure in general, I suspected two major contaminant factors, either the sodium chloride (NaCl) or the magnesium chloride (MgCl₂). Sodium chloride was the most abundant salt added to the SF-ASW. Based on its certification sodium chloride contains less than 0.002% of sulfate. Next suspect was magnesium chloride, and contains up to $\sim 0.004\%$. Likely, the contaminant source is the magnesium chloride.

4.5.4 18M Ω water experiments

As my goal is to develop a new ultra-low sulfate extraction method and establish the limits of this method, I proceeded next to replicate the ASW experiments using 18M Ω water amended with sulfate stock. In these experiments, four different sulfate concentrations were used. Sulfate concentrations, 0.5 mM, 0.1 mM, yielded results of $\delta^{34}\text{S} = 1.1 \pm 0.2\%$, $\delta^{34}\text{S} = 1.6 \pm 0.3\%$ (Figure

10A, Table 6). Sample replicates for 0.03 mM and 0.01 mM failed to produce a transferrable amount of barium sulfate. As a matter of routine, all isotope analyses are accompanied by the analysis of the instrumental yield of the sample. In simple terms, did the weight of the alleged sample produce an appropriate amount of sample gas as a result of combustion/pyrolysis? The answer for the 0.03 and 0.01 mM samples is ‘no’, suggesting again that not all the sample mass measured at the conclusion of the extraction method is barium sulfate.

To overcome this, I scaled up one of the lower concentration sample sets. Nominal volumes of initial samples used in the development of this method were ~45 ml. To ensure that I would recover enough actual barium sulfate, I increased the volume in the final set of experimental samples to ~200 ml. Sulfur isotope analysis from these samples yielded a result of $1.3 \pm 0.1\text{‰}$ (Table 6), a value in excellent agreement with the higher concentration samples in this experiment and the known value of the sulfate stock. Additionally, I measured the oxygen isotope composition of the barium sulfate from both the 0.5 mM samples and the large-volume 0.03 mM samples. These samples yielded oxygen isotope compositions of $9.2 \pm 0.4\text{‰}$ and $9.1 \pm 0.1\text{‰}$, respectively, again in unerring agreement with the known value of the sulfate stock solution used (Figure 10B, Table 3).

4.6 CONCLUSIONS – ROBUSTNESS OF NEW ULTRA-LOW SULFATE EXTRACTION METHOD

Although several methods exist for the extraction and analysis of low sulfate concentration solutions (Carmody et al., 1998; Gendre et al., 2016), my new method provides improvements over these methods on several levels. The original resin-method of Carmody et al. (1998) only worked for low-sulfate waters that were chloride free. Even with no chloride present, that method required 10s to 100s of liters of water of sample. While this resin method could produce enough

barium sulfate from a 0.01 mM sulfate sample, no oxygen isotope data results were presented for any of the test solutions. The latest resin method (Gendre et al., 2016) reports the reproducibility of oxygen isotope analysis of sulfate, with the specific target of ^{17}O analysis, however, the ‘low’ sulfate concentration of this study was actually 1 mM. My new method improves upon this by successfully processing samples that are 30-fold lower in concentration. Perhaps the tightest comparison with chloride-free solutions that can be made is to the work of Kang et al. (2014), which successfully analyzed 0.05 mM sulfate groundwater, but still required 1000 ml of sample to do so. Above all, my new method can handle high chloride concentration solutions, producing barium sulfate for the analysis of both sulfur and oxygen isotopes at concentrations as low as 0.03 mM, something that no other existing method can do.

4.6.1 Caveats of the method

Despite the overwhelming success of my new method development, there are still some considerations to reflect on. First is, although it can be demonstrated that there is no procedural blank with regards to sulfate that is picked up during the extraction procedure, some excess mass of some type is. This is problematic as it obscures whether the procedural yield is satisfactory or not. For example, in my ‘low’ sulfate concentration test samples, and particularly the 0.01 mM ASW extraction test samples, I should produce enough barium sulfate to have a small, but nicely visible portion of barium sulfate. While the measured weights suggest that the sample should be there, yield analysis for the isotope analysis side reveals that not enough barium sulfate is present. The positive result is that whatever step is resulting in a low barium sulfate yield on the preparatory side of the method, this inefficiency is not resulting in an isotopically shifted sample. Improvements in the extraction efficiency would remove the need to utilize the acetone transfer procedure and hence the need to combust the sample in a muffle oven to remove the excess carbon

blank induced by the use of the acetone. Improvements in efficiency would also reduce the need to increase sample size. Even though my 'large', 200ml sample is still 5x smaller than other methods, an improvement to the minimum required sample volume would expand the realm of sample types this method could be applied to.

5. PILOT STUDY – APPLICATION OF NEW ULTRA-LOW SULFATE EXTRACTION METHOD

To validate my new ultra-low sulfate extraction method in real life soil samples, I conducted a pilot study on arid soils in the El Paso region (Figure 11). Soil samples were collected at three sites, two samples were collected near the Indio Mountains Research Station, owned by UTEP located near Van Horn, Texas, another arid soil sample was collected on the ‘Lost Dog Trail’ hiking trail in El Paso (31.884524, -106.551486), and another soil sample was collected off of Highway 54 at mile-marker-1 (32.875700, -105.966427), approximately ~16 miles from The White Sands National Monument Park. Additionally, a Positive Control (PC) sample was obtained from a pecan farm near Las Cruces, New Mexico from Anna Ortiz, a Ph.D. candidate in the Department of Geological Sciences at UTEP. The main reason for sample contrast in the type of soil used for this experiment (sample close to White Sands, sample from an agricultural site and remote site in Van Horn, Texas) was to test sulfate availability from three different scenarios. It is important to take in consideration that the Highway 54, mile-marker-1 sample (Hwy54-I) is in close proximity to a large gypsum (calcium sulfate) source, the White Sands National Park where high concentrations of gypsum (fields of dunes of gypsum) are found. It is a logical conclusion that any dust originating from or settling nearby to this area will be rich in gypsum and so, very rich in sulfate. The Pecan farm agricultural sample (PC) represents a site where fertilizers and irrigation, both good sources of sulfate, are used constantly, and so, whether or not sulfate would have been found naturally in the soil prior to agricultural practices, sulfate is certainly present now. The Lost Dog Trail sample represents a typical arid soil for the El Paso region. Finally, the Indio Mountain Research Station samples (Indio Mtn I, 30.772777, -105.000277 & Indio Mtn II, 30.777222, -104.998888) represent a remote location, free from anthropogenic influence, and is a great example of an arid environment. The limited site selection for this study is not meant to

represent the wide range of arid soils present in the El Paso region. My arid soil samples were simply used for the first assessment of whether the sulfur and oxygen isotope composition of any sulfate present can be used to identify the source of sulfate to that arid soil, and if sulfate is present, to test if the isotope composition of that sulfate can be further used to assess the biogeochemical cycling of sulfur in soils.

5.1 SOIL COLLECTION AND PREPARATION

A surface soil sample (about 10 cm deep) was collected from the Highway 54, Indio Mountain Research Station, and Lost Dog Trail sites, making sure soil was collected in close proximity of vegetation. Then, samples were placed into “Ziploc” plastic bags, labelled, and brought back to the laboratory for analysis.

5.2 EXPERIMENT SET UP

I hypothesized both Hwy54-I and PC samples would contain a considerable amount of sulfate compared to the Lost Dog Trail and Indio Mtn I and II samples; Hwy54-I due to its proximity to White Sands National Park and PC sample due to the use of fertilized in pecan farm; and Lost Dog Trail and Indio I and II would have less sulfate due to how remote the sites are from an urban area and also due to its soil type (calcite).

To set up the soil sulfate extraction, four 50 ml centrifuge tubes were weighed and five grams of each soil site was added individually into each centrifuge tube. Five grams of Hwy54-I was added to one centrifuge, five grams of Indio Mtn I was added to another centrifuge tube, five grams of Indio Mtn II into another centrifuge tube, and five grams of PC sample into fourth centrifuge tube. Each centrifuge tube was weighed with soil and recorded. A sequential extraction procedure was

performed with these samples, to assess the variable availability of sulfate in the soil. Post-sequential leaching protocol will proceed with ultra-low sulfate extraction method.

5.3 METHODS – SEQUENTIAL LEACHING

In order to assess the pools of sulfate available in the soils collected for my pilot study, I employed a two-step sequential extraction method. The first step, the water ($18\text{M}\Omega\text{ H}_2\text{O}$) leaching recovers Readily Available Sulfate (RAS), the portion of sulfate that would become immediately bio-available after a rain event, likely resulting from the partial/incomplete dissolution of gypsum in the soil. The second step is a sodium chloride extraction step, which recovers what is termed Easily Soluble Sulfate (ESS). This step recovers the remainder of the sulfate present in the soil that would become bio-available over longer time-scales and includes mineral phases such as gypsum

5.3.1 Readily Available Sulfate (RAS)

Ultra-pure water ($18\text{M}\Omega\text{ H}_2\text{O}$) was added to each 50 ml centrifuge tube (with 5 grams of soil) to the 40 ml mark, the weight was recorded afterwards. The leaching process was employed for 24 hours using a revolver rotator. After 24 hours, samples were mixed by a using vortex to ensure that any RAS in the soil was completely dissolved. The samples were then centrifuged for 5 minutes at 3500 RPM speed. The supernatant was collected and filtered using a $0.45\text{ }\mu\text{m}$ filter and 50 ml syringe and placed into a different and clean 50 ml centrifuge tube. After the sample was filtered, $120\text{ }\mu\text{l}$ of HCl and 1 ml of BaCl_2 were added to each filtered sample and mixed. Samples were left sit overnight. After 24 hours, these samples underwent the ultra-low sulfate extraction method.

5.3.2 Easily Soluble Sulfate (ESS)

NaCl stock solution – To employ NaCl leaching of samples, a sodium chloride stock solution was prepared. My goal was to prepare a 2 M NaCl stock solution. First, 58.44 g of NaCl were added to a 500 ml graduate glass bottle. Then, ultra-pure water (18M Ω H₂O) was added to 500 ml mark in bottle. Let stir for 30 minutes or until NaCl is not visible anymore.

To 50 ml centrifuge with remaining soil, I added the 2 M NaCl stock solution to 40 ml mark in centrifuge tube and the same protocol as was used with the 18M Ω H₂O leaching procedure was followed with sodium chloride leaching process. The leaching process was employed for 24 hours using a revolver rotator. After 24 hours, samples were mixed by using vortex to ensure soil was completely suspended and all the solution was well mixed. Samples were then centrifuged for 5 minutes at 3500 RPM speed. The supernatant was collected and filtered using a 0.45 μ m filter and 50 ml syringe and placed into a new, clean 50 ml centrifuge tube. After sample was filtered, 120 μ l of HCl and 1 ml of BaCl₂ were added to each filtered sample and mixed. Samples were left sit overnight. After 24 hours, these samples underwent the ultra-low sulfate extraction method.

5.4 INITIAL RESULTS

No visible barium sulfate was observed in either the RAS or ESS leaching samples for the Lost Dog Trail and Indios Mtn I and II samples. For these samples, I proceeded with the application of my new ultra-low sulfate extraction method to see if any barium sulfate could be recovered and concentrated. Significant quantities of barium sulfate were visible in both Hwy54-I and PC samples at the initial barium sulfate precipitation stage and no further processing was required prior to isotope analysis.

These initial results qualitatively support my hypothesis that there should be detectable amounts of sulfate in the soil collected in proximity to White Sands and in the agricultural sample that had been exposed to fertilizers and irrigation, but that little to no sulfate would be present in the arid soils from the Lost Dog Trail and Indio Mountains samples. After the ultra-low sulfate extraction method was completed for the Lost Dog Trail and Indio Mtn I and II samples, sulfur isotope analysis was performed for all samples in the pilot study. No barium sulfate was recovered for the Lost Dog Trail sample, but small amounts (likely < 100 mg) of barium sulfate was recovered for the Indio Mtn samples.

Sulfur isotope analysis results (Figure 12) for Hwy54-I yielded a sulfur isotope composition of 11.0‰ for the RAS sample and ESS sample, a sulfur isotope composition of 10.8‰. These values are analytically indistinguishable from each other and likely represent the sulfur isotope composition of the gypsum sourced from White Sands. The agricultural sample (PC) yielded result of $\delta^{34}\text{S} = 5.5\text{‰}$ for the RAS sample and for the ESS sample, a sulfur isotope composition of 7.3‰. Interestingly, the bulk of sulfate in the PC sample was recovered from the RAS sample, indicating that the sulfate present is readily accessible, from either residual fertilizer or salts precipitated from irrigation waters, and not a gypsum/dust source, as also indicated by the distinct sulfur isotope composition of the RAS and ESS.

The Indio Mtn samples, although very small, yielded interesting sulfur isotope composition data. The RAS sulfur isotope composition was 2.6‰ and 3.9‰ for Indio Mtn I and II, respectively. The ESS samples yielded a $\delta^{34}\text{S} = 10.7\text{‰}$ for Indio Mtn I and 13.7‰ for Indio Mtn II. At first glance, these values might all appear significantly distinct from each other as nominal analytical precision for sulfur isotope analysis is maximum $\pm 0.5\text{‰}$. However, sulfur isotope analysis yield assessments indicate that the amount of barium sulfate actually present in the sample is extremely

low, on the order of 10 to 25%, (~20 to 40 μg of barium sulfate analyzed) pushing the limits of analytical accuracy. Taking a holistic view of the data, it can be seen that there are two sources of sulfate present in the Indio Mtn soils. The RAS is isotopically light ($\delta^{34}\text{S} = \sim 3\text{‰}$), consistent with what can be expected from sulfate that is precipitated from rainwater (Wu and Han, 2015; Torfs and Van Grieken, 1997). In contrast, the sulfate in the ESS fraction is heavier by 9‰ ($\delta^{34}\text{S} = \sim 12\text{‰}$), and looks a great deal like the gypsum sourced from White Sands, potentially indicating long-distance transport as dust. Hence, the soils at the Indio Mountain Research Station have two potential sources of sulfate, sulfate introduced during rain events and sulfate transported to the mountain soils in the form of gypsum dust.

5.5 RAIN EVENT EXPERIMENTS

Intrigued by the small, but detectable, amount of sulfate that is likely introduced to arid soils during rain events, I conducted an experiment to see if the microbially community present in the soils is responsive to sulfate addition after a rain event. For the purposes of this portion of the study, I focused on the oxygen isotope composition of sulfate as an indicator of microbial activity. Previous studies in phosphate cycling have shown that different microbial processes and enzyme systems leave distinct oxygen isotope composition signatures. These processes have the potential to be traced with the help of the oxygen isotope signature of phosphate (Tamburini et al. 2014, von Sperber et al. 2014, von Sperber et al. 2015). If the same is true for the biogeochemical cycling of sulfate, the expected natural variability in the stable oxygen isotope composition of sulfate opens the possibility to monitor soil sulfur transformations in the environment.

5.5.1 Experimental design

Soil collected from the Indio Mountains Research Station (Indio Mt I & II) were combined, sieved to remove rocks and root particles, and distributed into eight 50 ml centrifuge tubes with approximately 40 grams of soil per tube. The Lost Dog Trail sample was similarly sieved and distributed. A ‘rainwater’ solution was prepared with ultra-pure water, sulfate and $^{18}\text{O}\text{-H}_2\text{O}$ isotope label. A concentration of 200 mM sulfate was chosen for use in this experiment. This value is approximately 3x higher than the maximum concentration of sulfate that has been observed in rainwater across the continental U.S., but is not outside the range of reasonable sulfate concentrations in rainwater globally (Wu and Han, 2015). The stock rainwater solution was labeled to an $^{18}\text{O}\text{-H}_2\text{O}$ composition of $\delta^{18}\text{O} = \sim 1262 \text{ ‰}$. With this experiment design, the cycling of sulfate can be readily traced as any ‘regenerated’ sulfate will incorporate oxygen from water and as the water is strongly labeled with ^{18}O , this ^{18}O will show up in the oxygen isotope analysis of the sulfate.

Approximately 20 ml of rainwater stock were added to each soil sample. This mimicked a heavy rain event, where the soil surface was briefly flooded and percolation of the ‘rainwater’ was allowed to take place over 24 hrs. Samples were then processed at T0 – 24 hrs after the rain event, T1 – 5 days after the rain event, and T2 – 12 days after the rain event. Sample processing only included the RAS extraction step.

5.5.2 Rainwater experiment results

For both the Lost Dog Trail sample set and the Indio Mtn sample set, two general observations are shared between these sample sites. First, as time proceeds in the experiment the amount of sulfate that is extractable decreases. This is qualitatively observed in the amount of barium sulfate that is

produced by the RAS extraction (see Lost Dog Trail samples, Table 8). Second, the oxygen isotope composition of the sulfate recovered increases exponentially over time (Figure 13, 14). That is to say, sulfate is also being regenerated as time proceeds reflected by the increasing incorporation of oxygen from the ^{18}O -labeled water used in the experiment. In summary, sulfate is being utilized by the microbial community, but some sulfate is also being turned over by that community.

Distinct between the two sample sets is that the size of the response between the two sites is different (Table 8). Recall that no sulfate was recovered from the initial Lost Dog Trail sample. Yet as soon as this soil has been exposed to a rain event, the response is immediate and proceeds at a faster pace than what is observed for the Indio Mtn samples (Figure 13, 14), where sulfate was recovered initially. It would appear that there is a microbial community in the Lost Dog Trail soil that is more dependent on sulfate, or uses sulfate more efficiently. Once this soil experiences any input of sulfate, the microbial community is ready to ‘take-off’ and utilize the sulfate available. The microbial community in the Indio Mtn sample set responded similarly, but at a slower pace, perhaps indicating that the community was not starved to sulfate prior to the artificial rainwater event.

5.6 CONCLUSIONS OF THE RAINWATER EXPERIMENTS

The results of this pilot study provide proof-of-concept that my new ultra-low sulfate extraction method can be successfully applied to the study of sulfate cycling in natural samples, specifically in this instance, arid soils with extremely low sulfate concentrations. Sulfate extraction from arid soils with extremely low sulfate availability ($< 0.5 \mu\text{mol}/\text{gram soil}$) from the Indio Mountains reveals that there are at least two sources of bio-available sulfate present. Additionally, this new method, in combination with oxygen isotope labeling of water, has been successfully applied to the study of sulfate cycling in these soils with low sulfate availability. Experimentally, one can

differentiate between the relative rates of the microbial response to a sulfate input and rainfall event, studying the response to sulfate even in soils where no sulfate was previously detectable, such as with the Lost Dog Trail soil.

6. CONCLUSIONS AND FUTURE WORK

Improvements to the ultra-low sulfate extraction method can still be made, particularly with respect to the procedural yield and the apparent excess mass that obscures the true amount of barium sulfate recovered, thereby inhibiting gravimetric sulfate concentration calculations. One potential source of this excess mass might include non-sulfur residue from the filter paper. Future investigations could seek to shorten the amount of time that the filter paper spends in the DTPA solution as this is the most logical source where excess mass could be picked up.

With the completion of this thesis, two major achievements have been reached. First, I have established a new method for the extraction of low amounts of sulfate from saline solutions for isotope analysis. I have assessed the accuracy and precision of the method with respect to the measurement of sulfate concentrations, and sulfur and oxygen isotope analyses. Moreover, I know where the lower sulfate concentration limit for the applicability of this method currently is. I anticipate that other researchers will take advantage of this method for a wide range of applications on sulfur cycling, which ranges from the extraction of sulfate from diverse solutions, aerosols, as well as unlithified sediments to ancient rocks. The results from this portion of my thesis work will be published in a peer-reviewed journal.

Second, I have provided the proof of concept that ^{18}O isotope labeling of water can be successfully used to evaluate sulfur transformations in arid soils. The comparison between the different sites allowed me that there is a significant difference in soil sulfur cycling in the wide variety of arid environments. These findings provide the scientific community not only with a new tool – ^{18}O isotope labeling of water – but also with a new perspective on the role of microbes for desert sulfur cycling.

7. TABLES

Table 1. Example experimental design for ~45 ml test solutions for ultra-low sulfate extraction.

Target Sulfate Concentration (mM)	Sodium Sulfate Stock Solution Needed (g)
0.50	2.127
0.03	1.063
0.10	0.425
0.05	0.212
0.03	0.127
0.01	0.042

Table 2. Preliminary experiments testing the reproducibility of low sulfate extraction method using an ASW media.

Sample Preparation Date	Sample Analysis Date	Target Sulfate Concentration	Sample Analysis Label	IRMS Yield (%)	$\delta^{34}\text{S}$ VCDT (‰)	Oxygen Isotope Analysis Date	Sample Analysis Label	IRMS Yield (%)	$\delta^{18}\text{O}$ VSMOW (‰)
4/27/2016	7/6/2016	0.5 mM	A_MM0	93.6	1.1				
4/27/2016	7/6/2016	0.5 mM	B_MM0	102.2	1.4				
4/27/2016	7/6/2016	0.5 mM	C_MM0	83.2	1.4				
4/27/2016	7/6/2016	0.5 mM	D_MM0	99.3	1.4				
4/27/2016	7/6/2016	0.5 mM	E_MM0	97.5	1.8				
				average	1.4				
				stdev	0.3				
6/1/2016	7/6/2016	0.5 mM	1A_MM0	98.9	2.01				
6/1/2016	7/6/2016	0.5 mM	1B_MM0	98.0	2.01				
6/1/2016	7/6/2016	0.5 mM	1C_MM0	96.8	2.09				
6/1/2016	7/6/2016	0.5 mM	1D_MM0	92.5	2.26				
6/1/2016	7/6/2016	0.5 mM	1E_MM0	93.2	2.12				
				average	2.1				
				stdev	0.1				
6/20/2016	7/6/2016	0.5 mM	2A_MM0	99.6	2.11				
6/20/2016	7/6/2016	0.5 mM	2B_MM0	101.2	2.15				
6/20/2016	7/6/2016	0.5 mM	2C_MM0	101.0	2.15				
6/20/2016	7/6/2016	0.5 mM	2D_MM0	97.5	2.17				
6/20/2016	7/6/2016	0.5 mM	2E_MM0	91.9	1.98				
				average	2.1				
				stdev	0.1				
6/22/2016	7/6/2016	0.5 mM	A_0.5_MM0	99.9	2.21	4/26/2017	A_0.5_blue_ASW	105	8.8
6/22/2016	7/6/2016	0.5 mM	B_0.5_MM0	99.8	2.19	4/26/2017	B_0.5_blue_ASW	115	8.5
6/22/2016	7/6/2016	0.5 mM	C_0.5_MM0	103.6	2.41	4/26/2017	C_0.5_blue_ASW	105	8.7
				average	2.3				
				stdev	0.1				
6/22/2016	7/6/2016	0.25 mM	A_0.25_MM0	102.6	2.81	4/26/2017	A_0.25_C_0.25_blue_ASW	103	9.3
6/22/2016	7/6/2016	0.25 mM	B_0.25_MM0	99.9	2.88	4/26/2017	B_0.25_blue_ASW	104	9.3
6/22/2016	7/6/2016	0.25 mM	C_0.25_MM0	107.3	3.05				
				average	2.9		average all samples	106.5	8.9
				stdev	0.1		stdev	4.6	0.4

Note – yields were calculated based on results of pure barium sulfate standards.

Table 3. Isotope composition of sodium sulfate and sulfate stock solution.

Sample Preparation Date	Sample Analysis Date	Sample Analysis Label	IRMS Yield (%)	$\delta^{34}\text{S}$ VCDT (‰)
-	9/23/2016	MaMo_Na2SO4_stock	104	1.4
-	9/23/2016	MaMo_Na2SO4_stock	103	1.5
-	9/23/2016	MaMo_Na2SO4_stock	104	1.4
		average	103.7	1.4
		stdev	0.6	0.1
-	7/6/2016	Na2SO4_MMo	105	1.4
-	7/6/2016	Na2SO4_MMo	108	1.4
-	7/6/2016	Na2SO4_MMo	110	1.4
		average	107.9	1.4
		stdev	2.5	0.0
6/15/2016	2/28/2017	MMlgo_BaSO4_Stock_1	97	1.2
6/15/2016	2/28/2017	MMlgo_BaSO4_Stock_2	98	1.2
6/15/2016	2/28/2017	MMlgo_BaSO4_Stock_3	98	1.2
		average	97.6	1.2
		stdev	0.8	0.0

Sample Preparation Date	Sample Analysis Date	Sample Analysis Label	IRMS Yield (%)	$\delta^{18}\text{O}$ VSMOW (‰)
6/15/2016	4/25/2017	BaSO4_LSE	104	9.7
6/15/2016	4/25/2017	BaSO4_LSE	106	9.1
6/15/2016	4/25/2017	BaSO4_LSE	105	9.5
6/15/2016	4/25/2017	BaSO4_LSE	110	9.5
6/15/2016	4/25/2017	BaSO4_LSE	106	8.8
6/15/2016	4/25/2017	BaSO4_LSE	107	8.7
6/15/2016	4/25/2017	BaSO4_LSE	106	8.5
6/15/2016	<i>4/25/2017</i>	<i>BaSO4_LSE</i>	<i>108</i>	<i>9.8</i>
6/15/2016	4/26/2017	BaSO4_LSE	107	8.7
6/15/2016	4/26/2017	BaSO4_LSE	107	9.3
6/15/2016	4/26/2017	BaSO4_LSE	99	9.4
6/15/2016	4/26/2017	BaSO4_LSE	106	8.8
6/15/2016	4/26/2017	BaSO4_LSE	107	9.5
6/15/2016	4/26/2017	BaSO4_LSE	108	9.4
6/15/2016	4/26/2017	BaSO4_LSE	107	8.9
		average	106.1	9.1
		stdev	2.6	0.4

Note – value in red italics has been excluded from the average value calculation.

Table 5. Sources of sulfur contamination.

Preparation date	Sample Label	Weight empty 50ml centrifuge tube (g)	+SF-ASW (g)	Weight SF-ASW (g)	Weight empty 1.5ml tube (mg)	Weight 1.5ml tube + BaSO ₄ (mg)	Calculated BaSO ₄ (mg)	Calculated SO ₄ ²⁻ (mmol)	[SO ₄ ²⁻] mM	Sulfur Isotope Analysis Date	Sulfur Sample Analysis Label	$\delta^{34}\text{S}$ VCDT (‰)
10/6/2106	ASWa	13.8	54	40.2	1542.830	1543.116	0.286	0.0012	0.030	11/8/2016	MMlgo_ASWa	32.8
10/6/2106	ASWb	13.7	54.8	41.1	1541.443	1541.746	0.303	0.0013	0.032	11/8/2016	MMlgo_ASWb	37.5
10/6/2106	ASWc	13.8	54.3	40.5	1542.417	1542.760	0.343	0.0015	0.036	11/8/2016	MMlgo_ASWc	30.9
10/6/2106	18MOa	13.7	52.6	38.9	1562.625	1562.818	0.193	0.0008	0.021	11/8/2016	MMlgo_18MOhma	area too small to measure
10/6/2106	18MOb	13.8	53.1	39.3	1543.350	1543.532	0.182	0.0008	0.020			
10/6/2106	18MOc	14.00	52.7	38.7	1549.855	1550.049	0.194	0.0008	0.021	11/8/2016	MMlgo_18MOhmbc	area too small to measure
10/6/2016	control 1			40	1573.898	1573.953	0.055	0.0002	0.006			
10/6/2016	control 2			40	1543.517	1543.558	0.041	0.0002	0.004			
10/6/2016	control 3			40	1542.246	1542.176	-0.070	-0.0003	-0.007			

Table 6. Results of low sulfate extraction method with 18MΩ water.

Sample Preparation Date	Target Sulfate Concentration	Sulfur Isotope Analysis Date	Sulfur Sample Analysis Label	IRMS Yield (%)	$\delta^{34}\text{S}$ VCDT (‰)	Oxygen Isotope Analysis Date	Oxygen Sample Analysis Label	IRMS Yield (%)	$\delta^{18}\text{O}$ VSMOW (‰)
8/6/2016	0.5 mM	11/8/2016	MMIgo_0.5a	88.9	1.3	4/25/2017	a_0.5_18MOhm	104	9.6
8/6/2016	0.5 mM	11/8/2016	MMIgo_0.5b	94.6	1.0	4/25/2017	b_0.5_18MOhm	105	9.3
8/6/2016	0.5 mM	11/8/2016	MMIgo_0.5c	85.8	1.0	4/25/2017	c_0.5_18MOhm	103	8.8
			average	89.8	1.1		average	104.1	9.2
			stdev	4.5	0.2		stdev	1.1	0.4
8/6/2016	0.1 mM	11/8/2016	MMIgo_0.1a	89.9	1.5		NS		
8/6/2016	0.1 mM	11/8/2016	MMIgo_0.1b	80.8	1.9				
8/6/2016	0.1 mM	11/8/2016	MMIgo_0.1c	78.4	1.4				
			average	83.1	1.6				
			stdev	6.1	0.3				
8/6/2016	0.03 mM	11/8/2016	MMIgo_0.03a	17.5	3.8		NS		
8/6/2016	0.03 mM	11/8/2016	MMIgo_0.03b	38.2	6.0				
8/6/2016	0.03 mM	11/8/2016	MMIgo_0.03c	41.1	10.9				
			average	32.2	6.9				
			stdev	12.9	3.6				
8/6/2016	0.01 mM	11/8/2016	MMIgo_0.01a	28.1	12.1		NS		
8/6/2016	0.01 mM	11/8/2016	MMIgo_0.01b	16.8	27.1				
8/6/2016	0.01 mM	11/8/2016	MMIgo_0.01c	14.2	662.1				
			average	19.7	19.6				
			stdev	7.4	10.6				
high volume experiment (mis-label in IRMS software)									
11/8/2016	0.03 mM	2/28/2017	MMIgo_0.003_ap	99	1.4	4/25/2017	dp_0.03_18MOhm	107	9.1
11/8/2016	0.03 mM	2/28/2017	MMIgo_0.003_bp	101	1.3	4/25/2017	cp_0.03_18MOhm	105	9.1
11/8/2016	0.03 mM	2/28/2017	MMIgo_0.003_cp	98	1.1	4/25/2017	ep_0.03_18MOhm	108	9.2
			average	99.3	1.3		average	106.5	9.1
			stdev	1.9	0.1		stdev	1.7	0.1

NS = no sample available for analysis

Table 7. Results of pilot study.

Sample	Weight of soil (g)	Wt. of BaSO₄ from RAS leach (mg)	Wt. of BaSO₄ from EES leach (mg)	RAS Sulfur Sample Analysis Label	$\delta^{34}\text{S}$ VCDT (‰)	EES leach Sulfur Sample Analysis Label	$\delta^{34}\text{S}$ VCDT (‰)
Hwy54-I	5.0013	-	-	Hwy54_MQ	11.0	Hwy54_NaCl	10.8
Indio Mtn I	5.0327	0.518	0.145	MMlgo_IndioI_MQ	2.6	MMlgo_IndioI_NaCl	10.7
Indio Mtn II	5.0124	0.289	0.108	MMlgo_IndioI_MQ	3.9	MMlgo_IndioI_NaCl	13.7
PC	5.0289	-	-	MMlgo_PC_MQ	5.5	MMlgo_PC_NaCl	7.3

Table 8. Results of rainwater experiments. 'Rainwater' added 4/13/2017.

Sample	Weight of soil (g)	Wt. of BaSO₄ from RAS leach (mg)	Wt. of BaSO₄ from EES leach (mg)	RAS Sulfur Sample Analysis Label	$\delta^{34}\text{S}$ VCDT (‰)	EES leach Sulfur Sample Analysis Label	$\delta^{34}\text{S}$ VCDT (‰)
Hwy54-I	5.0013	-	-	Hwy54_MQ	11.0	Hwy54_NaCl	10.8
Indio Mtn I	5.0327	0.518	0.145	MMlgo_IndioI_MQ	2.6	MMlgo_IndioI_NaCl	10.7
Indio Mtn II	5.0124	0.289	0.108	MMlgo_IndioI_MQ	3.9	MMlgo_IndioI_NaCl	13.7
PC	5.0289	-	-	MMlgo_PC_MQ	5.5	MMlgo_PC_NaCl	7.3

8. FIGURES

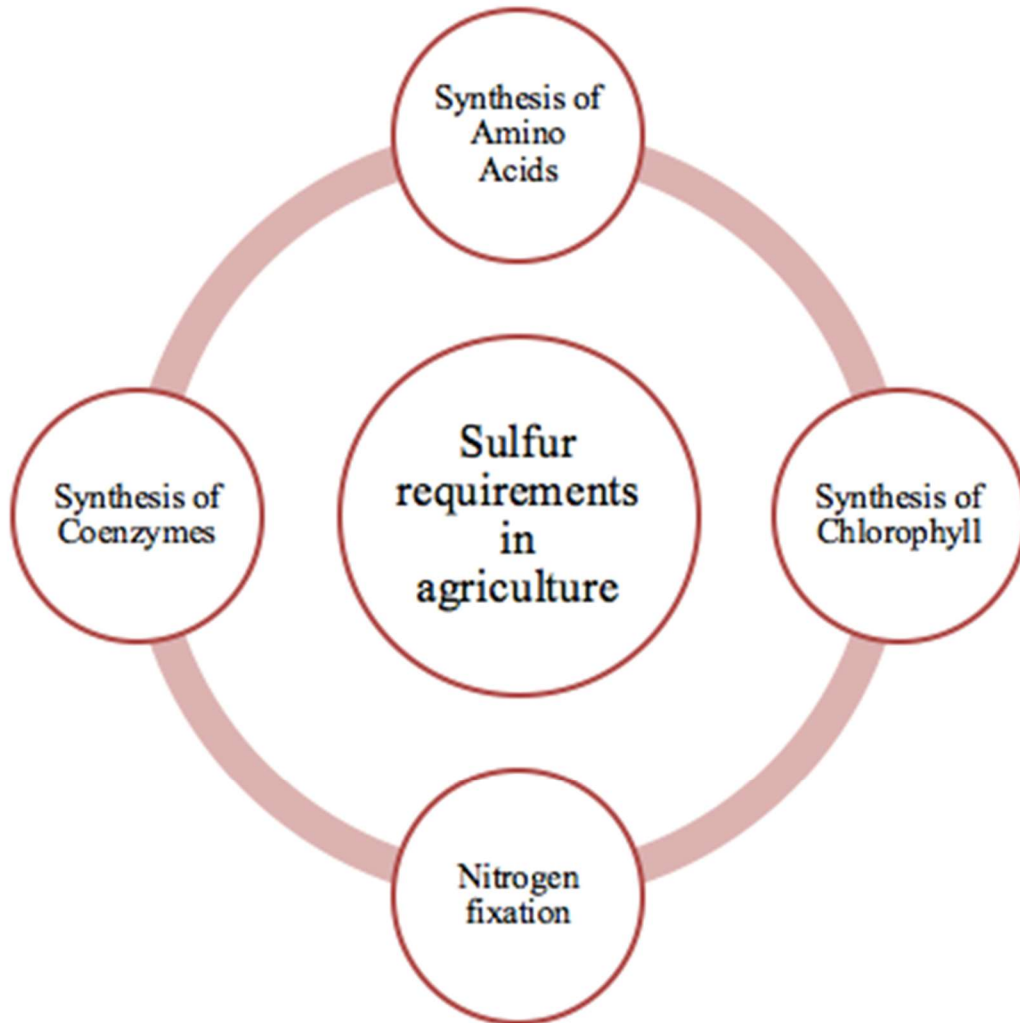


Figure 1. Importance of sulfur for organisms and agriculture.

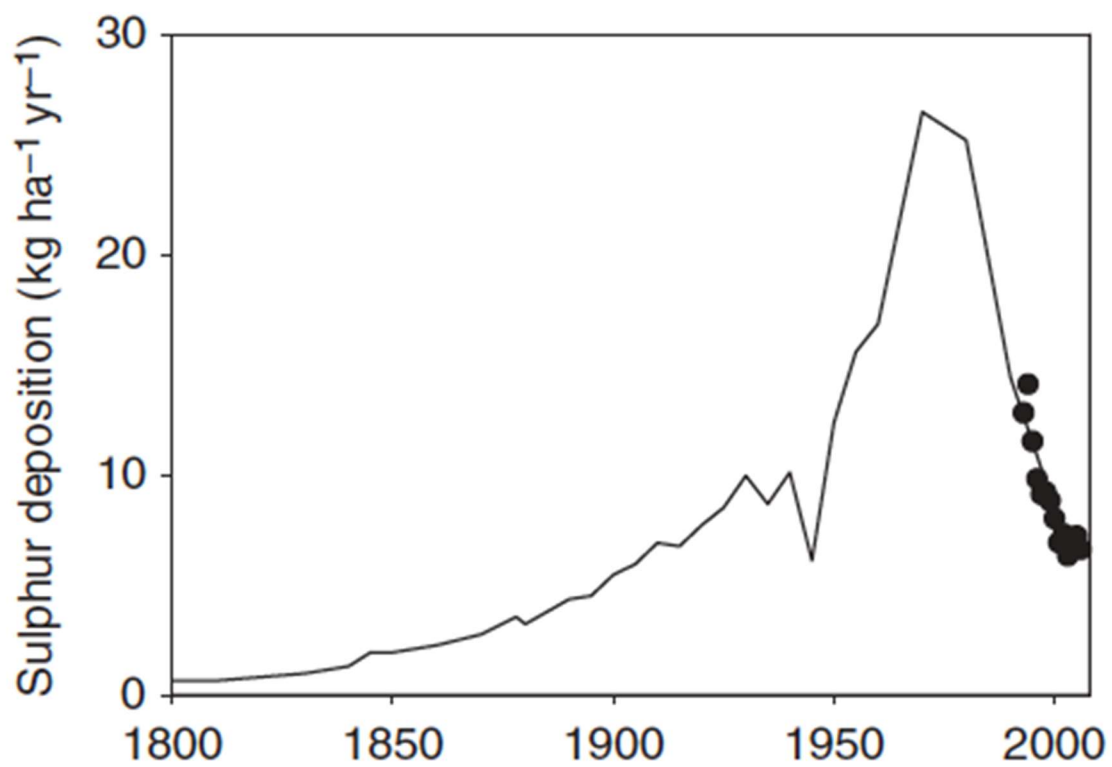


Figure 2. Sulfur deposition in Denmark through time (Eriksen, 2009).

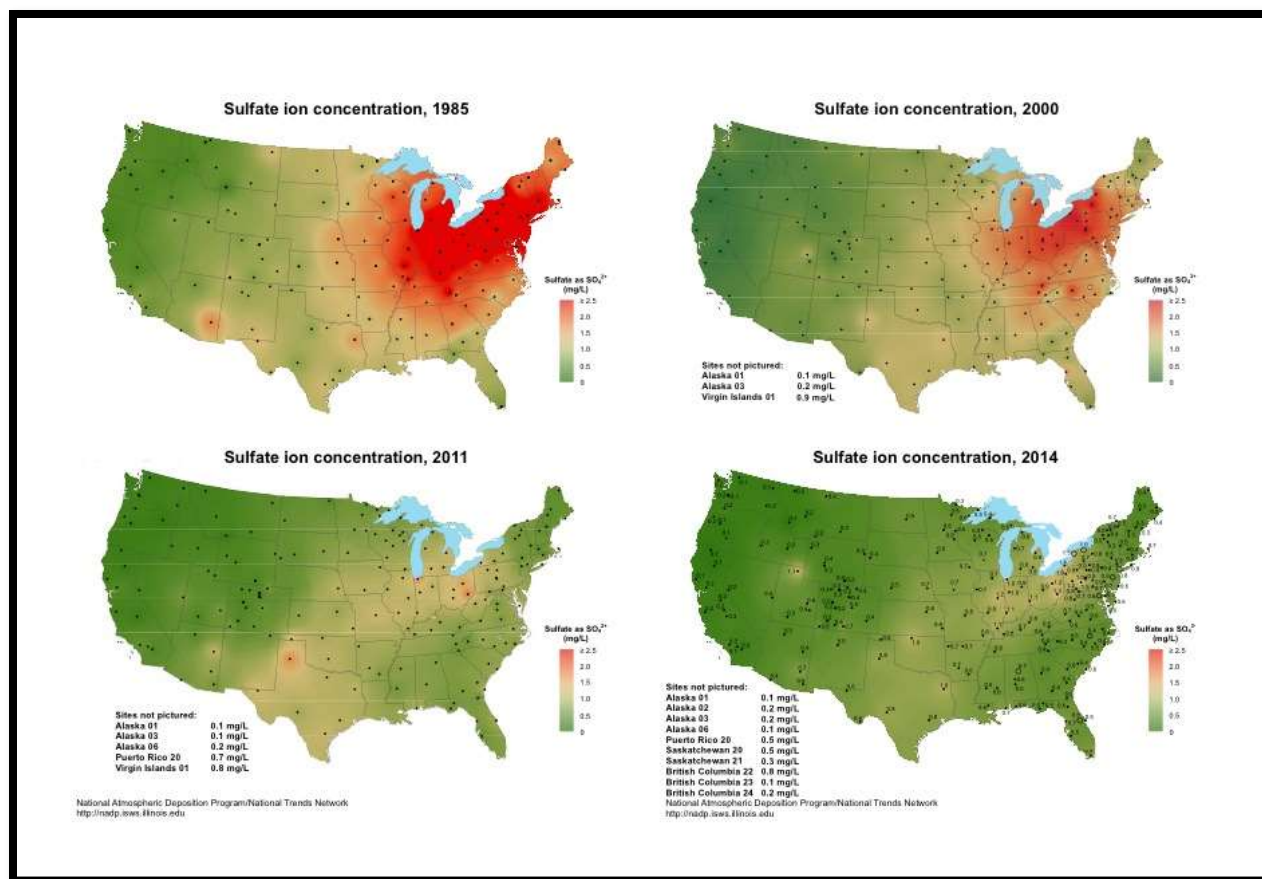


Figure 3. Sulfate ion concentration of precipitation-weighted mean from 1985 to 2014 in mg/L (<http://nadp.isws.illinois.edu>).

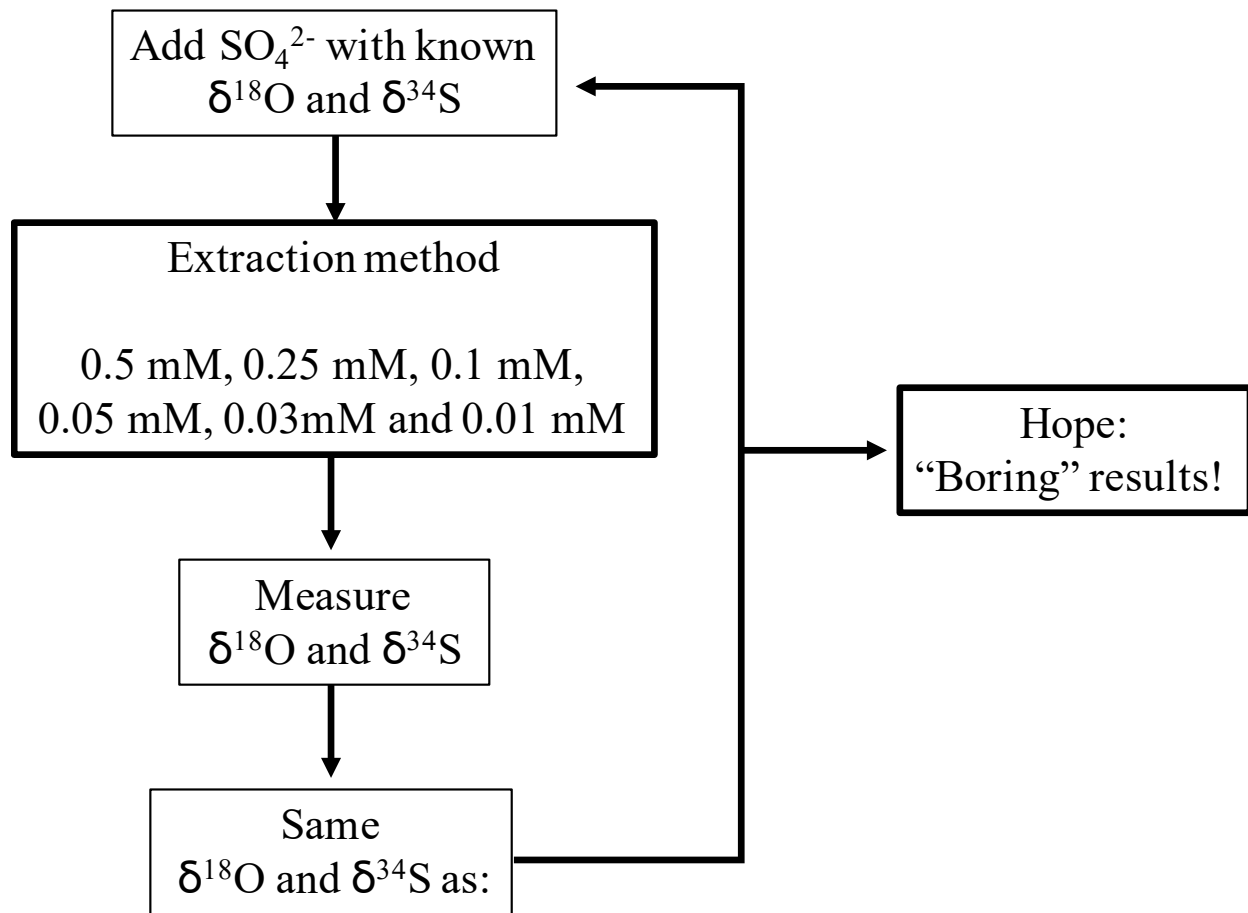


Figure 4. Concept design for testing new extraction method.

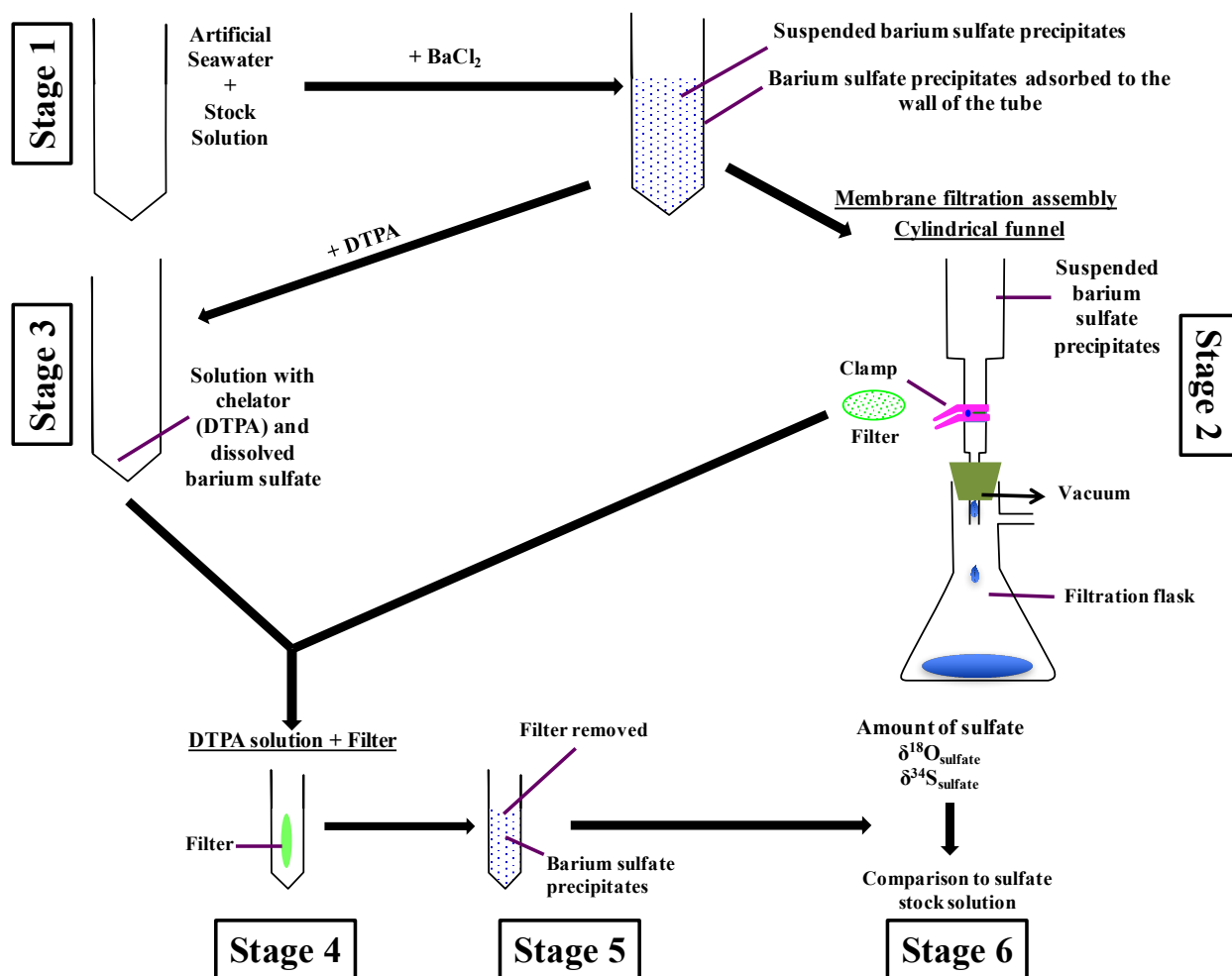


Figure 5. Sulfate extraction protocol for low sulfate concentrations.

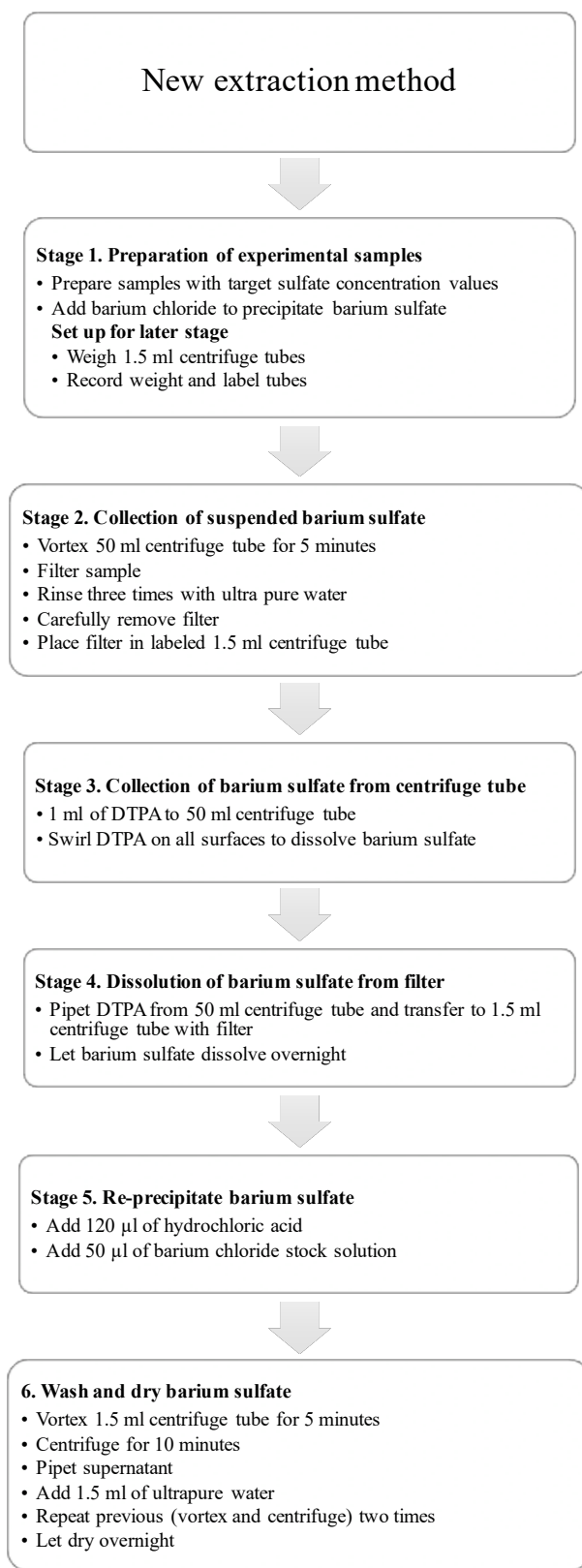


Figure 6. Flow chart for ultra-low sulfate extraction method testing.

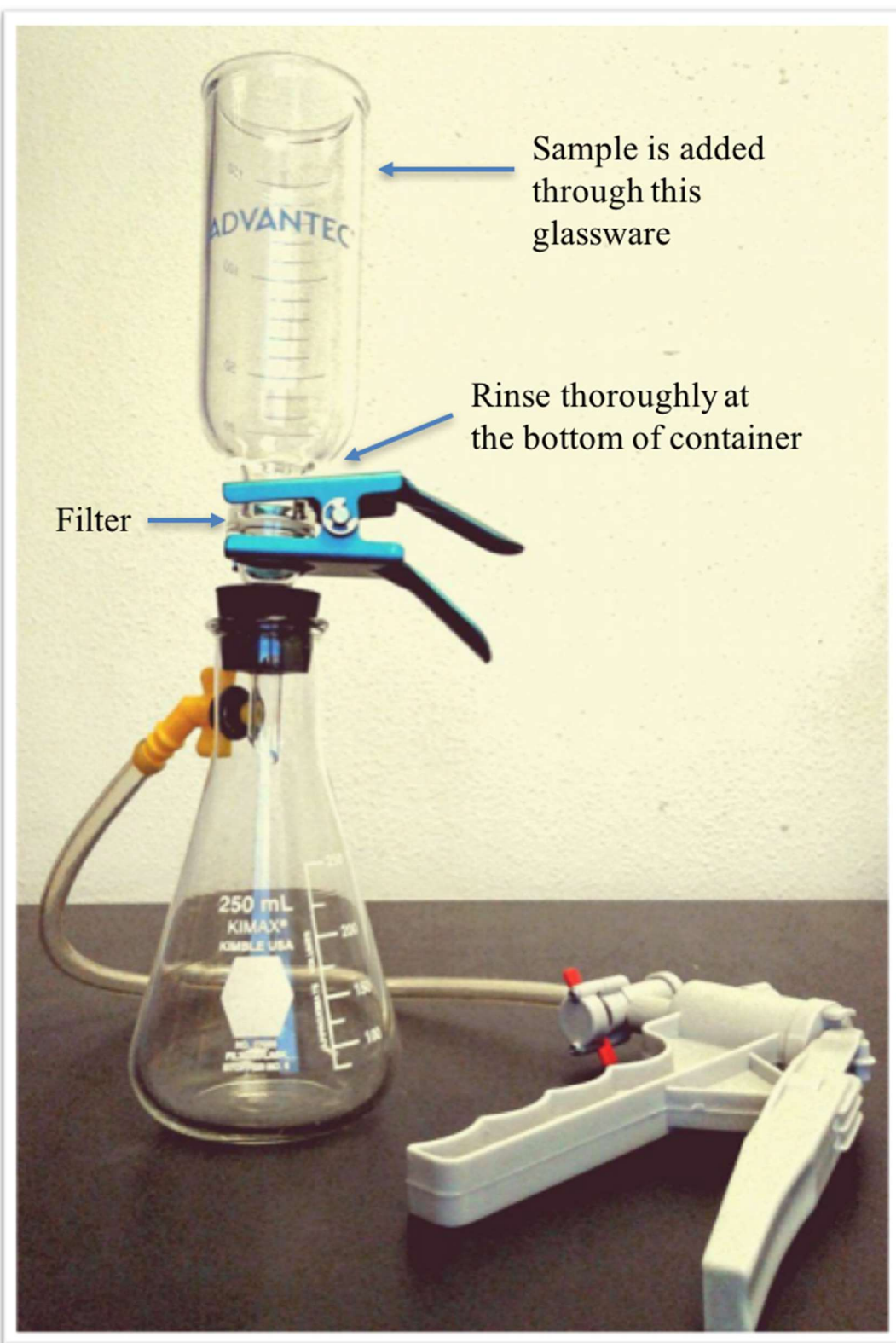


Figure 7. Important steps during filtration process.

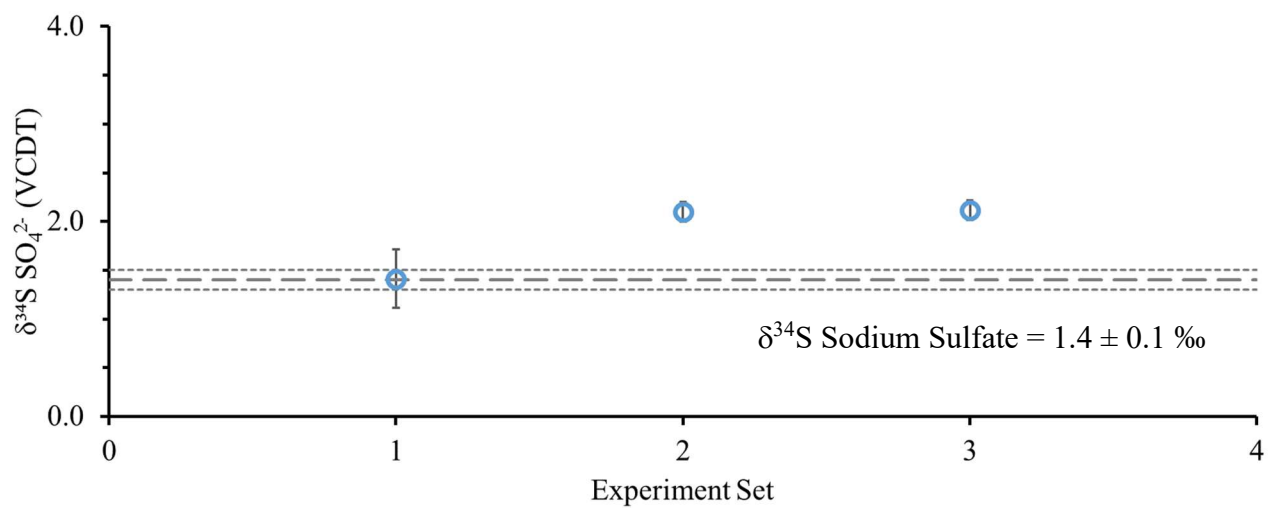


Figure 8. Preliminary extraction method results.
Long dash line represents average sulfur isotope composition of stock sodium sulfate. Short dashed lines are 1stdev of average.

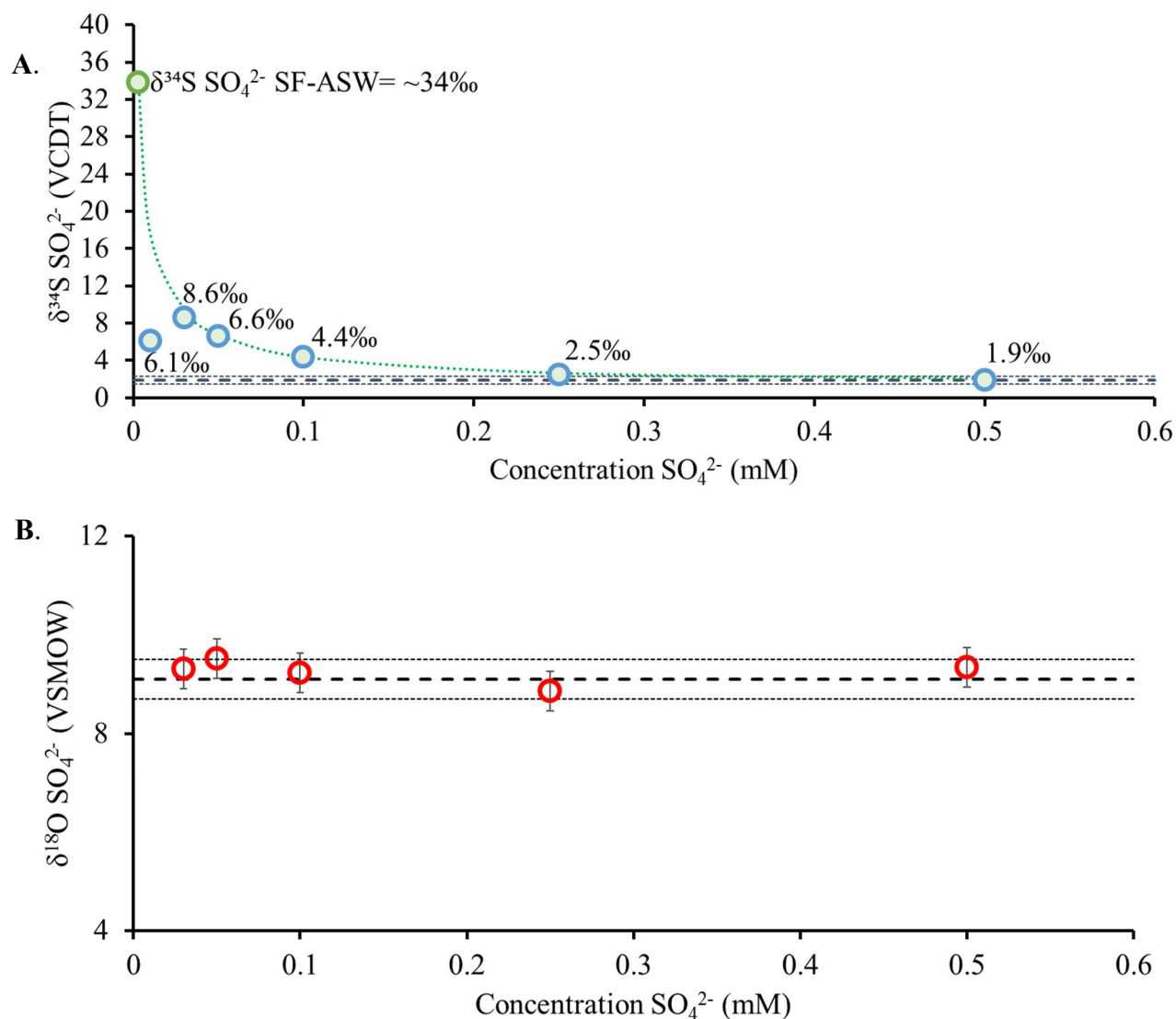


Figure 9. Results from variable concentration sulfate in ASW experiments for the sulfur isotope composition (A.) and oxygen isotope composition (B.) of sulfate.

Considering $\delta^{34}\text{S}$ of Na_2SO_4 =1.4‰, ASW=33.8‰ and $18\text{M}\Omega_{\text{water}}$ =122‰. Contamination amount: ASW=0.01 mM and $18\text{M}\Omega_{\text{water}}$ =0.0001 mM. Long dash line represents average isotope composition of stock sodium sulfate. Short dashed lines are 1stdev of average.

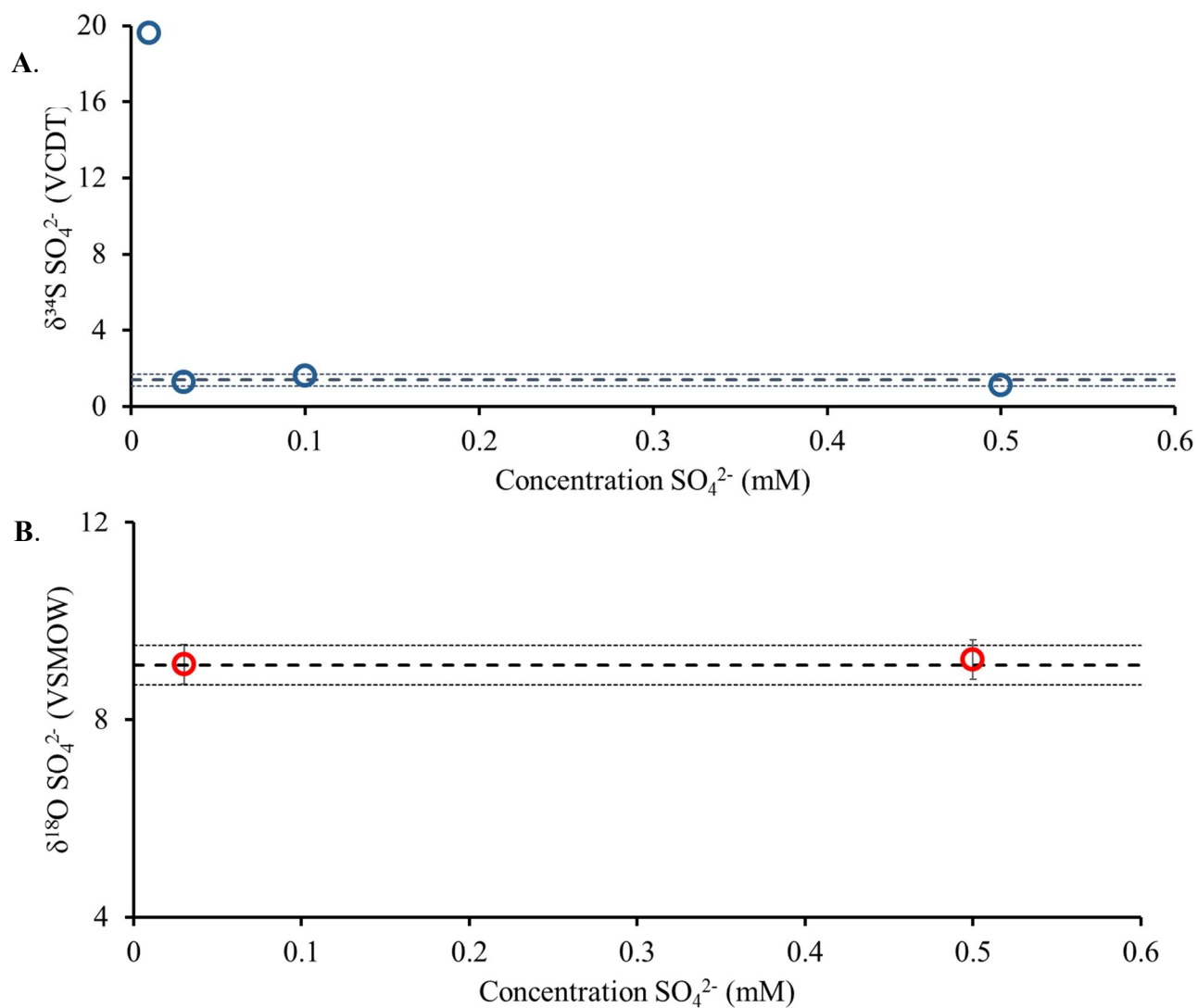


Figure 10. Results from variable concentration sulfate in ultra-pure water experiments for the sulfur isotope composition (A.) and oxygen isotope composition (B.) of sulfate. Long dash line represents average sulfur isotope composition of stock sodium sulfate. Short dashed lines are 1stdev of average.



Figure 11. Locations for sample collection in Pilot Study.

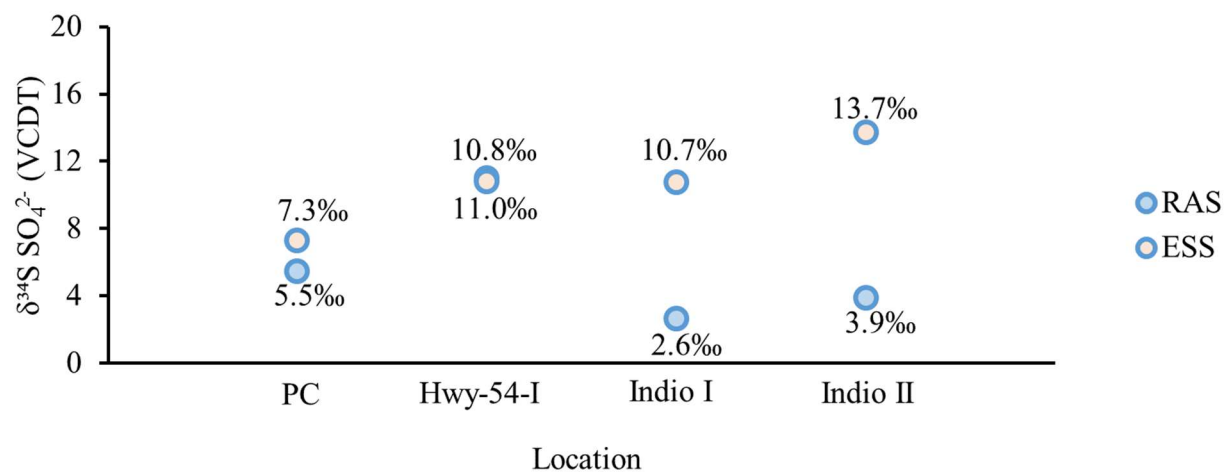


Figure 12. Measured $\delta^{34}\text{S}$ composition from sulfate extraction in Hwy54-I, Indio Mtn I, Indio Mtn II and Positive Control in sequential leaching process.

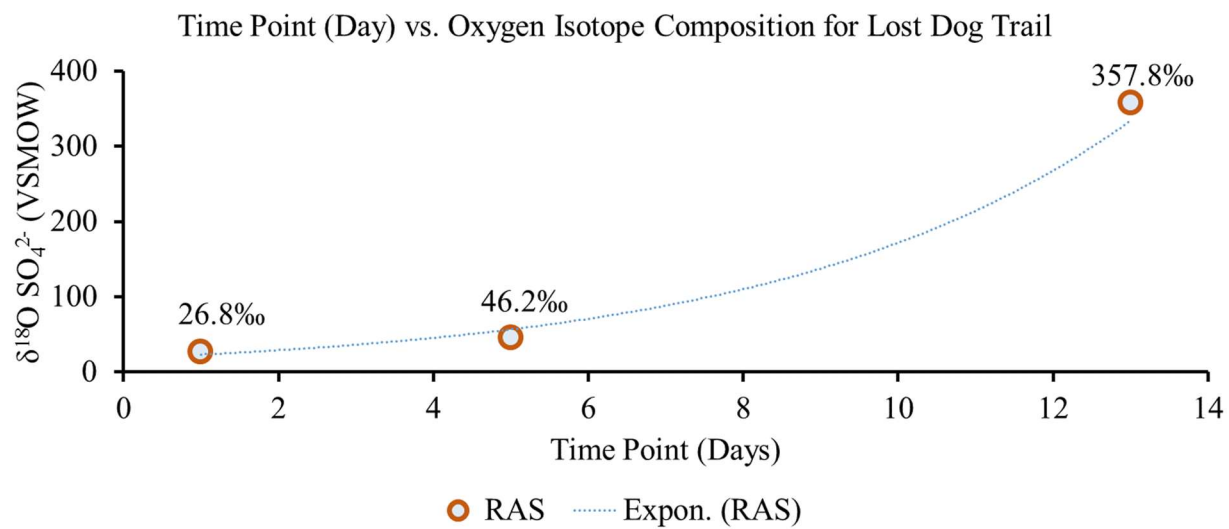


Figure 13. RAS leaching results. Three time points (Day 1, 5 and 13) vs. Oxygen Isotope Composition for Lost Dog Trail soil sample.

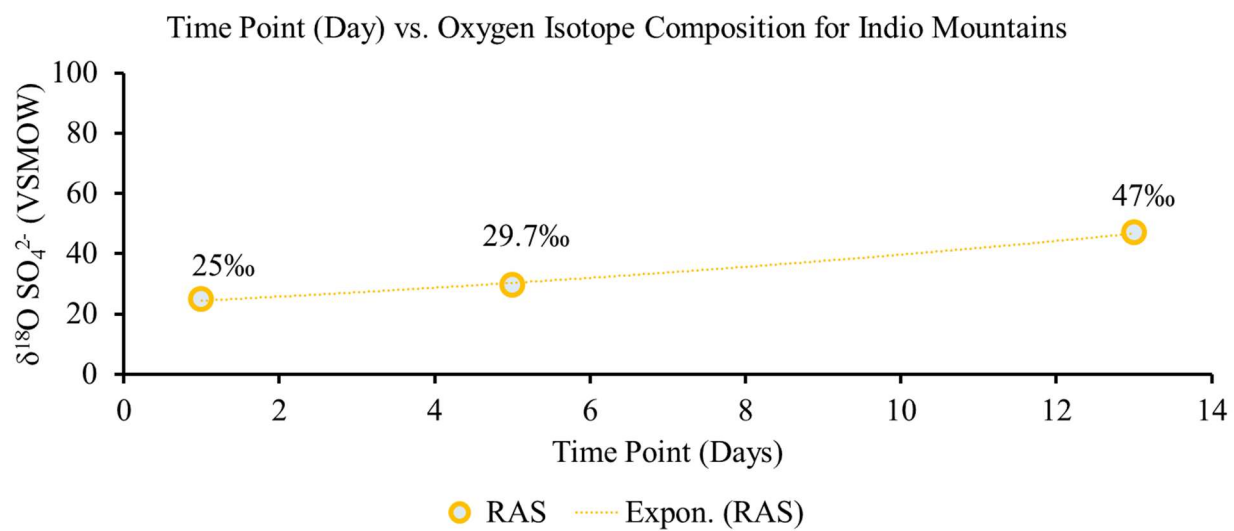


Figure 14. RAS leaching results. Three time points (Day 1, 5 and 13) vs. Oxygen Isotope Composition for Indio Mountain soil sample.

REFERENCES

- Arnold, G.L., Brunner, B., Müller, I.A., and Røy, H., 2014, Modern applications for a total sulfur reduction distillation method - what's old is new again: *Geochemical Transactions*, v. 15, no. 1, p. 4, doi: 10.1186/1467-4866-15-4.
- Bao, H., Michalski, G.M., and Thiemens, M.H., 2001, Sulfate oxygen-17 anomalies in desert varnishes: *Geochimica et Cosmochimica Acta*, v. 65, no. 13, p. 2029–2036, doi: 10.1016/S0016-7037(00)00490-7.
- Canfield, D.E., 2001, Biogeochemistry of Sulfur Isotopes: Reviews in Mineralogy and Geochemistry, v. 43, no. 1, p. 607–636, doi: 10.2138/gsrmg.43.1.607.
- Carmody, R.W., Plummer, L.N., Busenberg, E., and Coplen, T.B., 1998, Methods for collection of dissolved sulfate and sulfide and analysis of their sulfur isotopic composition: Open-File Report Report 97-234.
- Castellano, S.D., and Dick, R.P., 1991, Cropping and Sulfur Fertilization Influence on Sulfur Transformations in Soil: *Soil Science Society of America Journal*, v. 55, no. 1, p. 114, doi: 10.2136/sssaj1991.03615995005500010020x.
- Center for Sustainability and the Global Environment | SAGE | University of Wisconsin-Madison.
- Dai, A., 2011, Drought under global warming: a review: *Wiley Interdisciplinary Reviews: Climate Change*, v. 2, no. 1, p. 45–65, doi: 10.1002/wcc.81.
- Dracy, C., Eubanks, T.M., and Lowe, K.L., 2013, Thiosulfate-related microbial communities from four arid soils in the Southwestern United States: *Journal of Arid Environments*, v. 92, p. 59–62, doi: 10.1016/j.jaridenv.2013.01.007.
- Edwards, D.R., Lohman, D.C., and Wolfenden, R., 2012, Catalytic Proficiency: The Extreme Case of S–O Cleaving Sulfatases: *Journal of the American Chemical Society*, v. 134, no. 1, p. 525–531, doi: 10.1021/ja208827q.
- Eriksen, J., 2009, Chapter 2 Soil Sulfur Cycling in Temperate Agricultural Systems, *in* Agronomy, B.-A. in ed., Academic Press, p. 55–89.
- Eriksen, J., Murphy, M.D., and Schnug, E., 1998, The Soil Sulphur Cycle, *in* Schnug, E. ed., *Sulphur in Agroecosystems, Nutrients in Ecosystems 2*, Springer Netherlands, p. 39–73.
- Ganeshamurthy, A.N., and Nielsen, N.E., 1990, Arylsulphatase and the biochemical mineralization of soil organic sulphur: *Soil Biology and Biochemistry*, v. 22, no. 8, p. 1163–1165, doi: 10.1016/0038-0717(90)90045-2.
- Ghani, A., McLaren, R.G., and Swift, R.S., 1990, Seasonal fluctuations of sulphate and soil microbial biomass-S in the surface of a Wakanui soil: *New Zealand Journal of Agricultural Research*, v. 33, no. 3, p. 467–472, doi: 10.1080/00288233.1990.10428444.

- Ghani, A., McLaren, R., and Swift, R., 1993, The incorporation and transformations of ^{35}S in soil: Effects of soil conditioning and glucose or sulphate additions: *Soil Biology and Biochemistry*, v. 25, no. 3, p. 327–335, doi: 10.1016/0038-0717(93)90131-T.
- Goldhammer, T., Max, T., Brunner, B., Einsiedl, F., and Zabel, M., 2011, Marine sediment pore-water profiles of phosphate $\text{d}18\text{O}$ using a refined micro-extraction: *Limnology and Oceanography: Methods*, v. 9, p. 110–120, doi: 10.4319/lom.2011.9.110.
- Johnson, C.A., Mast, M.A., and Kester, C.L., 2001, Use of $^{17}\text{O}/^{16}\text{O}$ to trace atmospherically-deposited sulfate in surface waters: A case study in Alpine watersheds in the Rocky Mountains: *Geophysical Research Letters*, v. 28, no. 23, p. 4483–4486, doi: 10.1029/2001GL012966.
- Kang, P.-G., Mayer, B., and Mitchell, M.J., 2012, Comparison of sample preparation methods for stable isotope analysis of dissolved sulphate in forested watersheds: *Isotopes in Environmental and Health Studies*, v. 48, no. 3, p. 410–420, doi: 10.1080/10256016.2012.667810.
- Kang, P.-G., Mitchell, M.J., Mayer, B., and Campbell, J.L., 2014, Isotopic Evidence for Determining the Sources of Dissolved Organic Sulfur in a Forested Catchment: *Environmental Science & Technology*, v. 48, no. 19, p. 11259–11267, doi: 10.1021/es502563n.
- Kertesz, M.A., 2000, Riding the sulfur cycle – metabolism of sulfonates and sulfate esters in Gram-negative bacteria: *FEMS Microbiology Reviews*, v. 24, no. 2, p. 135–175, doi: 10.1016/S0168-6445(99)00033-9.
- Kertesz, M.A., and Mirleau, P., 2004, The role of soil microbes in plant sulphur nutrition: *Journal of Experimental Botany*, v. 55, no. 404, p. 1939–1945, doi: 10.1093/jxb/erh176.
- Klose, S., Moore, J.M., and Tabatabai, M.A., 1999, Arylsulfatase activity of microbial biomass in soils as affected by cropping systems: *Biology and Fertility of Soils*, v. 29, no. 1, p. 46–54, doi: 10.1007/s003740050523.
- Knapp, A.K., Beier, C., Briske, D.D., Classen, A.T., Luo, Y., Reichstein, M., Smith, M.D., Smith, S.D., Bell, J.E., Fay, P.A., Heisler, J.L., Leavitt, S.W., Sherry, R., Smith, B., et al., 2008, Consequences of More Extreme Precipitation Regimes for Terrestrial Ecosystems: *BioScience*, v. 58, no. 9, p. 811, doi: 10.1641/B580908.
- Le Gendre, E., Martin, E., Villemant, B., Cartigny, P., and Assayag, N., 2017, A simple and reliable anion-exchange resin method for sulfate extraction and purification suitable for multiple O- and S-isotope measurements: *Rapid Communications in Mass Spectrometry*, v. 31, p. 137–144, doi: 10.1002/rcm.7771.
- Lee, C.C.-W., Savarino, J., and Thieme, M.H., 2001, Mass independent oxygen isotopic composition of atmospheric sulfate: Origin and implications for the present and past atmosphere of Earth and Mars: *Geophysical Research Letters*, v. 28, no. 9, p. 1783–1786, doi: 10.1029/2000GL011826.

- Luo, W., Dijkstra, F.A., Bai, E., Feng, J., Lü, X.-T., Wang, C., Wu, H., Li, M.-H., Han, X., and Jiang, Y., 2015, A threshold reveals decoupled relationship of sulfur with carbon and nitrogen in soils across arid and semi-arid grasslands in northern China: *Biogeochemistry*, v. 127, no. 1, p. 141–153, doi: 10.1007/s10533-015-0174-4.
- Lynch, J.A., and Corbett, E.S., 1989, Hydrologic control of sulfate mobility in a forested watershed: *Water Resources Research*, v. 25, no. 7, p. 1695–1703, doi: 10.1029/WR025i007p01695.
- Mayer, B., Feger, K.H., Giesemann, A., and Jäger, H.-J., 1995a, Interpretation of sulfur cycling in two catchments in the Black Forest (Germany) using stable sulfur and oxygen isotope data: *Biogeochemistry*, v. 30, no. 1, p. 31–58, doi: 10.1007/BF02181039.
- Mayer, B., Fritz, P., Prietzel, J., and Krouse, H.R., 1995b, The use of stable sulfur and oxygen isotope ratios for interpreting the mobility of sulfate in aerobic forest soils: *Applied Geochemistry*, v. 10, no. 2, p. 161–173, doi: 10.1016/0883-2927(94)00054-A.
- O'donnell, A.G., Wu, J., and Syers, J.K., 1994, Sulphate-S amendments in soil and their effects on the transformation of soil sulphur: *Soil Biology and Biochemistry*, v. 26, no. 11, p. 1507–1514, doi: 10.1016/0038-0717(94)90092-2.
- Révész, K., and Coplen, T.B., 2007, Determination of the $\delta(34\text{S}/32\text{S})$ of low-concentration sulfate in water: RSIL lab code 1949, in Book 10, *Methods of the Reston Stable Isotope Laboratory*.
- Reynolds, J.F., Smith, D.M.S., Lambin, E.F., Turner, B.L., Mortimore, M., Batterbury, S.P.J., Downing, T.E., Dowlatabadi, H., Fernandez, R.J., Herrick, J.E., Huber-Sannwald, E., Jiang, H., Leemans, R., Lynam, T., et al., 2007, Global Desertification: Building a Science for Dryland Development: *Science*, v. 316, no. 5826, p. 847–851, doi: 10.1126/science.1131634.
- Scherer, H.W., 2009, Sulfur in soils: *Journal of Plant Nutrition and Soil Science*, v. 172, no. 3, p. 326–335, doi: 10.1002/jpln.200900037.
- Scherer, H.W., 2001, Sulphur in crop production — invited paper: *European Journal of Agronomy*, v. 14, no. 2, p. 81–111, doi: 10.1016/S1161-0301(00)00082-4.
- Schimel, D.S., 2010, Drylands in the Earth System: *Science*, v. 327, no. 5964, p. 418–419, doi: 10.1126/science.1184946.
- Shanley, J.B., Mayer, B., Mitchell, M.J., Michel, R.L., Bailey, S.W., and Kendall, C., 2005, Tracing Sources of Streamwater Sulfate During Snowmelt Using S and O Isotope Ratios of Sulfate and ^{35}S Activity: *Biogeochemistry*, v. 76, no. 1, p. 161–185, doi: 10.1007/s10533-005-2856-9.

- Tamburini, F., Pfahler, V., von Sperber, C., Frossard, E., and Bernasconi, S.M., 2014, Oxygen Isotopes for Unraveling Phosphorus Transformations in the Soil–Plant System: A Review: *Soil Science Society of America Journal*, v. 78, no. 1, doi: 10.2136/sssaj2013.05.0186dgs.
- Thode, H.G., 1991, Sulphur Isotopes in Nature and the Environment: An Overview, *in* *Stable Isotopes in the Assessment of Natural and Anthropogenic Sulphur in the Environment*, SCOPE 43, p. 1–26.
- Torfs, K.M., Van Grieken, R.E., and Buzek, F., 1997, Use of Stable Isotope Measurements To Evaluate the Origin of Sulfur in Gypsum Layers on Limestone Buildings: *Environmental Science & Technology*, v. 31, p. 2650–2655, doi: 10.1021/es970067v.
- Trust, B.A., and Fry, B., 1992, Stable sulphur isotopes in plants: a review: *Plant, Cell & Environment*, v. 15, no. 9, p. 1105–1110, doi: 10.1111/j.1365-3040.1992.tb01661.x.
- Turchyn, A.V., Tipper, E.T., Galy, A., Lo, J.-K., and Bickle, M.J., 2013, Isotope evidence for secondary sulfide precipitation along the Marsyandi River, Nepal, Himalayas: *Earth and Planetary Science Letters*, v. 374, p. 36–46, doi: 10.1016/j.epsl.2013.04.033.
- Von Sperber, C., Kries, H., Tamburini, F., Bernasconi, S.M., and Frossard, E., 2014, The effect of phosphomonoesterases on the oxygen isotope composition of phosphate: *Geochimica et Cosmochimica Acta*, v. 125, p. 519–527, doi: 10.1016/j.gca.2013.10.010.
- Von Sperber, C., Tamburini, F., Brunner, B., Bernasconi, S.M., and Frossard, E., 2015, The oxygen isotope composition of phosphate released from phytic acid by the activity of wheat and *Aspergillus niger* phytase: *Biogeosciences*, v. 12, no. 13, p. 4175–4184, doi: 10.5194/bg-12-4175-2015.
- Wu, Q., and Han, G., 2015, Sulfur isotope and chemical composition of the rainwater at the Three Gorges Reservoir: *Atmospheric Research*, v. 155, p. 130–140, doi: 10.1016/j.atmosres.2014.11.020.

APPENDIX

Table A1. Chemicals selected for artificial seawater

Solution	Chemical	Info	LOT #
DTPA Stock Solution	NaOH	Sodium Hydroxide pellets 98% NaOH 500 g Alfa-Aesar	K08Z056
	DTPA	Diethylenetriaminepentaacetic acid 98+% C ₁₄ H ₂₃ N ₃ O ₁₀ 250 g	C23Y018
SF-ASW	NaCl	Sodium Chloride ACS 99.0% Crystalline 2 kg Alfa-Aesar	P26B015
	MgCl ₂	Magnesium Chloride Hexahydrate ACS 99.0%-102% Crystalline	K07Z041
	CaCl ₂	Calcium Chloride di-hydrate 99% min Granular 500 g Alfa-Aesar	M20A038
	KCl	Potassium Chloride 99% 1000 g Alfa-Aesar	10179997
	KBr	Potassium Bromide Ward's Science Cat#: 9421004	2012071675
	NaHCO ₃	Sodium Hydrogen Carboate Puratonic 99.998% 10 g	10184273
	H ₃ BO ₃	Boric Acid 99+% 500 g	B10Y026
	SrCl ₂ ·6H ₂ O	Strontium Chloride Hexahydrate ACS 99.0%-103% Granular	T13B052
Sodium sulfate stock solution	Na ₂ SO ₄	Sodium Sulfate ACS 99.0% min granular 2 kg Alfa-Aesar	N11A005
BaCl₂ stock solution	BaCl ₂ ·H ₂ O	Barium Chloride, Dihydrate, Crystal J.T Baker	Batch#: 000006610
HCl	HCl	Hydrochloric Acid ACS Grace 3.8 L BDH	2014030624
Acetone	(CH ₃) ₂ CO	Acetone 4 L BDH	042114F

Table A2. Experimental setup.

Modified sulfate	0.5 mM	0.25 mM	0.1 mM	0.05 mM	0.03 mM	0.01 mM	ASW water	18M Ω water
Samples in Triplicate	A	A	A	A	A	A	A	A
	B	B	B	B	B	B	B	B
	C	C	C	C	C	C	C	C

For each sample, I used separate 50 ml centrifuge tubes. These tubes were weighed before adding any solution. Then, I filled them up with 40 ml of SF-ASW and weighed them again. Afterwards, a defined amount of sodium sulfate stock solution was added to each tube such that I obtain a series of final sodium sulfate concentrations, covering a range from 0.01 mM to 0.5 mM. After adding the aliquots of sodium sulfate stock solution, I weighed the centrifuge tubes again.

R1. DIETHYLENETRIAMINEPENTAACETIC ACID (DTPA) STOCK SOLUTION

Recipe for 0.05 M in 1 M NaOH in 1 L

Equipment

- 1000 ml graduated glass cylinder
- 1 L light shielded graduated glass bottle
- Magnetic stirrer
- Magnetic stir bar
- Balance maximum capacity: 4000 g for glassware weighing
- Balance maximum capacity: 110 g for chemical weighing
- Small spoon

Consumables

- Plastic weighing boats

Chemicals

- ~1000 ml 18M Ω _{water} (ultra-pure water)
- 40 g NaOH
- 20 g DTPA

Procedure

- Using balance and plastic weighing boats, weigh 40 g of NaOH and 20 g of DTPA and set aside for later use.
- Label plastic boats to make sure these are added correctly.
- Rinse 1000 ml graduated glass cylinder and 1 L graduated glass bottle with ultra-pure water thoroughly three times. Do not rinse with tap water.
- Add ~800 ml of ultra-pure water to 1000 ml graduated glass cylinder.
- Introduce magnetic stir bar to cylinder with ultra-pure water.
- Plug-in and turn magnetic stirrer on.

- Place cylinder with water and magnetic stir bar on magnetic stirrer. Manage to get magnetic stir bar work correctly to make sure that when chemicals are added, these are mixed properly.
 - Add 40 g of NaOH to graduated cylinder while magnetic stirrer in on. Let NaOH mix for 10 minutes. Ensure NaOH is completely dissolved before proceeding.
 - Add 20 g of DTPA to graduated cylinder with already mixed NaOH.
 - Let solution stir until all chemicals are completely dissolved and the solution is clear.
- Transfer solution to a 1 L light shielded graduated glass bottle. Must mark date on bottle.

Storage

Wrap container in aluminum foil. Container should be stored away from windows, in a closed cabinet if possible, out of direct light.

R2. DTPA CLEANING PROCEDURE

Equipment

- Glassware to be cleaned

Chemicals

- DTPA stock solution (amount depends on size of glassware, e.g. 20 ml of DTPA for a 500 ml graduated glass bottle)

Procedure

To use DTPA stock solution to clean glassware:

- Add DTPA solution to ensure an even coating on all surfaces of glassware.
- Glassware should be closed with DTPA solution inside.
- Gently swirl glassware and cover walls with solution.
- Rinse glassware three times with ultrapure water.

Attention

- Do not use tap water. For example, high concentrations of sulfate are found in the tap water in the Geological Sciences building at UTEP and if used, sample contamination is imminent.

R3. SULFATE-FREE ARTIFICIAL SEAWATER (SF-ASW)

Recipe for 2 L solution

Equipment

- DTPA cleaned 2000 ml graduated glass bottle
- Magnetic stirrer
- Magnetic stir bar
- Balance maximum capacity: 110 g for chemical weighing
- Small spoon

Consumables

- Plastic weighing boats

Chemicals

- ~2000 ml $18\text{M}\Omega_{\text{water}}$ (ultra-pure water)
- 49 g NaCl
- 22 g MgCl_2
- 3 g CaCl_2
- 1.4 g KCl
- 0.2 g KBr
- 0.34 g NaHCO_3
- 0.05 g H_3BO_3
- 0.05 g SrCl_2

Procedure

- Using balance and plastic weighing boats, weigh each chemical listed above. Label weighing boats to make sure each chemical is added correctly.
- Record actual weight of chemical as well as brand, catalog #, and Lot #.
- Add 1500 ml of ultra-pure water to 2000 ml graduated glass cylinder.
- Place magnetic stir bar into 2000 ml graduated bottle with water.

- Plug-in and turn magnetic stirrer on.
- Place graduated bottle on magnetic stirrer and manage to get magnetic stir bar work correctly to make sure that when chemicals are added, these are mixed properly.
- Start by adding the most abundant chemicals, proceeding to the least abundant chemicals.
- After adding all the chemicals, turn off magnetic stirrer and remove bottle.
- Add ultra-pure water to the 2000 ml mark in graduated glass bottle.
- Place bottle with water and chemicals on magnetic stirrer and make sure stir bar is working properly.
- Leave solution on magnetic stirrer overnight to ensure all the salts are completely dissolved.

Storage

Wrap container in aluminum foil. Store away from direct light.

R4. SODIUM SULFATE STOCK SOLUTION

Recipe for ~10 mM SO_4^{2-} in 500 ml

Equipment

- DTPA cleaned 500 ml graduated glass bottle
- Balance maximum capacity: 4000 g for glassware weighing
- Balance maximum capacity: 110 g for chemical weighing
- Small spoon

Consumables

- Plastic weighing boats

Chemicals

- ~500 ml $18\text{M}\Omega_{\text{water}}$ (ultra-pure water)
- ~0.673 g Na_2SO_4

Procedure

- Using balance and plastic weighing boats, weigh 0.673 g of Na_2SO_4 and set aside for future use.
- Place empty 500 ml graduated bottle on balance and tare.
- Add 0.673 g of sodium sulfate to 500 ml bottle.
- Add ultra-pure water to the 500 ml mark of the bottle.
- Record total weight of solution after adding water and chemical.
- Mix solution to ensure sodium sulfate is fully dissolved.

Storage

No special storage requirements.

R5. BARIUM CHLORIDE STOCK SOLUTION

Recipe for ~1 M BaCl₂ in 0.05 M HCl in 500 ml

Equipment

- DTPA cleaned 1000 ml graduated glass cylinder
- DTPA cleaned 500 ml graduated glass bottle
- Magnetic stirrer
- Magnetic stir bar
- Balance maximum capacity: 4000 g for glassware weighing
- Balance maximum capacity: 110 g for chemical weighing
- 0.5 - 5 ml pipette
- Small spoon

Consumables

- Plastic weighing boats
- 0.5 - 5 ml pipette tips

Chemicals

- ~600 ml 18M Ω _{water} (ultra-pure water)
- ~123 g BaCl₂
- ~2.5 ml HCl

Safety: use proper PPE when handling hydrochloric acid; gloves, lab coat and safety glasses. Hydrochloric acid must be added in an exhaust hood.

Procedure

- Using balance and plastic weighing boats, weigh 123 g of BaCl₂ and set aside.
- Add 100 ml of ultra-pure water to 1000 ml graduated glass cylinder.
- Connect and turn on magnetic stirrer.
- Place magnetic stir bar in cylinder with water and place on magnetic stirrer. Make sure magnetic stir bar is working correctly.

- Add previously weighed 129 g of barium chloride to cylinder as magnetic stirrer is on.
- Let stir for 5 minutes.
- Remove cylinder from magnetic stirrer.
- Place cylinder in an exhaust hood.
- Add 2.5 ml of hydrochloric acid to solution.
- Remove cylinder from exhaust hood.
- Add ultra-pure water to the 500 ml mark in the graduated glass cylinder.
- Place cylinder on magnetic stirrer and let solution stir overnight.
- Remove cylinder from magnetic stirrer and transfer barium chloride stock solution to a 500 ml graduated glass bottle.
- Label bottle with what it is, date it was prepared and initials of preparer.

Storage

No special storage requirements.

R6. RECIPE FOR ULTRA-LOW SULFATE EXTRACTION METHOD

Equipment

- Balance maximum capacity: 110 g
- 1.5 ml centrifuge machine VWR Kinetic Energy 26 Joules Galaxy Mini Centrifuge
- Filtration device (Figure 7, main text)
- Vortex machine
- 20 - 200 μ l pipette
- 100 - 1000 μ l pipette
- 0.5 - 5 ml pipette
- Tweezers

Consumables

- 1.5 ml micro centrifuge tube (need two per replicate)
- 50 ml centrifuge tube (need one per replicate)
- 25 mm 0.2 μ m filter (one per replicate), Millipore Express Plus[®] Membrane (GPWP02500), filter material=polyether sulfone (PES) – Attention: PES is the only material that will not dissolve in the DTPA solution.
- 20 - 200 μ l pipette tips
- 100 - 1000 μ l pipette tips
- 0.5 - 5 ml pipette tips

Chemicals

- 18M Ω_{water} (ultra-pure water) in a small squeeze bottle
- 1 ml DTPA stock solution per replicate
- 40 ml SF-ASW solution per replicate
- 1 ml BaCl₂ stock solution per replicate
- Na₂SO₄ stock solution (concentration dependent)
- 1.5 ml acetone per replicate
- 120 μ l HCl per replicate
- 50 μ l BaCl₂ per replicate for re-precipitation

Safety: use proper PPE when handling hydrochloric acid; gloves, lab coat and safety glasses.

Hydrochloric acid and acetone must be added in an exhaust hood.

Procedure

- Add 40 ml of SF-ASW to each 50 ml centrifuge tube.
- Add fixed Na_2SO_4 stock solution amount to each replicate.
- Add 1 ml of BaCl_2 stock solution to each replicate.
- Vortex each sample and let sit overnight.
- Record weight for empty 1.5 centrifuge tube.
- Replicate by replicate, vortex 50 ml centrifuge tube with suspended barium sulfate precipitate.
- Arrange filtration device as shown in Figure 7 main text, placing filter, make sure filter is placed correctly (dull side up). Using a clamp, secure filtration device.
- Carefully pour suspended barium sulfate, try aiming the center of glassware.
- Using pump, filter suspended barium sulfate.
- Rinse inside of glassware three times using ultra-pure water in a small squeeze bottle. Ensure ultra-pure water covers every surface of glassware. Use pump as glassware is rinsed with ultra-pure water, since water will collect on glassware.
- Rinse thoroughly with ultra-pure water. It is important to rinse thoroughly at the bottom of container of filtration device to ensure that all barium sulfate gets stuck to the filter paper and is not clinging to the walls of the filtration device.
- Remove clamp from filtration device.
- Open 1.5 ml centrifuge tube and place close to filtration device.
- Remove carefully filter from filtration device using tweezers. Do not disturb barium sulfate precipitates when removing filter.
- Using gloved fingers carefully roll filter and place into 1.5 centrifuge tube (tube with pre-recorded weight).
- Close 1.5 ml centrifuge tube and set aside for future use.

- Add 1 ml of DTPA stock solution to 50 ml centrifuge tube that initially contained suspended barium sulfate and SF-ASW.
- Swirl DTPA through 50 ml centrifuge to dissolve into DTPA making sure to cover all surfaces.
- Open previously used 1.5 centrifuge tube with filter inside.
- Pipet DTPA into 1.5 ml centrifuge tube with filter. Ensure that the volume of DTPA solution added to the 1.5 ml tube completely covers the filter. Continue with this procedure with the rest of samples.
- Let samples dissolve overnight.
- Carefully remove filter from 1.5 ml centrifuge tube using tweezers and place in remaining 1.5 ml centrifuge tube for storage.
- To the 1.5 ml centrifuge tube with the dissolved barium sulfate solution, add 120 μ l of hydrochloric acid. Perform this step in exhaust hood with proper PPE.
- Next add 50 μ l of barium chloride stock solution to ensure excess barium in solution. Continue this process with the rest of samples.
- Let solution re-precipitate barium sulfate overnight.
- Vortex each 1.5 ml centrifuge sample for 5 seconds to assure solution is in suspension and ready to centrifuge.
- Place samples in 1.5 ml centrifuge machine.
- Centrifuge 10 minutes.
- Carefully pipet supernatant from centrifuge tube.
- Add 1.5 ml of ultra-pure water.
- Vortex 1.5 centrifuge tube.
- Centrifuge for 10 minutes.
- Carefully pipet supernatant from centrifuge tube.
- Add 1.5 ml of ultra-pure water.
- Vortex 1.5 centrifuge tube.
- Centrifuge for 10 minutes.
- Carefully pipet supernatant from centrifuge tube.
- Add 1.5 ml of acetone.

- Vortex 1.5 centrifuge tube.
- Centrifuge for 10 minutes.
- Carefully pipet supernatant from centrifuge tube.
- Place 1.5 ml centrifuge tube in a rack with lid open.
- Cover with a large sheet of aluminum foil without touching tubes.
- Let samples dry overnight.

VITA

Marisela Montelongo Griego was born in Cd. Juarez, Chihuahua, Mexico. After graduating from Escuela Preparatoria Federal por Cooperación 2/3 “El Chamizal” she entered The University of Texas at El Paso (UTEP). In May 2013, she graduated with a Bachelor of Science in Environmental Science with a concentration in Biology. After graduation, she became an intern at the Environmental Services Department at the City of El Paso.

In Spring 2015 she entered UTEP to pursue her Master’s of Science in Environmental Science. In her first semester, she was awarded with the UTEP-EPA Air Quality Internship where she had the opportunity to assist the Office of Resilience and Sustainability at the City of El Paso. She maintained a part-time Teaching Assistant position during her Master’s.

After completing her Master’s thesis, Marisela will pursue a Ph.D. in Environmental Science and Engineering at The University of Texas at El Paso.

Email: mariselamontelongo4@gmail.com

This thesis was typed by the author, Marisela Montelongo